LABORATORY AND FIELD PERFORMANCE OF COTTON CONTAINING CRY1AC, CRY1F, AND BOTH CRY1AC AND CRY1F (WIDESTRIKE®) AGAINST BEET ARMYWORM AND FALL ARMYWORM LARVAE (LEPIDOPTERA: NOCTUIDAE)

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Abstract

The efficacy of transgenic cotton genotypes containing Cry1Ac, Cry1F, and Cry1Ac stacked with Cry1F (WideStrike®, Dow Agrosciences, Indianapolis, IN) were investigated during 2001-2003 against the beet armyworm, *Spodoptera exigua* (Hübner) (=BAW), and the fall armyworm, *Spodoptera frugiperda* (J. E. Smith) (=FAW), in laboratory bioassays and small experimental field plots. In all experiments, cotton containing Cry1F was more toxic to BAW and FAW larvae compared to cotton containing only Cry1Ac. In the majority of experiments, the addition of Cry1Ac to the Cry1F genotype had no increased effect on efficacy and certain biological parameters against BAW and FAW larvae compared to cotton containing only Cry1F. Furthermore, the presence or absence of an additive, synergistic, or antagonistic effect between Cry1Ac and Cry1F was not observed in these field and laboratory experiments.

Key Words: cotton, Cry1 genes, transgenic cotton, beet armyworm, fall armyworm, *Spodoptera* spp.

Resumen

La eficacia de los genotipos de algodón transgénicos que tienen Cry1Ac, Cry1F, y Cry1Ac combinados con Cry1F (WideStrike®, Dow Agrosciences, Indianapolis, IN) fueron investigados durante 2001-2003 contra del gusano trozador (BAW), *Spodoptera exigua* (Hübner), y el gusano cogollero (FAW), *Spodoptera frugiperda* (J. E. Smith), en bioensayos de laboratorio y en experimentos en parcelas pequeñas de campo. En todos los experimentos, el algodón que tenia Cry1F fue el más tóxico a las larvas de BAW y de FAW en comparación al algodón que tenia solamente Cry1Ac. En la mayoría de los experimentos, la adición de Cry1Ac al genotipo Cry1F no tuvo un incremento sobre la eficacia y ciertas parámetros biológicos contra las larvas de BAW y FAW comparado al algodón que tenia solamente Cry1F. Además, no se observó la presencia o ausensia de un efecto aditivo, sinergistico o antagónico entre el Cry1Ac y Cry1F en estos experimentos de campo y de laboratorio.

Since the first Cry1Ac *Bacillus thuringiensis* Berliner (Bt) cotton variety was commercialized in 1996 (Bollgard®, Monsanto Ag. Co., St. Louis, MO), there have been numerous advancements for insect control with transgenic technology. Current and experimental cotton varieties can contain Cry1Ac alone or they can be stacked with Cry2Ab (Bollgard® II, Monsanto Ag. Co.) or Cry1F (WideStrike®, Dow Agrosciences, Indianapolis, IN). Furthermore, a novel exotoxin from *B. thuringiensis* also is currently in development (VipCot®, Syngenta Crop Protection, Greensboro, NC).

The beet armyworm, *Spodoptera exigua* (Hübner) (=BAW), is an occasional but serious pest of various vegetable and row crops in the mid-southern United States of America (=Mid-South). Compared to other North American armyworm species, knowledge of the ecology of this pest in the Mid-South is limited. This pest has no known photoperiod or temperature induced diapause mechanism (Kim & Kim 1997), and it is able to overwinter by continuous generations in southern Florida and Texas. Therefore, initial populations of beet armyworms found throughout the Mid-South are believed to be the result of immigration from those areas (Mitchell 1979; Hendricks et al. 1995). Populations in the Mid-South are typically found in cotton after July 1, with higher populations on various wild hosts in the fall months (Adamczyk et al. 2003). Although larval feeding on cotton is primarily concentrated on foliage, larvae can cause devastating losses in yield (Hardee & Herzog 1997; Adamczyk et al. 1998).

The fall armyworm, *Spodoptera frugiperda* (J. E. Smith) (=FAW), also is a destructive migratory pest of many crops in the Western Hemisphere (Sparks 1979; Young 1979). Like the BAW, this pest has the potential to damage both conventional cotton bolls and Bollgard® cotton bolls (Adamczyk et al. 1998).

Although certain lepidopterous pests of cotton are controlled by Bollgard® cotton [e.g., tobacco budworms and pink bollworms, *Pectinophora gossypiella* (Saunders)], the Cry1Ac δ-endotoxin in

Bollgard® cotton is ineffective for control of BAW and FAW (Adamczyk et al. 1998; Henneberry et al. 2001). Consequently, outbreaks of BAW and FAW on Bollgard® often need full application rates of foliar insecticide treatments to keep these populations below economic injury levels (Hood 1997; Smith 1997). Efficacy data for BAW and FAW feeding on Bollgard II® is mainly limited to laboratory bioassays and small experimental field plots. However, the addition of Cry2Ab along with Cry1Ac appears to have improved the efficacy of Bollgard II® against both BAW and FAW (Adamczyk et al. 2001; Stewart et al. 2001). The purpose of the study was to examine the efficacy of Cry1Ac, Cry1F, and Cry1Ac stacked with Cry1F against BAW and FAW in laboratory bioassays and small experimental field plots.

Materials and Methods

Field Plots

From 2001-2003, experimental transgenic cotton varieties (Dow Agrosciences, Indianapolis, IN) were planted in research plots near Elizabeth, MS under a yearly Experimental Use Permit (EUP) (Table 1). In 2001, cotton was planted on May 23 and plots consisted of 2 rows (1.0 m centers) × 10.67 m. In 2002 and 2003, cotton was planted on May 13 and June 10, respectively, and plots consisted of 4 rows (1.0 m centers) × 12.20 m. All plots were arranged in a randomized complete block design with each variety replicated 8 times (twice in each block). Plots were irrigated once in 2002 and twice in 2001 and 2003. Only insecticides not active on Lepidoptera were applied to all plots throughout the season as dictated by local management practices.

Insects

Colonies of fall armyworms (FAW) and beet armyworms (BAW) were established from local migratory populations found in the Mississippi Delta. In May 2001, larvae (ca. 500) of FAW were collected from whorl-stage field corn near Stoneville, MS. FAW egg masses (ca. 20) were collected in August 2003 from royal paulownia, *Paulownia tomentosa* (thumb.) (Sieb. & Zucc. ex Steud.). BAW larvae (ca. 500 each year) were collected from redroot pigweed, *Amaranthus retroflexus* L., in June 2001 and July 2002. Both species were reared for one complete generation in the laboratory as described by Adamczyk et al. (1998), and the subsequent generation was utilized in either bioassays or field inoculations.

Table 1. Experimental transgenic cotton genotypes evaluated in 2001-2003.

Genotype	Cry genes	Years evaluated
MXB-7 (Cry1Ac)	1Ac	2001, 2002
MXB-9 (Cry1F)	1F	2001, 2002
MXB-13 (Cry1Ac/Cry1F or Widestrike®)	1Ac and 1F	2001-2003
PSC355 (conventional cotton isoline)	None	2001-2003

Field Experiments

Inoculations of BAW egg masses to plants for all varieties were conducted in 2001 and 2002. In the laboratory, egg masses were deposited on nylon cloth placed on the top of adult rearing cages (3.79-liter cardboard containers). For each inoculation, an egg mass of equal size (ca. 200-300 eggs/2.54-cm^2 cloth sample) was stapled to the underside of a mature leaf in all plots. Egg masses were spaced ca. 0.5 m from each other. Each plot received a total of 42 egg masses on July 10-12, 2001 and 56 egg masses on August 1-2, 2002. Eight days after inoculations (DAI), BAW populations were estimated with a standard 1.2-m drop cloth placed in the center row per plot (3 samples/plot). All recovered larvae within a plot were placed in 232-ml plastic containers and transported to the laboratory and weighed within 1 h after arrival. Prior to analysis, the coefficient of variation for the mean weights and number of larvae among genotypes was substantially improved by a log transformation. Both mean weights and numbers of BAW were analyzed by REML-ANOVA and were separated according to Fisher's Protected LSD (Littell et al. 1996; PROC MIXED, SAS Institute 2001).

In 2001, a minimum of 15 leaves (i.e., egg masses)/plot that showed evidence of successfully hatched neonates were visually examined after 9 DAI for BAW damage. Leaf damage was estimated with a categorical rating scale where 0% indicated no leaf damage while evidence of leaf consumption was given a value of 10%, 25%, or 50%. Prior to analysis, damage ratings appeared to be normally distributed, and a square-root transformation did not improve the coefficient of variation significantly among genotypes. Therefore, no transformation on this categorical data set was needed. Mean damage ratings were analyzed by REML-ANOVA, and means were separated according to Fisher's Protected LSD (Littell et al. 1996, PROC MIXED, SAS Institute 2001).

Bioassays

First-position flower buds (=squares) containing various transgenes (Table 1) were assayed in 2001 for bioactivity against BAW and FAW neonates. Individual squares were placed into a

Tight-Fit Lid sealing Petri dish (50 × 9 mm, BD Falcon® #351006, VWR International). For BAW, 3 larvae were placed in a dish containing a single square (5 dishes/plot) for a total of 120 larvae/genotype. For FAW, a single larva was placed in a dish containing a single square (10 dishes/plot) for a total of 40 larvae/genotype. Four days after exposure (DAE), larvae were prodded with a camel-hair brush and considered alive if coordinated movement was observed. Percent survival of neonates was analyzed by REML-ANOVA, and means were separated according to Fisher's Protected LSD (Littell et al. 1996; PROC MIXED, SAS Institute 2001).

In 2002, terminal leaves containing various transgenes (Table 1) were assayed for bioactivity against BAW neonates. Individual leaves were placed into a Tight-Fit Lid sealing Petri dish (50 × 9 mm, BD Falcon® #351006, VWR International). Three larvae were placed in a dish containing a single terminal leaf (5 dishes/plot) for a total of 120 larvae/genotype. At 3, 6, and 8 DAE, larvae were prodded with a camel-hair brush and considered alive if coordinated movement was observed. Percent survival of neonates was analyzed by REML-ANOVA, and means were separated according to Fisher's Protected LSD (Littell et al. 1996; PROC MIXED, SAS Institute 2001).

In 2003, terminal leaves containing only the Cry1Ac/Cry1F genotype (Table 1) were assayed for bioactivity against FAW neonates. A single larva was placed in a dish containing a single square or single terminal leaf (20 dishes/plot) for a total of 160 larvae/genotype. At 4 and 7 DAE, larvae were prodded with a camel-hair brush and considered alive if coordinated movement was observed. Percent survival of neonates was analyzed by REML-ANOVA, and means were separated according to Fisher's Protected LSD (Littell et al. 1996; PROC MIXED, SAS Institute 2001).

Results and Discussion

Field Experiments

Experimental cotton genotypes had differential affects on the survival of BAW larvae (Table 2). In 2001, the mean number of BAW larvae was significantly reduced in plots containing Cry1F and Cry1F stacked with Cry1Ac compared to the plots containing Cry1Ac alone or conventional cotton (PSC 355). The mean number of BAW larvae found in plots containing only Cry1Ac was not significantly different from the mean number of larvae found in conventional cotton. Therefore, the addition of Cry1Ac to cotton containing Cry1F had no significant effect in reducing the number of BAW larvae found in plots containing both these transgenes. However, in 2002 all transgenic cotton genotypes had significantly reduced mean BAW larval numbers as compared to the mean number of BAW larvae found in conventional cotton. Unlike 2001, cotton containing only Cry1Ac had significantly reduced BAW larval numbers as compared to the mean number of BAW larvae found in conventional cotton. In an adjacent experiment, higher parasitism rates of larvae from *Cotesia marginiventris* (Cresson) were observed in 2002 compared to 2001 (Adamczyk & Hardee 2002). This may have contributed to the lower numbers of recovered larvae in all plots in 2002 than in 2001. Sublethal affects of Cry1Ac (Henneberry et al. 2001) combined with increased parasitism may have resulted in the increased BAW mortality found in the Cry1Ac genotype in 2002 as compared to 2001. Another possibility is that different agronomic conditions (e.g., higher nitrogen levels) may have increased the level of Cry1Ac expressed in the plants which translated into the increased efficacy against BAW found in 2002 (Pierce et al. 1999).

Table 2. Number of BAW larvae found on various transgenic genotypes 8 days after egg inoculations in 2001 and 2002.

Genotype	Mean number of larvae ± SEM	
	2001	2002
PSC 355	37.8 ± 13.16 a	17.8 ± 9.44 a
Cry1Ac	31.0 ± 10.73 a	5.3 ± 1.70 b
Cry1F	4.8 ± 1.11 b	1.5 ± 0.50 c
Cry1Ac/Cry1F	6.3 ± 1.75 b	3.0 ± 0.71 bc
df	3, 9	3, 9
F value	16.20	13.43
($P > F$) ANOVA	<0.001	0.001

Means in a column followed by the same letter are not significantly different ($\alpha = 0.05$); Fisher's Protected LSD option of PROC MIXED (Littell et al. 1996; SAS Institute 2001). Means were log-transformed prior to analysis.

BAW larvae collected from all transgenic cotton genotypes had significantly lower mean weights as compared to BAW larvae collected from conventional cotton in 2001 and 2002 (Table 3). In addition, mean weights of BAW larvae were significantly lower when collected on cotton containing Cry1F as compared to BAW larvae collected from cotton containing only Cry1Ac. As with BAW larval numbers (Table 2), mean weights of BAW larvae collected from cotton containing both Cry1Ac and Cry1F were not significantly different from mean weights of BAW larvae collected from cotton containing only Cry1F for both years.

Leaf damage caused by BAW larvae was significantly lower in all transgenic cotton genotypes compared to conventional cotton in 2001 (Table 4). As with BAW larval weights (Table 3), larval damage was significantly lower in cotton containing Cry1F compared to cotton containing only Cry1Ac. Furthermore, BAW larval damage was

TABLE 3. WEIGHT OF BAW LARVAE 8 DAYS AFTER EGG INOCULATIONS ON VARIOUS TRANSGENIC GENOTYPES IN 2001 AND 2002.

	Mean weight of larvae (mg) ± SEM			
Genotype	No. weighed	2001	No. weighed	2002
PSC 355	94	17.4 ± 4.07 a	45	7.4 ± 1.40 a
Cry1Ac	114	6.5 ± 0.71 b	38	2.6 ± 0.36 b
Cry1F	19	3.3 ± 0.21 c	16	0.6 ± 0.23 c
Cry1Ac/Cry1F	21	2.3 ± 0.27 c	19	1.2 ± 0.18 c
df		3, 12		3, 9
F value		43.28		34.93
(*P* > *F*) ANOVA		<0.001		<0.001

Means in a column followed by the same letter are not significantly different ($\alpha = 0.05$); Fisher's Protected LSD option of PROC MIXED (Littell et al. 1996; SAS Institute 2001). Means were log-transformed prior to analysis.

not significantly different among the genotypes with Cry1F alone and Cry1F stacked with Cry1Ac.

Bioassays

Similar trends in larval survival on the various cotton structures from the different genotypes (Table 1) were found in the laboratory. The addition of Cry1Ac to the Cry1F genotype did not significantly reduce BAW or FAW larval survivorship when fed cotton squares compared to squares containing only Cry1F (Table 5). For both BAW and FAW, only squares that contained Cry1F significantly decreased larval survivorship compared to conventional cotton. This same trend was observed for BAW neonates fed cotton terminal leaves (Table 6). Survival of FAW larvae in 2003 was significantly lower when fed squares and terminal leaves of cotton containing both Cry1Ac and Cry1F than conventional cotton (note that Cry1Ac and Cry1F alone genotypes were not tested in 2003) (Table 7).

The *Cry1F* transgene apparently contributed more to total toxicity than the *cry1Ac* transgene in the Cry1Ac/Cry1F genotype (=WideStrike®) against BAW and FAW larvae. Luo et al. (1999) used a diet bioassay containing purified Cry1Ac or Cry1F and showed that Cry1F was >10× more toxic than Cry1Ac against BAW and FAW larvae. In Bollgard II®, the *cry2Ab* transgene is expressed throughout the plant at much higher levels than Cry1Ac (Greenplate et al. 2003). However, on an equal dose basis, Cry2Ab is more toxic against the soybean looper, *Pseudoplusia includens* (Walker), than Cry1Ac, while Cry1Ac is more toxic against the bollworm, *H. zea* (Sims 1997). Therefore, differences between toxicity of *cry1Ac* and *Cry1F* transgenes to BAW and FAW could be due to a higher titer of Cry1F in the plant compared to Cry1Ac, inherent toxicity differences between the two transgenes, or possibly a combination of both. However, the presence or absence of an additive, synergistic, or antagonistic effect between Cry1Ac and Cry1F was not observed in

TABLE 4. LEAF DAMAGE CAUSED BY BAW LARVAE 9 DAYS AFTER EGG INOCULATIONS ON VARIOUS TRANSGENIC GENOTYPES IN 2001.

Genotype	No. evaluated	Mean % leaf damage ± SEM
PSC 355	91	39.0 ± 1.79 a
Cry1Ac	95	32.2 ± 3.21 b
Cry1F	74	17.4 ± 1.76 c
Cry1Ac/Cry1F	76	18.5 ± 4.06 c
df		3, 9
F value		31.65
(*P* > *F*) ANOVA		<0.001

Means in a column followed by the same letter are not significantly different ($\alpha = 0.05$); Fisher's Protected LSD option of PROC MIXED (Littell et al. 1996;SAS Institute 2001).

TABLE 5. SURVIVAL OF BAW AND FAW LARVAE AT 4 DAE WHEN FED COTTON SQUARES FROM VARIOUS TRANSGENIC GENOTYPES IN 2001.

	Mean % survival ± SEM	
Genotype	BAW	FAW
PSC 355	76.7 ± 6.78 a	52.5 ± 4.79 a
Cry1Ac	70.0 ± 4.88 a	55.0 ± 8.66 a
Cry1F	44.2 ± 8.06 b	20.0 ± 10.80 b
Cry1Ac/Cry1F	43.3 ± 7.77 b	25.0 ± 9.57 b
df	3, 21	3, 12
F value	9.95	4.32
(*P* > *F*) ANOVA	<0.001	0.028

Means in a column followed by the same letter are not significantly different ($\alpha = 0.05$); Fisher's Protected LSD option of PROC MIXED (Littell et al. 1996; SAS Institute 2001).

TABLE 6. SURVIVAL OF BAW NEONATES WHEN FED COTTON TERMINAL LEAVES FROM VARIOUS TRANSGENIC GENOTYPES IN 2002.

	Mean % survival ± SEM		
Genotype	3 DAE[1]	6 DAE	8 DAE
PSC 355	83.3 ± 0.06 a	81.7 ± 0.08 a	78.3 ± 0.09 a
Cry1Ac	90.0 ± 0.04 a	86.7 ± 0.05 a	78.3 ± 0.03 a
Cry1F	80.0 ± 0.05 a	56.7 ± 0.10 b	48.3 ± 0.10 b
Cry1Ac/Cry1F	80.0 ± 0.03 a	55.0 ± 0.02 b	53.3 ± 0.03 b
df	3, 9	3, 9	3, 9
F value	0.92	7.35	6.90
(*P* > *F*) ANOVA	0.468	0.010	0.010

Means in a column followed by the same letter are not significantly different (α = 0.05); Fisher's Protected LSD option of PROC MIXED (Littell et al. 1996; SAS Institute 2001).
[1]Days after exposure to leaves.

TABLE 7. SURVIVAL OF FAW NEONATES WHEN FED COTTON SQUARES AND TERMINAL LEAVES FROM VARIOUS TRANSGENIC GENOTYPES IN 2003.

	Mean % survival ± SEM			
	Squares		Leaves	
Genotype	4 DAE[1]	7 DAE	4 DAE	7 DAE
PSC 355	61.9 ± 3.44 a	50.0 ± 4.79 a	75.0 ± 7.14 a	71.9 ± 5.34 a
Cry1Ac/Cry1F	25.0 ± 3.54 b	8.1 ± 1.57 b	65.0 ± 5.68 b	25.6 ± 3.71 b
df	1, 6	1, 3	1, 3	1, 3
F value	55.84	147.99	19.20	50.70
(*P* > *F*) ANOVA	<0.001	0.001	0.022	0.006

Means in a column followed by the same letter are not significantly different (α = 0.05); Fisher's Protected LSD option of PROC MIXED (Littell et al. 1996; SAS Institute 2001).
[1]Days after exposure to squares or leaves.

these field and laboratory experiments. Insuring that both transgenes provide dual protection against key lepidopterous pests is crucial for resistance management to transgenic crops (Gould & Tabashnik 1998).

DISCLAIMER

Mention of trade names or commercial products in this publication is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the U.S. Department of Agriculture.

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FIELD AND LABORATORY PERFORMANCE OF NOVEL INSECTICIDES AGAINST ARMYWORMS (LEPIDOPTERA: NOCTUIDAE)

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ABSTRACT

Beet armyworm, *Spodoptera exigua* (Hübner), and fall armyworm, *Spodoptera frugiperda* (J. E. Smith), are occasional pests of cotton, *Gossypium hirsutum* (L.), and soybean, *Glycine max* (L.) Merrill. These insects can be difficult to control due to insecticide resistance and larval behavior on plants. The objectives of these studies were to determine the efficacy of selected insecticides against native infestations of beet armyworm in cotton and soybean and to generate baseline dose-mortality responses for beet armyworm and fall armyworm adults to indoxacarb and pyridalyl in the adult vial test. Indoxacarb, pyridalyl, spinosad, methoxyfenozide, and emamectin benzoate controlled beet armyworm infestations up to 10 d after treatment compared to the non-treated control. Thiodicarb reduced beet armyworm densities up to three d after treatment. The LC_{50} values of indoxacarb and pyridalyl for beet armyworm and fall armyworm exceeded the highest concentrations tested (100-200 µg/vial) in the adult vial test. Dose-mortality values of indoxacarb and pyridalyl were higher than discriminating concentrations of cypermethrin, methomyl, profenofos, and endosulfan used in the adult vial test for monitoring tobacco budworm, *Heliothis virescens* (F.), and bollworm, *Helicoverpa zea* (Boddie), susceptibility in Louisiana and Texas. These results indicate that the adult vial test may not be the most efficient test method for indoxacarb and pyridalyl in insecticide susceptibility monitoring programs.

Key Words: Beet armyworm, *Spodoptera exigua*, fall armyworm, *Spodoptera frugiperda*, cotton, *Gossypium hirsutum*, soybean, *Glycine max*, insecticides.

RESUMEN

El gusano trozador, *Spodoptera exigua* (Hübner), y el gusano cogollero, *Spodoptera frugiperda* (J. E. Smith), son plagas ocasionales de algodón, *Gossypium hirsutum* (L.), y soya, *Glycine max* (L.) Merrill. Estos insectos son dificiles de controlar debido a la resistencia hacia el insecticida y el comportamiento de las larvas en las plantas. Los objectivos de estos estudios fueron para determinar la eficacia de los insecticidas seleccionados contra las infestaciones nativas del gusano trozador en algodón y soya y para obtener respuestas de dosis-mortalidad basicas para los adultos de gusano trozador y de gusano cogollero al indoxacarb y pyridalyl en pruebas de adultos en viales de prueba. El indoxacarb, pyridalyl, spinosad, methoxyfenozide, y emamectin benzoate controlaron las infestaciones del gusano trozador hasta 10 dias después del tratamiento comparado al control sin tratamiento. El Thiodicarb redujó las densidades del gusano trozador hasta 3 dias después del tratamiento. Los valores del CL_{50} de indoxacarb y pyridalyl para el gusano trozador y el gusano cogollero excedieron las concentraciones mas altas probadas (100-200 µg/vial) en la prueba de los adultos en viales de prueba. Los valores de dosis-mortalidad de indoxacarb y pyridalyl fueron mas altas que las concentraciones discriminantes del cypermethrin, methomyl, profenofos, y endosulfan usados en pruebas de adultos en viales para monitorear la susceptibilidad del gusano del brote de tobaco, *Heliothis virescens* (F.), y el gusano del elote del maíz, *Helicoverpa zea* (Boddie), en Louisiana y Texas. Estos resultados indican que la prueba de los adultos en viales posiblemente no es el método de prueba más eficaz para indoxacarb y pyridalyl en programas para monitorear la susceptibilidad de insecticidas.

Beet armyworm, *Spodoptera exigua* (Hübner), and fall armyworm, *Spodoptera frugiperda* (J. E. Smith), are occasional pests of cotton, *Gossypium hirsutum* (L.), and soybean, *Glycine max* (L.) Merrill, in the mid-southern and southeastern United States. Beet armyworm larvae feed primarily on foliage in cotton (Smith 1989, Leser et al. 1996) and soybean (Baldwin 1994). Beet armyworm can be difficult to control, and for many years the only effective insecticides were thiodicarb and chlorpyrifos. Their performance has varied considerably against beet armyworm in the Mid-South and southeastern United States. Thiodicarb and chlorpyrifos provided >65% control of beet armyworm

larvae in cotton in Texas (Smith 1985). In South Carolina, thiodicarb also provided 90% control of beet armyworm in cotton (Sullivan et al. 1991). Reed et al. (1994) reported <50% control of beet armyworm with both thiodicarb and chlorpyrifos in Mississippi cotton. In Louisiana, control of beet armyworm with thiodicarb and chlorpyrifos in cotton has been inconsistent (Burris et al. 1994; Graves et al. 1995; Mascarenhas et al. 1996). Spinosad (Dow Agrosciences, Indianapolis, IN), indoxacarb (E. I. DuPont de Nemours and Co., Wilmington, DE), and pyridalyl (Valent USA Corp., Walnut Creek, CA) are novel compounds that have demonstrated efficacy against many lepidopteran pests of cotton and soybean.

Fall armyworm larvae feed primarily on soybean foliage and are readily exposed to foliar insecticide applications (Baldwin 1994). In cotton, early instar fall armyworms occur in the lower portion of the plant canopy and feed on foliage (Ali et al. 1989, 1990). Therefore, control of early instar fall armyworms in cotton can be difficult because foliar insecticide applications generally do not penetrate the canopy sufficiently to reach the larvae. Older larvae move within the plant canopy to fruiting structures (Ali et al. 1990). These larger larvae feed inside fruiting structures which minimize their exposure to foliar insecticide applications. Fall armyworm larvae also become more tolerant to insecticides as larval size increases (Yu 1983; Mink & Luttrell 1989) making control even more difficult to achieve.

Insecticide resistance in key insect pests has become a significant problem in crop production. Surveying insect populations for changes in susceptibility to insecticides is an integral component of insecticide resistance management. Monitoring efforts should be initiated before a compound is widely used and while the frequency of resistant individuals is low (ffrench-Constant & Roush 1990). Determining the range of initial resistance frequencies among insect populations facilitates early detection of changes in susceptibility to an insecticide. Therefore, early establishment of resistance baselines are critical for successful implementation of insecticide resistance management strategies before field control failures become widespread. Baseline responses for laboratory and field strains of insects to novel compounds should be established to develop discriminating concentrations for monitoring programs and for historical reference values. Numerous states have implemented insecticide resistance monitoring programs for bollworm, *Helicoverpa zea* (Boddie), and tobacco budworm, *Heliothis virescens* (F.), in cotton. However, coordinated insecticide resistance monitoring programs in cotton have not been developed for beet armyworm and fall armyworm in the United States due to the sporadic occurrence of these pests in the Mid-South and southeastern United States.

The objectives of these studies were to evaluate the efficacy of selected insecticides against native infestations of beet armyworm in cotton and soybean, and to generate baseline dose-mortality responses for beet armyworm and fall armyworm adults to indoxacarb and pyridalyl in the adult vial test. These data will support insecticide recommendations and provide reference dose-mortality data for future monitoring programs.

Materials and Methods

Field Experiments

Field trials were conducted during 1998 and 2000 at the LSU Ag Center Macon Ridge Research Station (Franklin Parish, LA). Trials 1998 and 2000-B were conducted in cotton, while trial 2000-A was conducted in soybean. Plots were planted to the cotton varieties 'Stoneville LA 887' (Stoneville Pedigree Seed Co., Memphis, TN) and 'Phytogen 355' (Dow AgroSciences LLC, Indianapolis, IN) in 1998 and trial 2000-B, respectively. The soybean variety 'Pioneer 9631' (Pioneer Hi-Bred International, Inc., Des Moines, IA) was used in trial 2000-A. Plots were planted on 11 June 1998, 30 May in trial 2000-A, and on 28 June in trial 2000-B. Plots consisted of four rows on 1-m centers and 15.2 m long. Treatments were arranged in a randomized complete block design with four replications. Cultural practices recommended by the LSU AgCenter were followed to maintain plots in a consistent manner within each trial.

Insecticide treatments included the following: emamectin benzoate (Denim 0.16 Emulsifiable Concentrate (EC), 2.15% ai wt/wt, Syngenta Crop Protection, Greensboro, NC), indoxacarb (Steward 1.25 Suspension Concentrate (SC), 14.5% ai wt/wt, E. I. Du Pont de Nemours and Company, Wilmington, DE), methoxyfenozide (Intrepid 80 Wettable Powder (WP), 80% ai wt/wt, Dow AgroSciences LLC, Indianapolis, IN), pyridalyl (S-1812 4EC, 45% ai wt/wt, Valent USA Corporation, Walnut Creek, CA), Spinosad (Tracer 4SC, Dow AgroSciences LLC, Indianapolis, IN), and thiodicarb (Larvin 3.2 Flowable (F), 34% ai wt/wt, Bayer CropScience, Research Triangle Park, NC).

In 1998, treatments were applied on 14 and 17 August with a high-clearance sprayer and a CO_2-charged spray system calibrated to deliver 56.1 L per ha through TX-8 hollow cone nozzles (Spraying Systems Company, Wheaton, IL) (two per row) at 338 kPa. Treatments were applied on 11 and 14 Aug in trial 2000-A and trial 2000-B, respectively, with a high-clearance sprayer and a CO_2-charged spray system calibrated to deliver 56.1 L per ha through TX-8 hollow cone nozzles (two per row) at 359 kPa.

Treatment efficacy was determined 10 d after treatment (DAT), three and seven DAT, and two and seven DAT, respectively, in trials 1998, 2000-

A, and 2000-B. Larval density data were collected with a standard (38.1 cm) sweep net (25 sweeps per plot). Data for each trial were subjected to analysis of variance procedures and means separated according to Fisher's Protected Least Significant Difference (SAS Institute, 1990).

Laboratory Experiments

Insects tested were obtained from susceptible laboratory colonies maintained at the Louisiana State University Department of Entomology, Baton Rouge, LA. The beet armyworm colony was obtained from Ecogen, Inc. (Langhorne, PA) during 1994. This colony was originally established at the USDA-ARS Southern Insect Management Laboratory at Stoneville, MS before 1983. The fall armyworm colony was established in 1997 from collections in field corn, *Zea mays* L., and supplemented with additional individuals collected from field corn in 1999.

Larvae were fed an artificial wheat-germ and soybean protein diet described by King & Hartley (1985). Rearing conditions consisted of a 14:10 light-dark photoperiod, 23.9 to 29.4°C, and 80% relative humidity.

Samples of technical grade indoxacarb (E.I. DuPont de Nemours and Company, Wilmington, DE), pyridalyl (Valent USA Corporation, Walnut Creek, CA), spinosad (Dow AgroSciences, Indianapolis, IN), and cypermethrin (Chem Service, West Chester, PA) were used in adult vial tests. Procedures similar to those described by Plapp et al. (1987) for the adult vial test were used to evaluate the toxicity of indoxacarb, pyridalyl, and spinosad to beet armyworm and fall armyworm. Stock solutions of each compound were developed by dissolving technical grade insecticide in acetone. Dilutions from each stock solution were used to yield the desired concentrations. The interior surface of 20-ml scintillation vials was coated with insecticide by pipetting 0.5 ml of the appropriate insecticide solution into the vials. These vials were placed on a modified hot dog roller (heating element disconnected) until all of the acetone had evaporated. Vials were stored in a dark environment at ambient temperature (approximately 23.9°C) no longer than 21 d before being used in assays. All assays were conducted at ambient temperature (approximately 23.9°C). Washed (clean) non-treated vials were used as controls. Previous tests (J. B. Graves & B. R. Leonard, unpublished data) indicated no differences in mortality between washed non-treated vials and vials treated with acetone only.

Prior to testing, insects were segregated by sex based on dimorphic pupal characters. Pupae were placed into 3.78-L cardboard cartons containing a thin layer of vermiculite on the bottom. Newly eclosed adults were removed daily and placed into polypropylene cages (29.97 × 29.97 × 29.97 cm) (BugDorm, Megaview Science Education Services CO. Ltd., Taichung, Taiwan). Male and female moths were held in separate cages for ca. 24 h and provided 10% sugar water as a food source. Moths were placed into insecticide treated vials or control vials (1 moth per vial) and mortality was determined after 24 h of exposure. Moths were considered dead if they were incapable of sustained flight for at least 1.0 meter. The number and range of concentrations for each colony and compound combination are detailed in Table 1. Data were corrected for mortality in control vials (Abbott 1925) and analyzed by probit analysis with Polo PC (LeOra Software, Berkeley, CA). Differences were considered to be significant based upon non-overlap of the 95% confidence limits. Separate data analyses were conducted for males and females of the respective insect species for each compound. Data for males and females of the respective insect species for individual compounds were pooled when no significant differences were detected between sexes or LC_{50} or LC_{90} values exceeded the highest concentration tested. Data from adult vial tests are reported as µg of insecticide per vial.

TABLE 1. RANGE OF CONCENTRATIONS OF INDOXACARB, PYRIDALYL, SPINOSAD, AND CYPERMETHRIN TESTED AGAINST LABORATORY COLONIES OF INSECTS IN THE ADULT VIAL TEST.

Compound	Insect species	No. of concentrations	Concentration range (µg/vial)
Indoxacarb	Beet armyworm	7	10-200
	Fall armyworm	4	25-100
Pyridalyl	Beet armyworm	7	10-200
	Fall armyworm	4	25-100
Spinosad	Beet armyworm	15	1-100
	Fall armyworm	10	5-100
Cypermethrin	Beet armyworm	7	0.5-100
	Fall armyworm	5	5-100

Results and Discussion

Field Experiments

In trial 1998 (cotton), all insecticide treatments significantly reduced beet armyworm densities compared to the non-treated control at 10 DAT (Table 2). Beet armyworm densities were reduced by 5.6-fold, 5.7-fold, and 21.2-fold in plots treated with spinosad, indoxacarb, or pyridalyl, respectively, compared to those observed in the non-treated plots. In trial 2000-A (soybean), all insecticide treated plots had significantly lower densities of beet armyworm larvae compared to the non-treated plots at three DAT (Table 3). Plots treated with methoxyfenozide or emamectin benzoate had significantly fewer larvae compared to plots treated with indoxacarb, spinosad, or thiodicarb. Beet armyworm densities were 5.5-fold, 6.5-fold, and 17.9-fold lower in the indoxacarb, emamectin benzoate, and methoxyfenozide treated plots, respectively, compared to beet armyworm densities in the non-treated plots. At seven DAT, all insecticide treatments, except thiodicarb, significantly reduced beet armyworm densities compared to those observed in the non-treated control. In treated plots spinosad reduced beet armyworm densities 2.6-fold and indoxacarb reduced beet armyworms 4.8-fold compared to non-treated plots. No larvae were collected in plots treated with methoxyfenozide. In trial 2000-B (cotton), all insecticide treatments significantly reduced beet armyworm densities compared to those observed in the non-treated control at two DAT (Table 4). Plots treated with indoxacarb, spinosad, or emamectin benzoate had significantly fewer larvae than plots treated with methoxyfenozide. Beet armyworm densities in the spinosad and indoxacarb treated plots were 10.5-fold and 6.5-fold lower, respectively, compared to those in the non-treated plots. At seven DAT, all insecticide treatments significantly reduced larval densities compared to the non-treated control. Beet armyworm densities in the spinosad, indoxacarb, and methoxyfenozide treated plots were 30.2- fold, 11.9-fold, and 49.1-fold lower, respectively, compared to those in the non-treated plots.

Results of these studies are similar to those from Fitzpatrick et al. (1996); Terán-Vargas et al. (1997); Gore et al. (1999); Torrey et al. (1999) in which indoxacarb, spinosad, methoxyfenozide, and emamectin benzoate provided excellent control of beet armyworm infestations. Thiodicarb significantly reduced beet armyworm larval densities compared to the non-treated control at three DAT. At seven DAT, however, larval densities in the thiodicarb treated plots were not significantly different from those in the non-treated plots. These results for thiodicarb are similar to those reported by Mascarenhas et al. (1996) in which the performance of thiodicarb was inconsistent. Thiodicarb is no longer recommended for beet armyworm control in cotton in Mississippi and Louisiana (Bagwell et al. 2003; Layton 2004). The inconsistent performance of thiodicarb in these studies further supports its removal from insecticide recommendations for control of beet armyworm in cotton in Louisiana. Thiodicarb is recommended for use in soybeans against beet armyworm in Mississippi and Louisiana (Anonymous 2003; Baldwin et al. 2003) and for use in cotton and soybeans against beet armyworm in Arkansas (Johnson et al. 2002; Lorenz et al. 2002).

Laboratory Experiments

The LC_{50} values of indoxacarb and pyridalyl exceeded the highest concentration tested (200 µg per vial for indoxacarb and pyridalyl) for beet armyworm adults (Table 5). The LC_{50} values of spinosad (45.6 µg per vial) and cypermethrin (37.1 µg per vial) were not significantly different from each other. The LC_{90} values of indoxacarb, pyridalyl, spinosad, and cypermethrin for beet armyworm adults exceeded the highest concentrations tested

Table 2. Efficacy of selected insecticides against beet armyworm in cotton, 1998.

		No. beet armyworm larvae per 25 sweeps
Treatment	Rate per ha (kg AI)	10 DAT (± SE)
Indoxacarb	0.101	6.3 b ± 0.9
Pyridalyl	0.14	1.8 b ± 1.7
Spinosad	0.073	6.8 b ± 1.6
Emamectin Benzoate	0.011	4.0 b ± 3.3
Non-Treated	—	38.3 a ± 5.2
F		87.1
df		4,12
$P > F$		<0.01

Means followed by a common letter are not significantly different ($P \leq 0.05$ Fisher's Protected Least Significant Difference).

TABLE 3. EFFICACY OF SELECTED INSECTICIDES AGAINST BEET ARMYWORM IN SOYBEAN (TRIAL 2000-A).

Treatment	Rate per ha (kg AI)	No. beet armyworm larvae per 25 sweeps	
		3 DAT (± SE)	7 DAT (± SE)
Indoxacarb	0.101	5.2 bc ± 3.0	2.4 bc ± 1.5
Spinosad	0.045	8.0 bc ± 1.9	4.4 b ± 2.1
Methoxyfenozide	0.224	1.6 c ± 1.5	0.0 c ± 0.0
Thiodicarb	0.504	12.2 b ± 10.3	8.4 a ± 4.1
Emamectin Benzoate	0.011	4.4 c ± 2.1	2.2 bc ± 1.5
Non-Treated	—	28.6 a ± 11.1	11.4 a ± 6.2
F		14.1	10.3
df		5,20	5,20
P > *F*		<0.01	<0.01

Means within columns followed by a common letter are not significantly different ($P \geq 0.05$ Fisher's Protected Least Significant Difference).

(200 µg per vial for indoxacarb and pyridalyl, 100 µg per vial for spinosad and cypermethrin). The LC_{50} values of indoxacarb and pyridalyl for fall armyworm adults exceeded 100 µg per vial (highest concentration tested) (Table 6). The LC_{50} value of cypermethrin (31.0 µg per vial) was significantly lower than that of spinosad (69.3 µg per vial) for fall armyworm adults. The LC_{90} values of indoxacarb, pyridalyl, spinosad, and cypermethrin for fall armyworm adults exceeded 100 µg per vial (highest concentration tested).

In these studies, the LC_{50} values of indoxacarb and pyridalyl for beet armyworm and fall armyworm from laboratory colonies exceeded 100 µg per vial. These values were significantly higher compared to the discriminating concentrations of cypermethrin (5-10 µg per vial) (Plapp et al. 1987; Graves et al. 1989), methomyl (2.5-10 µg per vial), profenofos (10-40 µg per vial), and endosulfan (3-10 µg per vial) (Kanga et al. 1995; Graves et al. 1994) for tobacco budworm and bollworm. Andaloro et al. (2000) reported LC_{50} values >100 ppm for bollworm, tobacco budworm, and beet armyworm larvae exposed to glass surfaces treated with indoxacarb indicating that contact exposure to residues is not a primary route of intoxication for indoxacarb. Additionally, Wing et al. (2000) reported that indoxacarb is inactive and is metabolically activated into toxic metabolites. These metabolites are extremely active and block sodium channels in the insect nervous system. Information regarding the route of intoxication of pyridalyl has not been released.

These data compare the relative toxicity of indoxacarb and pyridalyl to that of other common insecticides against two *Spodoptera* species and comprise initial efforts to develop baseline data. These data also demonstrate that the adult vial test is not an efficient test procedure for use with indoxacarb and pyridalyl in resistance monitoring efforts, as opposed to pyrethroids and spinosad, which generally perform well in the adult vial test. These studies indicate that discriminating concentrations for indoxacarb and pyridalyl for use in the adult vial test would be extremely high. Coordinated resistance monitoring efforts

TABLE 4. EFFICACY OF SELECTED INSECTICIDES AGAINST BEET ARMYWORM IN COTTON (TRIAL 2000-B).

Treatment	Rate per ha (kg AI)	No. beet armyworm larvae per 25 sweeps	
		2 DAT (± SE)	7 DAT (± SE)
Indoxacarb	0.101	6.5 c ± 3.4	3.3 b ± 3.3
Spinosad	0.101	4.0 c ± 3.6	1.3 b ± 1.3
Methoxyfenozide	0.168	23.5 b ± 8.3	0.8 b ± 1.0
Emamectin Benzoate	0.008	8.0 c ± 1.6	5.8b ± 2.5
Non-Treated	—	42.0 a ± 18.3	39.3 a ± 10.5
F		13.2	43.1
df		4,12	4,12
P > *F*		<0.01	<0.01

Means within columns followed by a common letter are not significantly different ($P \geq 0.05$ Fisher's Protected Least Significant Difference).

TABLE 5. RESPONSES OF LABORATORY REARED BEET ARMYWORM ADULTS TO INDOXACARB, PYRIDALYL, SPINOSAD, AND CYPERMETHRIN IN THE ADULT VIAL TEST.

	N	Slope ± SE	LC_{50}	95% C.L.	LC_{90}	95% C.L.	χ^2, *df*	Regression equations
Indoxacarb	350	0.72 ± 0.22	>200[1]	NA[2]	>200[1]	NA[2]	4.08,5	Y = 0.72x + -0.21
Pyridalyl	350	0.10 ± 0.25	>200[3]	NA[2]	>200[3]	NA[2]	21.15,5[6]	Y = 0.10x + -1.87
Spinosad	893	1.87 ± 0.17	45.6	39.9-52.5	>100[4]	NA[2]	10.06,13[6]	Y = 1.87x + -3.11
Cypermethrin	176	1.14 ± 0.21	37.1	17.7-124.5	>100[5]	NA[2]	8.52,5	Y = 1.14x + -1.79

Concentrations expressed in µg insecticide per vial.
[1]Values exceeded 200 µg per vial (highest concentration tested), 200 µg per vial concentration resulted in 39.0% mortality.
[2]Confidence limits could not be calculated.
[3]Values exceeded 200 µg per vial (highest concentration tested), 200 µg per vial concentration resulted in 16.0% mortality.
[4]Values exceeded 100 µg per vial (highest concentration tested), 100 µg per vial concentration resulted in 68.3% mortality.
[5]Values exceeded 100 µg per vial (highest concentration tested), 100 µg per vial concentration resulted in 65.0% mortality.
[6]Significant χ^2 ($P = 0.05$).

TABLE 6. RESPONSES OF LABORATORY REARED FALL ARMYWORM ADULTS TO INDOXACARB, PYRIDALYL, SPINOSAD, AND CYPERMETHRIN IN THE ADULT VIAL TEST.

	N	Slope ± SE	LC_{50}	95% C.L.	LC_{90}	95% C.L.	χ^2, *df*	Regression equations
Indoxacarb	329	0.63 ± 0.39	>100[1]	NA[2]	>100[1]	NA[2]	5.99,2	Y = 0.63x + -2.10
Pyridalyl	166	2.26 ± 0.72	>100[3]	NA[2]	>100[3]	NA[2]	3.57,2	Y = 2.26x + -5.17
Spinosad	859	1.70 ± 0.22	69.3	44.8-134.0	NA[4]	NA[2]	35.79,8[6]	Y = 1.70x + -3.13
Cypermethrin	174	2.09 ± 0.35	31.0	24.6-42.4	NA[5]	NA[2]	2.45,3	Y = 2.09x + -3.11

Concentrations expressed in µg insecticide per vial.
[1]Values exceeded 100 µg per vial (highest concentration tested), 100 µg per vial concentration resulted in 27.3% mortality.
[2]Confidence limits could not be calculated.
[3]Values exceeded 100 µg per vial (highest concentration tested), 100 µg per vial concentration resulted in 25.7% mortality.
[4]Values exceeded 100 µg per vial (highest concentration tested), 100 µg per vial concentration resulted in 70.0% mortality.
[5]Values exceeded 100 µg per vial (highest concentration tested), 100 µg per vial concentration resulted in 88.9% mortality.
[6]Significant χ^2 ($P = 0.05$).

generally test hundreds to thousands of insects of a particular species annually. The high discriminating concentrations of indoxacarb and pyridalyl in the adult vial test would dramatically increase the cost of monitoring efforts.

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BEHAVIOR AND DISTRIBUTION OF THE TWO FALL ARMYWORM HOST STRAINS IN FLORIDA

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ABSTRACT

Fall armyworm is a significant agricultural pest in the United States, affecting most notably sweet corn and turf grass. While infesting much of North America, fall armyworms invading the eastern United States arise from annual migrations of populations wintering in southern Florida. It has long been noted that this seasonal geographical localization represents an opportunity for controlling this pest prior to its annual migration. However, such efforts have been hindered by the presence of two genetically distinct but morphologically identical strains that differ physiologically and behaviorally. The biology of the host strains is poorly understood and this lack of knowledge precludes accurate predictions of fall armyworm population behavior in the field. This paper reviews recent studies examining strain behavior and discusses the potential relevance of these results to the development of effective regional management strategies that can be used proactively to mitigate the economic impact of this pest.

Key Words: *Spodoptera frugiperda*, area wide management, corn, turf grass.

RESUMEN

El gusano cogollero es una plaga significativa para la agricultura de los Estados Unidos, que afecta mas notablemente el maíz dulce y pastos. Mientras que infestan la mayor parte de America del Norte, los gusanos cogolleros que invaden el este de los Estados Unidos provienen de las emigraciones anuales de poblaciones que pasan el inverno en el sur de Florida. Por un largo tiempo se ha notado que esta localización geográfica por estaciones climáticas representa una oportunidad para controlar esta plaga antes de la emigración anual. Sin embargo, estos esfuerzos han sido paralizados por la presencia de dos cepas genéticamente distintas pero morfológicamente identicas con una fisiología y un comportamiento diferentes. La biología de las cepas con respecto hospederos es pobremente conocida y es esta falta de conocimiento la que impide la predicción precisa del comportamiento de la población del gusano cogollero en el campo. Este articulo revisa los estudios recientes que han estudiado el comportamiento de las cepas y discute el potencial pertinente de estos resultados al desarrollo de estrategias de manejo regionales efectivos que pueden ser usados pro-activamente para mitigar el impacto económico de esta plaga.

The fall armyworm, *Spodoptera frugiperda* (J. E. Smith) is a periodic and significant economic pest in most of the continental United States, capable of causing substantial losses in maize, sorghum, forage grasses, turf grasses, rice, cotton, and peanut production (Luginbill 1928; Sparks 1979). Because fall armyworm do not survive conditions of prolonged freezing, most of the infestations in the continental U.S. derive from annual migrations of populations that over winter in southern Florida and southern Texas (Barfield et al. 1980). This localization of winter populations theoretically provides an opportunity to dramatically reduce the migratory population, as previously noted in a quote from E. F. Knipling: "The fall armyworm could serve as a model species for developing the concept of managing highly mobile pests by an organized attack on populations at a strategic time and place for the purpose of protecting crops in other and perhaps much larger areas at some later time in the seasonal cycle" (Knipling 1980). Unfortunately, the biological information necessary to develop an area-wide management strategy for this pest has been slow in coming, in large part because two morphologically identical but physiologically distinct host strains have complicated efforts to understand and predict fall armyworm behavior in the field. This paper reviews recent studies describing new methods of strain identification that greatly enhance our capacity to investigate and understand fall armyworm population biology. Preliminary results suggest that at least one strain, whose primary target is corn, might be particularly amenable to a regional management program.

Characteristics of the Two Host Strains

The existence of two strains was originally postulated after comparisons of electrophoretic pro-

tein variants from the wild identified genetically distinct subpopulations that were preferentially associated with either large grasses (designated corn-strain), such as corn and sorghum, or smaller grasses (designated rice-strain), such as rice and bermudagrass (Pashley 1986; Pashley 1988a; Pashley et al. 1985; Pashley et al. 1987a). This host plant specificity reflects nutritional adaptation, as rice-strain larvae feeding on corn displayed a slower rate of weight gain, longer developmental time, lower pupal weight, and reduced survival than when reared on bermudagrass (Pashley 1988b; Pashley et al. 1995; Veenstra et al. 1995). Whitford et al. (1988) also reported reductions in larval and pupal weight but did not observe differences in developmental time or survival. The effect on larval growth rate correlated with higher levels of mixed-function oxidase (an enzyme family involved in detoxification pathways) in the corn-strain. In contrast, the same set of studies showed that rearing corn-strain larvae on rice or bermudagrass had no consistent negative effect on larval development or fitness.

However, the observed nutritional variations are unlikely to completely account for the plant host bias exhibited by the strains. In behavioral preference tests, first instars of both strains strongly preferred corn to turf grass (Pashley et al. 1995; unpublished data). In addition, although both strains developed equally when reared on rice or turf grass, corn-strain larvae were rarely found on these plants in the field, present in only 3% of larvae collected from wild grass (McMichael & Prowell 1999). In contrast, and despite their adaptation to rice and bermudagrass, rice-strain larvae made up as much as 16% of the samples collected from corn plants. These results suggest that habitat specificity in strain distribution is probably due to adult behavior, the most obvious candidate being ovipositional host choice. However, because inconsistent results were obtained in the one major study testing this possibility, the biological basis for the plant host bias exhibited by the two strains remains unexplained (Whitford et al. 1988).

An important consideration for the management of this pest is that differences were found in the response of the two strains to chemical and biological agents. Rice-strain larvae were more susceptible than the corn-strain to several insecticides, including diazinon and carbaryl, while the reverse was true for carbofuran (Adamczyk et al. 1997; Pashley et al. 1987b). Similarly, the rice-strain was more susceptible than the corn-strain to transgenic *Bacillus thuringiensis* Berliner (Bt) cotton (Adamczyk et al. 1997). In addition, some bermudagrass cultivars bred for fall armyworm resistance showed differential effectiveness with respect to the two strains, with rice-strain larvae generally able to gain more weight and consume more plant material than their corn-strain counterparts (Jamjanya et al. 1990; Leuck et al. 1968; Lynch et al. 1983; Pashley et al. 1987a; Quisenberry & Whitford 1988). Clearly strain-identity must be taken into consideration when evaluating the effectiveness of new insecticides and "resistant" plant cultivars.

DNA Markers of Strain Identity

The fall armyworm strains are morphologically identical, making an unambiguous determination of strain identity difficult and largely limited to molecular methods. Restriction Fragment Length Polymorphisms (RFLPs) were identified in genomic DNA and formed patterns that could be segregated into two distinct groups generally consistent with the rice-strain and corn-strain populations derived from allozyme comparisons (Lu et al. 1992). Similarly, dendrograms produced by amplified fragment-length polymorphism analysis revealed two assemblages that were over 90% consistent with strain assignments based on host plant (McMichael & Prowell 1999). The same two groups could be identified by comparing variations in mitochondrial DNA (mtDNA) sequences, which was modestly more accurate than allozyme analysis at distinguishing strains (Lu & Adang 1996; Pashley 1989). In particular, an *MspI* restriction enzyme polymorphism was identified that was diagnostic of strain identity and for which a PCR-based detection method was developed (Levy et al. 2002; Lu & Adang 1996). These techniques allowed detection of the strain-specific RFLP from individuals exposed to outdoor conditions for up to two weeks after death (Meagher & Gallo-Meagher 2003; Fig. 1a).

The potential usefulness of this methodology to assay populations was demonstrated by the examination of adult males captured in pheromone traps. Specimens from cornfields during the spring growing season were tested by PCR with 72% (21/29) shown to carry the corn-strain marker (*mtC*). This contrasted with 39% (15/39) when the same traps were tested a few weeks after harvest. We compared these findings to collections made during the same time periods from traps placed in a pasture habitat containing primarily small grass species. During the pre-harvest and post-harvest periods, over 90% (18/19 and 17/18, respectively) carried the rice-strain (*mtR*) marker. Therefore, the distribution of the *COI* polymorphism in adult males correlates with the expected behavior of fall armyworm strains with respect to the local plant population.

Another genetic marker for strain identity is *FR* (for Fall armyworm Rice strain), a tandem-repeat sequence present in large clusters only in the rice-strain genome (Lu et al. 1994). We developed a PCR-based method for detecting *FR* sequences that allowed analysis from single individuals (Nagoshi & Meagher 2003b). When *FR* clusters are present in the template DNA, PCR amplifica-

tion produces a "ladder" of fragments resulting from the synthesis of different multiples of the repeated sequence, a consequence of the tandem repeat organization that allows a variety of amplification alternatives (Fig. 1b). In comparison, amplification of genomic DNA from the corn-strain produces between 0-3 bands, indicating the absence of large clusters. Through a series of genetic crosses we unambiguously mapped *FR* clusters to the sex chromosomes (Nagoshi & Meagher 2003a). This was consistent with earlier reports of sexual dimorphism in the numbers of *FR* clusters present in the rice-strain, suggestive of a order of magnitude more copies on the *Y* than on the *X* chromosome (Lu et al. 1994).

Interstrain Mating Behaviour

The persistence of genetic and physiological differences between the host strains strongly suggests barriers to matings between strains. However, a comparison of strain-specific esterase allozymes and mtDNA polymorphisms suggested that interstrain hybridization was occurring in wild populations (Prowell 1998). Between 11-16% of individuals carrying an allozyme marker of one strain had an mtDNA genotype of the other. One laboratory study indicated that this interbreeding may be limited in nature (Pashley & Martin 1987). When corn-strain females were mated to rice-strain males (C X R), no progeny were produced and no spermatophores were transferred. In contrast, the reciprocal mating of rice-strain females to corn-strain males (R X C) had fertility equal to control (within-strain) crosses. However, the hybrid R X C daughters produced failed to mate with males from either strain but were able to mate with their hybrid brothers, although with reduced fertility. In comparison, R X C hybrid males could fertilize females of either strain, although again fertility was somewhat reduced. These results suggest significant strain-specific mate selection, such that corn-strain females have a strong preference to males of the same strain or to hybrids, while rice-strain females are more promiscuous. However, this interpretation must be tempered by the failure of two subsequent studies to find similar directional and restricted interstrain mating behavior (Quisenberry 1991; Whitford et al. 1988). Instead, normal fertility was observed in both directions of interstrain crosses. We were also able to obtain fertile progeny from the mating of corn-strain females to rice-strain males in the laboratory, with no obvious differences in fecundity from within-strain crosses (unpublished results). The discrepancy between the mating experiments performed by different laboratories is unexplained, but suggests that laboratory culturing and conditions may easily confound strain-specific mate selection.

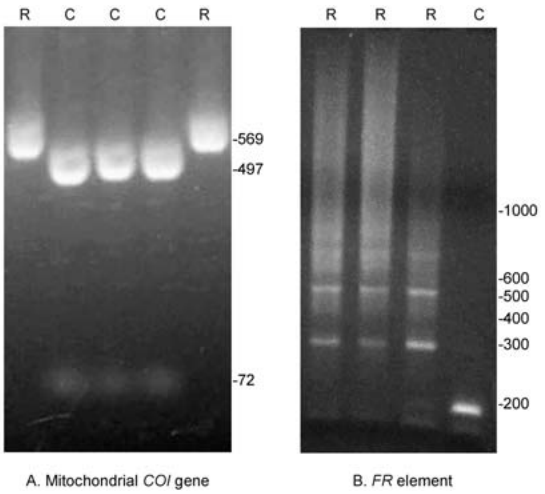

Fig. 1. Agarose gels displaying the diagnostic strain-specific DNA markers used to distinguish the host strains. Genomic DNA from individual adult males were individually amplified by PCR. (a). A portion of the mitochondrial *COI* gene was amplified. After digestion with *MspI*, a single band (569 bp) indicates the absence of the strain-specific *MspI* site, a characteristic of the rice-strain (mt^R) (R). Two smaller bands (497 bp and 72 bp) are produced if the *MspI* site is present, indicating a corn-strain (mt^C) (C) identity. (b). Three rice-strain individuals and one corn-strain individual were analyzed by PCR for the presence of *FR* clusters. PCR analysis produces a DNA ladder with an upper molecular weight smear in FR^+ specimens and 0-3 distinct bands in FR^0 samples. The gels were stained with ethidium bromide and photographed under ultraviolet illumination. Sizes are in base pairs.

Support for the existence of an assortative mating mechanism came from field studies in which virgin females of each strain were used to attract and capture males in the wild. Males of both strains exhibited a (60-75%) preference to females of the same strain, suggesting that pheromone differences might have a role in mate choice (Pashley 1993; Pashley et al. 1992). However, a more substantial difference was observed in studies examining the temporal partitioning of nocturnal mating activities (Pashley et al. 1992). Corn-strain females began calling (releasing pheromone) earlier in the scotophase than the rice-strain. Even more significant was the observation that strain-specific matings occurred at opposite times of the night with little overlap. The corn-strain mated during the first two-thirds of the evening while the rice-strain mated in the last third. Hence assortative mating might reflect divergence in the timing of strain-specific mating activity, with additional contributions coming from differences in pheromone attraction.

To measure the degree to which interstrain matings occur in the wild, we made use of the fact that our two strain-specific genetic markers undergo different but predictable inheritance pat-

terns. We designated the parental rice-stain marker configuration as mt^R FR^+ and the corn-strain as mt^C FR^0. With interstrain crosses, the mitochondrial marker should display a strictly maternal inheritance pattern while *FR* will exhibit sex linkage. This was confirmed by genetic crosses performed in the laboratory in which each set of parents was sacrificed and tested for their molecular genotypes after mating and oviposition (Nagoshi & Meagher 2003a). As predicted the mt^C FR^+ hybrid configuration was produced in males from a single cross between strains, while the reciprocal mt^R FR^0 hybrid combination was produced in males in two generations (Fig. 2). Therefore, if interstrain matings are common in the wild and there are no substantial fitness differences between genotypes, a random sampling of field-collected males should show substantial numbers of all four combinations. In particular, the number of mt^C FR^+ should approximate the number of mt^C FR^0 males. In contrast, if mating within the parental strains is highly preferred or substantially more productive, then the parental marker configurations (mt^C FR^0 and mt^R FR^+) should predominate. After a random sampling of field collected males from several southern Florida locations, we found 56% (199/356) of mt^R individuals had the hybrid configuration (mt^R FR^0), consistent with substantial R X C interstrain matings (Nagoshi & Meagher 2003a). In contrast, only 3% (4/144) of mt^C males were FR^+, the reciprocal hybrid pattern. These data strongly suggests that productive matings between corn-strain females and rice-strain males rarely occur in the wild, preventing the introduction of *FR* carrying X chromosomes into a corn-strain maternal background.

If the mt^R FR^0 field population is derived from interstrain hybridization, then we might expect to see some deviation in plant host preference from their "pure strain" mt^R FR^+ siblings. To examine this possibility, we compared the proportion of mt^R FR^0 individuals in the rice-strain pool collected from different habitats (Nagoshi & Meagher 2003a). A "primarily corn habitat" was arbitrarily defined as one where in which over 70% of the fall armyworm collected were mt^C, indicating that the plants in this time and area were attractive primarily to the corn-strain. Similarly, traps containing over 70% mt^R males were defined as "primarily grass habitats." The remainder formed the "mixed habitat" category. We found that the proportion of FR^0 males within the rice-strain pool increased to 83% (15/18) of the total rice-strain population trapped in corn habitats, compared to 61% (39/64) in areas biased to the rice-strain and 51% (23/45) in mixed habitats. Although the differences are only of borderline statistical significance, the trend is consistent with mt^R FR^0 males being less specific in their plant host preference than mt^R FR^+ males.

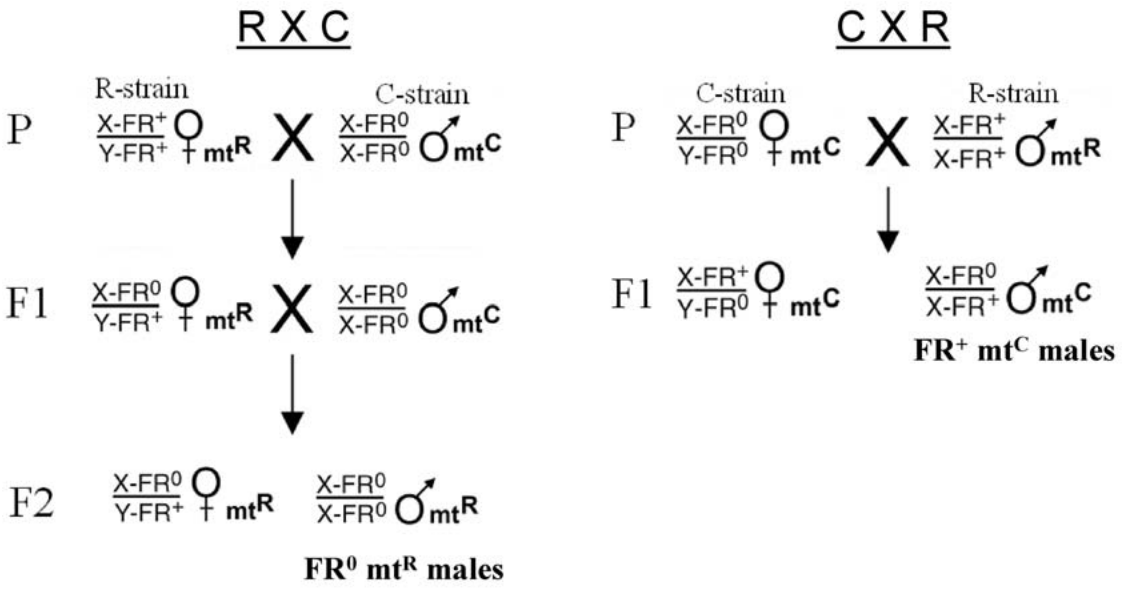

Fig. 2. Fall armyworm interstrain crosses designed to produce progeny with a hybrid marker configuration (Nagoshi & Meagher 2003b). *FR* clusters are on the sex chromosomes (X and Y) while the mitochondrial haplotype is maternally inherited. In Lepidopterans the female is the heterogametic sex. In the R X C mating, rice-strain females (mt^R FR^+) are mated to corn-strain males (mt^C FR^0). The hybrid female progeny are backcrossed to corn-strain males. All the resulting F_2 males will have the hybrid configuration (mt^R FR^0). The C X R mating represents the reciprocal cross. All F_1 males will be heterozygous for *FR* clusters and therefore identified as FR^+ by PCR. All will also be mt^C because of their corn-strain mother.

Undeveloped Areas do not Support High Fall Armyworm Populations

Critical to the development of an area-wide management strategy for fall armyworm is an understanding of the relative contributions of the various host plants to the overwintering and migrating populations of each strain (Knipling 1980). While several studies have examined sweet corn growing agricultural areas in Florida, other types of environments have not been examined in detail. To address this issue, we surveyed four different habitats that reflect some of the principal environments present in southern Florida (Meagher & Nagoshi 2004; Nagoshi & Meagher 2004). These are naturalized grassy wetlands and three types of developed areas: agricultural fields, managed turf grass (a sod farm and golf course), and urban.

We found that traps placed in geographically dispersed natural areas all showed very low capture rates throughout the year (Meagher & Nagoshi 2004). Over the 18-month test period the average for these traps was 0.4 captures/night/trap. Aside from unusually high numbers in two collections in February 2002, the average captures/night/trap never surpassed 5 for any two-week collection period (from March 2002 to July 2003). This occurred even though there was a high density and variety of short and tall grass species in the areas adjacent to these traps. Apparently these undeveloped habitats do not serve as high density refuges for fall armyworm at any time of the year, and are therefore unlikely to contribute substantially to the northward migrating populations in the spring or be a major source of the reinfestation of Florida agricultural areas in the fall and winter.

In contrast, 5-10-fold higher adult captures occurred in traps in a turf grass sod farm (average 8 captures/night/trap), agricultural fields (average 17 captures/night/trap), and urban developments (average 5 captures/night/trap). These results suggest that several types of human activities can lead to increases in the local fall armyworm population. It may be that unmanaged habitats are supportive of natural enemies that effectively control fall armyworm infestation or that the higher diversity of plant types in some way inhibits the establishment of high populations. Alternatively, developed areas may differ in the type, density, or quality of plant growth. In any case, identifying the environmental factors that make certain habitats unattractive to fall armyworm could have important benefits to the development of new control methods for this important agricultural pest.

The Corn-Strain is Primarily Found in Agricultural Areas

We used the strain-specific molecular markers combined with extensive pheromone trapping to examine the distribution of the strains in the different habitats (Meagher & Nagoshi 2004; Nagoshi & Meagher 2004). The rice-strain was found to be present in substantial proportions in all areas examined. This was particularly the case in the natural habitats and the sod farm, where over 90% of the captured males were of the rice-strain, though in the former the overall rate of capture was low (Fig. 3). In terms of numbers, the largest contributions came from agricultural areas and, not surprisingly, the turf grass-rich sod farm.

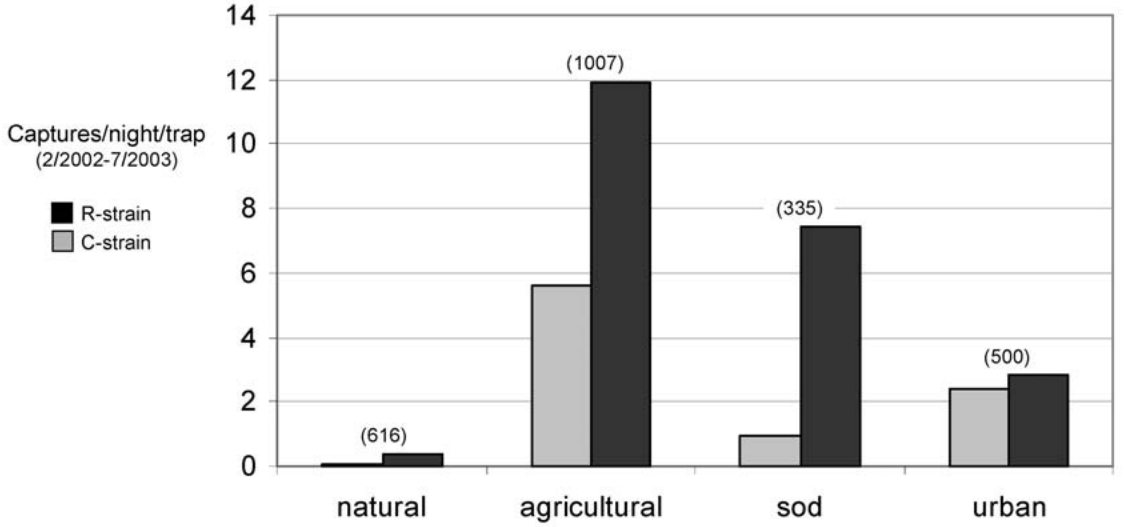

Fig. 3. Adult males captured per night in pheromone traps in four different habitats in southern Florida from February 2002 to July 2003. Fall armyworm numbers tested for strain identity at each habitat is in parenthesis. Data include those reported in Meagher & Nagoshi (2004) and Nagoshi & Meagher (2004).

By far the highest numbers of corn-strain captures occurred in the agricultural areas, where an average of nearly 6 corn-strain males was captured/night/trap, representing 32% of the total collection population at this site. Surprisingly, we also found a high proportion of the males trapped in urban areas were of the corn-strain, representing about half of the capture population. This was unexpected given that these sites did not have plant hosts in the vicinity known to be attractive to the corn-strain. However, the urban site averaged only a little over 2 corn-strain captures/night/trap, less than half of that observed in agricultural areas but still significantly (>95%) greater than observed in natural areas (Meagher & Nagoshi 2004). In comparison, although the overall fall armyworm population in the sod farm was relatively high (>8 captures/night/trap; Fig. 3), it contributed only modest amounts of corn-strain, averaging 1 capture/night/trap. Corn-strain captures in natural areas were inconsequential.

A critical question with respect to controlling fall armyworm infestation is to determine where the build up of strain populations occurs prior to the annual northward migration. This movement is at least in part dependent upon the timing of favorable weather patterns (Luginbill 1928; Mitchell et al. 1991); hence, a significant reduction in population numbers prior to the storm fronts could substantially reduce fall armyworm infestation in the eastern U.S. Population surveys in southern Florida cornfields typically show a rise in the overall fall armyworm population in the spring, followed by a rapid and prolonged decline during the summer months that presumably reflects the northward migration (Mitchell et al. 1991; Nagoshi & Meagher 2004; Pair et al. 1986). We conducted surveys of the four habitat types in the spring (February to May) of 2002 and 2003, just prior to the early summer migration period (Meagher & Nagoshi 2004). By far, the highest populations of corn-strain were found in the agricultural areas, displaying a 3-fold higher trap capture rate than the next highest (urban) site (Fig. 4a). Natural areas, the sod farm, and one season's data from a golf course indicated only minor contributions from these locales. In comparison, the rice-strain was found in substantial numbers in both agricultural and managed turf habitats, consistent with a broader host range (Fig. 4b). These results indicated that in the weeks prior to migration, the corn-strain population that will be the source of much of the corn damaging fall armyworm infestation in the eastern U.S. was mostly localized to the agricultural fields of southern Florida.

Comparisons of Seasonal Changes in Strain Populations

Because both strains are detected in regions where they are unlikely to survive the winter, it is expected that both migrate. However, given their different habitat preferences it would not be surprising to find strain-specific variations in migration behavior. This possibility was first suggested by studies in Louisiana that found the corn-strain population, first detected in the spring, reached a peak in density in early to mid-summer coincident with the maturation of the local corn crop (Pashley et al. 1987b). In comparison, the rice-strain population did not show substantial numbers until late summer, a period when the corn-strain population was nearly absent (Pashley et al. 1992). These differences suggest that the timing and/or magnitude of migration may not be the same for both strains.

If there are habitat-dependent or strain-specific factors that initiate migration, then we might expect the two strains to differ in the timing of their northward movement, which presumably can be detected by the sudden decline in capture numbers in southern Florida traps. We tested this by the examination of fall armyworm populations in agricultural fields predominated by sweet corn and tomatoes and a sod farm associated with turf grass (Meagher & Nagoshi 2004; Nagoshi & Meagher 2004). These sites were chosen because their respective plant populations allow clear predictions about the strain that should be attracted. Our data indicated that the corn- and rice-strains showed the same July-October population trough, although there is some evidence that the rice-strain decline begins earlier than the corn-strain (Fig. 5, arrows). This was even the case in comparisons between the rice-strain population in the sod farm with corn-strain in the agricultural habitat, indicating this population pattern was not dependent on the timing of sweet corn planting and harvesting. Instead, the decline in population appears to be due to some more general environmental or biological condition. Previous studies have attributed similar changes in the fall armyworm population to variations in plant quality and quantity resulting from the wet-dry seasonal cycle characteristic of tropical areas (Pair et al. 1986). For example, in studies performed in Mexico, high capture rates tended to occur 60-90 days after rainfall peaks, while intervals of least capture most frequently occurred 60-90 days after periods of least rainfall (Raulston et al. 1986). Alternatively, sharp declines in capture numbers during the year may be related to extremes in the daily minimum temperature. We found in our study that fall armyworm captures for both strains were lowest when the daily minimum temperature rose above 20°C (July-October) or fell below 5°C (January; see Nagoshi & Meagher 2004). There is precedence for correlations between daily temperature and field capture numbers for other insects (Butler et al. 1999; Cammell & Knight 1992; Scott et al. 2000; Souza & Carvalho 2002). It may be that an important fall armyworm behavior, such as mating or

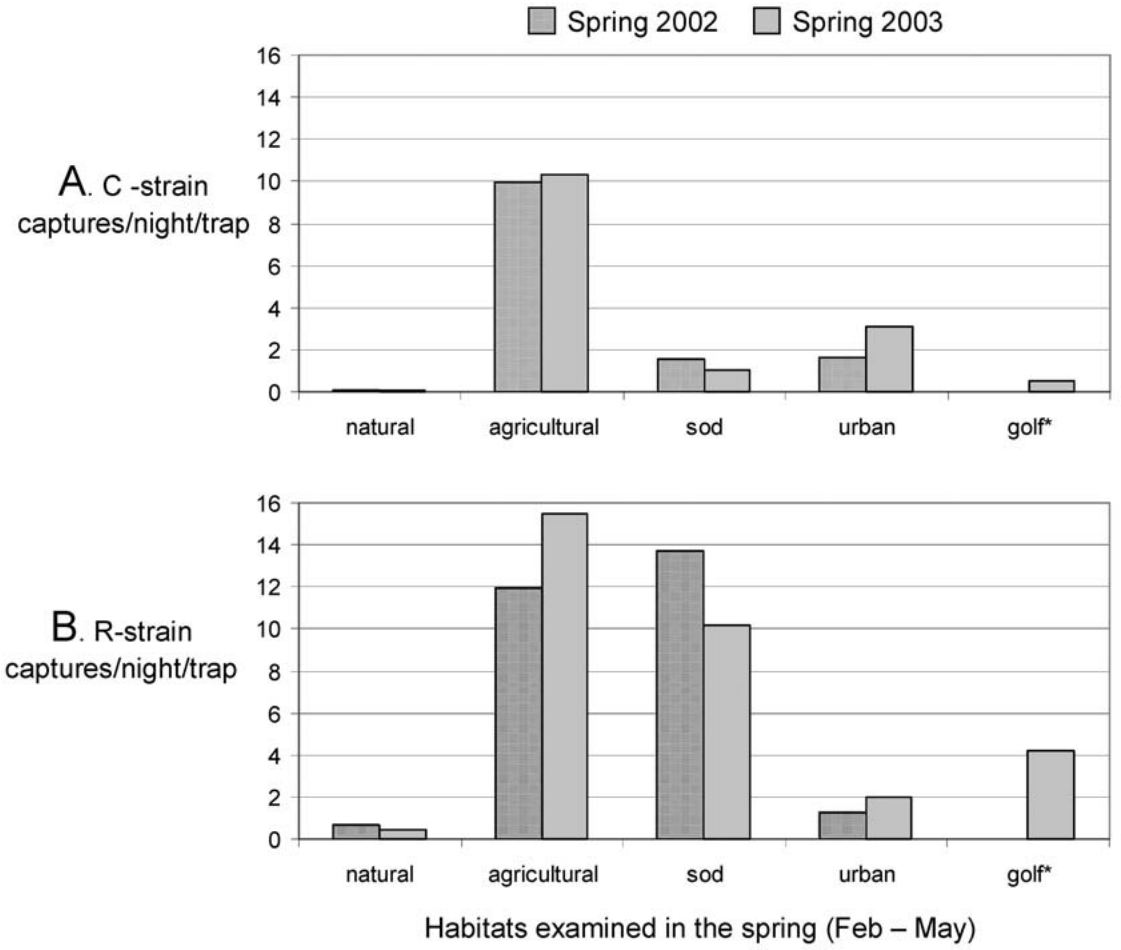

Fig. 4. Adult males captured per night in pheromone traps in four different habitats in southern Florida prior to the spring migration in 2002 and 2003 for corn-strain (a) and rice-strain (b) moths. Asterisk indicates only spring 2003 data available. Data include those reported in Meagher & Nagoshi (2004) and Nagoshi & Meagher (2004).

flight activity, is affected deleteriously by seasonal temperature extremes.

After the summer decline, fall armyworm populations begin increasing in the fall and winter in agricultural areas, coincident with the late year corn growing season. The timing of this increase was shown to correlate with weather and wind conditions conducive to southward migration, leading to the suggestion of a north-to-south return movement prior to the winter freeze (Mitchell et al. 1991; Pair et al. 1986; Pair et al. 1987). Our studies on strain distributions during this period led to the surprising observation that the fall population peak was due entirely to increases in rice-strain numbers (Nagoshi & Meagher 2004). Despite the presence of extensive sweet corn plantings in the agricultural trap areas from October to May, corn-strain numbers did not increase until February (Fig. 5, dashed line). The same pattern was observed in the fall of 2003 in the same and in additional agricultural test sites (Nagoshi & Meagher 2004; unpublished results). Interestingly, the rice-strain population in the sod farm site showed a similar population dynamic, indicating that this behavior is not specific to the agricultural planting and harvest cycle. Apparently, the presence of its preferred host plant and environmental conditions conducive to expansion of the rice-strain population were not sufficient to stimulate corn-strain increases during the fall. The reason for this is unknown, but these observations suggest that if there is a return migration in the fall, it is rice-strain specific.

Alternatively, there may be environmental factors that specifically prevent the expansion of either a migrant or indigenous corn-strain population late in the year. An intriguing, but purely speculative, explanation would be the existence of a corn-strain specific pathogen or natural enemy whose numbers increase during the year,

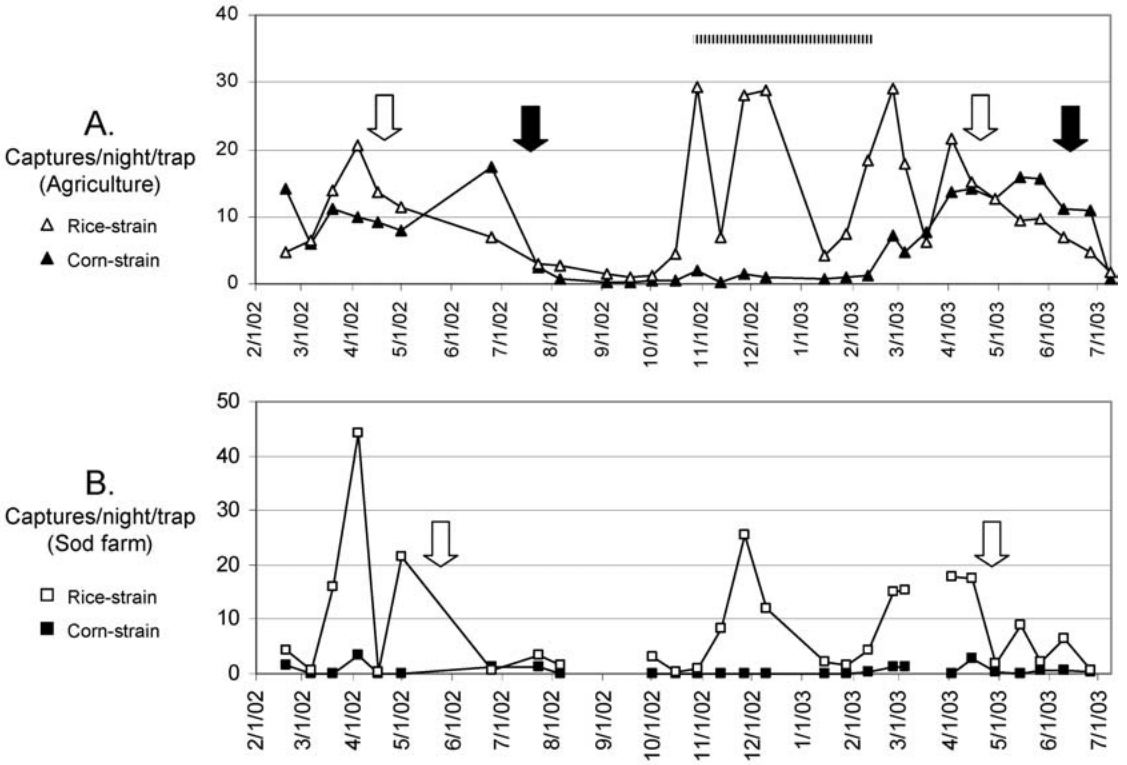

Fig. 5. Distribution of strains collected from pheromone traps in agricultural areas and a turf grass sod farm. The strain composition of each collection was determined by PCR and used to calculate the average number of each strain present per night per trap during each collection period. Arrows point to when the spring population begins to decline (open: rice-strain; filled: corn-strain). Dashed line indicates period of high fall armyworm numbers in the fall/winter associated with the rice-strain. Data include those reported in Nagoshi & Meagher (2004).

thereby suppressing reestablishment of a late season corn-strain, but not rice-strain, reinfestation. A crash in this putative population during the winter would then allow the corn-strain population peak observed every spring. It is clear that identifying the biological or environmental reasons for the relative absence of the corn-strain in the fall could have important ramifications for the development of strain-specific suppression methods.

Prospects for the Area-wide Control of Fall Armyworm Strains

Studies show that weather conditions prior to migration and changes in agricultural practices in southern Florida can mitigate significantly the duration and severity of fall armyworm damage in the eastern U.S. (Luginbill 1928; Westbrook & Sparks 1986). These observations suggest that changes in fall armyworm population dynamics in the overwintering area can significantly alter the magnitude of the northward migration. Unfortunately, the large amount of acreage, the broad host range and high mobility of the pest, the interspersion of urban development in the infested areas, and the presence of many ecologically-sensitive habitats make area-wide suppression of fall armyworm in southern Florida difficult. Current technology does not provide an affordable or environmentally benign method for the complete suppression of fall armyworm populations under these conditions. However, our studies show that the corn-strain has a relatively limited seasonal and geographical distribution in southern Florida. It may, therefore, be economically feasible to focus efforts over a limited time and space just prior to the normal migration period, with the objective to substantially reduce the corn-strain migrant population or delay migration such that the most vulnerable portions of the more northern corn growing seasons are missed.

Studies on the seasonal and geographical distribution of fall armyworm strains also have uncovered possible seasonal or habitat-specific factors that seem very effective in controlling the corn-strain. Both strains are largely absent in naturalized areas despite the presence of grass species that should be attractive to the rice-strain and laboratory evidence that corn-strain larvae are fully capable of normal development on these

substrates. We also observed surprisingly low numbers of corn-strain males captured in pheromone traps in all habitats tested during the fall, a period of extensive agricultural activity and substantial increases in the rice-strain capture population. The absence of a corn-strain population peak under conditions favorable to the rice-strain, and when host plants normally attractive to the corn-strain are present, suggests the existence of unknown factors effective in suppressing population numbers.

Current and ongoing studies on the strains indicate that a regional management program designed to mitigate or delay fall armyworm migration might be feasible. Still needed are longer term and more detailed information on the geographical and seasonal distribution of the fall armyworm strains in southern Florida, in particular the timing of the spring and fall population increases with respect to seasonal and environmental factors. We also need a more extensive understanding of strain-specific biology, including the mechanisms of strain-specific mating and ovipositional choice, and the effects of interstrain hybridization on behavior and physiology (in particular fertility, migration, plant host choice, and susceptibility to chemical and biological agents). While substantial research remains, we believe that information and techniques are now available that can address these issues and lead to the development of an effective area-wide management strategy.

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LABORATORY SELECTION FOR BEET ARMYWORM (LEPIDOPTERA: NOCTUIDAE) RESISTANCE TO METHOXYFENOZIDE

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ABSTRACT

Beet armyworms, *Spodoptera exigua* (Hübner), were artificially selected in the laboratory for resistance to the insect growth regulator, methoxyfenozide. A field collected beet armyworm colony was separated into three cohorts that were independently selected with three concentrations (0.033 ppm, 0.064 ppm, and 0.125 ppm) of methoxyfenozide incorporated into a meridic diet. These concentrations corresponded closely with the LC_{10} (0.033 ppm), LC_{50} (0.072 ppm), and LC_{90} (0.161 ppm), respectively, for the original colony. After seven generations of continuous exposure to methoxyfenozide, resistance in the colony selected at the low concentration did not increase significantly. In contrast, LC_{50} values increased 9.7- and 9.4-fold for the colonies selected at the moderate and high concentrations, respectively, over that of the original colony. Crosses between resistant and susceptible individuals indicated that the resistance was heritable. At 4 d after exposure, mortality of offspring from the reciprocal crosses was intermediate between mortality for the offspring from the parental crosses. When rated at 10 d, mortality of offspring from the reciprocal crosses was not different significantly from offspring from the cross between susceptible parents. These data will be important for developing a management program for beet armyworm resistance to methoxyfenozide.

Key Words: IPM, Intrepid, *Spodoptera exigua*, Insect Growth Regulator.

RESUMEN

Los gusanos trozadores, *Spodoptera exigua* (Hübner), fueron seleccionados artificialmente en el laboratorio para su resistencia al regulador de crecimiento de insectos, metoxifenozido. Una colonia del gusano trozador recolectada en el campo fue separada en tres cohortes que independientemente fueron seleccionados con tres concentraciones (0.033 ppm, 0.064 ppm, y 0.125 ppm) de metoxifenozido incorporadas en una dieta merídica. Estas concentraciones correspondieron de manera cercana con las concentraciones letales CL_{10} (0.033 ppm), CL_{50} (0.072 ppm), y CL_{90} (0.161 ppm), respectivemente, para la colonia original. Después de siete generaciones de ser expuesta continuamente al metoxifenozido, la resistencia en la colonia seleccionada a la concentración menor no aumentó significativamente. En contraste, los valores de CL_{50} aumentaron por 9.7 y 9.4 veces para las colonias seleccionadas de concentraciones moderadas y altas, respectivamente, sobre las de la colonia original. Los cruzes entre los individuos resistentes y susceptibles indicaron que la resistencia puede ser heredada. A los 4 días después de exponerlos, la mortalidad de la progenie de los cruzes recíprocos fue intermedia entre la mortalidad de la progenie de los cruzes de los padres. Cuando fue calibrada a los 10 dias, la mortalidad de la progenie de los cruzes recíprocos no fue significativamente diferente de la progenie de los cruzes entre los padres susceptibles. Estos datos serán importantes para desarrollar un programa de manejo para la resistencia del gusano trozador al metoxifenozido.

The beet armyworm, *Spodoptera exigua* (Hübner), is an occasional pest of cotton, *Gossypium hirsutum* L., in the southern United States. In general, beet armyworm populations remain low due to actions of natural enemies such as the parasitoid *Cotesia* spp., and various predators and pathogens (Mohaghegh et al. 2001; Bianchi et al. 2002). However, these beneficial insects are inadvertently disturbed with numerous insecticide applications for other pests. Cotton in the southern United States has numerous key pests such as the heliothine complex (tobacco budworm, *Heliothis virescens* (F.), and bollworm, *Helicoverpa zea* (Boddie)); tarnished plant bug, *Lygus lineolaris* Palisot de Beauvois, and numerous stink bug species (Heteroptera: Pentatomidae) that require multiple applications of broad spectrum insecticides annually to prevent economic losses. The introduction of Bollgard cotton has reduced the numbers of insecticide applications applied for tobacco budworm and bollworm (Williams 1996, 2001). However, Bollgard cotton is typically treated multiple times annually with pyrethroids to control bollworms, and organophosphates to control tarnished plant bugs and stink bugs. Those insecticides provide little control of beet armyworms, but can be damaging to beneficial arthropods. Consequently, beet armyworms generally only reach economically damaging levels after insecticides have been applied to control other pests. There are few insecticides currently labeled in cotton that provide effective, economi-

cal control of beet armyworms. When beet armyworm outbreaks occur, these insecticides are applied over large areas; thereby, providing intense selection for the development of resistance.

Methoxyfenozide (Intrepid 2F, Dow AgroSciences, Indianapolis, IN) is one of a few insecticides that provides effective control of beet armyworms in cotton. This insecticide is an insect growth regulator that acts as an agonist of 20-hydroxyecdysone, a key hormone in the molting process (Wing 1988). Methoxyfenozide affects only the larval stage of Lepidoptera (Wing et al. 1988). Intoxicated larvae cease feeding soon after ingestion and eventually die due to a premature molt. Although methoxyfenozide is highly effective against beet armyworm larvae, this compound has little impact on beneficial arthropods (Wing et al. 1988). Therefore, this insecticide is beneficial in integrated pest management systems and preservation of the insect growth regulators is an important concern.

Beet armyworms are inherently tolerant to many insecticides and have a high propensity for developing resistance to insecticides (Wolfenbarger 2002). Much of the research reported on beet armyworm resistance has represented compounds from the organochlorine, organophosphate, carbamate, and pyrethroid classes of insecticides. Little work has been conducted on beet armyworm resistance to insect growth regulators. However, Moulton et al. (2002) demonstrated that the necessary genetic variability needed for beet armyworms to develop resistance to the insect growth regulators, tebufenozide and methoxyfenozide, was present in populations from Thailand, and may be present in field populations from the United States. Because of this, proactive resistance management plans should be in place to ensure the sustainability of this insecticide. Baseline data are currently available for beet armyworm susceptibility to methoxyfenozide (Mascarenhas et al. 1998a, b). Those data are important for resistance management; however, information concerning the genetic heritability of resistance is not available. Understanding the heritability of beet armyworm resistance to methoxyfenozide will be necessary to design appropriate resistance management strategies. The current study was conducted to determine inheritance patterns of beet armyworm resistance to methoxyfenozide as a proactive step in the development of an effective resistance management plan.

Materials and Methods

A colony of beet armyworms was established from larvae collected on pigweed, *Amaranthus* spp., collected during the summer of 2002. This colony was maintained in the laboratory for five generations before the initiation of the experiment. A concentration-mortality bioassay was conducted with the commercial formulation of Intrepid 2F. The insecticide was incorporated into a meridic diet to determine the susceptibility of the colony before selection. Serial dilutions of Intrepid 2F and distilled water were made from a stock solution with a concentration of 1000 ppm of methoxyfenozide active ingredient. Dilutions ranged from 1.6 ppm to 100 ppm plus a non-treated control for initial bioassays. One ml of each solution was incorporated into 100 ml of meridic diet to obtain eight concentrations of treated diet that ranged from 0.016 ppm to 1.0 ppm. Treated and non-treated diet was dispensed into 29.5-ml plastic cups in 2.5-ml aliquots with a repeat pipetter. A total of 60 cups for each concentration was used for the bioassay. A single neonate beet armyworm was placed in each cup, and mortality was rated after 96 h. Mortality was scored as inability of larvae to move after being touched with a blunt probe. A concentration-mortality curve was generated with Probit Analysis and the LC_{50} and LC_{90} values were determined (PROC PROBIT, SAS Institute 1989).

The selection experiments were initiated in November of 2002. Three colonies were independently selected for resistance to methoxyfenozide with three different levels of selection pressure (low, moderate, and high). The concentrations included 0.032 ppm, 0.064 ppm, and 0.125 ppm for the low, moderate, and high levels of selection, respectively. These concentrations corresponded closely with LC_{10} (0.033 ppm), LC_{50} (0.072 ppm), and LC_{90} (0.161 ppm) values, respectively, obtained from the concentration-mortality curve of the original colony. Larvae were exposed to the insecticide by incorporating formulated Intrepid 2F into meridic diet as previously described. However, instead of using 29.5-ml plastic cups, approximately 20 ml of treated diet was dispensed into 236 ml cardboard cups. This facilitated the selection of a greater number of individuals on each concentration compared to making selections to an individual larva in small cups. Approximately 100 neonates were placed into each cup and allowed to feed for 96 h. After 96 h, surviving larvae were transferred individually onto non-treated diet in 29.5-ml cups. Only larvae that appeared to be developing normally (larvae that molted to the second instar) were selected. Dead and moribund larvae were discarded. Larvae were allowed to complete development on the non-treated diet. After pupation, beet armyworms from each colony (i.e., selection pressure) were mass mated (20 males and 20 females) in 3.79-L cardboard containers. At least ten mating containers were established for each colony.

Each of the beet armyworm colonies were exposed to selection at the respective concentrations for seven generations. After seven generations, reciprocal crosses were made with individuals from colonies selected at moderate

and high concentrations to individuals from another laboratory susceptible (LC_{50} = 0.08, slope = 0.72, df = 4, χ^2 = 1.13, P = 0.89) colony. This colony was originally collected from pigweed during 2001. Male beet armyworms from pheromone traps were incorporated into this colony during the summer of 2002. Reciprocal crosses between the laboratory selected colonies and laboratory susceptible colony were conducted during May of 2003. At least ten mating pairs were established for each of the parental colonies and their reciprocal crosses. The laboratory selected colonies were only crossed with the laboratory susceptible colony. Reciprocal crosses were not done between the colonies selected at the moderate and high concentrations.

Offspring from each mating pair (90 per pair) were exposed to a discriminating concentration (0.125 ppm) of formulated Intrepid 2F in meridic diet that corresponded to an LC_{90} for the original colony. Data for percent mortality of offspring was corrected for control mortality by Abbott's Formula (Abbott 1925) and analyzed with analysis of variance (PROC MIXED, Littell et al. 1996).

Results and Discussion

Beet armyworms exposed to moderate and high selection pressures developed 9.7- and 9.4-fold levels of resistance compared to the original colony within seven generations (Fig. 1). The LC_{50} value (95% fiducial limits) for the original colony was 0.07 (0.064-0.082) ppm (slope = 1.60, df = 4, χ^2 = 1.18, P = 0.88). The susceptibility of the colony at the low selection pressure did not change after seven generations of selection (LC_{50} = 0.07, slope = 0.95, df = 5, χ^2 = 8.55, P=0.13). Within seven generations, the LC_{50} value (95% fiducial limits) increased to 0.68 (0.427-1.106) ppm for the colony selected at the moderate selection pressure (slope = 0.93, df = 4, χ^2 =10.25, P = 0.04). Similarly, the

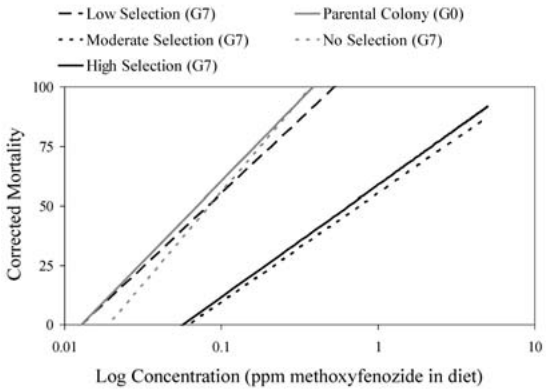

Fig. 1. Results of probit analysis for concentration-mortality curves of beet armyworm susceptibility to methoxyfenozide before and after laboratory selections.

LC_{50} value (95% fiducial limits) for the colony at the high selection pressure increased to 0.66 (0.554-0.809) ppm. The concentration-mortality curves and corresponding LC_{50} values were similar between the colonies selected at the moderate and high concentrations based on overlap of 95% fiducial limits. This may be an indication that the same mechanism of resistance was isolated from the two different selection pressures. Although the two selection pressures yielded similar LC_{50} values, the colony exposed to the high selection pressure appeared to be less variable in its response to the methoxyfenozide treated diet than the colony exposed to the moderate selection pressure. This is evidenced by the wide range of 95% fiducial limits observed with the colony selected at the moderate concentration. Also, the colony at the high selection pressure had a lower χ^2 value than the colony selected at the moderate concentration indicating that they were more homogeneous in their response to methoxyfenozide. This would be expected because the higher selection pressure should create a greater genetic bottleneck; whereas, the moderate selection pressure may have resulted in selection of other factors such as overall health and vigor in addition to the resistance trait.

Based on results from crosses between individuals from the colonies selected at the moderate and high selection pressures with individuals from the susceptible colony, the selected trait was determined to be heritable. However, the mode of inheritance is difficult to determine from these data. Mortality of larvae was significantly different among the different crosses for the colonies selected at the moderate (F = 60.46; df = 3, 22.2; P < 0.01) and high (F = 16.05; df = 3, 29; P < 0.01) pressures at 4 d (Fig. 2). When rated at 4 d, mortality of offspring from the parental crosses for the susceptible and resistant colonies averaged (SEM) 92.8 (2.53) percent and <29.0 (<8.85) percent, respectively. Offspring from the reciprocal crosses had intermediate levels of mortality.

Similar to mortality at 4 d, mortality at 10 d was significantly different among the crosses for the moderate (F = 12.25; df = 3, 32; P < 0.01) and high (F = 8.16; df = 3, 29; P < 0.01) selection pressures (Fig. 2). At 10 d; however, mortality was not significantly different among offspring from the reciprocal crosses and offspring from the parental cross for the susceptible colony. Mortality of offspring from the reciprocal crosses and the parental cross for the susceptible colony ranged from 82.9 (3.67) to 99.1 (0.38) percent for the colony selected at the moderate pressure. Mortality of offspring from the cross between resistant parents averaged 54.3 (9.7) percent. For the colony selected at the high pressure, mortality of offspring from the reciprocal crosses and the parental cross for the susceptible colony ranged from 88.4 (3.75) to 99.1 (0.38) percent; while, mortality of off-

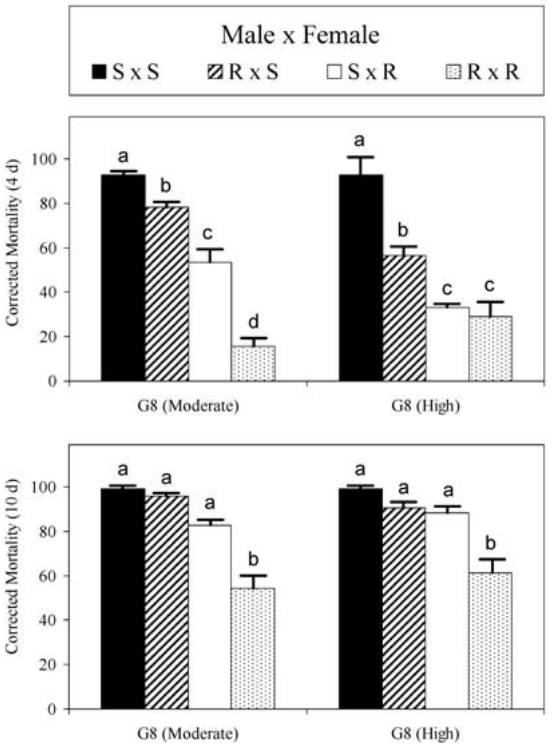

Fig. 2. Mortality of offspring from reciprocal crosses on a discriminating concentration of methoxyfenozide.

spring from the parental cross of the resistant colony averaged 61.4 (8.6) percent.

Based on results of these laboratory selections, the potential for beet armyworms to develop resistance to methoxyfenozide does exist. Beet armyworm larvae from a field-collected colony developed approximately 10-fold level of resistance in a relatively short period of time (seven generations) in the current study. Moulton et al. (2002) isolated a beet armyworm strain from Thailand with 320- to 340-fold level of resistance to methoxyfenozide compared to a laboratory strain. In a similar study, a sex linkage was determined for a beet armyworm strain exhibiting resistance to several insecticides (Wolfenbarger 2002). In that experiment, resistance to fenvalerate and methomyl was associated with the two X chromosomes of the male resistant strain and not the Y chromosome of the female (Wolfenbarger 2002). In contrast, ratings at 4 d from crosses in the current study suggest that the Y chromosome of the female may be important in resistance development. However, when mortality was rated at 10 d, sex-linkage of the trait was not apparent; therefore, no definitive conclusions can be made concerning inheritance of the trait selected in these experiments. More research is necessary to isolate strains of beet armyworms with varying levels of resistance to methoxyfenozide so that they can be crossed with susceptible individuals for multiple generations to determine inheritance of resistance. This information will be important for proactive management of beet armyworm resistance to methoxyfenozide.

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LARVAL DEVELOPMENT OF FALL ARMYWORM (LEPIDOPTERA: NOCTUIDAE) ON DIFFERENT COVER CROP PLANTS

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ABSTRACT

A series of laboratory and field experiments were conducted to compare larval development, feeding behavior, and ovipositional preference of fall armyworm (*Spodoptera frugiperda*) on a standard host plant, a standard cover crop plant, and two candidate cover crop plants. The results indicate that larvae from different rearing cultures and host strains developed comparably on corn and sorghum-sudangrass, but generally developed poorly on cowpeas and sunnhemp. Larval and ovipositional experiments also suggested a preference for either corn or sorghum-sudangrass. Field plantings of cowpeas and sunnhemp in two locations were ignored by fall armyworm in favor of corn. These studies suggest that cowpeas and sunnhemp have the potential to reduce stepping stone nursery populations of fall armyworm by lengthening developmental time and increasing larval mortality.

Key Words: *Spodoptera frugiperda*, Cowpeas, Sunnhemp.

RESUMEN

Una serie de experimentos de laboratorio y de campo fueron realizados para comparar el desarrollo de las larvas, el comportamiento de alimentación, y la preferencia de la oviposición del gusano cogollero (*Spodoptera frugiperda*) en una planta hospedera típica, una planta de cobertura típica, y dos plantas candidatas para ser usadas en cobertura. Los resultados indicaron que las larvas de diferentes crias y cepas de hospederos desarrollaron similarmente en maíz y sorgo-pasto de sudan, pero en general desarrollaron pobremente en caupí y en "sunn-hemp" (*Crotalaria juncea*). Los experimentos sobre las larvas y la oviposición también sugerieron que hay una preferencia para maíz o sorgo-pasto de sudan. Las siembras de campo con caupí y *Crotalaria* en dos localidades fueron ignoradas por el gusano cogollero en favor de maíz. Estos estudios sugerieron que la caupí y *Crotalaria* tienen un potencial para reducir las poblaciones del gusano cogollero en los viveros de piedra escalonados por alargar el tiempo de desarrollo y al aumentar la mortalidad de las larvas.

Fall armyworm, *Spodoptera frugiperda* (J. E. Smith) is a polyphagous, migratory insect that moves northward each season from overwintering areas in southern Florida (Luginbill 1928; Mitchell 1979; Pair et al. 1986; Westbrook & Sparks 1986; Mitchell et al. 1991) and southern Texas/northern Mexico (Luginbill 1928; Raulston et al. 1986; Pair et al. 1991). Populations from the overwintering areas in southern Florida move into secondary source areas or "stepping-stone nurseries" located in northern Florida and southern Georgia in April and May, and it is believed that populations from these areas can increase and add to the numbers of moths moving northward (Mitchell 1979; Pair & Westbrook 1995).

Fall armyworm is composed of two sympatric and morphologically identical strains that are defined by their host plant preferences (Pashley et al. 1985; Pashley 1986). One strain was identified from populations feeding on corn and sorghum (corn strain) and the other strain was identified from populations feeding on rice and forage grasses (rice strain). The two strains can be distinguished by strain-specific allozyme variants and genetic markers (Lu et al. 1994, Lu & Adang 1996; McMichael & Prowell 1999).

Corn (*Zea mays* L.) and other host plants, including vegetables and cover crops, may allow fall armyworm populations of both strains to increase during spring in the stepping stone nurseries. In northeastern Florida, over 12,000 ha of vegetable crops, primarily potatoes (*Solanum tuberosum* L.) and cabbage (*Brassica oleracea* L. var. *capitata*) are grown during January to May in three counties (St. John's, Flagler, and Putnam) (Aerts & Nesheim 2000; Larson Vasquez & Nesheim 2000). After vegetable harvest, cover crops such as sorghum-sudangrass (*Sorghum bicolor* (L.) Moench, a sorghum/sudan hybrid) generally are planted. Sorghum-sudangrass is a warm-season annual grass

hybrid that is used in Florida as a green manure cover crop following harvest of winter vegetables (Chambliss 2002). These plants are used to produce biomass, contribute nitrogen to the soil, increase soil organic matter, and prevent soil erosion (Chambliss et al. 2003; Rich et al. 2003). Benefits of cover crops in regards to biomass production, nitrogen yield, and crop yield have been shown for both large vegetable production systems (Creamer & Baldwin 2000) and small subsistence farming systems (Jeranyama et al. 2000).

In northern and southern Florida large populations of fall armyworm can develop in fields planted to sorghum-sudangrass (ERM, unpublished data; Pair & Westbrook 1995). Therefore, alternative cover crops that are poorer host plants for fall armyworm may reduce migrating populations. This research was conducted to compare population development and feeding behavior of fall armyworm host strains on a standard host plant (corn), a standard cover crop plant (sorghum-sudangrass) (SSG), and two candidate cover crop plants (iron-clay cowpeas and sunnhemp). Cowpeas [*Vigna unguiculata* (L.) Walpers spp. *unguiculata*] is a warm-season annual legume that alone or mixed with SSG can be used as a rotation or cover crop with vegetables (Miller et al. 1989). Sunnhemp (*Crotalaria juncea* L.) is a warm-season legume that is used as a cover crop in alternation with vegetable crops (Duke 1981; Li et al. 2000; Rich et al. 2003).

Materials and Methods

Larval Feeding Experiments

Larvae for this experiment were from two sources. 'Tifton' larvae were from a laboratory culture reared on artificial diet and shown to carry the mitochondrial marker of corn strain (Meagher & Gallo-Meagher 2003; Nagoshi & Meagher 2003). This culture originated from individuals received from Dr. James Carpenter, USDA-ARS, Tifton, GA and was reared on a pinto bean artificial diet according to the procedures of Guy et al. (1985). 'Ona' larvae were from a culture of individuals collected in July 2002 from the Range Cattle Research and Education Center, Ona, FL. This culture was reared on grasses (bermudagrass, *Cynodon dactylon* (L.) Pers. and stargrass, *C. nlemfuensis* Vanderyst var. *nlemfuensis* 'Florona') and has the rice strain mitochondrial marker (Nagoshi & Meagher 2003).

Plants 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 growth leaves were selected from plants aged ca. 3 wk. old for cowpeas, SSG, and corn, and 6 wk. old for sunnhemp. Plant foliage was placed on filter paper discs (Whatman®, 90 mm) moistened with ca. 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° with a 14:10 photoperiod. The filter paper in each petri dish was moistened daily with ca. 1 ml of deionized water for the first 10 days. Larvae were supplied with a continuous amount of fresh plant material until time of pupation. Larval weights were measured at 10 days. Development time (in days) from neonate to pupa, pupal weight, and sex were recorded at pupation. For each host plant, 15-30 larvae were arranged in three 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 plants.

Larval Choice Experiments

The larval choice experiments were designed to evaluate the feeding preference of larvae on leaf sections of four host plants. Rice strain larvae from the Ona culture were used along with corn strain larvae collected from a population near Hague, FL. This culture was reared on greenhouse-grown corn and was in culture for < four generations. Plant culturing and leaf selection was similar to that used for the larval feeding experiment, except that leaves were trimmed to a uniform size (ca. 2 × 5 cm).

Four separate experiments were completed. First, a four-choice experiment compared sections from the four host plants. One section of each plant was randomly placed on filter paper discs moistened with ca. 3 ml deionized water and sections were placed ca. 2 cm from the center along the outer edge of the petri dish. Twenty neonate larvae from Ona or Hague cultures were placed in the center of each petri dish, which were then placed in an incubator at 23.9 ± 2° with a 14:10 photoperiod. Counts were made after 24 h by observing the number of larvae on or under each leaf section. The second experiment was a two-choice test comparing corn to each of the other plants using Ona larvae.

Results from the first two experiments led us to test two possible explanations for the reduced plant host specificity of the rice strain culture. The third experiment examined whether the feeding experience of the parents influenced the plant host preference of the next generation. Rice strain larvae that completed development on each host plant were mated and resulting progeny were tested against all four plants. The fourth experiment was a four-choice test that used the progeny of larvae that selected each host plant in Experiment 1. Therefore, larvae that selected corn, cowpeas, SSG, or sunnhemp were reared on that plant and their progeny used in the bioassay.

All experiments contained 10 replications. Analysis of variance of square root-transformed data (PROC MIXED, Contrasts, Littell et al. 1996) was used to examine variation among plants.

Oviposition Choice Experiments

In addition to larval preference, the differential distribution of fall armyworm on different plant types could result from selective ovipositional behavior by adult females. Oviposition choice experiments were conducted in a greenhouse using plastic swimming pools as a cage. The bioassay consisted of a plastic swimming pool (109.2 cm d × 12 cm h) containing 22.7 kg of commercially available sand. Hardware cloth (1.27 cm l, 25.4 cm h) was curled and placed in the sand along the inside edge of the pool. Gray window screen was placed on top of the hardware cloth, and a second inverted swimming pool was placed on top of the screen to form a 'sandwich'.

All tests used our Gainesville laboratory culture reared on artificial diet and shown to carry the mitochondrial marker of corn strain (Meagher & Gallo-Meagher 2003). Greenhouse-grown plants (same size and age as plants used above) were placed outside for 3 d before being used. One plant of each host was placed randomly in a circle inside the unit. Ten male and female moths (<5 d old) were placed along with a 10% honey-sugar solution feeding station in the center of the unit. The screen and top pool were placed on top of the hardware cloth enclosing the moths. In this way, moths could not oviposit on the smooth surface of the top or bottom pools. Plants were sampled 48 h later for eggmasses. Experiment 1 was completed June–August 2001 and used 10 separate cages (replications); experiment 2 was completed in October 2001 and used 8 replications. All data were transformed by a square root (y + 0.5) transformation and means were separated with the Contrasts statement in PROC MIXED (Littell et al. 1996).

Field Experiments

Corn, cowpeas, SSG, and sunnhemp were planted in four randomized complete blocks at a field site in Gainesville on 19 June 2001 and at the University of Florida Vegetable Research Station, Hastings on 8 July 2001. Each plot was four rows wide by 30.5 m long. An additional treatment of green manure (SSG, incorporated into the soil 60 d after planting) was added to the Hastings test. Standard agronomic practices were used except no insecticides were applied. All plots were sampled beginning in late July and ending in early October for evidence of plant damage and the presence of larvae. Plants were sampled for larvae by checking a randomly selected one-meter row at each observation point. Analysis of variance of square root-transformed data (PROC MIXED, Contrasts, Littell et al. 1996) was used to examine variation among treatments.

Results

Larval Feeding

Differences between Tifton (corn strain) and Ona (rice strain) cultures were found for larval weight, pupal weight, larval development time, and survival, and there was a significant interaction between culture and host plant ($P < 0.0001$) (Table 1). Therefore results from each culture were statistically analyzed separately. For the Tifton culture, the largest larvae were found on corn, where the average weight was higher than that of larvae cultured on SSG or cowpeas ($P < 0.001$). Larvae grown on sunnhemp were significantly smaller, averaging approximately 21% of

Table 1. Larval weight, pupal weight, larval development time, and survival of Tifton (corn strain) and Ona (rice strain) fall armyworm larvae fed different host plants. Means with the same uppercase (between cultures) or lowercase (among plants within a culture) letter are not significantly different, $P > 0.05$.

Culture	Plant	Larval wt. (mg)	Pupal wt. (mg)	Development time (days)	% Survival
Tifton		50.4 ± 4.1 B	182.4 ± 4.2 A	20.9 ± 0.3 B	62.2 ± 0.07 B
	corn	83.5 ± 8.8 a	160.3 ± 6.8 b	19.1 ± 0.5 a	62.2 ± 0.12 b
	SSG	51.2 ± 6.5 b	209.6 ± 5.7 a	19.7 ± 0.3 a	95.6 ± 0.04 a
	cowpeas	34.5 ± 5.0 bc	151.9 ± 8.9 b	23.3 ± 0.6 b	51.1 ± 0.06 b
	sunnhemp	18.0 ± 3.9 c	190.6 ± 7.4 a	23.4 ± 0.5 b	40.0 ± 0.04 b
Ona		109.0 ± 6.5 A	158.8 ± 2.2 B	18.1 ± 0.3 A	77.2 ± 0.05 A
	corn	122.2 ± 6.1 b	166.8 ± 3.0 a	15.6 ± 0.2 b	91.1 ± 0.02 a
	SSG	209.6 ± 12.9 a	176.2 ± 3.1 a	14.3 ± 0.2 a	86.7 ± 0 ab
	cowpeas	62.5 ± 6.4 c	151.0 ± 4.7 b	20.5 ± 0.5 c	67.8 ± 0.04 b
	sunnhemp	41.9 ± 4.6 c	140.6 ± 4.1 c	22.1 ± 0.4 d	63.3 ± 0.13 b

the weight from those on corn (Table 1). A somewhat different result was observed with pupal weights. The heaviest pupae were those developing on SSG and sunnhemp, with those from corn and cowpeas being about 20-25% smaller. Larvae feeding on cowpeas and sunnhemp took longer to develop than those on corn and SSG, and had lower survival than those on SSG.

The rice strain was similar to the corn strain in that the smallest larvae, longest development time, and highest mortality were associated with development on cowpeas and sunnhemp (Table 1). There was unusually high early larval growth on SSG, with an average weight about 70% greater than found on corn. However this early growth on SSG did not lead to an exceptionally high pupal weight.

Larval Choice

Larvae from the more recently derived corn strain culture (Hague) selected corn over SSG and were rarely found on cowpeas ($P < 0.0001$, Table 2). No larvae were found on sunnhemp. In comparison, rice strain larvae from the Ona culture displayed an equal preference for corn and SSG compared to sunnhemp and cowpeas (Table 2). The two-choice bioassays with rice strain larvae comparing corn with the other plants showed only a difference between corn and cowpeas (Table 3).

Ona larvae whose parents were reared on the different host plants were generally found more on corn and SSG and less on cowpeas, no matter what the host plant was of the parents (Table 4). The exception was larvae from SSG-reared parents, which showed equal preference for all plants. Ona larvae whose parents selected different host plants were generally found more on corn or SSG and less on cowpeas (Table 5). Larvae from parents that selected sunnhemp were an exception, choosing that plant at a similar frequency as corn or SSG.

TABLE 2. NEONATE FALL ARMYWORM LARVAE FROM HAGUE OR ONA CULTURES TESTED IN CHOICE BIOASSAYS COMPARING FOUR HOST PLANTS. MEANS (AMONG PLANTS WITHIN A CULTURE) WITH THE SAME LETTER ARE NOT SIGNIFICANTLY DIFFERENT, $P > 0.05$.

Culture	Plant	Larvae per section
Hague	corn	14.6 ±1.3 a
	SSG	4.9 ± 1.3 b
	sunnhemp	0 ± 0 c
	cowpeas	0.2 ± 0.1 c
Ona	corn	8.3 ± 0.8 a
	SSG	6.3 ± 0.8 a
	sunnhemp	2.6 ± 0.8 b
	cowpeas	1.8 ± 0.7 b

TABLE 3. TWO-CHOICE BIOASSAYS OF ONA FALL ARMYWORM NEONATES COMPARING CORN TO SSG, SUNNHEMP, OR COWPEAS. MEANS IN EACH COMPARISON WITH THE SAME LETTER ARE NOT SIGNIFICANTLY DIFFERENT, $P > 0.05$.

Plant	Larvae per section
Corn	3.2 ± 0.7 a
SSG	4.2 ± 1.9 a
Corn	8.6 ± 0.8 a
Sunnhemp	5.2 ± 1.0 a
Corn	11.6 ± 1.5 a
Cowpeas	2.6 ± 0.5 b

Oviposition Choice

The first study performed during the summer resulted in few eggmasses and no significant difference among plants (Fig. 1). Oviposition during October was higher, with more eggmasses found on corn and SSG plants and fewer on cowpeas or sunnhemp. These results indicate that at least under greenhouse conditions and during the autumn season, females can show a preference for corn and SSG over the other plants.

Field Experiment

In Hastings, larval populations were found primarily on corn plants (12.9 ± 2.6 larvae per 1 m-row) compared to SSG (2.1 ± 0.5) or SSG with

TABLE 4. FOUR-CHOICE BIOASSAYS WITH ONA FALL ARMYWORM NEONATES WHOSE PARENTS WERE REARED ON CORN, SSG, SUNNHEMP OR COWPEAS. MEANS (WITHIN A GROUPING) WITH THE SAME LETTER ARE NOT SIGNIFICANTLY DIFFERENT, $P > 0.05$.

Plant parents reared on	Plant	Larvae per section
corn	corn	4.6 ± 1.0 a
	SSG	6.9 ± 1.3 a
	sunnhemp	3.6 ± 0.8 b
	cowpeas	2.8 ± 0.5 b
SSG	corn	5.9 ± 1.1 a
	SSG	5.2 ± 0.9 a
	sunnhemp	3.7 ± 0.8 a
	cowpeas	3.4 ± 1.4 a
sunnhemp	corn	8.0 ± 0.8 a
	SSG	7.8 ± 1.1 a
	sunnhemp	1.4 ± 0.5 b
	cowpeas	1.6 ± 0.6 b
cowpeas	corn	6.7 ± 1.5 a
	SSG	5.4 ± 0.9 a
	sunnhemp	3.6 ± 0.6 ab
	cowpeas	1.8 ± 0.7 b

TABLE 5. FOUR-CHOICE BIOASSAYS WITH F_1 ONA FALL ARMYWORM NEONATES WHOSE PARENTS SELECTED CORN, SSG, SUNNHEMP OR COWPEAS IN FIRST TEST. MEANS (WITHIN A GROUPING) WITH THE SAME LETTER ARE NOT SIGNIFICANTLY DIFFERENT, $P > 0.05$.

Plant parents selected	Plant	Larvae per section
corn	corn	5.4 ± 0.5 a
	SSG	5.4 ± 0.6 a
	sunnhemp	6.6 ± 0.9 a
	cowpeas	0.9 ± 0.3 b
SSG	corn	7.1 ± 1.0 a
	SSG	3.8 ± 0.8 b
	sunnhemp	5.7 ± 0.8 ab
	cowpeas	1.9 ± 0.4 c
sunnhemp	corn	4.7 ± 1.0 ab
	SSG	6.2 ± 0.6 a
	sunnhemp	5.0 ± 0.8 ab
	cowpeas	3.1 ± 0.6 b
cowpeas	corn	6.7 ± 0.6 a
	SSG	5.5 ± 0.8 ab
	sunnhemp	3.2 ± 0.7 bc
	cowpeas	2.8 ± 0.7 c

green manure (1.9 ± 0.5) (Fig. 2). No fall armyworm larvae were found on cowpeas or sunnhemp. The Gainesville plots provided the same information, as more larvae were collected on corn (26.0 ± 3.8) compared to SSG (3.5 ± 1.0). Very few larvae were collected on sunnhemp (0.17 ± 0.1) or cowpeas (0.08 ± 0.06). It was surprising that few larvae were found on SSG, but the results suggest that when both corn and SSG are present in the same area, fall armyworm will show a strong bias to the former.

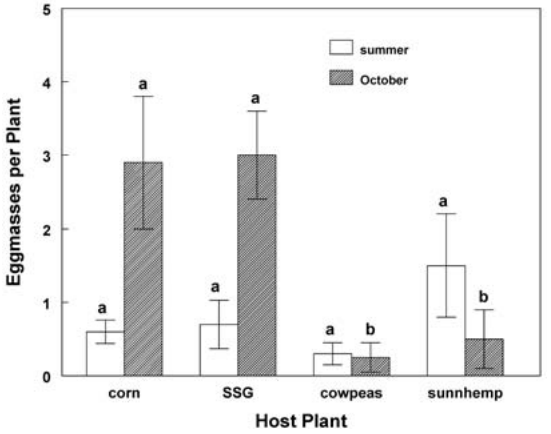

Fig. 1. Adult moths tested in swimming pool bioassays comparing oviposition on four host plants in summer and October experiments. For each test, columns with the same letter are not significantly different, $P > 0.05$.

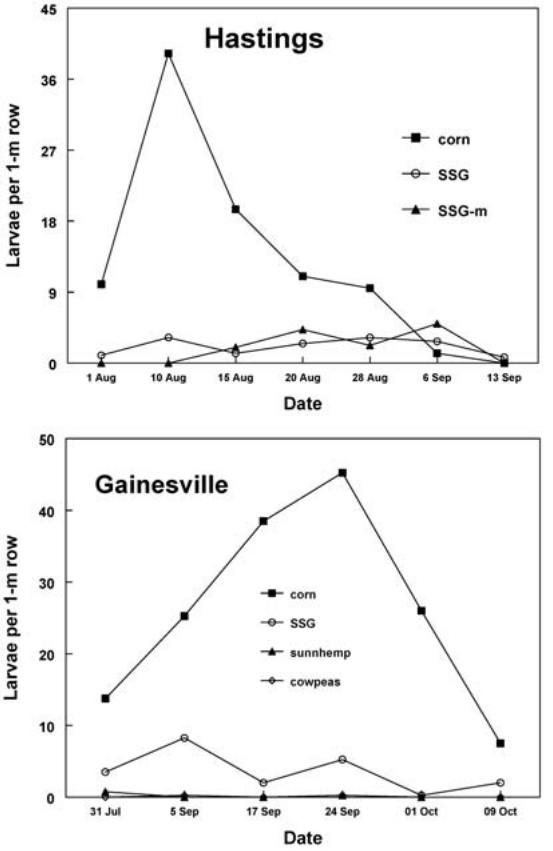

Fig. 2. Number of fall armyworm larvae collected from 1-m row of host plant in experiments conducted at Hastings and Gainesville, FL, 2001.

DISCUSSION

Previous studies have compared the development of the two host strains on different food sources, most notably corn and turf grass (Pashley 1988; Whitford et al. 1988; Pashley et al. 1995; Veenstra et al. 1995). These showed several strain-specific differences with respect to larval and pupal weights, consumption rates, development times, and mortality. It was surprising that only 62.2% of the Tifton larvae feeding on corn survived to the pupal stage. Although the Tifton culture shows the mitochondrial marker for corn strain, it was a laboratory culture that historically developed on meridic diet and may have exhibited feeding behavior effects of colonization (Mason 1987).

In general, rice strain larvae were larger and the development period shorter than the corn strain on all four host plants. This differs from previous reports indicating that when grown on corn, corn strain larvae were larger (Pashley et al. 1995). Apparently substantial variation can occur

with different cultures and experimental conditions. Despite these potential difficulties, our larval feeding studies consistently indicated that the two strains find cowpeas and sunnhemp to be a less favorable food source than corn or SSG.

Results from the larval choice experiments suggest strain differences in the preference of larvae to particular plant hosts. While both strains were attracted to corn, the corn strain preference appeared to be stronger and more specific. The result with corn strain larvae is consistent with a previous study that showed that larvae demonstrated a strong preference for corn over turf grass (Pashley et al. 1995).

For rice strain larvae, parental feeding on corn or SSG did not lead to increased bias of their progeny for these plants. Similarly, the larvae from parents raised on cowpeas or sunnhemp showed no increased preference to these plants. These results indicate that parental feeding history does not significantly influence the feeding preference of the progeny. Experiment 4 tested whether the variation in the rice strain culture was due to genetic polymorphisms, e.g., the presence of subpopulations with heritable biases to different plants. If this were the case, then larvae from parents that selected a particular host should be predisposed to the same bias, resulting in an increased proportion of that generation choosing the same plant. Results showed no clear evidence of strong heritability for plant preference. Therefore, it appears that the reduced specificity of rice strain larvae to corn compared to the corn strain represents an innate strain-specific difference.

Fall armyworm oviposition has been described on corn (Thomson & All 1983) and cotton (Ali et al. 1989), with differential preferences reported both within and among crops (Combs & Valerio 1980; Pitre et al. 1983). Only one previous study examined oviposition preference between host strains, with results showing a strong preference for corn strain moths to oviposit on corn and rice strain moths to oviposit on bermudagrass (Whitford et al. 1988). Recent research suggests that tactile cues may be more important than plant volatiles (Rojas et al. 2003), consistent with observations of egg masses on many non-plant objects such as vinyl flags (Thomson & All 1982), plastic bucket traps, and vehicle mirrors (RLM, unpublished). Our results indicate that a seasonal and/or environmental context must be taken into account when interpreting ovipositional studies.

A previous study described fall armyworm infestation in a forage sorghum field in southern Florida (Pair & Westbrook 1995). With 64% of the 200-ha field infested, the authors calculated hypothetically that the field could have produced 320 million adults/ha if 50% of the larvae survived to adulthood. The amount of SSG grown in Florida is not known, but almost 122,000 ha of vegetables were planted in 2002 (Anonymous 2003). If 50% of the vegetable land is planted to a SSG cover crop, over 19 trillion adult moths are potentially produced (320 million adults per ha $\times$ 61,000 ha). Therefore, large numbers of moths are potentially produced that could migrate to northerly areas or remain in local areas to later infest future crops.

Our experiments suggest that cowpeas and sunnhemp have the potential to reduce these populations of fall armyworm by lengthening developmental time and increasing larval mortality. Furthermore, these alternative cover crops are much less attractive to fall armyworm larvae and adults, and so may limit the concentration of this pest when corn is unavailable. Reducing the endogenous fall armyworm population in cover crops could both mitigate the level and delay the timing of infestation during the subsequent corn-growing season while simultaneously interfering with migration. While a potentially effective and economical means of cultural control, substantial additional research is needed to determine the influence of cover crops on the population dynamics of fall armyworm and thereby the potential effect of changes in cover crop choice. In particular, we need to determine the extent to which different cover crops serve as refuges for fall armyworm populations throughout the year and in particular during periods when corn is unavailable.

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NATURAL DISTRIBUTION OF HYMENOPTERAN PARASITOIDS OF *SPODOPTERA FRUGIPERDA* (LEPIDOPTERA: NOCTUIDAE) LARVAE IN MEXICO

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ABSTRACT

A survey of parasitoids of fall armyworm (FAW), *Spodoptera frugiperda* (J. E. Smith), larvae was conducted in six Mexican states during August and September 2000. Thirteen genera of hymenopteran parasitoids were recovered representing the following 3 families, Braconidae: *Aleoides*, *Chelonus*, *Cotesia, Glyptapanteles*, *Homolobus*, and *Meteorus*; Ichneumonidae: *Campoletis*, *Eiphosoma*, *Ophion*, and *Pristomerus*; and Eulophidae: *Aprostocetus*, *Euplectrus,* and *Horismenus*. Out of 5591 FAW larvae collected, 772 produced parasitoids, for a parasitism rate of 13.8%. The highest rate of parasitism from a single collection was 42.2%, representing three species of parasitoids in Michoacán. *Chelonus insularis* Cresson was the most widely distributed species occurring in 45.3% of the locations. *Pristomerus spinator* (F.), and *Meteorus laphygmae* (Viereck), exhibited the highest rates of parasitism for a single collection with 22.2% and 22.1%, in Sinaloa, and Michoacán, respectively. The results supported the hypothesis that natural distribution and rates of parasitism of FAW larvae may be related to more diverse habitats with more forests, orchards, and pastures near to cornfields.

Key Words: fall armyworm, *Chelonus*, *Pristomerus*, *Meteorus*, *Ophion*, *Campoletis*, corn, survey.

RESUMEN

Se llevó a cabo un inventario de parasitoides de larvas del gusano cogollero, *Spodoptera frugiperda* (J. E. Smith) (FAW) colectadas principalmente de maizales en estado de verticilio en seis estados mexicanos durante Agosto y Septiembre de 2000. Trece géneros de parasitoides himenópteros fueron recuperados, representando a tres familias, Braconidae: *Aleoides*, *Chelonus*, *Cotesia*, *Glyptapanteles*, *Homolobus*, y *Meteorus*); Ichneumonidae: *Campoletis*, *Eiphosoma*, *Ophion*, y *Pristomerus*; y Eulophidae: *Aprostocetus*, *Euplectrus*, y *Horismenus*. De un total de 5591 larvas colectadas, 772 produjeron parasitoides, para una tasa de parasitismo de 13.8%. La tasa de parasitismo más alta para una colecta simple fué de 42.2%, representando a tres especies de parasitoides in Michoacán. La especie más ampliamente distribuida fué *Chelonus insularis* Cresson, presentándose en 45.3% de las localidades inventariadas. *Pristomerus spinator* (F.), y *Meteorus laphygmae* (Viereck), mostraron las tasa más altas de parasitismo para una colecta simple con 22.2% y 22.1%, en Sinaloa, y Michoacán, respectivamente. Los resultados apoyan la hipótesis de que la distribución natural y las tasas de parasitismo pueden estar relacionadas a lo diverso de los hábitat con la cercanía de más bosques, huertas y pastizales a los maizales.

Translation provided by the authors.

The therapeutic approach of killing pest organisms with toxic chemicals has prevailed as a pest control strategy for over 50 years (Lewis et al. 1997). In the 1950s environmental effects of persistent organochlorine insecticides such as DDT began to be observed. Currently, in agricultural pest control, the adverse effects of the use of insecticides are leading scientists to search for alternatives to chemical control of insect pests based on health, environmental, wild life, and economic concerns (Johnson et al. 1998; Mattsson et al. 2000; Solomon & Schettler 2000).

Native insects and pathogens are normal parts of functioning agro-ecosystems and can profoundly influence the agricultural structure, species composition, and diversity. Agro-ecosystems

exhibit high biodiversity, mainly influenced by crops, weeds, microorganisms, and arthropods, but these factors are also influenced by geographical location, soil, and climatic characteristics, as well as human factors. Scientific evidence suggests that biodiversity can be used for improved pest management (Altieri 1991). The increased use of beneficial insects and interference with the colonization of fall armyworm in multiple cropping systems have prevented outbreaks in Latin America (Altieri 1994).

The fall armyworm (FAW), *Spodoptera frugiperda* (J. E. Smith), is a voracious pest inflicting damage to a multiplicity of annual crops in the Americas, and it is commonly controlled with synthetic insecticides, although insecticide resistance has been observed and is a concern (Yu 1991, 1992). Moreover, two strains of FAW have been identified according to their host preference, a corn-associated strain that feeds principally on corn, and a rice-associated strain that feeds primarily on forage grasses and rice (Pashley et al. 1987). Both FAW strains exhibited differences in resistance to chemical and biological insecticides (Adamczyk et al. 1997; López-Edwards et al. 1999), and have differences in their genetic population structure and population ecology (Pashley 1988; Lu & Adang 1996; Bossart & Prowell 1998; Levy et al. 2002; Meagher & Gallo-Meagher 2003; Nagoshi & Meagher 2003). These differences between FAW strains complicate the management of this pest.

Biological control is a highly desirable alternative to insecticides for controlling FAW infestations (Gross & Pair 1986). The value of parasitoids in reducing larval populations of this noctuid has long been recognized (Luginbill 1928; Vickery 1929). In order to develop a better understanding of the natural distribution of the FAW parasitoid complex and natural enemies, surveys have been carried out in different regions of Mexico (Carrillo 1980; Lezama-Gutiérrez et al. 2001; Molina-Ochoa et al. 2001, 2003a).

Here, we report the natural distribution of parasitoids of FAW larvae collected from whorl-stage corn, grain sorghum, forage sorghum, and Sudan grass fields from five Mexican states in the Pacific coast and one state in the Gulf of Mexico, during the summer of 2000.

Materials and Methods

During August and September of 2000, *S. frugiperda* larvae were collected from whorl-stage corn, grain and forage sorghum, and Sudan grass fields in 64 locations in the Mexican Pacific coast states of Sinaloa, Nayarit, Jalisco, Colima, and Michoacán, and in the Gulf of Mexico state of Veracruz. Egg masses and pupae were not collected.

FAW larvae were individually placed into 30-cc plastic cups with pinto bean diet (Burton & Perkins 1989), and held in the laboratory (Laboratory of Biological Control, Universidad de Colima, Facultad de Ciencias Biológicas y Agropecuarias, Tecomán, Colima, México) for emergence of parasitoids (Molina-Ochoa et al. 2001). Adult parasitoids were placed in 70% ethanol and then submitted to the USDA/ARS Systematic Entomology Laboratory, Beltsville, MD for identification. Collection size ranged from 33 to 119 FAW larvae. The number collected was corrected by subtracting the number that died from injury or unknown causes during the first few days after collection before calculating percent parasitism. Mortality due to pathogens and parasitic nematodes has been previously reported (Molina-Ochoa et al. 2003a).

Collection dates, geographic location, altitude, crop, sample size and total parasitism of FAW larvae in six Mexican states are presented in the Table 1. A Garmin GPS III Plus˜ was used for obtaining the coordinates and altitude data.

Results and Discussion

Out of 5591 FAW larvae collected, 772 produced parasitoids, for a parasitism rate of 13.8%. These parasitoids represented 13 genera from three families of Hymenoptera: six Braconidae, four Ichneumonidae, and three Eulophidae. Nine of the 64 collections produced no parasitoids, six of 12 collections from whorl-stage corn in Michoacán, two of 13 in Jalisco, and only one of 11 in Colima. The highest rates of parasitism in each state were found in C4 (33.3%) in Colima, J12 (21.1%)in Jalisco, M12 (14.4%) in Michoacán, N9 (18.9%) in Nayarit, S5 (27.4%) in Sinaloa, and V4 (11.5%) in Veracruz (Table 1). The most diverse collections of parasitoids were found in the locations C5, J12, and N9 with 5, 4, and 4 species, respectively, (Tables 2 and 3). The collection from S5 produced the highest rate of parasitism for a single species with 22.1%; the braconid *Meteorus laphygmae* Viereck was the most common parasitoid collected from Sudangrass. Other parasitoids in that collection were the eulophid *Euplectrus plathypenae* Howard (2 individuals), and the ichneumonid *Ophion flavidus* Brulle (1 individual). The braconid *C. insularis* occurred in 29 of the 64 collections from the six states, and it was the most widely distributed parasitoid. Another important braconid was *M. laphygmae*, occurring in 21 of the 64 collections. The ichneumonid parasitoids, *O. flavidus*, and *Pristomerus spinator* F., occurred in 18, and 17 of the 64 collections, respectively. *E. plathypenae* was the most important and widely distributed eulophid, occurring in 16 of the 64 collections (Tables 2 and 3).

Chelonus insularis was the most widely distributed parasitoid of FAW larvae in this survey, occurring in all the six Mexican States, and it was the braconid species with the second highest parasitism rate per location with 16.7%. Thus, *C. in-*

TABLE 1. GEOGRAPHIC LOCATION, DATE, ALTITUDE, CROP (*), SAMPLE SIZE (*N*), AND TOTAL PERCENT *SPODOPTERA FRUGIPERDA* LARVAE PARASITIZED IN SIX MEXICAN STATES (**) DURING 2000.

Code	Date	Location	Coordinates	Alt (m)	*	N	Percentage parasitized
C1	08/04	El poblado, Coquimatlán	19°3.698'N 103°47.722'W	422	C	90	17.8
C2	08/04	Pueblo Juárez, Coquimatlán	19°10.752'N 103°54.634'W	279	C	90	4.4
C3	08/04	Amachico, Coquimatlán	19°10.667'N 103°56.351'W	328	C	90	12.2
C4	08/06	Los mezcales, Comala	19°20.811'N 103°47.176'W	608	C	90	33.3
C5	08/06	El remate, Comala	19°24.825'N 103°47.639'W	817	C	90	13.3
C6	08/06	Carrizalillo, Quesería	19°25.389'N 103°41.000'W	1550	C	90	1.1
C7	08/06	Quesería	19°23.362'N 103°34.882'W	1304	c	90	10.0
C8	08/06	Villa de Alvarez	19°17.201'N 103°47.030'W	515	c	90	4.4
C9	08/06	Juluapan, Villa de Alvarez	19°18.890'N 103°49.611'W	539	c	90	4.4
C10	08/07	Tepames, Colima	19°08.231'N 103°37.996'W	519	c	90	0.0
C11	08/07	Estapilla, Colima	18°59.549'N 103°31.140'W	304	c	90	21.1
J1	08/08	Ciudad Guzmán	19°40.011'N 103°28.830'W	1557	c	90	0.0
J2	08/15	Los pinitos, Tonila	19°25.343'N 103°32.447'W	1326	c	90	2.2
J3	08/15	Pialla, Tuxpan	19°27.293'N 103°28.514'W	1079	c	90	0.0
J4	08/15	Atenquique, Tuxpan	19°31.778'N 103°27.851'W	1338	c	90	1.1
J5	08/17	Canoas, Zapotiltic	19°34.073'N 103°27.324'W	1391	c	90	3.3
J6	08/17	Apastepe	19°38.060'N 103°30.950'W	1709	c	90	1.1
J7	08/17	Teocuitatlán	20°07.035'N 103°32.704'W	1369	c	90	10.0
J8	08/17	Zacoalco de Torres	20°11.988'N 103°33.806'W	1425	c	90	4.4
J9	08/17	Acatlán de Juárez	20°25.362'N 103°33.406'W	1575	c	96	2.1
J10	08/17	Tlajomulco de Zúñiga	20°29.396'N 103°28.298'W	1607	c	92	4.3
J11	08/18	Zapopan	20°43.129'N 103°29.041'W	1670	c	90	4.4
J12	08/18	Magdalena	20°53.008'N 103°55.477'W	1496	c	93	21.5
J13	08/23	Crucero de Magdalena	20°56.300'N 104°02.509'W	1386	c	92	2.2
M1	08/09	Totolán	19°58.890'N 102°40.183'W	1590	c	90	0.0
M2	08/09	Santa Inés Tocumbo	19°44.502'N 102°34.967'W	1630	c	90	1.1
M3	08/09	Peribán	19°33.106'N 102°26.586'W	1475	c	90	1.1
M4	08/10	Cointzio	19°41.609'N 101°16.398'W	1932	c	90	0.0

*Corn (c), gran sorghum (gs), forage sorghum (fs), and Sudan grass (sg).
**Colima (C), Jalisco (J), Michoacan (M) Nayarit (N), Sinaloa (S), and Veracruz (V).

TABLE 1. (CONTINUED) GEOGRAPHIC LOCATION, DATE, ALTITUDE, CROP (*), SAMPLE SIZE (*N*), AND TOTAL PERCENT *SPODOPTERA FRUGIPERDA* LARVAE PARASITIZED IN SIX MEXICAN STATES (**) DURING 2000.

Code	Date	Location	Coordinates	Alt (m)	*	N	Percentage parasitized
M5	08/10	Cerro "La Esperanza"	19°41.223'N 101°18.980'W	1998	c	90	1.1
M6	08/11	Tejabán	19°13.342'N 101°53.714'W	587	c	90	0.0
M7	08/11	Carretera a Nueva Italia	19°03.290'N 102°02.458'W	442	c	90	0.0
M8	08/11	Presa de Zicuirán	18°56.191'N 101°54.650'W	292	c	63	0.0
M9	08/11	El ceñidor, Nueva Italia	18°59.651'N 102°11.577'W	350	c	57	1.8
M10	08/12	La Guadalupe Parácuaro	19°07.472'N 102°12.519'W	540	fs	90	1.1
M11	08/12	Las yeguas Parácuaro	18°57.308'N 102°16.733'W	359	fs	90	1.1
M12	08/12	El cirián, Nueva Italia	18°53.661'N 102°07.483'W	255	c	90	42.2
N1	08/18	Santa María del Oro	21°20.121'N 104°40.174'W	1160	c	90	3.3
N2	08/18	El rincón, Tepic	21°32.472'N 104°56.123'W	849	c	96	10.4
N3	08/18	El pichón, Tepic	21°33.479'N 104°56.937'W	774	c	95	4.2
N4	08/19	Xalisco	21°19.601'N 104°55.060'W	1042	c	107	2.8
N5	08/19	El refilión, Xalisco	21°19.407'N 104°55.323'W	964	c	90	8.9
N6	08/19	Compostela	21°17.858'N 104°54.044'W	920	c	93	1.1
N7	08/19	La presa, Compostela	21°13.714'N 104°52.162'W	928	c	90	1.1
N8	08/20	Las lumbres, Acaponeta	22°20.795'N 105°18.141'W	48	C&gs	60	5.0
N9	08/23	Seboruco	21°20.850'N 104°40.749'W	1134	c	90	18.9
N10	08/23	Ahuacatlán	21°06.331'N 104°27.427'W	1120	c	90	5.6
S1	08/21	Bacurimi, Culiacán	24°51.668'N 107°29.478'W	70	gs	97	4.1
S2	08/21	La campana, Culiacán	24°58.415'N 107°33.517'W	143	gs	100	5.0
S3	08/21	Pericos, Mocorito	25°03.574'N 107°39.547'W	80	gs	95	9.5
S4	08/21	Rancho viejo, Mocorito	25°06.033'N 107°43.165'W	89	gs	98	13.3
S5	08/22	Aguapepito, Mocorito	25°03.861'N 107°39.547'W	68	sg	95	27.4
S6	08/22	Comanito, Mocorito	25°09.006'N 107°39.645'W	91	gs	95	3.2
S7	08/22	La poma, Badiraguato	25°15.749'N 107°40.739'W	157	c	100	13.0
S8	08/22	La majada, Badiraguato	25°14.076'N 107°39.781'W	145	c	92	7.6
V1	09/02	Seis de Enero, Xalapa	19°34.115'N 96°50.207'W	950	c	91	6.6
V2	09/02	Altolucero, Almolonga	19°35.063'N 96°47.384'W	908	c	33	12.1

*Corn (c), gran sorghum (gs), forage sorghum (fs), and Sudan grass (sg).
**Colima (C), Jalisco (J), Michoacan (M) Nayarit (N), Sinaloa (S), and Veracruz (V).

TABLE 1. (CONTINUED) GEOGRAPHIC LOCATION, DATE, ALTITUDE, CROP (*), SAMPLE SIZE (*N*), AND TOTAL PERCENT *SPODOPTERA FRUGIPERDA* LARVAE PARASITIZED IN SIX MEXICAN STATES (**) DURING 2000.

Code	Date	Location	Coordinates	Alt (m)	*	N	Percentage parasitized
V3	09/02	Actopan	19°34.623'N 96°48.589'W	775	c	64	3.1
V4	09/02	Los González, Actopan	19°31.894'N 96°41.294'W	432	c	113	11.5
V5	09/02	Bocana, Actopan	19°24.416'N 96°36.731'W	311	c	119	4.2
V6	09/03	El volador, Coatepec	19°21.594'N 96°51.037'W	709	c	90	3.3
V7	09/03	Palmillas	19°12.293'N 96°46.221'W	702	c	59	6.8
V8	09/03	Tierra Colorada	19°13.255'N 96°21.916'W	46	c	45	4.4
V9	09/04	Cerro gordo	19°25.252'N 96°39.566'W	443	c	45	8.9
V10	09/04	La cumbre	19°23.320'N 96°38.807'W	366	c	66	6.1

*Corn (c), gran sorghum (gs), forage sorghum (fs), and Sudan grass (sg).
**Colima (C), Jalisco (J), Michoacan (M) Nayarit (N), Sinaloa (S), and Veracruz (V).

sularis is one of the most abundant natural enemies of fall armyworm larvae in the Western Coast and Gulf of Mexico. *Chelonus insularis* has been reported as an important parasitoid controlling FAW populations in the US (Lugingill 1928; Vickery 1929). Ashley (1986) and Andrews (1988) listed *C. insularis* occurring in Central America and the US, highlighting its role as parasitoid of FAW in southern Florida where 63% of the FAW larvae were attacked. Recently, Molina-Ochoa et al., (2003b) reported *C. insularis* syn. *C. texanus* as the braconid with the broadest distribution in Latin America, including South America (Uruguay and Venezuela), the Caribbean Basin (Trinidad and Puerto Rico), and the US. In that inventory *Chelonus* sp. is also reported in Brazil, Mexico, and Peru. Lewis and Nordlund (1980) emphasized its role considering it as an excellent candidate for the following augmentative approaches: a) release throughout its overwintering zone; b) early-season colonization, and c) direct therapeutic release on target crops.

In a previous survey, Molina-Ochoa et al. (2001) commented on the importance and need of more study in Mexico on the taxonomy of the genus *Chelonus* (P. M. Marsh, pers. comm.).

Meteorus laphygmae occurred in 21 of the 64 collections. The highest rate of parasitism for a single location was obtained in S5 with 22.1%. This parasitoid occurred in all of the collections from Sinaloa, and the rate of parasitism ranged from 2.1 to 22.1%. *Meteorus laphygmae* was also collected in Colima, Nayarit, Michoacán, Jalisco, and Veracruz occurring in 45.5%, 30%, 25%, 10%, and 8.3% of the collections, respectively. This braconid was reported by Ashley (1986) occurring in the Continental US, exhibiting its greatest impact on FAW collected from grass. Other reports were made by Alvarado-Rodríguez (1987) in Sinaloa, Mexico attacking *Spodoptera exigua* (Hübner) infesting tomatoes with a parasitism rate of 9.0%. A similar rate of parasitism was reported by Molina-Ochoa et al. (2001) in a single collection of FAW larvae made in El Mante, Tamaulipas with 10.3%. Molina-Ochoa et al. (2003b) listed several reports from countries of Central and South America, such as Honduras, Nicaragua, Mexico, Chile, Colombia, and Suriname, where *M. laphygmae* was collected from other crops such as maize, rice, cotton, sorghum, peanuts, and Bermudagrass, and was one of the most prevalent parasitoids in South America.

Low rates of occurrence and parasitization of *Cotesia* sp. probably *marginiventris* (Cresson), *Glyptapanteles* sp. probably *militaris* (Walsh), *Aleiodes* sp., and *Homolobus* sp. probably *mellea* (Cresson) were recorded. They were found in 5, 2, 1, and 1 of the 64 collections, respectively.

Cotesia sp. occurred in Colima, Jalisco, Nayarit with lower parasitization rates than 2.3%. Similar rates were reported by Molina-Ochoa et al. (2001) in a previous survey conducted in four Mexican States. This parasitoid is reported attacking FAW larvae in Argentina, Brazil, Chile, Honduras, Lesser Antilles, Mexico, Nicaragua, Puerto Rico, Suriname (Molina-Ochoa et al. 2003b), but it has been often reported as a parasitoid of FAW in the US (Ashley 1986) with parasitization rates of 6.3% on FAW larvae collected from maize (Riggin et al. 1993) and from less than 1% to 40% collected from maize and Bermudagrass, respectively (Ashley et al. 1983).

TABLE 2. PERCENTAGE OF *SPODOPTERA FRUGIPERDA* LARVAE PARASITIZED BY EACH SPECIES OF BRACONIDAE AT EACH LOCATION.

	Braconidae					
Code*	*Aleoides*	*Chelonus*	*Cotesia*	*Glyptapanteles*	*Homolobus*	*Meteorus*
C1	0.0	3.3	0.0	0.0	0.0	2.2
C2	0.0	0.0	0.0	0.0	0.0	1.1
C3	0.0	7.8	0.0	0.0	0.0	0.0
C4	0.0	16.7	1.1	0.0	0.0	0.0
C5	0.0	1.1	1.1	0.0	0.0	4.4
C6	0.0	0.0	0.0	0.0	0.0	0.0
C7	0.0	1.1	1.1	0.0	0.0	0.0
C8	0.0	2.2	0.0	0.0	0.0	1.1
C9	0.0	1.1	0.0	0.0	0.0	2.2
C10	0.0	0.0	0.0	0.0	0.0	0.0
C11	0.0	14.4	0.0	0.0	0.0	0.0
J1	0.0	0.0	0.0	0.0	0.0	0.0
J2	0.0	0.0	0.0	0.0	0.0	0.0
J3	0.0	0.0	0.0	0.0	0.0	0.0
J4	0.0	0.0	0.0	0.0	0.0	0.0
J5	0.0	0.0	0.0	0.0	0.0	0.0
J6	0.0	1.1	0.0	0.0	0.0	0.0
J7	0.0	3.3	0.0	0.0	0.0	1.1
J8	0.0	4.4	0.0	0.0	0.0	0.0
J9	0.0	0.0	0.0	0.0	0.0	0.0
J10	0.0	3.3	0.0	0.0	0.0	0.0
J11	0.0	1.1	0.0	0.0	0.0	0.0
J12	0.0	15.1	1.1	0.0	0.0	0.0
J13	0.0	1.1	0.0	0.0	0.0	0.0
M1	0.0	0.0	0.0	0.0	0.0	0.0
M2	0.0	0.0	0.0	0.0	0.0	1.1
M3	0.0	0.0	0.0	0.0	1.1	0.0
M4	0.0	0.0	0.0	0.0	0.0	0.0
M5	0.0	0.0	0.0	0.0	0.0	0.0
M6	0.0	0.0	0.0	0.0	0.0	0.0
M7	0.0	0.0	0.0	0.0	0.0	0.0
M8	0.0	0.0	0.0	0.0	0.0	0.0
M9	0.0	0.0	0.0	0.0	0.0	1.8
M10	0.0	1.1	0.0	0.0	0.0	0.0
M11	0.0	0.0	0.0	0.0	0.0	1.1
M12	0.0	14.4	0.0	0.0	0.0	0.0
N1	0.0	0.0	0.0	0.0	0.0	0.0
N2	1.0	1.0	0.0	8.3	0.0	0.0
N3	0.0	1.1	0.0	0.0	0.0	2.1
N4	0.0	0.0	0.0	1.9	0.0	0.9
N5	0.0	1.1	0.0	0.0	0.0	2.2
N6	0.0	0.0	0.0	0.0	0.0	0.0
N7	0.0	0.0	0.0	0.0	0.0	0.0
N8	0.0	5.0	0.0	0.0	0.0	0.0
N9	0.0	5.6	2.2	0.0	0.0	0.0
N10	0.0	2.2	0.0	0.0	0.0	0.0
S1	0.0	0.0	0.0	0.0	0.0	2.1
S2	0.0	1.0	0.0	0.0	0.0	4.0
S3	0.0	0.0	0.0	0.0	0.0	8.4
S4	0.0	1.0	0.0	0.0	0.0	12.2
S5	0.0	0.0	0.0	0.0	0.0	22.1
S6	0.0	0.0	0.0	0.0	0.0	3.2

Aleiodes sp., *Chelonus* sp. Probably *insularis* Cresson, *Cotesia* sp. probably *marginiventris* Cresson, *Glyptapanteles* sp. probably *militaris* Walsh, *Homolobus* sp. probably *mellea* Cresson, *Meteorus* sp. probably *laphygmae* Viereck.

TABLE 2. (CONTINUED) PERCENTAGE OF *SPODOPTERA FRUGIPERDA* LARVAE PARASITIZED BY EACH SPECIES OF BRACONIDAE AT EACH LOCATION.

	Braconidae					
Code*	*Aleoides*	*Chelonus*	*Cotesia*	*Glyptapanteles*	*Homolobus*	*Meteorus*
S7	0.0	2.0	0.0	0.0	0.0	10.0
S8	0.0	0.0	0.0	0.0	0.0	6.5
V1	0.0	3.3	0.0	0.0	0.0	1.1
V2	0.0	0.0	0.0	0.0	0.0	0.0
V3	0.0	0.0	0.0	0.0	0.0	0.0
V4	0.0	0.0	0.0	0.0	0.0	0.0
V5	0.0	0.0	0.0	0.0	0.0	0.0
V6	0.0	1.1	0.0	0.0	0.0	0.0
V7	0.0	0.0	0.0	1.7	0.0	0.0
V8	0.0	0.0	0.0	0.0	0.0	0.0
V9	0.0	0.0	0.0	0.0	0.0	0.0
V10	0.0	0.0	0.0	0.0	0.0	0.0

Aleiodes sp., *Chelonus* sp. Probably *insularis* Cresson, *Cotesia* sp. probably *marginiventris* Cresson, *Glyptapanteles* sp. probably *militaris* Walsh, *Homolobus* sp. probably *mellea* Cresson, *Meteorus* sp. probably *laphygmae* Viereck.

Glyptapanteles sp. was found in Nayarit in two collections, N2 and N4, with parasitization rates of 8.3% and 1.9%, respectively, and in one location in Veracruz (V7) with 1.7% of parasitism rate. Rohlfs & Mack (1985), and Cave (1993) reported the occurrence of this parasitoid attacking FAW larvae in the US and Honduras, collected from sorghum and maize, respectively. Steffey (2001) reported *G. militaris* attacking armyworms and other caterpillars in Illinois. He speculated that this braconid and other natural enemies could suppress armyworm populations and keep them well below economic levels. Recently, Reis et al. (2003) suggested that the parasitoid may be well adapted to the Azorean agricultural systems in Portugal, characterized by prevalence of the grass, *Lolium perenne* L., throughout the year. The armyworm, *Pseudaletia unipuncta* (Haworth) when fed on fresh leaves of *L. perenne* is the most suitable host for the mass rearing of this braconid.

Aleiodes sp. occurred only in one collection in Nayarit (N2), and *Homolobus* sp. was found in Michoacán (M3), and their parasitism was lower than 1.2%. Ruíz-Cancino (1991) reported species of *Rogas* (Syn: *Aleiodes*) occurring in "La Reserva de la Biosfera *El Cielo*" in Tamaulipas, Mexico, and the family Braconidae is the second more abundant with 10% of the individuals, these braconids were attacking insect pest of annual, perennial and ornamental crops. *Aleiodes laphygmae* was reported by Molina-Ochoa et al. (2001) with a low parasitism rate (0.3%) on FAW larvae in Tamaulipas, Mexico. This braconid, *A. laphygmae* was the most abundant parasitoid attacking FAW larvae (12.8% parasitism) in South Georgia (Riggin et al. 1993).

Homolobus sp. probably *mellea* (Cresson), syn: *Zele mellea* (Cresson) was previously found in small numbers attacking FAW larvae in Honduras (Cave 1993), Nicaragua (Huis 1981) and the US (Vickery 1929; Wilson 1933; Riggin et al. 1992), but was not previously reported in Mexico. Parasitism by this species was low (1.1%), but finding it contributes to our knowledge on the occurrence and diversity of beneficial insects affecting FAW populations in Michoacán.

The ichneumonid parasitoids, *O. flavidus, P. spinator,* and *C. flavicincta* were the most frequently reared species in 18, 17, and 14 of the 64 collections, respectively. *Ophion flavidus* was recovered in more locations in Michoacán, and Colima (5 and 4 locations, respectively), but the highest parasitism rate for a single location was obtained in Colima (C7) with 6.7%. Similar results were reported by Molina et al. (2001), and Riggin et al. (1993). Recently, Molina-Ochoa et al., (2003b) listed the occurrence of *O. flavidus* in Argentina, Brazil, Honduras, Mexico, Nicaragua, and the US. Ashley et al. (1983) reported that *Ophion* sp. attacked FAW larvae developing on volunteer corn and Paragrass at Homestead, Florida. Gross & Pair (1991) emphasized that the tachinid *Archytas marmoratus* (Townsend) and *O. flavidus* provide opportunities for advancing biological strategies for managing FAW, with the development of economical methods for mass-propagation.

P. spinator was the second most widely distributed ichneumonid parasitoid. It was recovered in 17 of the 64 collections, 7 in Colima, 2 in Jalisco, 4 in Michoacán, and Nayarit, but this species was not recovered from Sinaloa, and Veracruz. The highest rate of parasitism for a single location was obtained in Michoacán (M12) with 22.2%. *Pristomerus spinator* has been reported in Mexico occurring in Quintana Roo, Tamaulipas (Carrillo 1980), and Michoacán, Colima, and Jalisco (Molina-Ochoa et al.

TABLE 3. PERCENTAGE OF *Spodoptera frugiperda* LARVAE PARASITIZED BY EACH SPECIES OF ICHNEUMONIDAE AND EULOPHIDAE AT EACH LOCATION.

	Ichneumonidae				Eulophidae		
Code*	C.f	E.v	O.f	P.s	*A*.sp	*E*.p	*H*.sp
C1	0.0	0.0	0.0	12.2	0.0	0.0	0.0
C2	0.0	2.2	0.0	1.1	0.0	0.0	0.0
C3	0.0	0.0	0.0	4.4	0.0	0.0	0.0
C4	0.0	1.1	0.0	14.4	0.0	0.0	0.0
C5	0.0	0.0	5.6	1.1	0.0	0.0	0.0
C6	1.1	0.0	0.0	0.0	0.0	0.0	0.0
C7	0.0	0.0	6.7	1.1	0.0	0.0	0.0
C8	0.0	0.0	1.1	0.0	0.0	0.0	0.0
C9	0.0	0.0	1.1	0.0	0.0	0.0	0.0
C10	0.0	0.0	0.0	0.0	0.0	0.0	0.0
C11	0.0	0.0	0.0	6.7	0.0	0.0	0.0
J1	0.0	0.0	0.0	0.0	0.0	0.0	0.0
J2	0.0	0.0	0.0	2.2	0.0	0.0	0.0
J3	0.0	0.0	0.0	0.0	0.0	0.0	0.0
J4	0.0	0.0	1.1	0.0	0.0	0.0	0.0
J5	0.0	0.0	0.0	3.3	0.0	0.0	0.0
J6	0.0	0.0	0.0	0.0	0.0	0.0	0.0
J7	1.1	0.0	4.4	0.0	0.0	0.0	0.0
J8	0.0	0.0	0.0	0.0	0.0	0.0	0.0
J9	2.1	0.0	0.0	0.0	0.0	0.0	0.0
J10	1.1	0.0	0.0	0.0	0.0	0.0	0.0
J11	3.3	0.0	0.0	0.0	0.0	0.0	0.0
J12	3.2	0.0	2.1	0.0	0.0	0.0	0.0
J13	0.0	0.0	0.0	0.0	0.0	1.1	0.0
M1	3.3	0.0	1.1	0.0	0.0	0.0	0.0
M2	2.2	0.0	0.0	3.3	0.0	0.0	0.0
M3	0.0	0.0	3.3	1.1	0.0	0.0	0.0
M4	0.0	0.0	1.1	1.1	0.0	0.0	0.0
M5	1.1	0.0	0.0	0.0	0.0	0.0	0.0
M6	0.0	3.3	0.0	0.0	0.0	0.0	0.0
M7	0.0	0.0	0.0	0.0	0.0	0.0	0.0
M8	0.0	0.0	0.0	0.0	0.0	1.6	0.0
M9	0.0	0.0	1.8	0.0	0.0	0.0	0.0
M10	0.0	0.0	1.1	0.0	0.0	1.1	0.0
M11	0.0	2.2	0.0	0.0	0.0	0.0	0.0
M12	0.0	5.6	0.0	22.2	0.0	0.0	0.0
N1	2.2	0.0	0.0	1.1	0.0	0.0	0.0
N2	0.0	0.0	0.0	0.0	0.0	0.0	0.0
N3	0.0	1.1	0.0	0.0	0.0	0.0	0.0
N4	0.0	0.0	0.0	0.0	0.0	0.0	0.0
N5	1.1	0.0	1.1	3.3	0.0	0.0	0.0
N6	0.0	0.0	1.1	0.0	0.0	0.0	0.0
N7	1.1	0.0	0.0	0.0	0.0	0.0	0.0
N8	0.0	0.0	0.0	0.0	0.0	0.0	0.0
N9	3.3	0.0	0.0	7.8	0.0	0.0	0.0
N10	2.2	0.0	0.0	1.1	0.0	0.0	0.0
S1	0.0	0.0	2.1	0.0	0.0	0.0	0.0
S2	0.0	0.0	0.0	0.0	0.0	0.0	0.0
S3	0.0	0.0	1.1	0.0	0.0	0.0	0.0
S4	0.0	0.0	0.0	0.0	0.0	0.0	0.0
S5	0.0	0.0	1.1	0.0	0.0	4.2	0.0
S6	0.0	0.0	0.0	0.0	0.0	0.0	0.0

C.f = *Campoletis flavicincta* Ashmead, E.v = *Eiphosoma vitticolle* Cresson, O.f = *Ophion flavidus* Brulle, P.s = *Pristomerus spinator* Fabricius, *A*.sp. = *Aprostocetus* sp., *E*.p = *Euplectrus plathypenae* Howard, *H*.sp. = *Horismenus* sp.

TABLE 3. (CONTINUED) PERCENTAGE OF *Spodoptera frugiperda* LARVAE PARASITIZED BY EACH SPECIES OF ICHNEUMONIDAE AND EULOPHIDAE AT EACH LOCATION.

	Ichneumonidae				Eulophidae		
Code*	C.f	E.v	O.f	P.s	*A*.sp	*E*.p	*H*.sp
S7	0.0	0.0	0.0	0.0	0.0	1.0	0.0
S8	0.0	0.0	0.0	0.0	0.0	1.1	0.0
V1	0.0	0.0	0.0	0.0	0.0	2.2	0.0
V2	0.0	0.0	0.0	0.0	3.0	6.1	3.0
V3	0.0	0.0	0.0	0.0	0.0	3.1	0.0
V4	0.0	0.0	0.0	0.0	0.0	11.5	0.0
V5	0.0	0.0	0.0	0.0	0.0	4.2	0.0
V6	0.0	0.0	0.0	0.0	0.0	2.2	0.0
V7	0.0	0.0	0.0	0.0	0.0	5.1	0.0
V8	0.0	0.0	0.0	0.0	0.0	4.4	0.0
V9	0.0	0.0	0.0	0.0	0.0	8.9	0.0
V10	0.0	0.0	0.0	0.0	0.0	6.1	0.0

C.f = *Campoletis flavicincta* Ashmead, E.v = *Eiphosoma vitticolle* Cresson, O.f = *Ophion flavidus* Brulle, P.s = *Pristomerus spinator* Fabricius, *A*.sp. = *Aprostocetus* sp., *E*.p = *Euplectrus plathypenae* Howard, *H*.sp. = *Horismenus* sp.

2001). Two collections from Michoacán during 1998 and 2000 exhibited the highest parasitism rates for a single location (El Hueso, and El Cirián, Nueva Italia) with 12.7%, and 22.2%, respectively. The ichneumonid was previously reported from Brazil, Honduras, Mexico, Nicaragua, and the US (Molina-Ochoa et al. 2003b).

Campoletis flavicincta was found in 14 of 64 collections, one in Colima, 5 in Jalisco, 3 in Michoacán, and 5 in Nayarit, but it was not recovered in Sinaloa, and Veracruz. *Campoletis flavicincta* had an overall parasitism range from 0 to 3.3%. The highest parasitism rate for a single location was obtained in N9. In a previous survey conducted by Molina-Ochoa et al. (2001), *C. flavicincta* accounted for 23% of parasitism in El Batillero, Michoacán, a location surrounded by avocado orchards and pine forest near to Apo, Michoacán; however, the FAW larvae from nearby locations in this survey (M1 and M2) showed low parasitism rates (3.3%, and 2.2%, respectively) by this parasitoid. It appears that, *C. flavicincta,* prefers or was associated with locations with high altitude; in this survey, it was found in locations with altitudes with an average of 1417 meters, as well as in locations near forests mainly constituted with pine and oak trees. Molina-Ochoa et al. (2003b) reported *C. flavicincta* occurring in Brazil, Honduras, Mexico, Nicaragua, and the US. This species was also reported attacking beet armyworm larvae fed on cotton in Georgia, USA (Ruberson et al. 1993, 1994).

Eiphosoma vitticole was the ichneumonid with the most limited distribution in this survey, found in 6 of the 64 collections. *E. vitticole* occurred in 2 locations in Colima, 3 locations in Michoacán, and 1 location in Nayarit. The highest rate of parasitism for a single location was recorded in M12 with 5.6%. This species showed low parasitism rates, and it was not found in Jalisco, Sinaloa, and Veracruz. It was collected from locations with an average altitude of 472m, with a range between 255 and 744m. Pair et al. (1986) reported the occurrence of *E. vitticole* in Texas, and Tamaulipas, Mexico. It also has been reported from Bolivia, Brazil, Colombia, Honduras, and Nicaragua (Molina-Ochoa et al. 2003b)

Three species of eulophid parasitoids were found in this survey, *Aprostocetus* sp., *Euplectrus plathypenae* Howard, and *Horismenus* sp. *Euplectrus plathypenae* was the most widely distributed eulophid, occurring in 16 of the 64 collections. It was found in Veracruz in all collections (10), Sinaloa in 3 collections, 2 in Michoacán, and one in Jalisco. Molina-Ochoa et al. (2001) reported a parasitism rate of 8.3% by *E. plathypenae* in a single collection in El Mante, Tamaulipas, similar rates in several locations in Veracruz, and low rate of about 1% in Michoacán. We also did not find levels higher than 1.6% in Michoacán; however, we found a range of parasitism in Sinaloa between 1% and 4.2%. The highest level of parasitism for a single location was obtained in the location V4 with 11.5%. Montoya-Burgos (1980) reported natural parasitism of about 15% by *Euplectrus* sp. against L2 FAW developing on corn in Veracruz. *Euplectrus plathypenae* is an important and well distributed parasitoid in the tropical Americas, and the US (Molina-Ochoa et al. (2003b).

The other eulophids, *Aprostocetus* sp. and *Horismenus* sp., occurred only in the location V2, with a parasitism rate of 3.0% for both species. This is the first report of *Aprostocetus* sp. and *Horismenus* sp. as parasitoids of FAW larvae. *Aprostocetus* sp. has been reported as a hyperparasitoid of *Gelechia senticetella* (Stgr.) (Lepidoptera:

Gelechiidae) fed on *Juniperus excelsa* in Bulgaria (Mirchev et al. 2001). *Aprostocetus* sp. also was reported as an egg parasitoid of mango leafhoppers (Fasih & Srivatava 1990). *Aprostocetus diplosis* Crawford is a parasitoid of *Stenodiplosis sorghicola*, a dipterous pest of sorghum in Brazil (Campos et al. 1998). *Horismenus* sp. has been reported to be a parasitoid of prepupae and pupae of the Citrus leafminer, *Phyllocnistis citrella* (Lepidoptera: Gracillariidae) in Mexico (Perales et al. 1996, Bautista-Martínez et al. 1998). Coffelt & Schultz (1993) mentioned that it is very common to find species of this genus acting as hyperparasitoids.

Our results demonstrate that hymenopteran parasitoids of FAW differentially occurred throughout the six Mexican states surveyed. However, this may have been influenced by the size of the FAW larvae collected. The hymenopteran parasitoids caused significant mortality of FAW larvae in most of the localities of this survey. It is important to highlight the occurrence and role on the FAW larval mortality caused by the braconids, *C. insularis*, and *M. laphygmae,* the ichneumonids, *O. flavidus*, *P. spinator,* and *C. flavicincta*, as well as the eulophid *E. plathypenae*. Our findings agree with Ashley (1986) in that no single parasitoid species exerted significant mortality throughout a major portion of the range of FAW. Another important aspect to note is the need for more taxonomic studies on two genera, *Chelonus* and *Meteorus*, which are important sources of mortality for FAW larvae.

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MITOCHONDRIAL DNA VARIATION AND DISTRIBUTION OF THE SUBTERRANEAN TERMITE GENUS *RETICULITERMES* (ISOPTERA: RHINOTERMITIDAE) IN ARKANSAS AND LOUISIANA

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ABSTRACT

Limited information exists on genetic variation and distribution of *Reticulitermes* from the south central United States. Focusing on molecular sequence data from the mitochondrial DNA 16S gene, this study records the distribution and genetic variation of *Reticulitermes* species in Arkansas and updates the current distribution in a neighboring State, Louisiana. Termite samples were collected from the field, subjected to DNA analysis with Polymerase Chain-Reaction (PCR), and sequenced. *Reticulitermes* sp. sequence data were aligned, genetic distances recorded, and their respective haplotypes were evaluated for possible geographic structure. From 35 Arkansas counties, 59 *R. flavipes*, 13 *R. hageni*, and seven *R. virginicus* were identified. In Arkansas, 11 mitochondrial haplotypes were observed in *R. flavipes*, three in *R. hageni* and three in *R. virginicus*. Among the 12 Louisiana parishes sampled, 13 *R. flavipes*, three *R. virginicus*, and one *R. tibialis* were identified with six, three, and one haplotypes for each species, respectively. Genetic variation among the *R. flavipes* haplotypes from both States ranged from 0.2 to 0.9%. *Reticulitermes flavipes* haplotype diversity observed in Arkansas and Louisiana was lower than observed in Texas and Oklahoma.

Key Words: 16S rRNA gene, DNA sequence, genetic variation, population genetics, *Reticulitermes*, termite.

RESUMEN

La información existente sobre la variación genética y la distribución de *Reticulitermes* en el sur central del los Estados Unidos es limitada. Enfocandose en los datos de las secuencias moleculares del gene 16S del ADN mitocondrial, este estudio registra la distribución y la variación genética de *Reticulitermes* spp. en Arkansas y pone al dia la distribución actual en el estado vecino, Louisiana. Las muestras de termitas recolectadas del campo, fueron sujetas al análisis de ADN por la reacción en cadena de la polímerasa (RCP), y secuenciadas. Los datos de secuenciación genética para *Reticulitermes* spp. fueron alineados, las distancias genéticas registradas, y sus haplotipos respectivos fueron evaluados para su posible estructura geográfica. De los 35 condados de Arkansas, 59 *R. flavipes*, 13 *R. hageni*, y siete *R. virginicus* fueron identificados. En Arkansas, 11 haplotipos de mitocondria fueron observados en *R. flavipes,* tres en *R. hageni* y tres en *R. virginicus.* Entre las 12 regiones de Louisiana muestreadas, 13 *R. flavipes*, tres *R. virginicus*, y una *R. tibialis* fueron identificados con seis, tres, y un haplotipos por cada especie, respectivamente. La variación genética entre los haplotipos de *R. flavipes* de ambos estados fue de 0.2 hasta 0.9%. La diversidad de los haplotipos de *Reticulitermes flavipes* observada en Arkansas y Louisiana fue menor de la que fue observada en Texas y Oklahoma.

In Arkansas and Louisiana, subterranean termites cause millions of dollars of damage annually. Damage caused by subterranean termite activity probably exceeds $2.5 billion annually in the United States (Anonymous 2003) and $22 billion globally (Su 2002). Several structurally important species in the genus *Reticulitermes* are common throughout the southeastern U.S. (Weesner 1965). Messenger et al. (2002) completed a comprehensive survey of termites in Louisiana, providing information for the likely occurrences of *Reticulitermes* spp. in Arkansas. Previously, Snyder (1954) listed four species in Arkansas: *Reticulitermes flavipes* (Kollar), *R. virginicus* Banks, *R. hageni* Banks, and *R. tibialis* Banks. All four species have been documented in Louisiana, including *R. tibialis*, which was recently discovered (Messenger 2003). Information on the distribution of *Reticulitermes* spp. in Arkansas has become available through a current national termite survey, which confirms that *Reticulitermes* spp. are common throughout the State (Messenger 2003). Moreover, surveys from the neighboring States of Texas and Oklahoma have included valuable information on *Reticulitermes* distribution and genetic composition (Austin et al. 2004a, b).

Previous genetic studies have primarily focused on phylogenetic relationships among *Reti-*

culitermes species from the eastern United States and Western Europe (Jenkins et al. 1998, 2001; Marini & Mantovani 2002; Uva et al. 2003; Ye et al. 2003). Recently, Austin et al. (2004a, b) have conducted the first comprehensive genetic surveys of *Reticulitermes* sp. for Texas and Oklahoma using DNA sequencing of a portion of the mitochondrial DNA (mtDNA) 16S rRNA gene. We investigated the extent of genetic variation within and among Arkansas and Louisiana *Reticulitermes* relative to Texas and Oklahoma (Austin et al. 2004[ab]), evaluated these genetic markers for identifying species, and updated the geographical distribution of these taxa.

Materials and Methods

Termites were collected in Arkansas and Louisiana and preserved in 100% ethanol (Table 1). We solicited the assistance of Pest Management Professionals (PMPs) throughout both States to determine which species are most frequently recovered from infested structures. We provided collection kits, and PMPs returned samples to our laboratory for analysis. From various geographic zones throughout Arkansas and Louisiana (Table 1), 96 samples were used for molecular analysis.

When alates or soldiers were available, *Reticulitermes* sp. were morphologically identified to species by applying the keys of Krishna & Weesner (1969); Scheffrahn & Su (1994); Hostettler et al. (1995); and Donovan et al. (2000). In addition, all samples were identified to species with mtDNA 16S sequences (Szalanski et al. 2003). All of the morphological species identifications agreed with the molecular species identifications. Three additional taxa (Table 1) were included as outgroup taxa to corroborate relationships within the genus for phylogenetic analysis. Voucher specimens preserved in 100% ethanol are maintained at the Arthropod Museum, Department of Entomology, University of Arkansas, Fayetteville, AR.

DNA was extracted from alcohol-preserved specimens dried on filter paper according to Liu & Beckenbach (1992) and Jenkins et al. (1999) from individual worker termites with the Puregene DNA isolation kit D-5000A (Gentra, Minneapolis, MN). Extracted DNA was resuspended in 50 µl of Tris:EDTA and stored at -20°C. Polymerase chain reaction (PCR) was conducted with the primers LR-J-13007 (5'-TTACGCTGTTATCCCTAA-3') (Kambhampati & Smith 1995) and LR-N-13398 (5'-CGCCTGTTTATCAAAAACAT-3') (Simon et al., 1994). These PCR primers amplify an approximately 428-bp region of the mtDNA 16S rRNA gene. The PCR reactions were conducted with 1 µl of the extracted DNA (Szalanski et al. 2000), having a profile consisting of 35 cycles of 94°C for 45 s, 46°C for 45 s and 72°C for 60 s. Amplified DNA from individual termites was purified and concentrated with minicolumns according to the manufacturer's instructions (Wizard PCRpreps, Promega). Samples were sent to The University of Arkansas Medical School DNA Sequencing Facility (Little Rock, AR) for direct sequencing in both directions. GenBank accession numbers were AY603499 to AY603509 for termite DNA sequence haplotypes new to this study. Consensus sequences for each sample were obtained with Bioedit 5.09 (Hall 1999), and sequences were aligned by CLUSTAL W (Thompson et al. 1994). Mitochondrial DNA haplotypes were aligned by MacClade v4 (Sinauer Associates, Sunderland, MA). Haplotype distribution between populations, number of haplotypes, number of unique haplotypes, haplotype diversity (h), and nucleotide diversity (pi) were calculated with DNAsp v3.51 (Rozas & Rozas 1999).

The distance matrix option of PAUP* 4.0b10 (Swofford 2001) was used to calculate genetic distances according to the Kimura 2-parameter model of sequence evolution (Kimura 1980). Mitochondrial 16S sequences from the desert subterranean termite *Heterotermes aureus* (Snyder) (GenBank AY380299) and Formosan subterranean termite *Coptotermes formosanus* Shiraki (GenBank AY558910) were added to the *Reticulitermes* DNA sequences as outgroup taxa. Maximum parsimony analysis on the alignments were conducted with PAUP* 4.0b10 (Swofford 2001). Gaps were treated as missing data. The reliability of trees was tested with a bootstrap test (Felsenstein 1985). Parsimony bootstrap analysis included 1,000 resamplings and used the Branch and Bound algorithm of PAUP*. Because no previous accounts of the abundance of *Reticulitermes* in Arkansas have been published, we compiled all available data from existing sources and noted them on our distribution map (Fig. 1).

Results

The DNA sequencing of the 16S rDNA amplicon revealed an average size of 428 bp. The average base frequencies were A = 0.41, C = 0.23, G = 0.13, and T = 0.23. From 35 Arkansas counties 59 *R. flavipes*, 11 *R. hageni*, and seven *R. virginicus* were identified based on species diagnostic nucleotide sites from Szalanski et al. (2003) (Table 1). In Arkansas, 11 haplotypes were observed in *R. flavipes*, three in *R. hageni* and three in *R. virginicus* (Table 1, Fig. 1). Among the 12 Louisiana parishes sampled, 13 *R. flavipes*, three *R. virginicus*, and one *R. tibalis* were identified with six, three, and one haplotypes for each species, respectively (Fig. 2, Table 1).

Nine nucleotide sites were variable among the 11 *R. flavipes* haplotypes (Table 2), and Tajima-Nei distances (Tajima & Nei 1984) among the *R. flavipes* haplotypes ranged from 0.2 to 0.9% (Table 3). The most common haplotypes were F and G with 32 and 9 representatives, respectively.

TABLE 1. COLLECTION DATA, AND HAPLOTYPES FOR ARKANSAS AND LOUISIANA *Reticulitermes* AND OUTGROUP TAXA.

Species	City	County/Parish	State	Haplotype	n
R. flavipes	Amity	Clark	AR	F	1
	Brinkley	Monroe	AR	F	1
	Cave City	Sharp	AR	F	1
	Clifty	Madison	AR	F	1
		Polk	AR	F	1
	Cushman	Independence	AR	F	1
	Strickler	Washington	AR	F	1
	Fayetteville	Washington	AR	F	2
	El Dorado	Union	AR	F	1
	Eureka Springs	Carroll	AR	F	1
	Ft. Smith	Sebastian	AR	F	1
	Harrison	Boone	AR	F	1
	Jasper	Newton	AR	F	1
	Marvell	Phillips	AR	F	1
	McGehee	Desha	AR	F	1
	Mineral Springs	Howard	AR	F	1
	Nashville	Howard	AR	F	1
	Paragould	Greene	AR	F	1
	Stuttgart	Arkansas	AR	F	1
	Warm Springs	Randolph	AR	F	1
	Hardin	Jefferson	AR	F	1
	Hoxie	Lawrence	AR	F	1
	Walnut Ridge	Lawrence	AR	F	1
	Newport	Jackson	AR	F	1
	Hot Springs	Garland	AR	F	1
	Baton Rouge	E. Baton Rouge	LA	F	2
	Houma	Terrebonne	LA	F	1
	Cut Off	Lafourche	LA	F	1
	Delhi	Richland	LA	F	1
	Port Sulphur	Plaquemines	LA	F	1
	West Monroe	Ouachita	LA	J	1
	Ashdown	Little River	AR	M	1
	Ft. Smith	Sebastian	AR	M	1
	Warren	Bradley	AR	M	1
	Fayetteville	Washington	AR	M	1
	Pine Bluff	Jefferson	AR	M	1
	Shreveport	Caddo	LA	M	1
	Sulphur	Calcasieu	LA	M	1
	Camp Robinson	Pulaski	AR	G	1
	Glenwood	Pike	AR	G	1
	Jonesboro	Craighead	AR	G	1
	N. Little Rock	Pulaski	AR	G	1
	Newport	Jackson	AR	G	1
	Piggot	Clay	AR	G	1
	Morrilton	Conway	AR	G	1
	Shreveport	Caddo	LA	G	1
	Jonesboro	Jackson	LA	G	1
	Fayetteville	Washington	AR	Q	1
	Harrison	Boone	AR	Q	1
	Hoxie	Lawrence	AR	Q	1
	Newport	Jackson	AR	Q	1
	Walnut Ridge	Lawrence	AR	Q	1
	Fayetteville	Washington	AR	T	1
	Hoxie	Lawrence	AR	W	1
	Jonesboro	Craighead	AR	P	1
	Lake Charles	Calcasieu	LA	P	1
	Lake Dardanelle	Pope	AR	R	1

TABLE 1. (CONTINUED) COLLECTION DATA, AND HAPLOTYPES FOR ARKANSAS AND LOUISIANA *Reticulitermes* AND OUT-

Species	City	County/Parish	State	Haplotype	n
	Jonesboro	Craighead	AR	R	1
	Strickler	Washington	AR	R	1
	Fayetteville	Washington	AR	R	1
		Madison	AR	R	1
	Searcy	White	AR	R	1
		Faulkner	AR	S	1
	Chauvin	Terrebonne	LA	S	1
	Little Rock	Pulaski	AR	V	1
	Pocahontas	Randolph	AR	V	2
	Sheridan	Grant	AR	V	1
	Sherwood	Pulaski	AR	V	1
	Ft. Smith	Sebastian	AR	U	1
R. hageni	Eureka Springs	Carroll	AR	H1	1
	Fayetteville	Washington	AR	H1	1
	Weddington	Washington	AR	H1	3
	Pocahontas	Randolph	AR	H1	1
	Conway	Faulkner	AR	H1	1
		Polk	AR	H1	1
	Clifty	Madison	AR	H1	1
	Fayetteville	Washington	AR	H2	1
	Marmaduke	Greene	AR	H2	1
	Strickler	Washington	AR	H3	1
	Hamburg	Ashley	AR	H3	1
R. virginicus	Fayetteville	Washington	AR	V1	4
	Fayetteville	Washington	AR	V2	1
	Fayetteville	Washington	AR	V3	1
	Morgan Mtn.	Franklin	AR	V1	1
	Minden	Webster	LA	V1	1
	Raceland	Lafourche	LA	V4	1
	Delcambre	Vermilion	LA	V5	1
R. tibialis	Sulphur	Calcasieu	LA	T8	1
Coptotermes formosanus	Rockwall	Lamar	TX	outgroup	
Heterotermes aureus			AZ	outgroup	

Within *R. hageni*, one nucleotide site was variable between the two observed haplotypes. Haplotype diversity for *R. flavipes* from Arkansas was 0.759, and 0.782 for Louisiana (Table 4). Both States had high levels of genetic diversity. Tajima's test resulted in non-significant P values ($P < 0.05$) in both States leading to the acceptance of the null-hypothesis of neutrality for the mtDNA rRNA 16S gene (Table 4).

Bootstrap analysis of the aligned *Reticulitermes* species and the outgroup taxa resulted in a consensus tree with several distinct branches (Fig. 3). These distinct clades included *R. flavipes*, *R. tibialis*, *R. hageni*, and *R. virginicus*. No genetic relationship was observed among *R. flavipes* haplotypes.

DISCUSSION

This study represents the first attempt to update the current geographic distribution of *Reticulitermes* spp. and genetically categorize the genus *Reticulitermes* in Arkansas. At the same time, this is the first molecular description of *Reticulitermes* spp. from Louisiana, albeit on a limited scale.

Populations of nearly all species, social or otherwise, exhibit at least some degree of genetic differentiation among geographic locales (Ehrlich & Raven 1969). One of the purposes of the research presented herein was to estimate the baseline genetic variation which occurs both within and among *Reticulitermes* spp. in Arkansas and Louisiana. By combining haplotype observations in the present study with the studies of Austin et al. (2004a) from Texas and Austin et al. (2004b) from Oklahoma, we had hoped to observe some type of spatial continuity which may not have been revealed otherwise. As with animal populations, additional genetic structure normally is to be expected over increasing spatial scales, where populations can show additional differentiation due to spatial habitat structure, isolation by distance, or other factors (Avise 1994). There was no

Fig. 1. Species distribution of *Reticulitermes* haplotypes in Arkansas. Samples obtained from the National Termite Survey, but not subjected to genetic analysis are marked as "◆" *R. flavipes*, "●" *R. virginicus*, "■" *R. hageni.* Letters indicating haplotypes are given in Table 1.

Fig. 2. Species distribution of *Reticulitermes* haplotypes in Louisiana. Letters indicating haplotypes are given in Table 1.

apparent consistency of haplotype occurrence for *Reticulitermes* spp. in this study based on geography. However, we found genetic divergence values similar to those detected in our previous works (Austin et al. 2004a, b).

Of the four species of *Reticulitermes* presented in this study, *R. flavipes* was the most common, followed by *R. hageni* and *R. virginicus*. Haplotype F was the most common haplotype of *R. flavipes* observed in Arkansas, and represented 26 of the 79 (44%) samples from Arkansas. This haplotype is also present in both Texas and Oklahoma but in smaller distributions: 12 and 13% for Oklahoma and Texas, respectively. In Oklahoma and Texas, the most abundant *R. flavipes* haplotypes are L (23%) and G (28%), respectively (Austin et al. 2004a, b).

A haplotype or allele is defined by one unique form of the gene and differs from any other haplotype by at least one nucleotide. Haplotype diversity or gene diversity quantifies the number of haplotypes in relation to their relative frequency to each other, and is the probability that two sequences randomly selected from a population are different (Nei 1987). Haplotype diversity for *R. flavipes* from Arkansas and Louisiana was lower than Texas or Oklahoma (Austin et al. 2004a, b).

Tajima's D (Tajima 1989) is a standard test for the neutrality of a gene region. This is a useful measure because nucleotide diversity (Pi) calculations are dependant upon the infinite alleles model that assumes gene neutrality. Values of D indicate not only if natural selection is influencing gene frequencies but the type of selection pressure in operation. Negative values of D can indicate re-

TABLE 2. HAPLOTYPE VARIATION AT 9 NUCLEOTIDE SITES AMONG *Reticulitermes flavipes* FROM ARKANSAS AND LOUISIANA.

Haplotype	55	97	122	131	162	168	179	270	271
F	G	A	A	A	G	G	C	T	T
G	.	.	T	G	.	A	.	.	C
J	.	.	.	G	.	A	.	.	.
M	.	.	.	.	.	A	.	.	.
P	.	.	.	.	.	A	.	.	C
Q	.	.	.	.	.	.	T	.	.
R	.	.	.	.	.	A	.	.	.
S	.	.	.	.	A	A	.	.	.
T	.	.	.	.	.	A	.	C	C
U	.	.	.	.	.	.	.	C	.
V	.	.	.	.	A	.	.	.	.

TABLE 3. GENETIC DIVERGENCE AMONG RETICULITERMES FLAVIPES HAPLOTYPES (HAP) FROM ARKANSAS AND LOUISIANA.

Hap	G	T	P	U	V	F	Q	R	S	M	J
G	—										
T	0.009	—									
P	0.007	0.002	—								
U	0.009	0.005	0.007	—							
V	0.009	0.009	0.007	0.005	—						
F	0.007	0.007	0.005	0.002	0.002	—					
Q	0.009	0.009	0.007	0.005	0.005	0.002	—				
R	0.007	0.007	0.005	0.007	0.007	0.005	0.007	—			
S	0.007	0.009	0.007	0.009	0.005	0.007	0.009	0.002	—		
M	0.005	0.005	0.002	0.005	0.002	0.005	0.002	0.005	—		
J	0.002	0.007	0.005	0.007	0.007	0.005	0.007	0.005	0.007	0.002	—

cent expansions in population size while positive values indicate recent population bottlenecks. Tajima's D value was negative for both Arkansas and Louisiana, indicating that *R. flavipes* in these States may be expanding in population size. A similar result was observed from *R. flavipes* from Oklahoma, while Texas *R. flavipes* have a positive D value, indicating a recent population bottleneck. The null hypothesis of this test is that the gene region of interest is neutral. If the D value is significant ($P < 0.05$) then the null hypothesis of neutrality may be rejected. Tajima's test resulted in non-significant p values ($P > 0.05$) for Arkansas and Louisiana. This was also observed in Texas and Oklahoma (Austin et al. 2004a, b) leading to the acceptance of the null-hypothesis of neutrality for the mtDNA rRNA 16S gene.

The phylogenetic relationships of *Reticulitermes* evaluated in this study are consistent with those from neighboring states (Austin et al. 2004a, b) and in other areas (Austin et al. 2002; Jenkins 1998, 1999, 2001). Extensive collecting in Louisiana has produced only one sample of *R. tibialis* from Sulphur, Calcasieu Parish, which borders Texas. Although Snyder (1954) lists *R. tibialis* as occurring in Arkansas, we have not yet recovered this species to date.

We speculate that because *R. tibialis* was generally not recovered in our collecting efforts, an eastern transition zone (from east to west) for the distribution of this species may exist in the proximity of Calcasieu Parish, Louisiana**.** This species is known to prefer more arid climates which can be more readily found in Texas and Oklahoma where *R. tibialis* has been more frequently recovered. More intensive collecting efforts should be performed to validate this hypothesis.

The lack of a geographical haplotype continuity of *Reticulitermes* suggests that (1) we require samples from larger geographic zones, (2) we need to increase the number of samples sequenced, or (3) the observed lack of spatial continuity from *Reticulitermes* may be attributed to anthropogenic origins. To evaluate the latter element, evaluation of haplotype frequency from undisturbed habitats (e.g., protected forests) should be compared with our current data, which largely reflects samples obtained from urban landscapes. Selected sites in undisturbed locations are being evaluated for intensive collecting efforts in future studies where more comprehensive statistical measures can be applied, and a better overall understanding of population dynamics may be addressed than in the current study.

TABLE 4. GENETIC DIVERSITY PARAMETERS FOR EACH POPULATION OF *RETICULITERMES FLAVIPES* FROM TEXAS, OKLAHOMA, ARKANSAS, AND LOUISIANA.

Population	n	No. haplotypes	No. unique haplotypes	Haplotype diversity (h)	Nucleotide diversity (Pi)	Tajima's[a] D value (*P*-value)
TX[b]	69	13	3	0.864	0.0071	+0.6021 ns
OK[c]	41	10	1	0.811	0.0048	-0.6349 ns
AR	59	11	3	0.759	0.0044	-0.3488 ns
LA	13	6	0	0.782	0.0044	-0.0688 ns

[a]A non-significant value for this test indicates that the null hypothesis of neutrality in the 16S rRNA gene cannot be rejected. Non-significance of test when $P > 0.10$.

[b,c]Data from Austin et al. (2004a, b).

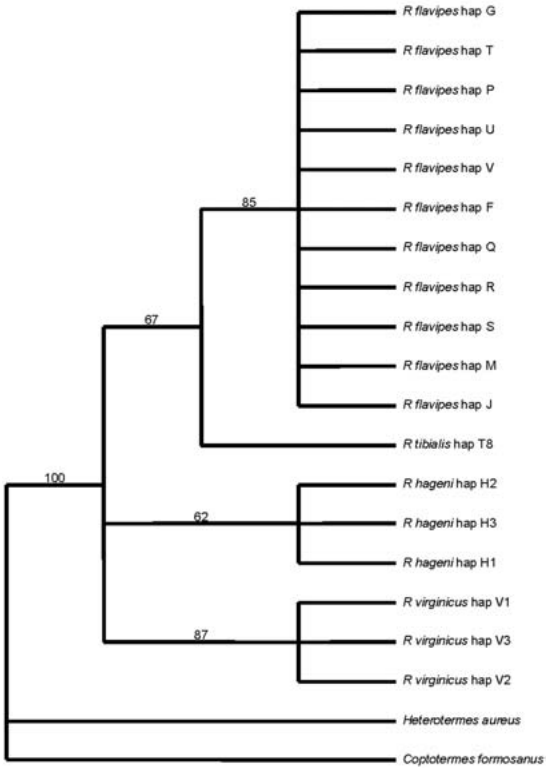

Fig. 3. Single most parsimonious tree based on the 16S rRNA gene during a branch and bound search with PAUP*. Bootstrap values for 1,000 replicates are listed above the branches supported at ≥50%.

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SYNERGISTIC AND INHIBITORY INTERACTIONS BETWEEN METHYL EUGENOL AND CUE LURE INFLUENCE TRAP CATCH OF MALE FRUIT FLIES, *BACTROCERA DORSALIS* (HENDEL) AND *B. CUCURBITAE* (DIPTERA: TEPHRITIDAE)

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ABSTRACT

Males of the oriental fruit fly, *Bactrocera dorsalis* (Hendel) and the melon fly, *B. cucurbitae* (Coquillett), are attracted to methyl eugenol (ME) and cue lure (CL), respectively. These lures, when mixed with a toxicant, are widely used to detect and suppress populations of these agricultural pests. The objective of this study was to assess the effectiveness of (1) traps baited with both ME and CL (mixed or presented separately on adjacent wicks), and (2) traps baited with a single lure but placed in the same tree as a trap containing the alternate lure (1 or 3 m apart). Jackson traps were placed in a mixed orchard on Oahu, Hawaii, and the numbers of released (marked) and wild males were recorded. Traps baited with ME and CL (mixed or separate) captured significantly fewer *B. dorsalis* males than traps baited with ME alone. CL placed 1 m from ME-baited traps in the same tree also reduced the number of *B. dorsalis* males captured. Conversely, ME appeared to increase capture of *B. cucurbitae* males, and traps baited with the 2 lures (mixed but not separate) captured significantly more released males than traps baited with CL alone. Also, ME placed 1 m (but not 3 m) from CL-baited traps increased the trap catch of released *B. cucurbitae* males. Results are discussed from the perspectives of management and evolution of *Bactrocera* species.

Key Words: *Bactrocera* spp., oriental fruit fly, melon fly, methyl eugenol, cue-lure, Diptera.

RESUMEN

Los machos de la mosca oriental de la fruta, *Bactrocera dorsalis* (Hendel) y de la mosca del melón, *B. cucurbitae* (Coquillett), son atraídos al eugenol metil (EM) y al atrayente "cue lure" (CL), respectivemente. Estos atrayentes, cuando estan mezclados con una toxina, son usados en muchas lugares para detectar y suprimir las poblaciones de estas plagas agrícolas. El objectivo de este estudio fue para avaluar la eficacia de 1) trampas de cebo con ambos EM y CL (mezcladas o presentadas separadamente en mechas adyacentes) y 2) trampas de cebos con un solo atrayente pero puestos en el mismo arból de una trampa que tiene un atrayente alternativo (separadas por 1 o 3 metros). Las trampas "Jackson" fueron puestas en un huerto mezclado en Oahu, Hawaii, y el número de los machos liberados (marcados) y los machos naturales fueron registrados. Las trampas con EM y CL (mezclados o separados) capturaron significativamente menos machos de *B. dorsalis* que las trampas con solo EM. Tambien, el CL puesto 1 m de las trampas con EM en el mismo arból redujó el número de los machos de *B. dorsalis* capturados. Al contrario, el EM aparece que se aumenta el número capturado de los machos de *B. cucurbitae*, y las trampas con los dos atrayentes (mezclados pero no separados) capturaron significativamente más machos liberados que las trampas con solo CL. También, el EM puesto 1 m (pero no 3 m) de las trampas cebadas con el CL aumentó el número capturado de machos de *B. cucurbitae* liberados. Se discuten los resultados desde el punto de vista de manejo y evolución de las especies de *Bactrocera*.

The genus *Bactrocera* contains approximately 440 species distributed primarily in Southeast Asia, the South Pacific, and Australia (White & Elson-Harris 1992). Males of many *Bactrocera* species are attracted to either methyl eugenol (4-allyl-1,2-dimethoxybenzene) or cue lure [4-(4-acetoxyphenyl)-2-butanone] (Drew & Hooper 1981). Methyl eugenol (ME hereafter) is a widely distributed plant natural product and occurs in over 200 plant species representing 32 families (Tan & Nishida 1996). Cue lure (CL hereafter) has not been isolated as a natural product but is rapidly hydolyzed to form raspberry ketone (RK hereafter), which is found in a variety of plants (Metcalf 1990). In Hawaii, the site of the present study, two major fruit fly pests are the Oriental fruit fly, *B. dorsalis* (Hendel), and the melon fly, *B. cucurbitae* (Coquillett), males of which respond to ME and CL, respectively.

Control programs against *Bactrocera* species use male lures to both detect and suppress pest populations. For example, ME- and CL-baited traps are monitored year-round in southern California to detect the presence of incipient infestations of *B. dorsalis* and *B. cucurbitae*, respectively (Gilbert & Bingham 2002). In a well-known case

of male annihilation, Steiner et al. (1965) eradicated *B. dorsalis* from Rota Island by distributing thousands of fiber board blocks soaked with ME and a toxicant. Although a less powerful attractant than ME, and hence less effective at male annihilation, CL (plus a toxicant) has similarly been used to suppress populations of *B. cucurbitae* prior to the start of sterile release programs (e.g., Miyako Island, Kuba et al. 1996).

Several studies have investigated the effectiveness of traps baited with a mixture of ME and CL for possible use in detection and suppression programs in areas containing both ME- and CL-responding *Bactrocera* species. Combining lures in such a manner could potentially reduce the number of traps needed in a given area and the associated manpower required to service them. In addition, combining lures may reduce the amount of pesticide used, thus lessening economic and environmental costs. Previous studies suggest an asymmetry in the effectiveness of ME + CL mixtures for ME- versus CL-responding species. On one hand, data uniformly show that CL, either mixed with or placed immediately adjacent to ME, reduces trap capture of ME-responding species (Hooper 1978; Vargas et al. 2000). In contrast, data regarding lure mixtures and the response of CL-responding species have been inconsistent. Data on *B. cucurbitae* from Taiwan (cited by Hooper 1978) showed that adding ME to CL nearly doubled the number of males captured compared with traps baited with CL alone. In contrast, Hooper (1978) found that the mixture of ME + CL reduced trap capture of CL-responding species in Queensland, Australia. However, Hooper (1978) did report an increase in male numbers for traps in which ME and CL were applied to separate wicks in the same trap. More recently, Vargas et al. (2000) found that lure mixtures containing at least 25% CL by volume attracted similar numbers of *B. cucurbitae* males as traps baited solely with CL.

The objective of the present study was to provide additional data on the responsiveness of *B. dorsalis* and *B. cucurbitae* males to traps baited only with ME or CL, respectively, relative to traps containing the 2 lures mixed together or on separate wicks. In addition, we monitored the effect of ME on CL, and vice versa, on trap captures when 2 traps containing the respective lures were placed in the canopy of the same tree.

Materials and Methods

Study Site

Fieldwork was conducted during January - November, 2003, at the University of Hawaii Agricultural Experiment Station, Waimanalo, Oahu, in a mixed fruit orchard that contained mango (*Mangifera indica* L.), guava (*Psidium guajava* L.), orange (*Citrus sinensis* (L.) Osbeck), lime (*C. aurantiifolia* (Christm.) Swingle), and breadfruit (*Artocarpus altilis* (Parkins.) Fosb.) along with other non-host trees. Daily maximum and minimum temperatures ranged between 25-33°C and 19-25°C, respectively, during this period.

Study Insects

Males of *B. dorsalis* and *B. cucurbitae* were from laboratory colonies maintained by the USDA-ARS Tropical Fruit, Vegetable, and Ornamental Crop Laboratory, Honolulu, since the mid-1980s and mid-1960s, respectively (D. McInnis, personal communication). We obtained *B. cucurbitae* as pupae and *B. dorsalis* as eggs, which were placed on standard larval medium (Tanaka et al. 1969) in plastic containers over vermiculite for pupation. In both species, males were collected within 48 h of eclosion (males from laboratory colonies attain sexual maturity at 6-7 days of age, Vargas et al. 1984), held in screen-covered plastic buckets (volume 5 liters; 100-150 males per bucket), and provided water and food (a sugar-yeast hydrolysate mixture, 3:1 v/v). Flies were maintained at 23-27°C and 60-90% RH under a natural photoperiod (approximately 12:12 L:D). One day before release, we marked males by cooling them for several minutes and placing a dot of enamel paint on the thorax. This procedure had no obvious adverse effects, and males resumed normal activities within minutes of handling. When used in the field experiments, males of both species were 10-19 days old.

Effect of CL on Trap Catch of *B. dorsalis*

We conducted 4 experiments to assess the effect of CL on the capture of *B. dorsalis* males in ME-baited traps. Lures (2 ml of either mixed or pure lures, see below) were applied to cotton wicks (1.2 cm diameter, 4.0 cm length) with a pipette. Wicks were placed individually in perforated, plastic baskets, which were suspended inside Jackson traps above a sticky insert resting on the trap floor. In each experiment, we placed traps in 8 trees arranged in a circle (radius 40 m) about a central tree, which served as the release point. In any given replicate, 4 of the trees contained a single ME-baited Jackson trap. The remaining 4 trees contained the following: in Experiment 1, a single trap baited with a mixture of CL and ME (2 ml of each lure were mixed, and the mixture was apportioned equally between 2 wicks held in 2 separate baskets); in Experiment 2, a single trap baited with CL and ME applied separately to 2 wicks (and housed in separate baskets); in Experiment 3, 2 traps baited with CL and ME, respectively, placed 1 m apart; or, in Experiment 4, 2 traps baited with CL and ME, respectively, placed 3 m apart. Traps were placed 2 m above ground in shaded locations within the

tree canopy. For a given replicate, adjacent test trees contained different treatments (i.e., ME only, or some combination of ME and CL).

For a given replicate, we set out the traps between 0900-1000 h and then released 300 males at the central release point. Flies were released by placing 2 buckets (150 males per bucket) on the ground beneath the release tree and gently removing the screen cover from the bucket. The buckets were not tapped or shaken, and the flies exited the bucket on their own volition. Traps were collected 48 h after release, and the numbers of released (marked) and wild (unmarked) *B. dorsalis* males were recorded. In general, successive releases were separated by an interval of 2-4 days to allow previously released flies time to disperse from the study area. The same test trees were used in all experiments, but the treatment assigned to a particular tree was alternated between successive replicates in a given experiment. Ten replicates were conducted for each experiment.

Effect of ME on Trap Catch of *B. cucurbitae*

We repeated Experiments 1-4 to assess the effect of ME on capture of *B. curcubitae* males in CL-baited traps. These experiments followed the procedures described above, except that 300 *B. cucurbitae* males were released, and CL was used to bait traps on trees that had only a single male attractant present. Experiments involving release of *B. cucurbitae* were replicated 8 times except Experiment 4 for which 6 replicates were performed.

Statistical Analysis

For both species, pairwise comparisons were made by the 2-tailed *t*-test. Raw data were used for both species as the assumptions of normality and homoscedasticity were met, except for Experiment 1 with *B. cucurbitae* for which a $\log_{10}$ transformation of the raw data was performed to meet these criteria. Calculations were performed with SigmaStat Statistical Software (Version 2.0).

Results

Data regarding trap captures of *B. dorsalis* males are presented in Table 1. Traps containing both ME and CL captured significantly fewer *B. dorsalis* males than traps baited with ME only. This result was evident for released and wild males both when the lures were mixed together (Experiment 1) and when they were presented separately (i.e., on different wicks) in the same trap (Experiment 2). In addition, CL-baited traps placed 1 m from ME-baited traps on the same tree reduced trap catch of released *B. dorsalis* males relative to ME-baited traps occurring singly on trees (Experiment 3). A similar result was obtained for wild males, but in this case the effect was only marginally significant. No reduction in trap catch of *B. dorsalis* males (released or wild) was apparent when CL- and ME-baited traps were separated by 3 m on the same tree (Experiment 4). Wild males of *B. cucurbitae* were not found in any trap baited solely with ME in any of the experiments.

Data from the experiments involving *B. cucurbitae* are presented in Table 2. Traps containing mixed lures (Experiment 1) captured significantly more released *B. cucurbitae* males than traps baited with CL only, while a similar, but marginally significant, result was found for wild males. When CL and ME were presented separately (i.e., on different wicks) in the same trap (Experiment 2), a marginally significant increase in released males was recorded, but no effect was noted for wild males. The mean number of released *B. cucurbitae* males captured in CL-baited traps placed 1 m from ME-baited traps was significantly greater than that recorded for CL-baited traps placed singly on trees, but no difference was evident for wild males (Experiment 3). ME-baited traps placed 3 m from CL-baited traps had no effect on trap catch of wild or released males (Experiment 4). Wild males of *B. dorsalis* were not found in any trap baited solely with CL in any of the experiments.

Discussion

Management

The present results for *B. dorsalis* are consistent with those of previous studies (Hooper 1978; Vargas et al. 2000), which showed that when presented in the same trap as ME (mixed or separate), CL significantly reduced capture of *B. dorsalis* males. Our study further showed that trap catch of *B. dorsalis* males was reduced (though the effect was only marginally significant for wild males) even when CL was placed 1 m (but not 3 m) from ME-baited traps. With respect to *B. cucurbitae*, the present findings agree with the Taiwan study (cited by Hooper 1978) in which the 2 lures mixed together increased capture of *B. cucurbitae* males (though the effect was only marginally significant for wild males). Also, when ME was placed 1 m from CL-baited traps, there was an increase in the number of released (but not wild) males captured. There were, however, no significant effects detected when ME was presented separately in the same trap or 3 m away from CL-containing wicks. Thus, in general, the inhibitory effect of CL on capture of *B. dorsalis* males appears greater than the synergistic effect of ME on capture of *B. cucurbitae* males. The absence of *B. dorsalis* males in traps baited with CL only and *B. cucurbitae* males in traps baited with ME confirms earlier laboratory results (Metcalf et al. 1983).

TABLE 1. NUMBER OF RELEASED AND WILD *B. DORSALIS* MALES CAPTURED IN JACKSON TRAPS BAITED WITH METHYL EUGENOL ONLY AND PLACED SINGLY ON TREES (DESIGNATED ME) VERSUS JACKSON TRAPS BAITED WITH A METHYL EUGENOL-CUE LURE MIXTURE (ME+CL, EXPERIMENT 1), METHYL EUGENOL AND CUE LURE PRESENTED SEPARATELY (ME+CL, EXPERIMENT 2), OR METHYL EUGENOL ONLY BUT PLACED ON A TREE WITH CUE LURE-BAITED JACKSON TRAP EITHER 1 M ME+CL, EXPERIMENT 3) OR 3 M (ME+CL, EXPERIMENT 4) APART. VALUES REPRESENT MEANS ±1 SD; 10 REPLICATES WERE CONDUCTED FOR EACH EXPERIMENT. SIGNIFICANCE LEVELS (df = 18 IN ALL TESTS): $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$, $^{ms}p < 0.10$, $^{ns}p > 0.10$.

Experiment		Trap type		
A. Released males	Cue lure placement relative to methyl eugenol	ME	ME+CL	t
1	Same trap, same wick	36.8 (14.2)	8.6 (3.4)	6.1^{***}
2	Same trap, separate wick	36.6 (16.7)	19.3 (12.1)	2.2^{*}
3	Separate trap, 1 m away	39.8 (13.9)	22.2 (13.0)	2.9^{**}
4	Separate trap, 3 m away	31.5 (17.4)	33.7 (21.9)	0.2^{NS}
B. Wild males				
1	Same trap, same wick	182.0 (76.7)	81.1 (56.6)	3.3^{**}
2	Same trap, separate wick	239.2 (65.8)	143.8 (47.4)	3.7^{**}
3	Separate trap,1 m away	128.2 (54.3)	82.9 (49.9)	1.8^{MS}
4	Separate trap, 3 m away	199.5 (69.6)	159.1 (79.7)	1.0^{NS}

These data imply that where *B. dorsalis* (or another ME-responding species) is dominant, the most effective trapping procedure would involve placement of separate ME and CL traps in different trees. However, where *B. cucurbitae* (or another CL-responding species) is most abundant, mixing ME with CL in the same trap may be most effective. In addition, the price of ME is only about 20% that of CL (J. Knapp, pers. comm.), and therefore use of a ME-CL mixture could reduce costs considerably.

Lure Interactions and Evolution

Plants containing ME or RK are attractive to *Bactrocera* males, apparently because they signal the presence of pheromone precursors. Males of *B. dorsalis* that feed on ME (Nishida et al. 1988) or flowers containing ME (Nishida et al. 1997) contain ME metabolites in the rectal gland, the site of pheromone synthesis and storage. Likewise, males of *B. cucurbitae* that feed on CL (Nishida et al. 1990) or plants containing RK (Nishida et al. 1993) sequester RK in their rectal gland. To the extent that the incorporation of ME or RK in the sex pheromone promotes a species-specific response, we might expect males of *B. dorsalis* and *B. cucurbitae* to possess specific receptors only for ME and RK/CL, respectively, and (as reported by Drew & Hooper 1981) to respond to either ME or RK/CL but not both.

The present study shows clearly, however, that males of *B. dorsalis* and *B. cucurbitae* respond to the "alternate" lure when ME and CL are mixed or presented in close proximity. Although pheromones, and not plant volatiles, are involved, similar types of interspecific interactions are well-documented for moths (Phelan 1992) and bark beetles (Byers 1995). It seems unlikely that the same single receptor cells are responding to both compounds, resulting in competitive blocking on the surface of the receptor cell membrane. Rather, as described for other instances of olfactory interaction (Mustaparta 1984), the compounds probably activate different receptor cells and the concurrent transduction from these different cells interrupts, in the case of *B. dorsalis*, or enhances, in the case of *B cucurbitae*, the response at a more central neurophysiological level.

Why the between-lure interaction is inhibitory for *B. dorsalis* but synergistic for *B cucurbitae* is unknown but may reflect evolutionary relationships. Based on biochemical changes accompanying the evolution of higher plants, it appears that both ME and RK are derived from the same, widely distributed compound (*p*-hydroxycinnamic acid), and speciation within the Dacinae subsequently led to ME- and RK/CL-responding species (Metcalf 1979). Because contemporary RK/CL-responding species also respond to this ancestral compound, whereas ME-responding species do not, it further appears that the RK/CL-responding species are more closely related to the ancestral dacines and that ME-responding species are derived taxa (Metcalf et al. 1983).

If ME-sensitive species arose from an RK-responding lineage, then there may have been strong selection for RK inhibition in the incipient species to avoid hybridization (and unsuccessful reproductive attempts) with females from basal, RK-sensitive species. Once again, data from pheromonal interactions are illustrative. Specific components of the pheromone of *Heliothus virescens* inhibit response by *H. zea* and vice versa (Stadelbacher et al. 1983). Cross-inhibition is highly adaptive in this case, as heterospecific matings

TABLE 2. NUMBER OF RELEASED AND WILD *B. curcubitae* MALES CAPTURED IN JACKSON TRAPS BAITED WITH CUE LURE ONLY AND PLACED SINGLY ON TREES (DESIGNATED CL) VERSUS JACKSON TRAPS BAITED WITH A CUE LURE-METHYL EUGENOL MIXTURE (CL + ME, EXPERIMENT 1), CUE LURE AND METHYL EUGENOL PRESENTED SEPARATELY (CL + ME, EXPERIMENT 2), OR CUE LURE ONLY BUT PLACED ON A TREE WITH A METHYL EUGENOL-BAITED JACKSON TRAP EITHER 1 M (CL + ME, EXPERIMENT 3) OR 3 M (CL + ME, EXPERIMENT 4) APART. VALUES REPRESENT MEANS ±1 SD (LOG_{10} TRANSFORMED DATA WERE USED IN T TEST IN EXPERIMENT 1). EIGHT REPLICATES WERE CONDUCTED FOR EXPERIMENTS 1-3, AND 6 REPLICATES WERE CONDUCTED FOR EXPERIMENT 4. SIGNIFICANCE LEVELS ($df = 14$ IN EXPERIMENTS 1-3 AND 10 IN EXPERIMENT 4): $^{*}p < 0.05$, $^{**}p < 0.01$; $^{ms}p < 0.10$; $^{ns}p > 0.10$.

Experiment		Trap type		
A. Released males	Cue lure placement relative to methyl eugenol	ME	ME+CL	*t*
1	Same trap, same wick	16.8 (5.2)	33.3 (11.2)	3.1*
2	Same trap, separate wick	14.9 (8.1)	22.9 (9.2)	1.8^{MS}
3	Separate trap, 1 m away	10.2 (3.2)	20.3 (8.2)	3.2**
4	Separate trap, 3 m away	15.0 (5.9)	17.5 (6.1)	0.7^{NS}
B. Wild animals				
1	Same trap, same wick	29.6 (17.2)	42.7 (21.0)	1.9^{MS}
2	Same trap, separate wick	40.5 (13.8)	32.7 (13.2)	1.4^{NS}
3	Separate trap 1 m away	55.8 (23.4)	54.0 (21.1)	0.3^{NS}
4	Separate trap, 3 m away	57.3 (48.4)	46.2 (24.9)	0.5^{NS}

are unsuccessful due to incompatible genitalia (Stadelbacher et al. 1983). The synergism noted between ME and CL apparently reflects the absence of any evolutionary cost to *B. cucurbitae* of responding to this chemical blend, which, in turn, may reflect the infrequency with which ME and RK co-occur in the same plant species. Without natural exposure to an ME-RK blend, males of *B. cucurbitae* have not evolved avoidance of this stimulus. Comparing the relative strength of inhibitory and synergistic effects supports the reliance on evolutionary costs to explain the observed interactions, namely that the inhibitory effect of CL on ME is stronger (reflecting the high cost of inviable matings) than is the synergistic effect of ME on CL (reflecting the absence of benefits).

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EFFECTS OF SUCROSE IN ADULT DIET ON MORTALITY OF MALES OF *ANASTREPHA SUSPENSA* (DIPTERA: TEPHRITIDAE)

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ABSTRACT

Survival of adult male Caribbean fruit flies, *Anastrepha suspense* (Loew) fed sucrose and protein in the form of hydrolyzed brewers yeast was studied under greenhouse conditions. Flies fed either a 3:1 mixture of sucrose and protein (optimal) or just sugar from the day of adult eclosion showed no appreciable mortality during the 14-day test period. However, flies fed just protein, or those that were not provided with sugar or protein showed rapid rates of mortality, with 50% mortality occurring at 1.87 and 1.53 days, respectively, and 95% mortality occurring at 2.8 and 2.5 days. Switching flies from the optimal diet to either the protein-only diet or nothing at 7 or 11 days after emergence resulted in values of 50% and 95% mortality, respectively, that were similar to those for flies reared from eclosion on either just protein or nothing. No significant mortality occurred among males maintained on the optimal or sugar-only diets or when flies were shifted from the optimal diet to only sugar at either day 7 or 11 after emergence. These data demonstrate that the flies have an absolute requirement for carbohydrate in the adult diet. Additionally, the results indicate that the flies are incapable of converting of amino acids from protein hydrolysate into precursors useful for generating metabolic energy in sufficient amounts to sustain life.

Key Words: Caribbean Fruit Fly, dietary sucrose, carbohydrate, protein.

RESUMEN

La sobrevivencia de los machos adultos de la mosca del Caribe alimentados con sucrosa y proteina en la forma de levadura hidrolizada de cerveza fue estudiada bajo condiciones de invernadero. Las moscas alimentadas con una mezcla de 3:1 sucrosa y proteina (óptima) o solo azucar desde el día de la eclosión del adulto no mostraron mortalidad apreciable durante el período de pruebas de 14 dias. Sin embargo, las moscas alimentadas solo con proteina, o las moscas no proveidas con azucar o proteina mostraron una tasa rápida de mortalidad, con 50% mortalidad ocurriendo a los 1.87 y 1.53 días, respectivamente, y 95% mortalidad ocurriendo a los 2.8 y 2.5 días. Cambiando las moscas de una dieta óptima a cualquier de las dietas de solo proteina o nada a los 7 o 11 días después de la emergencia resultó en valores de mortalidad de 50% y 95%, respectivamente, que fueron similares a los datos para las moscas criadas desde la eclosión en la dieta de sola proteina o nada. No mortalidad significativa ocurrió entre los machos mantenidos en las dietas óptima o de solo azucar o cuando la dieta de las moscas fue cambiada de la dieta óptima a la dieta de solo azucar a los 7 o 11 días después de la emergencia. Estos datos demuestran que las moscas tienen un requisito absoluto para el carbohidratado en la dieta del adulto. Además, los resultados indican que las moscas no fueron capaces de convertir los aminoácidos de proteína-hidrolizada a los precursores útiles para generar la energía metabolica en cantidades suficientes para mantener la vida.

The Caribbean fruit fly, *Anastrepha suspensa* (Loew), became established in south Florida in the early 1960s and spread rapidly through most of southern and central Florida. The rapid spread of the pest was due to its host range which includes at least 80 different fruit and vegetable hosts commonly found in Florida (Swanson & Baranowski 1972). Although many of the host plants are not of economic importance, the ability to infest over-ripe grapefruit and oranges has resulted in quarantine restrictions being placed on shipment of not only citrus but also other fruits including tomato, bell pepper, lychee, mango, avocado, guava and carambola (Greany & Riherd 1993). In order to overcome shipment restrictions the state of Florida developed and implemented the Caribbean Fruit Fly Pest Management System (CFFPMS) that has resulted in state certification of "Fly Free Zones" (Greany & Riherd 1993). Although the CFFPMS has been effective and Florida fruit is being shipped around the world, eradication of the fly would significantly improve the agricultural economics of citrus and vegetable production in Florida.

One of the most effective and environmentally sound methods for eradication of pest insects is the sterile insect technique (SIT) pioneered by Knipling (1955). It was first implemented effectively to eradicate the screwworm fly from Florida (Baumhover et al. 1955; Knipling 1959). SIT has

been demonstrated to be an effective tool for suppression and eradication of a number of species of tephritid flies, particularly when coupled with other control techniques (Kakinohana et al. 1997; Steiner et al. 1965, 1970; Wong et al. 1992). Indeed, SIT commonly is used for both direct and prophylactic control of Mediterranean (*Ceratitis capitata* (Wiedemann)) and Mexican (*Anastrepha ludens* Loew) fruit flies in the continental United States and it has been tested for control of the Caribbean fruit fly (Burditt et al. 1975; Holler & Harris 1993). Control is achieved in SIT by mass release of sterile males which mate with wild females. Females that mate with sterile males do not produce viable eggs, and, over time, this results in population decline and possibly eradication. The key to optimizing efficacy of SIT is to produce sterile males that compete as well as, or out compete, wild males in mating opportunities with wild females. Although SIT is an effective population management tool, it is expensive in terms of both money and time. Thus, cost benefits must be clearly defined in order to balance the needs for effective control with funding constraints. One of the more significant costs associated with SIT protocols for the Caribbean fruit fly is the need to hold mass reared adult flies for as many as 7 days prior to release because the mass-produced strains require time to become sexually mature (Teal et al. 2000, and references therein). Minimizing the costs associated with adult holding while still optimizing reproductive performance of the flies is clearly a key element in improving efficacy of SIT management protocols. One way to do this is to minimize the expense associated with feeding adult flies prior to release (see Martinez et al. 1987). Although it is well known that these flies require food as adults and that reproduction and effective sexual signaling are positively impacted by consumption of both carbohydrate and protein (Epsky & Heath 1993; Landolt & Davis-Hernandez 1993; Landolt & Sivinski 1992) little is known about the absolute necessities for either protein or sugar in the adult diet. We were interested in determining if adult males of the Caribbean fruit fly required sugars, protein or both for survival. The following reports the results of studies demonstrating that carbohydrate is absolutely essential for adult survival.

Materials and Methods

Insect Cultures

Caribbean fruit flies were obtained as pupae from a culture maintained at the Division of Plant Industry, Florida Department of Agriculture and Consumer Services, Gainesville, Florida. Pupae were housed in screen cages in a greenhouse maintained at 23-27°C and 50-70% RH, and natural light conditions (Teal et al. 2000). Newly eclosed adults were removed daily, separated by sex and placed into cages immediately after emergence. Females were not used and males were held in a greenhouse that contained no adult female flies and maintained under the above conditions. All flies were provided with water dispensed from a cotton wick pushed through the top of a covered plastic cup containing 25 ml of water. Water cups were changed weekly.

Diets vs Survival

For initial studies we assessed the effects of providing adult males with only water or water plus diets containing either sugar plus protein, only sugar or only protein. The sugar plus protein diet was composed of a cake containing a 3:1 mixture of sucrose and brewers yeast hydrolysate (ICN Biochemicals, #103304). This diet was considered to be the "optimal diet" and was used as the standard diet to which all other diets were compared. The sugar-only diet was a sugar cube and the protein-only diet was a cake composed of yeast hydrolysate. Groups of 25 males were caged in 30-cm^3 screen cages on the day of eclosion and provided with one of the above diets. Survivorship was recorded every 24 h. We also conducted an experiment in which we maintained the males on the optimal diet and then switched to only water, or sugar-, or protein-only diets on either 7 or 11 days after eclosion. We compared the survival of flies that were switched to new diets with those maintained on the optimal diet. A one way analysis of variance was used to test the effect of diet on survival, and separate analyses were conducted for flies that were 0, 7, or 11 d old at the time they were switched to the test diets. Fisher's least significant difference test ($P = 0.05$) was used for separation of means.

Results and Discussion:

Results of studies in which we provided males with only water or water plus diets containing either sugar plus protein (optimum diet), or only sugar, or only protein from the day of adult eclosion showed that sugar was absolutely essential for adult survival. Flies fed water only or water plus protein all died within 96 h but essentially all flies provided with water plus either only sugar or sugar plus protein lived for the duration of the experiment (Tables 1 to 3). In fact, in other experiments in which we fed flies sugar and water from the day of eclosion we found no significant mortality for as long as three weeks ($n = 6$ groups, 25 males; mean mortality at 21 days = 6.7% ± 3.67). Transferring flies to protein plus water, or water only, after feeding for 7 days on the optimal diet resulted in significant mortality (ca. 50%) on day 9, and more than 85% males were dead by the end of the 10th day (Tables 1 and 2). However,

TABLE 1. MEAN SURVIVORSHIP OF FLIES ON EACH DAY AFTER BEING FED ONLY PROTEIN. FLIES WERE EITHER PROVIDED WITH ONLY PROTEIN FROM THE DAY OF ECLOSION OR SWITCHED FROM THE OPTIMAL SUGAR PLUS PROTEIN DIET TO ONLY PROTEIN ON EITHER THE SEVENTH OR ELEVENTH DAY AFTER ADULT ECLOSION. ALL FLIES WERE PROVIDED WITH WATER.

Days after feeding only protein	Mean survivorship of flies fed only protein from day of eclosion*	Mean survivorship of flies switched to protein on day 7*	Mean survivorship of flies switched to protein on day 11*
0	100 A	100 A	100 A
1	91.5 A	94.8 A	92.4 A
2	33.0 B	39.7 B	8.0 C
3	8.5 C	8.7 C	2.4 C
4	1.5 C	1.0 C	0.0 C

*Means in the same column or row followed by the same letter are not significantly different by Fisher's least significant difference test ($P = 0.05$) applied after ANOVA indicated differences among the means.

flies switched to sugar plus water or maintained on the optimal diet had no significant mortality through day 15 (Table 3). Similarly transferring flies from the optimal diet on the 11th day to protein plus water or only water resulted in greater than 90% mortality on day 13 and essentially all were dead by day 14 (Tables 1and 2). Mortality was negligible for males maintained on the optimal diet or when switched to sugar plus water through day 15 (Table 3). When we compared survival with respect to time after switching diets we found that survival at each day was no different for flies maintained on protein only from eclosion or if they were transferred from the optimal to protein on either day 7 or 11 (ANOVA followed by Fisher's LSD, $P = 0.05$) (Table 1). However, when flies were transferred to only water on day 7 fewer flies died on the second day after transfer than if they were transferred to water on day 11 (ANOVA followed by Fisher's LSD, $P = 0.05$) (Table 2). Survivorship at other ages was not different. There was no significant mortality when flies were maintained on either the optimal diet or when switched to sugar plus water at 11 days.

A number of studies have demonstrated that the addition of protein to a carbohydrate source is important for optimizing reproductive success in adult *Anastrepha* species. For example, protein dietary supplements have been shown to have positive effects on ovarian maturation and fecundity in females of *A. serpentina*, *A. ludens*, *and A. obliqua* (Jacome et al. 1999; Aluja et al. 2001a; Mangan 2003) and improves sexual performance of males of at least four species including *A. suspensa* (Epsky & Heath 1993) *A. serpentina*, *A. striata*, and *A. obliqua.* (Aljua et al. 2001b). Thus, dietary protein appears to be an important component for reproductive success in all *Anastrepha* spp. However, apart from reports that sugars are necessary dietary requirements (Bateman 1972), important for long term survival (Jacome et al. 1999), and that starvation, by removal of both protein and carbohydrate sources from the adult diet, results in rapid morality, there is little information on the dietary need of sugar alone or if protein can take the place of sugar as an adult nutrient. Results of our study indicate clearly that carbohydrates are absolute dietary requirements, not only among adult males that have not reached sexual maturity, but also among sexually mature adult males. The results demonstrate that adult male Caribbean flies carry few resources forward from pupation to the adult stage that can be tapped for energy utilization and store only lim-

TABLE 2. MEAN SURVIVORSHIP OF FLIES ON EACH DAY AFTER ALL FOOD WAS WITHHELD. FLIES WERE EITHER STARVED FROM THE DAY OF ECLOSION OR SWITCHED FROM THE OPTIMAL SUGAR PLUS PROTEIN DIET TO NO FOOD ON EITHER THE SEVENTH OR ELEVENTH DAY AFTER ADULT ECLOSION. ALL FLIES WERE PROVIDED WITH WATER.

Days after removing food	Mean survivorship of flies starved from day of eclosion*	Mean survivorship of flies switched to no food on day 7*	Mean survivorship of flies switched to no food on day 11*
0	100 A	100 A	100 A
1	81 B	90.7 A, B	85.6 A, B
2	21.5 D	54.6 C	8.0 D, E
3	4.0 E	5.8 D, E	1.6 E
4	0.0 E	0.5 E	0.0 E

*Means in the same column or row followed by the same letter are not significantly different by Fisher's least significant difference test ($P = 0.05$) applied after ANOVA indicated differences among the means.

TABLE 3. MEAN SURVIVORSHIP OF FLIES ON EACH DAY AFTER BEING FED ONLY SUGAR. FLIES WERE EITHER PROVIDED WITH ONLY SUGAR FROM THE DAY OF ECLOSION OR SWITCHED FROM THE OPTIMAL SUGAR + PROTEIN DIET TO JUST SUGAR ON EITHER THE SEVENTH OR ELEVENTH DAY AFTER ADULT ECLOSION. ALL FLIES WERE PROVIDED WITH WATER.

Daysafter feeding sugar only	Mean survivorship of flies starved from day of eclosion*	Mean survivorship of flies switched to no food on day 7*	Mean survivorship of flies switched to no food on day 11*
0	100	100	100
1	100	100	100
2	100	99.8	100
3	100	98.6	98
4	99.3	96.6	94.7

*Means in the same column or row are not significantly different in an ANOVA or Fisher's least significant difference test (P = 0.05). Mean survivorship of flies fed protein plus sugar for the first 4 days was 98.67%; for 11 days it was 99.6%, and for 15 days it was 99.6%.

ited energy reserves as adults despite being provided with far more food, in the form of hydrolyzed yeast protein, than is required. This suggests strongly that flies maintain only limited supplies of glycogen in muscle and fat body tissue and have limited capacity to convert lipids from the fat body into energy. Perhaps more surprising is the apparent inability of flies to convert dietary protein resources to energy in the absence of carbohydrate as is evidenced by the fact that flies provided with only protein or switched from the complete diet to protein alone at 7 or 11 days exhibited the same rates of survival as males provided with water only. This is intriguing given that the protein fed to males was rich in amino acids such as proline, alanine, aspartate, and glutamate which are readily converted by some insects to pyruvate or oxaloacetate or α-ketoglutatrate for use in the Krebs cycle. Indeed, complete catabolism of one mole of proline can yield 14 moles of ATP and in some Diptera, such as the Tsetse fly (*Glossina morsitans*, Westwood), proline is the major source for ATP generation (Bursell 1981). This suggests strongly that transamination of normally important amino acids is not a primary method of substrate production for the Krebs cycle of these flies.

The absolute requirement for sugar and inability of the flies to utilize protein for metabolic energy may reflect a physiological adaptation to environmental conditions. In the tropics, where these flies evolved, sugars from fruit and nectar are available at all times of the year but protein is a relatively limited resource (Bateman 1972; Hendrichs et al. 1993). Indeed, protein dietary supplements have been shown to have positive effects on ovarian maturation and fecundity in *A. serpentina*, *A. ludens*, and *A. obliqua* (Jacome et al. 1999; Aluja et al. 2001a; Mangan 2003) indicating that dietary protein is a critical component for egg production and, consequently, reproductive success in all *Anastrepha* species. Thus, the flies have probably developed a physiological strategy in which they utilize the most available food source, sugars, to insure survival, and take advantage of limited protein resources when available to achieve sexual maturity (Bateman 1972).

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A SIMPLE AND EFFECTIVE CYLINDRICAL STICKY TRAP FOR FRUIT FLIES (DIPTERA: TEPHRITIDAE)

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ABSTRACT

A sticky trap for fruit flies was developed that is 2.5× more effective than yellow panel traps of equal surface area for capture of Mexican fruit flies (*Anastrepha ludens* (Loew)). The trap consists of a slightly conical yellow cardboard cylinder coated on the outside surface with trapping adhesive. In trapping efficacy, these stand-alone cylinders were equivalent to plastic Liquibaitor trap tops with similar cylinders fitted over the trap top with the sticky surface facing outward. Liquibaitor trap tops with cylinders mounted on the inside with their sticky surfaces facing inward were ineffective, and Liquibaitor tops with cylinders both inside and outside were not more effective than those with the sticky surface only on the outside. Besides the increased attractiveness of the stand-alone cylinders with the sticky surface outside, advantages of this design are that lures can be suspended from the trap hanger inside the cylinder where they do not contact the sticky surface, sticky cylinders can be changed in the field without disturbing lures that are suspended from the hangers, and traps can be stacked like Dixie cups for storage and transport.

Key Words: Mexican fruit fly, *Anastrepha ludens*, trap design, integrated pest management.

RESUMEN

Una trampa pegajosa para moscas de las frutas que es 2.5× más efectiva que las trampas de paneles amarillas de igual área de superficia para capturar la mosca mexicana de la fruta *(Anastrepha ludens* Loew) fue desarrollada. La trampa consiste de un cilindro de cartón un poco cónico de color amarillo con la superficie exterior cubierta con un pegamento para atrapar las moscas. En cuanto la eficacia de las trampas, estos cilindros que se sostienen solos fueron equivalentes a las trampas de tapa "Liquibaitor" con cilindros similares puestos sobre la trampa con la superficie pegajosa hacia afuera. Las trampas de tapa "Liquibator" montadas con la superficie pegajosa hacia adentro fueron inefectivas y las tapas de "Liquibator" con cilindros con la superficie exterior y la interior pegajosa no fueron mas efectivas que las trampas con solo la superficie exterior pegajosa. Aparte de que estos cilindros tienen la superficie exterior pegajosa y se pueden sostener solos y atrapan mas moscas, este diseño tiene la ventaja de que los señuelos pueden ser suspendidos de un gancho puesto dentro del cilindico donde no tiene contacto con la superficie pegajosa, se puede cambiar los cilindros pegajosos en el campo sin disturbar los señuelos que estan suspendidos de los ganchos y además puede guardar y transportar las trampas una encima la otra como vasos de la marca "Dixie".

Detection with traps is the first line of defense against exotic fruit flies and a critical element in programs to control resident species (Robacker & Landolt 2002). Two principal types of traps are in general usage: those that induce flies to land and become trapped on a sticky surface, and those that lure flies into an enclosed space where they drown in a liquid reservoir or contact a killing agent. Which type works better depends on the fly species and type of lure. Each type has found numerous niches in fruit fly programs around the world. Because of the need for earlier and more reliable detection to improve fruit fly control, development of better traps of both types is ongoing in many labs and agencies concerned with fruit fly management.

Synthetic lures for Mediterranean fruit fly (*Ceratitis capitata* Wiedemann) and various *Anastrepha* such as the Mexican fruit fly (*A. ludens* (Loew)) have been invented during the last decade (Biolure, Suterra, Inc., Bend, OR; *Anastrepha* Fruit Fly Lure, IPM Tech, Portland, OR). Whereas traditional baits for detection of *Anastrepha* were liquid suspensions that required McPhail-type traps, these new lures can be readily used with either enclosed traps or sticky traps. One of these lures (Biolure) has been used successfully in Multilure traps (Florence Agri Investment, Inc., Miami, FL) (Thomas et al. 2001) and other McPhail-type traps with liquid reservoirs including Liquibaitor traps (International Pheromone, South Wirral, UK) (Epsky et al. 1999; Katsoyannos et al. 1999; Papadopoulos et al. 2001). Although agencies charged with fruit fly trapping may actually prefer to use a dry trap, a change to dry traps is unlikely unless they are at least as attractive as existing McPhail-type wet traps. At this time, no commercially available

dry traps can match trapping efficacy of McPhail-type traps.

Our goal is to develop a more effective sticky trap. In this work, we investigated effect of trap shape. It is well known that trap shape affects attractiveness of sticky traps. Although numerous shapes have been tested, little has been published on shapes other than panels and spheres (Katsoyannos 1989; Epsky & Heath 1998). Nakagawa et al. (1978) showed that cylinders were among the least attractive shapes to Mediterranean fruit flies. However, Heath et al. (1997) described a highly effective cylindrical sticky trap that was constructed with paper coated with an extremely tacky dry adhesive (Atlantic Paste and Glue Co., Inc., Brooklyn, NY). The relative effects of the cylindrical shape and the highly sticky surface on performance of the trap were not evaluated.

Because the cylindrical trap developed by Heath et al. (1997) was so effective, we wanted to re-investigate effectiveness of cylindrical traps. In this work we evaluate the cylindrical shape with a standard sticking agent rather than the dry adhesive used by Heath et al. (1997). In one experiment we investigated the effect of having the sticky surface on the inside vs. outside of cylinders mounted on the top of Liquibaitor trap tops. In the second experiment, we evaluated stand-alone cylinders vs. cylinders mounted on Liquibaitor trap tops. Traps were compared with a commercially available sticky trap for catching irradiated, laboratory-colony Mexican fruit flies released into a citrus orchard.

Materials and Methods

Experimental Traps

Four cylindrical trap types were constructed of yellow cardboard obtained from IPM Tech (Portland, OR) coated with Stickem Special (Seabright Laboratories, Emeryville, CA). For three of these types, the cardboard was formed into cylinders that fit snugly either inside or on the outside of the plastic top of a Liquibaitor trap (often referred to as an International Pheromone McPhail trap). The three trap types constructed this way consisted of Liquibaitor trap tops with sticky cylinders inside, outside, or both inside and outside.

The fourth trap was a stand-alone (without a Liquibaitor trap top) cylinder with the sticky coating on the outside, of the same dimensions as used to fit over the outside of the Liquibaitor trap top (Fig. 1). The trap is slightly conical with a top diameter of 13.5 cm, a bottom diameter of 16 cm, and a height of 13.5 cm. The total sticky surface area (618 cm^2) was approximately equal to that (644 cm^2) of a Pherocon AM trap (Trece, Inc., Salinas, CA). A wire with a loop in the center was fastened across the top diameter of the cylinder. The loop in the wire served for hanging the trap.

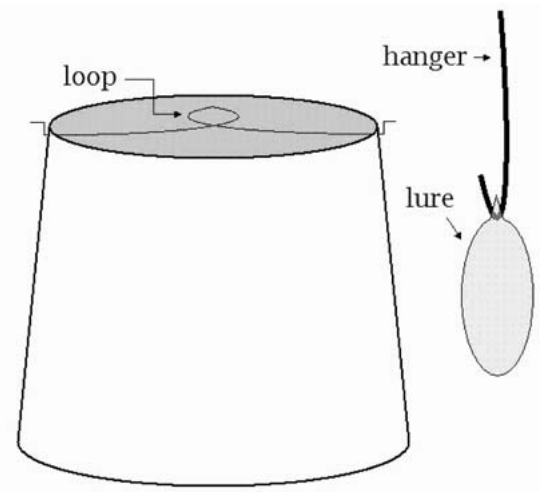

Fig. 1. Stand-alone sticky cylindrical trap for fruit flies constructed with yellow cardboard coated with Stickem Special (top diameter, 13.5 cm; bottom diameter, 16 cm; height, 13.5 cm). The lure and trap (at the loop in the wire) can be suspended separately from the trap hanger.

Insects

Mexican fruit flies (*Anastrepha ludens*) were used to evaluate the effectiveness of the trap. Flies were from a culture that originated from yellow chapote fruit, *Casimiroa greggii* (Rutaceae), collected in Nuevo Leon, Mexico, in 1987. Flies were irradiated, due to quarantine laws, with 70-92 Grays (Cobalt 60) 1 to 2 days before adult eclosion. Mixed-sex groups of 180-200 flies were kept in 473-ml cardboard cartons with screen tops until used in tests. Laboratory conditions for holding flies were 22 ± 2°C, 50 ± 20% relative humidity and photophase from 0630 to 1930 hours provided by fluorescent lights. Flies were fed sugar and water until they were released in test plots 3 to 8 days after eclosion.

Field Evaluations

The purpose of these experiments was to test the efficacy of the experimental traps against the Pherocon AM (no bait) trap. Pherocon AM traps are rectangular (14 × 23 cm) yellow cardboard panels coated with an adhesive similar to Stickum Special. Experiments were conducted in a mixed citrus orchard located near the laboratory in Weslaco, Texas. The orchard contained several varieties of orange, lemon, and tangerine trees of various ages. One row of Dancy tangerine (*C. reticulata*) was chosen for tests since it contained relatively large (2-3 m height) fruit-bearing trees. IPM Tech *Anastrepha* Fruit Fly Lures were suspended inside of Liquibaitor trap tops or from the hanger of the sticky cylinder traps at the loop in

the wire. Pherocon AM (no bait) traps with IPM Tech *Anastrepha* Fruit Fly Lures attached to the trap hangers were used as the control. Traps were located one to a tree, north of center, at 1-2 m height. Trapped flies were counted and all of the traps were replaced each week. Lures were used for the duration of each of the two field experiments. Each week approximately 3000 flies were released onto trees in a row adjacent to the test row so as to create a uniform distribution of flies near the test trees. The first experiment was a test of the three trap designs with sticky cylinders on Liquibaitor trap tops compared with Pherocon traps. Three linear blocks of four consecutive trees were used in the row, with one buffer tree between blocks. Each of the three blocks contained one each of the four trap types. Trap types were randomized within each block the first time traps were put into the orchard, and then moved sequentially within each block when traps were serviced once per week. Eighteen replicates of each trap type were tested (3 blocks × 6 service weeks).

The second experiment tested sticky cylinders without Liquibaitor trap tops (Fig. 1) against sticky cylinders on the outside of Liquibaitor trap tops, and Pherocon traps. Four linear blocks of three consecutive trees were used in the row, with a buffer tree between blocks. Each of the four blocks contained one each of the three trap types. Procedure was the same as in the previous experiment. Twenty-eight replicates of each trap type were tested (4 blocks × 7 service weeks).

Statistical Analyses

The experimental design for both experiments was a randomized complete block. Replications over time (service weeks) were treated like replications over space (blocks of trees) for the purpose of statistical analyses. Data were subjected to analysis of variance with SuperANOVA (Abacus Concepts 1989).

Results and Discussion

Results of the experiment testing sticky cylinders on Liquibaitor trap tops are shown in Table 1. The analysis of variance was highly significant ($F = 11.4$; $df = 3,68$; $P < 0.0001$). Liquibaitor trap tops with sticky cylinders on the inside captured fewer flies than Pherocon traps. Traps with sticky cylinders on the outside did not differ in attractiveness from those with cylinders both inside and outside. Both of these designs were more attractive than Pherocon traps. Trap types did not differ regarding percentage of females captured.

Fly captures on stand-alone sticky cylinder traps did not differ from those on sticky cylinders fitted on the outside of Liquibaitor trap tops ($F = 8.9$; $df = 2,81$; $P < 0.001$) (Table 2). Both designs were more attractive than Pherocon traps. Trap types did not differ regarding percentage of females captured.

Cylindrical sticky traps with the sticky surface on the outside, either stand-alone or mounted on Liquibaitor trap tops, captured about 2.5×more Mexican fruit flies than Pherocon panel traps of approximately the same sticky surface area. These results indicate that cylinders are more attractive than panels.

As discussed in the introduction, Heath et al. (1997) described a highly effective cylindrical sticky trap made with an extremely tacky dry-adhesive paper. This trap captured twice as many Mexican fruit flies and Mediterranean fruit flies as glass McPhail traps with the same lures. Relative importance of cylindrical shape and the trapping adhesive were not evaluated, however, it now seems likely that the great effectiveness was at least partly due to the shape.

The cylinder traps with their sticky surfaces inside Liquibaitor trap tops were designed to function like a dry version of a McPhail trap. In both types of traps, flies must enter from below as they approach the attractive volatiles coming from either the liquid reservoir or the lure suspended inside the trap top. The poor performance of these traps was unexpected based on the historical effectiveness of McPhail traps. However, Heath et al. (1997) also reported poor captures of Mexican fruit flies with an open-bottom cylindrical dry trap that required flies to enter from the bottom or through small holes in the side.

Despite great promise, the cylindrical sticky trap described by Heath et al. (1997) was never produced commercially, possibly because small

Table 1. Mexican fruit fly captures per week on sticky cylinders attached inside or outside of Liquibaitor trap tops compared with Pherocon traps.[1,2]

Test trap	Males	Females	Total
Pherocon	1.3 ± 0.3 a	2.2 ± 0.4 b	3.5 ± 0.5 b
Sticky cylinder inside	0.3 ± 0.2 a	0.2 ± 0.2 a	0.5 ± 0.2 a
Sticky cylinder outside	4.0 ± 0.8 b	5.4 ± 1.1 c	9.4 ± 1.7 c
Sticky cylinders inside and outside	3.2 ± 0.5 b	4.2 ± 0.6 c	7.4 ± 0.9 c

[1]All traps were baited with an IPM Tech *Anastrepha* Fruit Fly Lure.
[2]Means (±SE) in the same column followed by the same letter are not significantly different by Fisher's protected LSD test ($P < 0.05$).

TABLE 2. MEXICAN FRUIT FLY CAPTURES PER WEEK ON TWO TYPES OF STICKY CYLINDER TRAPS COMPARED WITH PHEROCON TRAPS.[1,2]

Test trap	Males	Females	Total
Pherocon	1.7 ± 0.3 a	2.4 ± 0.4 a	4.1 ± 0.6 a
Sticky cylinder on Liquibaitor trap top	5.1 ± 0.8 b	4.7 ± 0.5 b	9.8 ± 1.1 b
Sticky cylinder	5.1 ± 0.7 b	5.0 ± 0.6 b	10.0 ± 1.2 b

[1]All traps were baited with an IPM Tech *Anastrepha* Fruit Fly Lure.
[2]Means in the same column followed by the same letter are not significantly different by Fisher's protected LSD test ($P < 0.05$).

birds and lizards were sometimes trapped due to the extreme stickiness (Heath et al. 1997). Also, the sticky surface of these traps adheres flies so well that fly damage on removal renders identification difficult (T. Holler, USDA-APHIS, pers. comm). Also, experiments with panel traps made with the same paper (Robacker & Heath 2001) indicated that rain damages both the adhesive and the paper, making the traps ineffective.

The stand-alone cylindrical sticky trap developed in this work has numerous features that enhance its effectiveness and ease of use. First, the looped wire spanning the diameter of the top of the trap provides a point for attachment of the hanger and suspension of a lure in the center. If the lure is suspended from the trap hanger, then the disposable sticky trap body can be easily replaced without disengaging the lure. Further, the lure does not become sticky because it never comes in contact with the sticky surface of the trap. This is important because commercial synthetic lures are manufactured so as to last several months. Also, because the trap is slightly conical, the sticky surface can be covered with wax paper and traps can be stacked like Dixie cups for shipping and transport to the field. This feature gives this trap an advantage over spheres and non-conical cylinders. With regard to the adhesive, neither the cardboard nor the Stickem Special trapping adhesive are damaged by rain and trapping of birds or other small animals has not been observed. Most importantly, the cylindrical shape makes it much more attractive than yellow panel traps, greatly improving detection of Mexican fruit flies and perhaps other species of Tephritidae.

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SEASONAL AND NOCTURNAL FLIGHT ACTIVITY OF *SPODOPTERA FRUGIPERDA* MALES (LEPIDOPTERA: NOCTUIDAE) MONITORED BY PHEROMONE TRAPS IN THE COAST OF CHIAPAS, MEXICO

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ABSTRACT

We determined seasonal and nocturnal flight activity of *Spodoptera frugiperda* (J. E. Smith) males with traps baited with pheromone in the coast of Chiapas, Mexico. A total of 3015, 3065, and 838 males were captured in 2000, 2001, and 2002, respectively. Pheromone trap catches decreased approximately 72% during 2002 with respect to 2000 and 2001. One of five experimental sites caught 90% of the total captured. The pattern of trap captures was quite variable among years and sites. In general, the flight activity of *S. frugiperda* males was seasonal, with two distinctive peaks in trap captures during the year. Males were caught during all hours of scotophase, however, most males were captured during the first 7 h. Highest peak capture was between 1900-2000 h. Trap captures were positively correlated with wind speed and temperature, and negatively correlated with relative humidity. Significantly more males were captured at wind speeds of 100-200 and >200 m/min than at wind speeds of 0-100 m/min.

Key Words: *Spodoptera frugiperda*, pheromones, monitoring, seasonal activity, nocturnal activity, Mexico.

RESUMEN

La actividad de vuelo estacional así como la actividad nocturna de machos de *Spodoptera frugiperda* (J. E. Smith) fue determinada usando trampas cebadas con feromona en la costa de Chiapas, México. Un total de 3015, 3065 y 838 machos fueron capturados en 2000, 2001 y 2002, respectivamente. La captura de las trampas cebadas con feromona, disminuyó aproximadamente en un 72% en 2002 con respecto al 2000 y 2001. El perfil de captura de machos de *S. frugiperda* fue muy similar en 2000 y 2001, pero diferente al del 2002. En los dos primeros años se obtuvieron grandes capturas de machos de *S. frugiperda* en los meses de enero a marzo. Por el contrario en 2002, las mayores capturas se obtuvieron en agosto. Del total de machos capturados, más del 90% fueron capturados en un sitio experimental y el 10% restante en las otras localidades estudiadas. En cuanto al estudio de la actividad nocturna de los machos de *S. frugiperda* con trampas cebadas con feromona, se encontró que la captura se inició desde las primeras horas y se mantuvo durante toda la noche. Sin embargo, la mayoría de los machos fueron capturados durante las primeras siete horas de la noche, alcanzando el mayor pico de captura entre las 1900-2000 h. Las capturas obtenidas con las trampas correlacionan positivamente con la velocidad del viento y la temperatura, y de manera negativa con la humedad relativa. Se capturaron de manera significativa más machos cuando la velocidad del viento fue de 100-200 y mayor de 200 m/min, que cuando fue de 0-100 m/min.

Translation provided by the authors.

The fall armyworm (FAW), *Spodoptera frugiperda* (J. E. Smith) is indigenous to the tropical regions of the western hemisphere from Argentina to the United States of America. This species is considered a generalist feeder, feeding on a very wide host range of plants in several families, with preference for grasses. In Mexico, FAW is one of the most important pests of corn and sorghum, although occasionally it attacks other crops. It frequently is controlled through the use of insecticides. The misuse of insecticides has led to resistance of *S. frugiperda* to several insecticides in Mexico (Pacheco-Covarrubias 1993) and to possible harmful effects on human health and the environment (Tinoco & Halperin 1998). Alternatives for managing this pest are currently being explored in Mexico, including the use of agents of biological control, cultural techniques, host plant resistance and pheromones (Malo et al. 2001; Cisneros et al. 2002; Mendez et al. 2002; Farias-Rivera et al. 2003; Molina-Ochoa et al. 2003).

Pheromones can be used for direct control of some insect pests (mass trapping, kill and lure, and mating disruption) and for monitoring pest populations. Pheromone-monitoring traps can be used for detection of a particular species, timing of control measures, and economic risk assessment of that pest (Wall 1990). In the case of *S. frugiperda*, pheromone has been used mainly to monitor male activity, in determining migration,

seasonal dynamics, and spatial distribution (Tingle & Mitchell 1979; Waddill et al. 1982; Starrat & McLeod 1982; Pair et al. 1986; Adams et al. 1989; Mitchell et al. 1989). The possibility of using pheromone as a direct way of control has been less explored (Mitchell et al. 1974a).

FAW is a key pest of corn in Chiapas, Mexico, but information on moth seasonal dynamics and flight patterns is lacking for this region. The objective of this study was to provide information on seasonal and nocturnal flight activity of FAW males in the coast of Chiapas with traps baited with sex pheromone. The information obtained could be integrated to the control measures to manage *S. frugiperda* infestations in an effective manner.

Materials and Methods

Seasonal Flight Activity

We selected five sites in three municipalities at the coast of Chiapas, Mexico (Fig. 1). This region experiences a humid temperate climate with heavy rain in the summer, with average annual rainfall of 2,063 mm, with a rainy season normally occurring from late May through November. The average annual temperature is 26°C, and April and May are the hottest months. Most of the land is flat, but there are rolling hills in the Northeast region. The most common soil types are luvisol, nitosol andosol, and planosol. A great diversity of plants are cultivated, including annual (e.g., corn, sorghum and soybean) and perennial crops (e.g., mango, coffee, and cacao). Many hectares are dedicated to cattle ranching, with less space devoted to pigs and poultry. Two of the sites selected were located in the Tapachula municipality. In the first site, "Los Toros" (14°48'N, 92°19'W, 40 masl), 45 ha of soybean are cultivated once a year (June to October). Mango orchards and native trees surround this area. In this site, traps were placed 15 m away from a living fence of *Jatropha curcas* L.

The second site is known as "El Manzano" (14°44'N, 92°19'W, 20 masl), where about 700 ha are devoted to cultivation of two crops annually. Sorghum or corn is cultivated from January to May, and is watered by a sprinkler irrigation system. Soybean is cultivated during the rainy season from July to October. In this site, traps were placed 200 m away from a cashew orchard. Two additional sites were selected in the municipality of Suchiate; the first known as "20 de Noviembre" (14°42'N, 92°16'W, 20 masl), where 108 ha are dedicated to cultivate star grass (*Cynodon nlemuensis* Vanderyst) for feeding cattle. Mango orchards and native trees surround the area. In this site, traps were placed 200 m away from a patch of native trees. In the second site called "Ciudad Hidalgo" (14°40'N, 92°10'W, 25 masl), 13 ha are devoted to cultivation of corn once a year from June to September. Banana plantations, mango orchards and native trees surround the area. In this site, traps were placed 20 m away from a patch of native trees. The fifth site known as "Chincuyo" (14°51'N, 92°11'W, 155 masl) was located at the municipality of Tuxtla Chico, where 2 ha of corn are cultivated during the rainy season from June to October. Cacao and lemon orchards surround the site. Traps were placed 20 m away from a lemon orchard.

At each site, two Scentry *Heliothis* traps were placed 1.5 m above the ground on wooden stakes, spaced more than 30 m apart. Each trap was baited with a *S. frugiperda* pheromone bubble cup lure (Chemtica, Costa Rica) that was replaced monthly. Moths were collected every 10 d, counted, and numbers recorded by trap and locality. Trapping was conducted from January 2000 to December 2002, resulting in a total of 103 observation dates.

Nocturnal Flight Activity

This experiment was conducted at El Manzano locality in Tapachula. The experimental field was planted with sorghum ('V-M') variety sown at a density of 50,000 plants/ha with row spacing of 0.75 m. Four Scentry *Heliothis* traps were placed at intervals of 100 m in a straight line inside the field, starting with the first trap 100 m from the crop edge. Traps baited with a *S. frugiperda* pheromone formulated as a bubble cup (Chemtica, Costa Rica) were hung approximately 1.5 m above the ground on wooden stakes. When the experiment began, sorghum plants were 25 cm high. Traps were emptied each h, beginning at 1800 h (30 min before sunset) until 6000 h (10 min before dawn). Males caught in each h were killed with ethyl acetate and counted. Before each observational night, traps were cleaned and rotated along the line to remove possible trap bias on male capture. In addition, temperature, relative humidity, wind direction, and wind speed each 5 min were recorded 2 m above ground level at the edge of the field. The experiment was conducted during 5 nights, from 10-14 February, 2003.

Statistical Analysis

Analysis of variance (ANOVA) was used to determine if the number of males caught varied through the night. Data were transformed by ln (X + 1) before ANOVA, to correct for heterogeneity of variances. A possible correlation between the number of males trapped and the meteorological parameters recorded was examined by Spearman rank correlation analysis. Data from all traps were used for the correlation analysis because preliminary analysis showed that trap position did not influence the numbers of males captured.

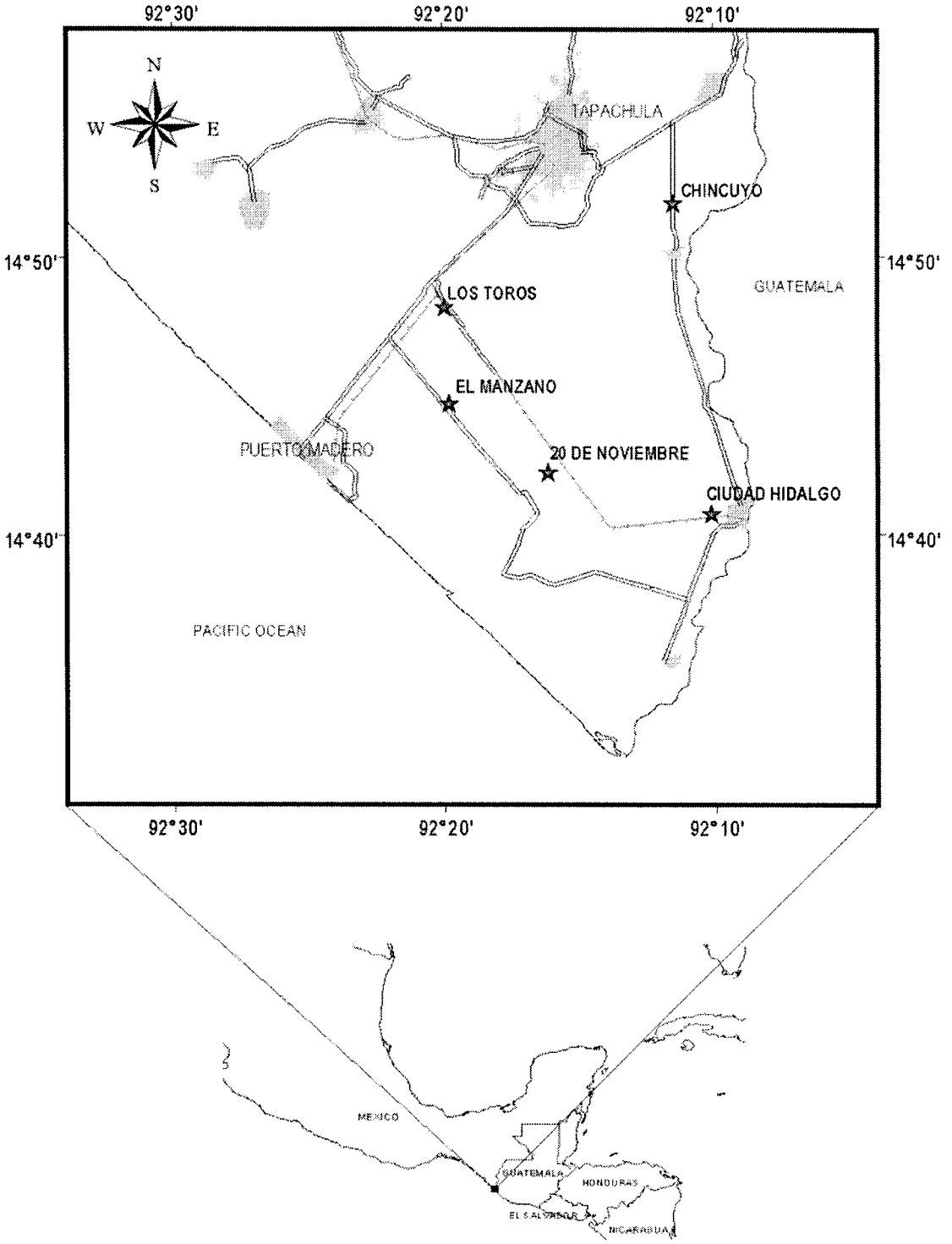

Fig. 1. Map showing where pheromone traps were placed in Soconusco region, Chiapas, Mexico during 2000-2002. The sites sampled are marked with black stars.

Also, wind speed was categorized as light (0-100 m/min), moderate (100-200 m/min), or strong (>200 m/min), and an ANOVA performed among the traps captures across wind-speed categories. When statistical significance was found after ANOVA, treatment means were separated by the Tukey test procedure. The level of probability considered significant in all analysis was $P \leq 0.05$.

Results

Seasonal Flight Activity

A total of 6770 *S. frugiperda* males was captured during the three years of trapping. A total of 3015, 3065, and 838 males was captured in 2000, 2001, and 2002, respectively. Pheromone trap catches decreased approximately 72% during 2002 compared to 2000 and 2001. The El Manzano site caught 93.4% of all males captured among the sites. The pattern of trap captures was quite variable among years and sites (Fig. 2). For example, the pattern of trap captures in El Manzano was quite similar during 2000 and 2001, but slightly different in 2002. In the first two years, the flight activity of *S. frugiperda* males was seasonal, with two distinctive peaks in trap catches. The first peak occurred from January to March during the dry season, and the second peak between June and September, during the rainy season. During 2002, there were also two peaks, the first between January and February, and the second one occurred in August. In the other sites the flight activity of *S. frugiperda* was also bimodal, the first and higher peak occurred between June and July, whereas a smaller peak was observed between September and November at the end of the rainy season. No moths were caught from January to April during the three years in the experimental sites Chincuyo, 20 de Noviembre and Los Toros, except that a small number of males was caught at Los Toros in 2001 (Fig. 2).

Nocturnal Flight Activity

The trap position within the field did not affect the number of males captured during the experiment ($F = 0.7$; $df = 3, 16$; $P = 0.57$). The nocturnal activity of *S. frugiperda* males began at sunset and they were active throughout the night, although the number of males trapped varied significantly through time ($F = 4.5$; $df = 11, 228$; $P < 0.0001$). Most males were captured during the first 7 h, with the highest catches between 1900 and 2000 h (Fig. 3). Trap captures were positively correlated with wind speed ($r = 0.29$, $P = 0.03$) and temperature ($r = 0.32$, $P = 0.016$), and negatively correlated with relative humidity ($r = -0.32$, $P = 0.016$). More males were captured at wind speeds of 100-200 and >200 m/min than at wind speed of 0-100 m/min ($F = 4.9$; $df = 2, 50$; $P = 0.01$) (Fig. 4).

Discussion

Our results show great variation in the number of *S. frugiperda* males caught among the different experimental sites, with the "El Manzano" site capturing most of the insects. Capture of adults at this site was bimodal, the greatest number captured from January to March, in the dry season, with a second smaller peak between June to September, during the rainy season. In general, our results from this site are in agreement with those reported by Raulston et al. (1986), who investigated the population trends of *S. frugiperda* along the Mexican Gulf Coast, the Isthmus of Tehuantepec, and the Yucatan Peninsula with Harstack pheromone traps. They found that low numbers of males were caught during the midportions of the year, while peak captures occurred either early or late in the year. In contrast, the highest captures occurred during the rainy season and a few males were captured in the dry season at the other trap sites. These results agree with those of Mitchell et al. (1991), who reported that in the tropics, *S. frugiperda* populations have a tendency to vary with seasonal changes in rainfall, with the lowest populations recorded during the dry seasons. In French Guiana, the highest populations of adults and larvae of *S. frugiperda* occur during the rainy season and the lowest in the dry seasons (Silvain & Hing 1985). Our study was conducted in a relatively small area and factors such as temperature and rainfall are expected to be quite similar and therefore these hardly could explain the difference in traps captures among experimental sites. Gutierrez-Martinez et al. (1989) reported that temperature and rainfall did not affect the captures of *S. frugiperda* males in the central area of Chiapas State, Mexico. On the contrary, the wind speed and direction changes from place to place, and this variation could explain why traps placed at El Manzano captured more males than other sites. Also, "El Manzano" is not surrounded by native or cultivated trees (e.g., mangoes) as was the case with the other experimental sites, which may constitute a barrier for both insects and pheromone dispersion. It has been documented that the distinct plume of pheromone is constructed from the pheromone source and a large proportion of males entering it can reach the source when the wind is strong (Lewis & Macaulay 1976). Mitchell et al. (1991) reported that favorable wind currents contributed to the distribution of *S. frugiperda* into and from different areas in the USA. However, the most important factor affecting trap captures seems to be the availability of host plants. For example, average host area in El Manzano was 6.8, 16.4, 56.7, and 368.5 times higher than in 20 de Noviembre, Los Toros, Ciudad Hidalgo and Chincuyo, respectively. Also, at El Manzano, a greater number of *S. frugiperda* males/trap/day was

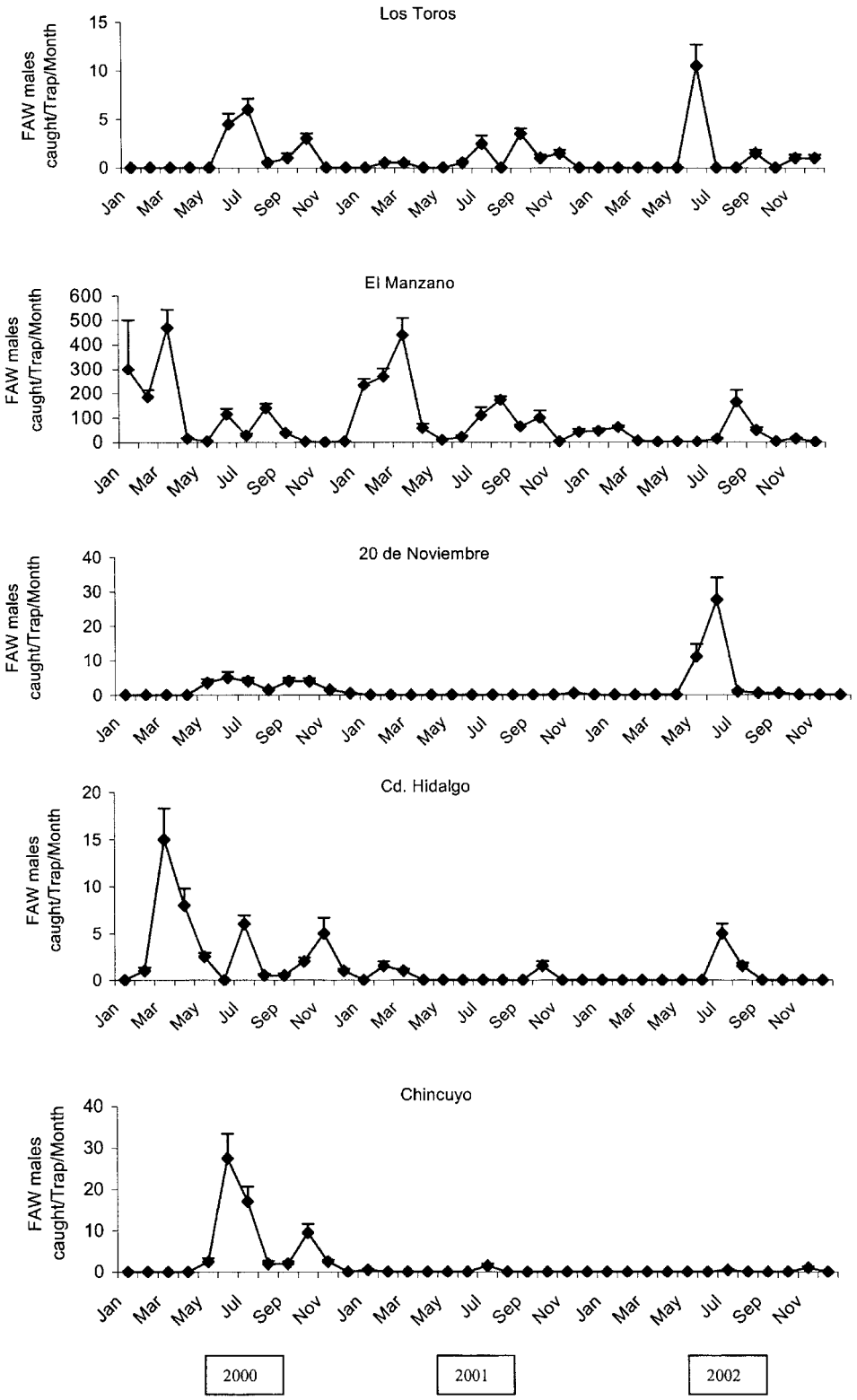

Fig. 2. Mean number of *S. frugiperda* males caught with pheromone traps at five sites in Soconusco region, Chiapas, Mexico during 2000-2002. Lines extending from each dot are standard error of the mean.

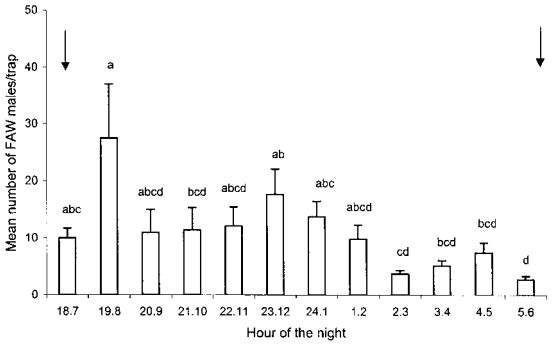

Fig. 3. Nocturnal flight of *S. frugiperda* males (mean ± SE) caught with pheromone traps during five successive nights from 10-14 February, 2003. Arrows indicate sunset and sunrise. Values (mean ± SE) followed by the same letter indicate no significant differences at the 5% level according to the Tukey test.

caught when crops (sorghum or corn and soybean) were present than when crops were absent (Mean ± SE = 5.8 ± 0.7 and 3.4 ± 0.7, respectively) ($t = 2.1$, $df = 93$, $P = 0.04$). Gutierrez-Martinez et al. (1989) showed that the first *S. frugiperda* males are captured right after plant emergence, with the highest frequency from the 10th through the 41st day of emergence, when the plant is more susceptible. Pair et al. (1986) reported that the availability and amounts of susceptible stages of corn planted in more northerly areas may be the most important factors determining the magnitude of *S. frugiperda* populations each year throughout the southeastern states of the USA. In other moth species, it also has been shown that host availability influences trap captures (Slosser et al. 1987; Parajulee et al. 1998).

We found that *S. frugiperda* populations were lower in 2002 than in 2000 and 2001 despite the fact that cropping patterns and total crop area did not vary during the three-year study. Thus, host availability cannot explain the decrease of FAW populations in 2002. One possible explanation is that climatic factors affected the populations. Pair et al. (1986) reported that *S. frugiperda* populations were lower in 1984 than in 1983 or 1985 in the southeastern states of USA. The authors mentioned that one possible cause of the diminution of populations in 1984 was the colder temperature, 0°C or lower recorded in winter 1983 and spring 1984 in the study area. It is known that 0°C kill *S. frugiperda* life stages and their host plants (Luginbill 1928). In our case, the minimal temperatures recorded in winter were about 19°C. Thus, low temperature does not seem to explain the decrease of populations in 2002. Rainfall is another possible factor to explain the lower capture rates of *S. frugiperda* males in 2002. Van Huis (1981) reported that heavy and light rain kill significant numbers of early instars of FAW. Data from a meteorological station located 10 km away from El Manzano show that it rained in December 2001, but not in December 1999 and 2000. Thus, it is possible that this rain killed *S. frugiperda* larvae, which reduced adult populations.

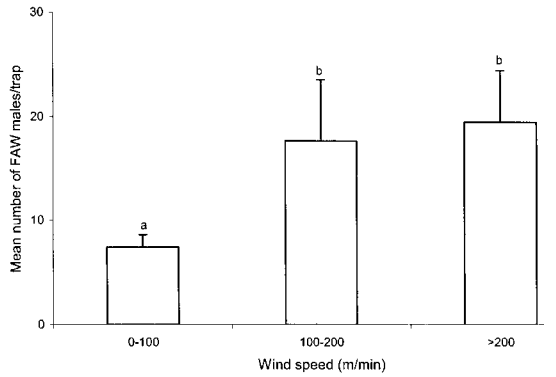

Fig. 4. Effect of wind speed on *S. frugiperda* capture with pheromone traps. Values (mean ± SE) followed by the same letter indicate no significant differences at the 5% level according to the Tukey test.

We found that *S. frugiperda* males were caught throughout the night but trap captures among night intervals was variable. Most males were captured during the first 7 h, reaching the highest capture peak between 1900 and 2000 h. That males responded to pheromone traps during the whole night could be due to the fact that we used synthetic lures which continuously emit pheromone and response could be independent from female calling periodicity. Mitchell et al. (1974b), who used *S. frugiperda* virgin females as lures, found that males responded to traps from 0.5 h before sunset to 1.5 h before sunrise, which is in agreement with our results.

Raina & Menn (1987) indicated that synchronous timing of calling behavior of females and flight activity of males in Lepidoptera could provide a high probability of mate finding with a minimum expense of energy. We do not have information about the diel periodicity of calling behavior of *S. frugiperda* females in the field, but under laboratory conditions, 2-d-old virgin females started to call 90 min after lights were turned off and individuals called throughout the night. Females stopped calling as soon as lights were turned on. Older females began to call 15 min after lights were turned off and calling peaked 3 h after lights were off (Reyes-Galvez 1999). In another study, mating pairs of *S. frugiperda* were observed throughout the night, although the percentage of moths in copulation peaked 3-4 h into the dark cycle (Simmons & Marti 1992). Temporal differences between peak calling, mating, and peak male flight may be attributed to differences in temperatures and photoperiod regimes. In other moth species, the flight

time of males is well synchronized with female calling (Cardé 1974; Sasaki & Riddiford 1984; Cibrian-Tovar & Mitchell 1991). However, in some cases, the calling behavior of females and the activity cycle of males seems not to be well coordinated because the peak of male flight does not coincide with the peak of female calling (Sanders 1971; Cardé 1974). For instance, females of *Choristoneura fumiferana* (Clemens) started calling 4.5 h before sunset and the peak of females calling was reached 2.5 h later. On the other hand, males were caught at all times during the 24 h period and the peak catch occurred 1.5 h after sunset. These results suggest that factors other than the release of pheromone have strong influence on the activity of *C. fumiferana* males (Sanders 1971).

Acknowledgments

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OVIPOSITION PREFERENCE OF *HOMALODISCA COAGULATA* FOR TWO *CITRUS LIMON* CULTIVARS AND INFLUENCE OF HOST PLANT ON PARASITISM BY *GONATOCERUS ASHMEADI* AND *G. TRIGUTTATUS* (HYMENOPTERA: MYMARIDAE)

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ABSTRACT

Oviposition preference of *Homalodisca coagulata* (Say) and two of its mymarid egg parasitoids, *Gonatocerus ashmeadi* Girault and *G. triguttatus* Girault, for two *Citrus limon* L. cultivars ('Eureka' and 'Lisbon') was investigated. In laboratory oviposition choice tests, the number of leaves containing *H. coagulata* egg masses, the number of *H. coagulata* egg masses, and the total number of *H. coagulata* eggs were significantly higher at 187.2%, 204.2%, and 181.7%, respectively, on 'Eureka' versus 'Lisbon'. In the field, there was no significant difference in the number of *H. coagulata* motiles counted in five-minute searches of foliage on 'Eureka' and 'Lisbon' trees, and numbers of leaves with old (emerged) and new (unemerged) *H. coagulata* egg masses were equivalent between field-planted cultivars. In the laboratory, parasitism of *H. coagulata* egg masses by *G. ashmeadi* and *G. triguttatus* was 18.6% and 23.2% higher, respectively, for eggs laid on 'Eureka' leaves compared to 'Lisbon', but these differences were not significant. Leaf surface morphology and thickness of leaf cell layers of both lemon cultivars were compared with scanning electron microscopy (SEM). SEM demonstrated that total leaf thickness and the thickness of the palisade layer was 19.2% and 38.6% higher, respectively, in 'Eureka' leaves compared to 'Lisbon', and that *H. coagulata* egg placement was between the lower epidermis and spongy parenchyma layer for both cultivars. Furthermore, 'Lisbon' leaves had a smooth underside, whereas 'Eureka' leaves had many small ridges. The thickness and rough surface of 'Eureka' leaves may be beneficial for *H. coagulata* oviposition. However, additional research is required to further investigate whether leaf characteristics or xylem chemistry are responsible for *H. coagulata* oviposition choice. For mass rearing programs with lemons as host plants, it is recommended that the 'Eureka' cultivar be used in preference to 'Lisbon' because *H. coagulata* prefers this cultivar for oviposition and parasitoid foraging is not adversely affected.

Key Words: *Homalodisca coagulata*, Hemiptera, Cicadellidae, Hymenoptera, Mymaridae, oviposition preference, host plant influence, *Citrus limon*.

RESUMEN

La preferencia para la oviposición de *Homalodisca coagulata* (Say) y dos de sus parasitoides de huevos mymaridos, *Gonatocerus ashmeadi* Girault y *G. triguttatus* Girault, en dos variedades de *Citrus limon* L. ('Eureka' y 'Lisbon') fue investigada. En pruebas del laboratorio donde pueden escoger el lugar de la oviposición, el número de las hojas que tuvieron masas de huevos de *H. coagulata*, el número de masas de huevos de *H. coagulata*, y el número total de huevos de *H. coagulata* fueron significativamente mas altos a 187.2%, 204.2%, y 181.7%, respectivamente, en la variedad 'Eureka' versus 'Lisbon'. En el campo, no hubo una diferencia significativa en el número de *H. coagulata* moviles contados en una busqueda de 5 minutos del follaje de las variedades de 'Eureka' y 'Lisbon' y el número de hojas con masas de huevos de *H. coagulata* viejas (emergidas) y nuevas (no emergidas) fueron equivalentes entre las variedades sembradas en el campo. En el laboratorio, el parasitismo de las masas de huevos de *H. coagulata* por *G. ashmeadi* y *G. triguttatus* fue 18.6% y 23.2% mas alta, respectivamente, para los huevos puestos en hojas de 'Eureka' comparado con 'Lisbon', pero estas diferencias no fueron significativas. La morfologia de la superficie de la hoja y el grueso de las tapas de celulas de la hoja de ambas variedades de limón fueron comparados con un microscopio electronico de barrido (SEM). El SEM demostró que el grueso total de la hoja y el grueso de la capa empalizada fue 19.2% y 38.6% mas alto, respectivamente, en hojas de 'Eureka' comparado a las hojas de 'Lisbon' y que la postura de los huevos de *H. coagulata* fue entre la epidermis menor y la capa del parénquima esponjosa para ambas variedades. Además, las hojas de 'Lisbon' tienen el envés liso, mientras que el envés en las hojas de 'Eureka' tienen un gran número de pequeñas estrías. El grueso y la superficie áspera de las hojas de 'Eureka' puede ser benéfico para la oviposición de *H. coagulata*. Sin embargo, se requiere de investigación adicional para saber si las caracteristicas de la hoja o la química del xilema son responsables para la selección del sitio de oviposición de *H. coagulata*. Para los programas

de cria en masa usando limones como plantas hospederas, se recomienda que se use la variedad 'Eureka' en preferencia a 'Lisbon' por que *H. coagulata* prefiere esta variedad para la oviposición y la actividad forrajera del parasitoide no es afectada adversamente.

The glassy-winged sharpshooter, *Homalodisca coagulata* (Say) (Hemiptera: Cicadellidae), is native to southeastern U.S.A. and northeastern Mexico, and following its establishment in California in the 1980s, it has become a significant threat to agricultural and ornamental industries due to its ability to spread the plant pathogenic bacterium, *Xylella fastidiosa* Wells et al. (Hopkins & Adlerz 1988; Purcell & Saunders 1999; Purcell & Feil 2001; Hopkins & Purcell 2002; CDFA 2003; Hoddle et al. 2003). A major classical biological control program has been launched in California against *H. coagulata*, including the mass rearing and release of two mymarid egg parasitoids, *Gonatocerus ashmeadi* Girault and *G. triguttatus* Girault. *Citrus limon* L. (lemon) has been chosen as a host plant for *H. coagulata* rearing and related experiments because it is one of the preferred hosts for *H. coagulata* in southern California and research on this pest is predominately conducted in agro-ecosystems where citrus dominates (Perring et al. 2001). However, several lemon cultivars exist, and two, 'Eureka' and 'Lisbon', are the most common commercial cultivars. It has been well documented that leafhoppers demonstrate significant oviposition preferences between plant species and between cultivars of the same species (McClure 1980; Stiling 1980; Singh & Agarwal 1988; Catindig et al. 1996; Sharma & Singh 2002). However, when this work was conducted it was not known which cultivar of *C. limon H. coagulata* preferred for oviposition under artificial rearing conditions. Consequently, the research undertaken here sought to determine the oviposition preference of *H. coagulata* for two *C. limon* cultivars, 'Eureka' and 'Lisbon', and whether *G. ashmeadi* and *G. triguttatus* exhibited preferences for *H. coagulata* eggs laid on 'Eureka' or 'Lisbon' leaves. To assist with interpretation of preference data collected from the laboratory, we conducted field surveys of *H. coagulata* life stages on 'Eureka' and 'Lisbon' lemons and compared leaf surface characteristics and measured leaf cell layers of both cultivars with scanning electron microscopy (SEM).

Materials and Methods

Homalodisca coagulata Oviposition Preference in the Laboratory

'Lisbon' and 'Eureka' trees approximately two years of age and grafted to *Macrophylla* sp. rootstock were obtained from C & M Nurseries, Nipomo, CA. Five trees of each cultivar were pruned to 60 cm in height (mean no. leaves per tree = 43 ± 5.7 for 'Eureka' and 37 ± 8.4 for 'Lisbon'). The trees were potted into 4-litre containers and fertilized every two weeks with Miracle-Gro (20 ml/3.5 liters of water, Scotts Miracle-Gro Products Inc., Marysville, OH). Each of five cages (30 × 60 × 35 cm) received one tree of each cultivar in a randomized design. Cages with trees were held in a greenhouse at 26° ± 2°C and 30-40% RH under natural light. Forty female and ten male *H. coagulata* were collected from the field and placed into cages holding experimental plants. Every two days, approximately 25 field collected female and five male *H. coagulata* were added to each cage. The number of leaves with *H. coagulata* eggs, the number of egg masses, and the number of eggs laid on each cultivar were recorded daily. This study was conducted over the period May 10-24, 2001. Each combination of date and replicates was treated as a block, and a paired comparison *t*-test at the 0.05 level of significance was applied to the data in SAS (SAS 1990).

Homalodisca coagulata Oviposition Preference in the Field

A five-minute search for *H. coagulata* eggs on foliage 1-2 m above the ground was conducted on six 'Eureka' and six 'Lisbon' trees planted as part of a completely randomized block design variety trial at the University of California, Riverside Agricultural Operations Area. Surveyed trees were 17 years of age and cultivar scions were grafted to *Macrophylla* sp. rootstock. Old (emerged) and new (unemerged) *H. coagulata* egg masses found during time searches were harvested from trees and placed in labeled plastic bags. Additionally, a one-minute search for adult *H. coagulata* and nymphs was conducted on foliage within a 1-2 m band above ground around the tree for each replicate.

For collected leaves with emerged eggs, the number of *H. coagulata* egg masses, *H. coagulata* eggs per egg mass, emerged *H. coagulata* nymphs, solitary parasitoid emergence holes, unemerged nymphs and parasitoids, and 'unemerged unknowns' (those that could not be identified) were recorded for each lemon cultivar. Leaves with unemerged egg masses were held at 26° ± 2°C and 30-40% RH under a L14:10D photoperiod in 130-ml plastic vials (40-dram Plastic Vial, Thornton Plastics, Salt Lake City, UT) filled with deionized water and 3 ml of antiseptic [(Listerine Antiseptic Mouthwash, Pfizer Inc., New York, NY) (to prevent bacterial rot)] for two weeks to allow *H. coagulata* nymphs and parasitoids to emerge before recording data. This sampling and rearing protocol was repeated every two weeks over the period July 12-October 18, 2001. The total number of

H. coagulata (adults and nymphs) counted on cultivars and the number of leaves with *H. coagulata* egg masses was log transformed prior to analysis. Data were compared between cultivars by two-way ANOVA in SAS (SAS 1990).

For emerged egg masses, percentage parasitism [(the number of solitary parasitoid emergence holes + the number of unemerged parasitoids)/(number of *H. coagulata* eggs) × 100], percentage nymphs [(the number of emerged *H. coagulata* nymphs + unemerged nymphs)/(number of eggs) × 100] and percentage unknowns [(unknowns/number of eggs) × 100] were calculated. The number of *H. coagulata* eggs was square-root transformed, and egg, *H. coagulata* nymph, parasitism, and unknowns data were compared between cultivars by two-way ANOVA. For unemerged egg masses, percentage parasitism, percentage nymphs and percentage unknowns were calculated as above. The number of *H. coagulata* eggs was square-root transformed, and all data were compared between cultivars by Friedman's χ^2 test. All statistical tests were performed in SAS and means presented here are back-transformed.

Gonatocerus ashmeadi and *G. triguttatus* Oviposition Preference

Parasitoid colonies were maintained at the University of California, Riverside at 26° ± 2°C and 30-40% RH under a L14:10D photoperiod in cages (50 × 40 × 40 cm) on *H. coagulata* eggs laid on 'Eureka' leaves. Colonies were provisioned with honey-water solution (3:1 Natural uncooked honey, Wild Mountain Brand, Oakland CA). Harvested *H. coagulata* egg masses on excised leaves were removed from colonies and checked daily for parasitoid emergence to assure uniform age for choice experiments. One 'Eureka' and 'Lisbon' leaf containing approximately 15 *H. coagulata* eggs (~24-48 h of age) per leaf were placed through holes in a lid of a 130-ml plastic vial filled with deionized water and 3 ml of antiseptic. A second 130-ml plastic vial with ventilation [three 2-cm holes (one on the bottom, and one on each of two sides) covered with mesh netting (80 µm Jelliff Corporation, Southport, CT)] was inverted and attached to the lid of the vial holding the water and leaves. One newly emerged mated naïve female parasitoid (~24 h old) was placed inside the inverted vial that covered the test material and left for 1 h to forage and oviposit at 26° ± 2°C and 30-40% RH under 1.2 ± 0.2 log lumens/sqm light before being removed. This set up was replicated 15 times for *G. ashmeadi* and *G. triguttatus*. Vials containing leaves with egg masses exposed to parasitoids were held at 26° ± 2°C and 30-40% RH under a L14:10D photoperiod for three weeks to allow parasitoids to emerge. Percentage parasitism [(the total number of emerged and unemerged parasitoids/total number of *H. coagulata* eggs) × 100] was calculated for each 'Eureka' and 'Lisbon' leaf. Females that were not mated (producing male only progeny) were excluded from the analysis. Data were transformed by square root and arcsine transformation and compared between 'Eureka' and 'Lisbon' cultivars by paired *t*- tests at the 0.05 level of significance in SAS (SAS 1990). Means presented here are back-transformed.

Investigation of Leaf Characteristics with SEM

One ~2-mm wide leaf section was cut from the middle of each of 10 fully expanded 'Eureka' and 'Lisbon' leaves (fifth leaf down from the growing tip of branches from containerized trees) with a new razor blade and fixed in 2% gluteraldahyde in 0.1 M phosphate buffer for 2 h. Leaf pieces were washed in buffer and post fixed in 1% osmium tetroxide in 0.1 M phosphate buffer for 2 h. Leaf pieces were then washed in distilled water and dehydrated in an ethanol series and critical point dried. Prepared leaf pieces were mounted on SEM stubs and coated with gold-palladium. Photographs taken at ~800 × magnification for each of the 10 samples for each cultivar of the vertical distribution of leaf cell layer were used to calculate mean thickness of the waxy cuticle, epidermis, palisade layer, and spongy parenchyma layer. Leaf parameters were compared between cultivars by two sampled *t*-test in SAS (SAS 1990). In addition, SEM was used to examine and photograph the intact leaf surfaces. Finally, 'Eureka' and 'Lisbon' leaves with *H. coagulata* egg masses (sourced from the "*H. coagulata* oviposition preference laboratory trial" above) were prepared for SEM as previously described, and examined to determine where the placement of eggs under the leaf cuticle on the undersides of leaves occurred.

Results

Homalodisca coagulata Oviposition Preference in the Laboratory

In the paired choice studies, the number of leaves containing *H. coagulata* egg masses, the number of *H. coagulata* egg masses, and the total number of *H. coagulata* eggs were 187.2%, 204.2% and 181.7% greater, respectively, on 'Eureka' trees compared with 'Lisbon' (leaves: $t = 4.95$, $df = 76$, $P < 0.005$; egg masses: $t = 4.99$, $df = 76$, $P < 0.005$; total eggs: $t = 4.38$, $df = 76$, $P<0.005$) (Fig. 1).

Homalodisca coagulata Oviposition Preference in the Field

There was no significant difference in the total number of *H. coagulata* nymphs and adults and the number of leaves with *H. coagulata* egg masses between 'Eureka' and 'Lisbon' cultivars (Table 1). For emerged egg masses in the field, the number of

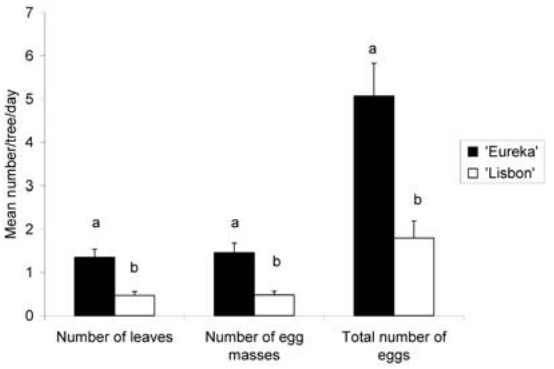

Fig. 1. The mean number of leaves containing *H. coagulata* egg masses, the number of *H. coagulata* egg masses and the total number of *H. coagulata* eggs laid on 'Eureka' and 'Lisbon' lemon trees in the laboratory [different letters indicate significant ($P < 0.05$) differences between cultivars; error bars indicate ± SEM].

H. coagulata egg masses, total *H. coagulata* eggs, percentage parasitism and percentage unknowns were not significantly different between the two cultivars (Table 1). However, the percentage nymphs was significantly higher (5.8% greater) on 'Lisbon' trees compared with 'Eureka' (0%) (Table 1). For egg masses harvested in the field and returned to the laboratory, there were no significant differences in emergence rates between both cultivars for all parameters measured (Table 1).

Gonatocerus ashmeadi and *G. triguttatus* Oviposition Preference

In paired oviposition preference tests in the laboratory, parasitism by *G. ashmeadi* and *G. triguttatus* was respectively 61.7% ± 8.5 and 35.4% ± 12.2 on 'Eureka, and on 'Lisbon' parasitism was 43.1% ± 9.9 and 17.2% ± 7.6, respectively, for *G. ashmeadi* and *G. triguttatus*. These differences were not significant ($t = 1.3$, $n = 15$, $P = 0.22$ and $t = 1.17$, $n = 15$, $P = 0.26$).

Investigation of Leaf Characteristics with SEM

Mean total leaf thickness was higher (19.2%) for 'Eureka' leaves (156.8µm ± 6.2) compared to 'Lisbon' (131.5µm ± 7.3) ($t = 2.63$, $df = 18$, $P < 0.05$) (Fig. 2). The mean thickness of the palisade layer was thicker ($t = 2.32$, $df = 11.1$, $P < 0.05$) in 'Eureka' (38.6%) leaves compared to 'Lisbon' (Fig. 2). There was no difference in cell layer thickness between the cultivars for cuticle ($t = 0.39$, $df = 18$, $P = 0.70$), epidermis ($t = 0.60$, $df = 18$, $P = 0.56$), and spongy parenchyma layers ($t = 1.31$, $df = 18$, $P = 0.21$) (Fig. 2). The underside of 'Lisbon' leaves was smooth, whereas 'Eureka' had many small ridges (Fig. 3). *Homalodisca coagulata* egg placement was between the lower epidermis and spongy parenchyma layer for both cultivars (Fig. 4).

Discussion

Results from this study showed that in the laboratory, the number of *H. coagulata* egg masses laid on 'Eureka' was 204.2% higher than on 'Lisbon'. This indicates that when given a choice under artificial rearing conditions, *H. coagulata* preferred 'Eureka' over 'Lisbon' for oviposition, and suggests that this cultivar should be used for research projects that require large numbers of *H. coagulata* eggs laid on lemon leaves that can be harvested from young potted plants.

Table 1. Mean numbers of *Homalodisca coagulata* egg masses, and percentage nymph and percentage parasitoid emergence from *H. coagulata* eggs collected from field planted 'Eureka' and 'Lisbon' lemon trees.

Mean variable/tree/sampling event	'Eureka' ± SEM	'Lisbon' ± SEM	Significance
Number of *H. coagulata* nymphs and adults	30.9 ± 1.6	33.0 ± 2.2	$F = 0.96$, $df = 18$, $P = 0.33$
Number of old and new leaves selected	21.0 ± 1.0	24.0 ± 1.1	$F = 3.86$, $df = 1,8$ $P = 0.06$
Emergence from egg masses in the field			
Number of old masses	31.1 ± 2.0	35.1 ± 2.1	$F = 1.87$, $df = 1,2$ $P = 0.18$
Number of old eggs	196.5 ± 13.9	212.8 ± 12.9	$F = 1.04$, $df = 1,12$ $P = 0.31$
Percentage parasitism	42.8 ± 2.4	37.8 ± 2.1	$F = 2.34$, $df = 1,12$ $P = 0.13$
Percentage nymphs	26.5 ± 2.0	32.4 ± 1.7	$F = 5.53$, $df = 1,12$ $P < 0.05$
Percentage unknowns	16.8 ± 1.2	19.5 ± 1.3	$F = 2.39$, $df = 1,12$ $P = 0.13$
Emergence from egg masses in the lab			
Number of new masses	1.3 ± 0.2	1.2 ± 0.2	$\chi^2 = 1.29$, $df = 1$, $P = 0.26$
Number of new eggs	8.0 ± 1.5	7.6 ± 1.5	$\chi^2 = 0.18$, $df = 1$, $P = 0.67$
Percentage parasitism	53.9 ± 7.6	48.9 ± 6.8	$\chi^2 = 1.29$, $df = 1$, $P = 0.26$
Percentage nymphs	5.8 ± 3.8	0.0 ± 0.0	$\chi^2 = 3.00$, $df = 1$, $P = 0.08$
Percentage unknowns	37.2 ± 7.6	43.0 ± 7.1	$\chi^2 = 0.14$, $df = 1$, $P = 0.71$

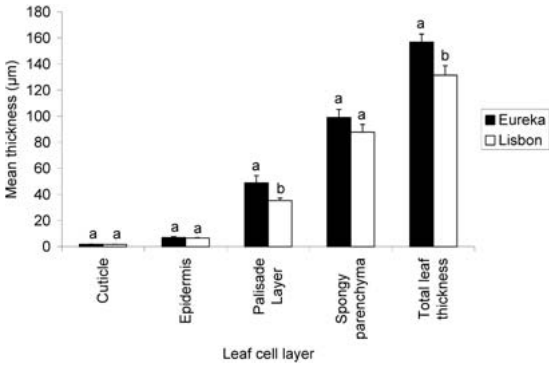

Fig. 2. Mean thickness (µm ± SEM) of the cuticle, epidermis, palisade and spongy parenchyma leaf cell layers within 'Eureka' and 'Lisbon' lemon cultivars [different letters indicate significant ($P < 0.05$) differences between cultivars].

The reasons for this demonstrated *H. coagulata* oviposition preference are unknown. Scanning electron microscopy (SEM) showed that the underside of 'Lisbon' leaves was smooth, whereas 'Eureka' had many small ridges on the cuticle that may provide female *H. coagulata* with enhanced tarsal grip during oviposition. However, the depth of these ridges was not quantified. Results also showed that the thickness of the palisade layer was significantly higher (38.6%) for 'Eureka' leaves compared to 'Lisbon'. A thicker leaf structure may be more favorable for *H. coagulata* oviposition.

Female *H. coagulata* preferentially oviposit on lower leaf surfaces, and SEM showed that egg placement occurs between the lower epidermis and spongy parenchyma layer for both lemon cultivars. A thick palisade layer, which is positioned at the top of the leaf, may afford some protection of *H. coagulata* eggs from adverse conditions. For example, solar radiation falls onto the upper leaf surfaces so a thicker palisade layer may insulate eggs from excessive sun exposure. However, the experimental design used in the current study did not standardize 'Eureka' and 'Lisbon' trees to possess equivalent numbers of leaves, or surface areas, which may have influenced oviposition preference by *H. coagulata* because of varying light intensity. Other factors that influence leafhopper oviposition preference, such as plant chemistry, leaf vein characteristics, number of branches, and trichome densities (Singh & Agarwal 1988; Lit & Bernardo 1990; Denno & Roderick 1991; Andersen et al. 1992; Sharma & Sharma 1997; Sharma et al. 1999; Sharma & Singh 2002) were not investigated in this study and may warrant further research if it becomes necessary to explain in detail factors influencing *H. coagulata* oviposition preferences.

Field results showed that there were no significant differences in the total number of *H. coagulata* nymphs and adults, and the number of leaves with *H. coagulata* egg masses between 'Eureka' and 'Lisbon' cultivars. Consequently the oviposi-

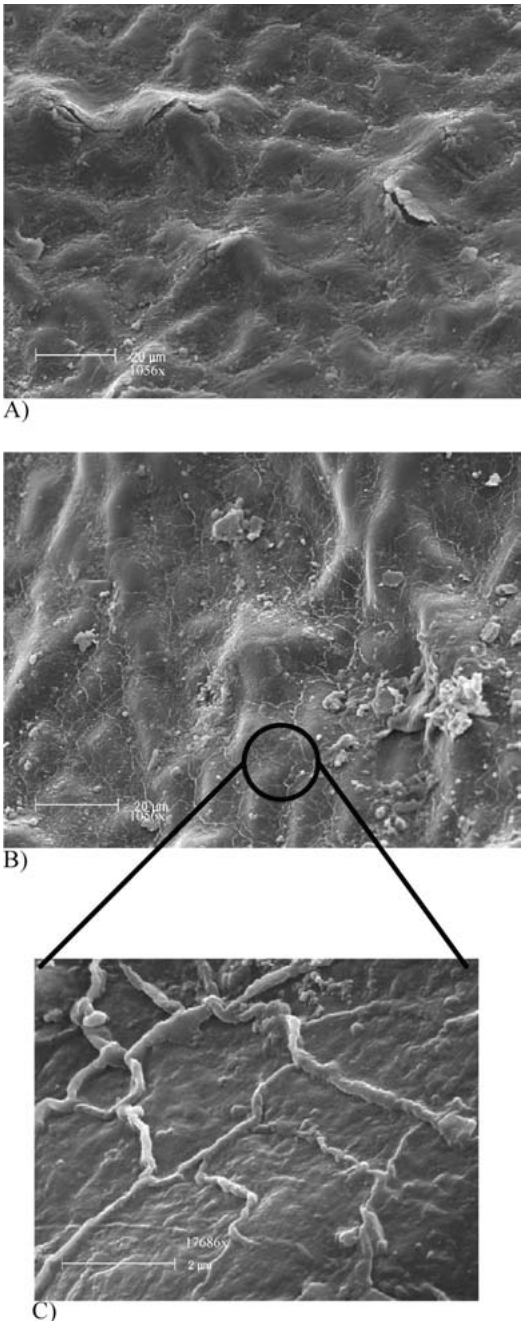

Fig. 3. SEM photographs of the lower leaf surface of 'Lisbon' and 'Eureka' *Citrus limon* cultivars: (A) 'Lisbon' with smooth leaf surface; (B) 'Eureka' leaf surface with many small ridges on the cuticle that may enhance *H. coagulata* tarsal grip during egg laying; (C) Close up of small ridged on 'Eureka' leaves that were not present on 'Lisbon'.

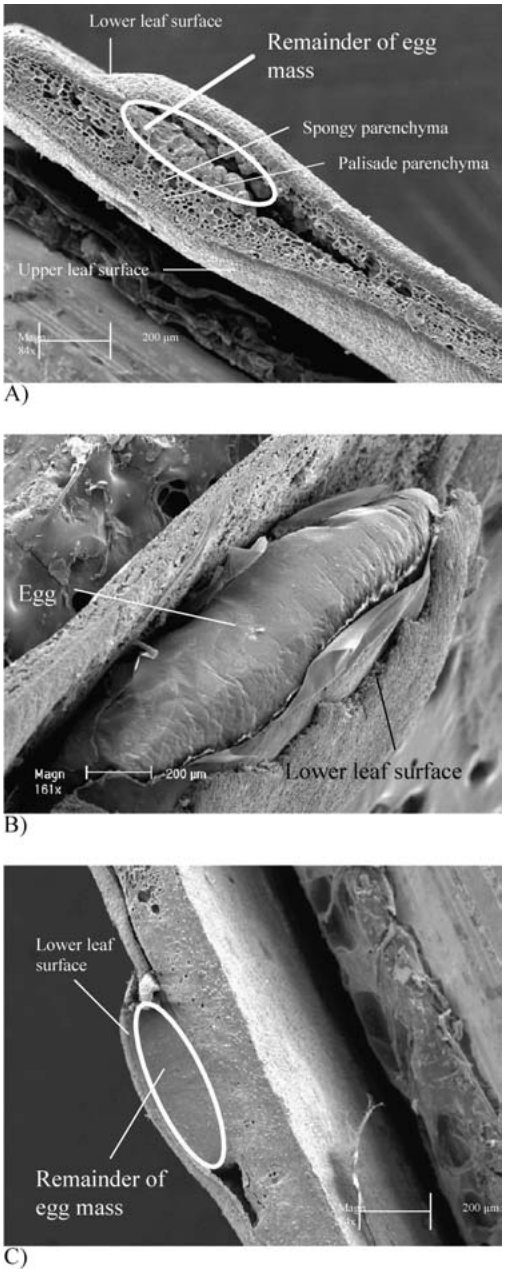

Fig. 4. SEM photographs showing placement of *H. coagulata* egg mass within 'Eureka' and 'Lisbon' lemon leaves: (A) 'Eureka' leaf—egg sliced latitudinally, shows placement of egg between epidermis and spongy parenchyma; (B) 'Eureka' leaf—egg sliced longitudinally, shows placement directly beneath epidermis; (C) 'Lisbon' leaf—egg sliced latitudinally, shows placement of egg between epidermis and spongy parenchyma (note: egg protein filled leaf cells).

tion preference for 'Eureka' demonstrated in the laboratory was not confirmed in the field. In fact, field results showed that significantly more (5.9%) *H. coagulata* nymphs successfully emerged from 'Lisbon' leaves compared with 'Eureka' (leaf thickness of field-planted trees was not investigated in this study). The lack of congruence between laboratory and field studies may be due to differences in environmental factors and tree age between field-planted (~17 years of age) and small containerized trees (~2 years of age). Environmental aspects, such as sunlight, water, soil and nutrients, may affect leaf characteristics and influence host oviposition and utilization (DeReffye et al. 1995), while xylem chemistry varies drastically with plant age and phenology (Andersen et al. 1992, 1995a,b). It has been demonstrated that *H. coagulata* is very sensitive to changes in nutritional quality (Brodbeck et al. 1990, 1999), and in California Toscano et al. (2003) showed that *H. coagulata* numbers were over 6-fold higher on young citrus trees compared to older trees. It is also possible that given the very high densities of ovipositing females in citrus at the time this field survey was conducted less preferred lemon cultivars were being used due to shortages of oviposition sites on the most preferred cultivars.

Parasitism by *G. ashmeadi* and *G. triguttatus* of *H. coagulata* eggs masses on 'Eureka' and 'Lisbon', were not significantly different. The lack of statistical significance suggests that using 'Eureka' trees for future experiments with these parasitoids should not detrimentally influence parasitism rate data or fecundity estimates, and demonstrates that parasitoids were not conditioned to oviposit preferentially on 'Eureka' over 'Lisbon' even though they had been reared on 'Eureka'.

Summary

Based on the results of oviposition preference studies conducted in the laboratory we conclude that the lemon cultivar 'Eureka' is preferred over 'Lisbon' for oviposition by field-collected *H. coagulata* females. This cultivar preference by *H. coagulata*, however, was not observed in the field. Furthermore, the mymarid parasitoids *G. ashmeadi* and *G. triguttatus* do not exhibit cultivar preferences indicating that the use of 'Eureka' will not adversely affect studies on the reproductive and developmental biology of *Gonatocerus* spp. It is recommended that for mass rearing programs that require the use of small young containerized lemons to maximize *H. coagulata* egg mass or *Gonatocerus* spp. production the 'Eureka' cultivar should be used in preference to 'Lisbon'.

Acknowledgments

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BIOLOGY OF *ZAGELLA DELICATA* (HYMENOPTERA: TRICHOGRAMMATIDAE), AN EGG PARASITOID OF THE SHARPSHOOTER *TAPAJOSA RUBROMARGINATA* (HEMIPTERA: CLYPEORRHYNCHA: CICADELLIDAE) IN ARGENTINA

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ABSTRACT

Research on biological control of glassy-winged sharpshooter (GWSS) *Homalodisca coagulata* (Say) started in the 1990s. This sharpshooter, vector of Pierce's disease bacteria *Xylella fastidiosa* Wells, expanded its geographical distribution and it has become a very serious pest problem in several crops, especially grapes, in southern California. In 2000, a survey of sharpshooter egg parasitoids was initiated in Argentina. Fourteen species of egg-parasitoids were collected. We report here on laboratory studies of adult longevity, oviposition preference, sex ratio, and development time of *Zagella delicata* De Santis. Field result of the incidence on its hosts and seasonal occurrence also are provided. *Zagella delicata* produced one adult per host egg. The overall results indicated that 72.5% of the sharpshooter eggs exposed to *Z. delicata* were parasitized. Wasps emergence was 43.8%. In host plant searching preference tests, *Z. delicata* females parasitized 66.7% of host eggs on sugar cane, 57.0% of eggs on corn and 4.5% on citrus leaves. The development time (from oviposition to adult emergence) averaged 23.5 ± 1.2 days. The average adult longevity was 10.3 ± 5.8 days. Females lived longer than males (females: 12.2 ± 5.6 days, males: 6.2 ± 3.7 days). The sex ratio in the laboratory was 1: 2.1 (males/females). In a hyperparasitism test, no adults of *Z. delicata* emerged from eggs previously exposed to *Gonatocerus tuberculifemur*. Seasonal sampling carried out in San Miguel de Tucumán showed that *Z. delicata* occurred from spring to fall, with maximum abundance at the beginning of the spring, where 57.2% out of the 1568 sampled eggs were parasitized. Field and laboratory data suggest that *Z. delicata* could be a prospective biological control agent against other, exotic, proconiine sharpshooters including *H. coagulata*. However, the efficiency of *Z. delicata* is restricted to habitats dominated by grasses.

Key Words: glassy-winged sharpshooter, *Homalodisca coagulata*, Trichogrammatidae, egg parasitoid, biological control, *Zagella delicata*.

RESUMEN

Las investigaciones sobre el control biológico de *Homalodisca coagulata* (Cicadellidae: Proconiini) fueron iniciadas en la década del 90. Esta chicharrita, vector de la bacteria *Xylella fastidiosa*, expandió su área de distribución geográfica y aumentó su abundancia transformándose en una seria plaga de varios cultivos, especialmente en la vid, en el sudeste de California. En el año 2000 fue iniciada una exploración de parasitoides de huevos de chicharritas proconiinas en Argentina, donde fueron colectadas 14 especies de parasitoides. Aquí se reportan los resultados de campo y laboratorio de uno de estos parasitoides, *Zagella delicata* De Santis, aportando información sobre su bionomía (longevidad de adultos, preferencia de oviposición, proporción de sexos y duración del desarrollo), incidencia sobre su hospedador en el campo, y ocurrencia estacional. *Zagella delicata* produjo un solo adulto por huevo. En general, parasitó el 72.5% de los huevos y emergiendo avispas del 43.8% de los huevos parasitados. En las pruebas de preferencia de oviposición de planta hospedadora, las hembras de *Z. delicata* parasitaron 66.7% de los huevos en caña de azúcar, 57.0% de los huevos en maíz y fueron atacados 4.5% de los huevos depositados en *Citrus*. La duración del tiempo de desarrollo (desde huevo a adulto) fue de 23 ± 1.2 días. La longevidad promedio de los adultos fue de 10.3 ± 5.8 días. Las hembras vivieron más que los machos (hembras: 12.2 ± 5.6; machos: 6.2 ± 3.7 días). La proporción de sexos en el laboratorio fue de 1: 2.1 (machos/hembras). En los estudios de hyperparasitismo, ningún adulto de *Z. delicata* emergió de huevos

previamente expuestos al mymárido *Gonatocerus tuberculifemur*. El muestreo estacional realizado en San Miguel de Tucumán mostró que *Z. delicata* aparece desde la primavera hasta el otoño, con máxima abundancia a principios de la primavera, donde el 57.2% de los 1568 huevos muestreados estaban parasitados. Los estudios de campo y laboratorio sugieren que *Z. delicata* tiene potencialidades como agente de control biológico de otras chicharritas proconiinas exóticas incluyendo a *H. coagulata*. Sin embargo, *Z. delicata* está circunscripta a hábitats dominados por gramíneas.

Translation provided by the authors.

In the early 1990s, the glassy-winged sharpshooter *Homalodisca coagulata* (Say) (Hemiptera: Cicadellidae) established in California, USA. It has become a very serious problem as an efficient vector of Pierce's disease (*Xylella fastidiosa* Wells) (Blua et al. 1999). The insect is native to the southeastern USA and northeastern Mexico. Although parasitization of *H. coagulata* can reach 100% in California, it seems that natural enemies cannot control the pest (Triapitsyn et al. 1998; Triapitsyn & Phillips 2000). Although the glassy-winged sharpshooter appears to be adapting to California environment, it is not certain that native parasitoids will be as effective there as they are in their co-evolved native range. Thus, egg parasitoids of closely related hosts were sought from areas in South America where subclimate types and habitats were similar to that in California.

In order to identify candidates for a neo-classical biological control program against this pest, a survey of proconiine sharpshooter egg parasitoids was initiated in South America in 2000 (Jones 2001). Most of the collection efforts were carried out in northwestern Argentina with sentinel egg masses of *Tapajosa rubromarginata* (Signoret), a native South American proconiine sharpshooter. *Tapajosa* is close related to *Homalodisca*; both genera have the posterior meron exposed and are included in the same group due to their phylogenetic proximity (Young 1968). This survey resulted in collections of nine different species of the genus *Gonatocerus* Nees (Hymenoptera: Mymaridae), at least one species of *Paracentrobia* Howard, two species of *Oligosita* Walker, and two species of *Zagella* Girault (Hymenoptera: Trichogrammatidae).

To date, there are nine described species of *Zagella* in the New World; three are from the Nearctic region (Triapitsyn 2003), and six from the Neotropical region (De Santis 1957, 1970, 1997). The only known hosts of a *Zagella* species are *H. coagulata* and *Oncometopia orbona* (Fabricius) (Cicadellidae: Proconiini) for *Z. spirita* (Girault) in the USA (Triapitsyn & Phillips 2000; Triapitsyn 2003). Of the two *Zagella* species collected from *T. rubromarginata* eggs in Argentina, *Z. delicata* was the most abundant in terms of frequency of occurrence and population density. The other, tentatively identified as *Z. platensis* (De Santis) by Triapitsyn, was much less abundant.

Zagella delicata De Santis was described from a single female without information on its host association(s) (De Santis 1970). Detailed taxonomic and biological studies are indispensable for a biological control program. Essential biological information about a *Zagella* species has not been available before this study on the biology, geographic distribution, and hosts. We report the results of laboratory and field studies on *Z. delicata*, providing information on its bionomics (i.e., adult longevity, oviposition preference, sex ratio, development time), the incidence of its host in the field, and seasonal occurrence of the parasitoid. The possibilities of using this egg parasitoid as a potential agent for biological control of *H. coagulata* in the USA are discussed.

Materials and Methods

The studies on development time, sex ratio, adult longevity, and oviposition of *Z. delicata* on eggs of *T. rubromarginata* laid on different host plants were carried out at the USDA-ARS South American Biological Control Laboratory in Hurlingham, Buenos Aires Province, and at PROIMI, San Miguel de Tucumán, Tucumán Province. Field studies included (a) a seasonal sampling of egg masses in Tucumán Province for the entire 2002 growing season to estimate parasitoid incidence and occurrence, and (b) a survey with sentinel eggs in a wide geographical range between 22 and 42º LS in Argentina.

Laboratory Studies

The initial stock of *Z. delicata* was obtained by collecting 68 egg masses of *T. rubromarginata* on Johnson grass, *Sorghum halepense* (L.) Person, in an open field in Tafi Viejo, Tucumán Province, in January 2001. Additional collections of egg masses of *T. rubromarginata* on Johnson grass were made when necessary in a soccer field near PROIMI in San Miguel de Tucumán. The colony of *Z. delicata* was reared in the laboratory of PROIMI. About 5-7 wasps, both males and females, were placed in 20-cm high × 2-cm diameter glass tubes with 1-2 egg masses (6-15 eggs) of *T. rubromarginata* and left until the wasps died (approximately in 5-7 days. The eggs used in the experiment were 24-48 h old and were laid on the edge of the distal portions of corn leaves. For aer-

ation, the glass tube top was either closed by a nylon mesh or the hole was fitted with cotton plugs that were moistened with water and honey as needed. After exposure, the egg masses were checked daily to ensure the freshness of the leaves until adult wasps emerged. Percentage of parasitism of the exposed eggs and percentage of wasps emerged were calculated on the two generations obtained in the laboratory. The effects of host plant species on the preference of *Z. delicata* to parasitize eggs of *T. rubromarginata* was tested on *Citrus* sp. (11 egg masses, 148 eggs), sugar cane (3 egg masses, 51 eggs) and corn (5 egg masses, 100 eggs). The experiments were conducted at room temperature (24.5 ± 6.2°C, 70-80% RH, photoperiod 14:10 h. L/D), and the colony was maintained under the same conditions.

Host eggs that changed to brownish or reddish after 5-7 d were considered "parasitized eggs", and those that developed eyespots of the host's nymphs were considered "unparasitized". The number of sharpshooter nymphs that hatched from the unparasitized eggs was counted daily. After 25 d, when parasitoid emergence was nearly complete, each leaf was dissected and the remaining host eggs were counted. By this time, most *Z. delicata* adults had either emerged or attained the pupal stage, so it was easy to distinguish parasitized eggs from unparasitized ones. The percentage of parasitism was calculated as follows:

% parasitism = (number of parasitized eggs/ number of host eggs offered) × 100.

Percentage of wasp emergence was calculated as follows:

% of wasp emergence = (number of wasps emerged/ number of offered eggs) × 100.

Test of Hyperparasitism

In field-collected egg masses of *T. rubromarginata*, it was common to observe the adults of *Z. delicata* and *Gonatocerus* spp. emerging from the same egg mass. *Zagella delicata* emerged about 10-15 days after *Gonatocerus*, suggesting it could be a hyperparasitoid of the latter, or that it is not an obligate primary parasitoid (Triapitsyn 2003). Considering that *Z. delicata* might be used as biocontrol agent, mixtures of primary parasitoids and hyperparasitoids often lead to erroneous host parasitoid records (Noyes 1994). We tested whether *Z. delicata* could develop in the eggs of *T. rubromarginata* parasitized by *Gonatocerus*. Seventy-two hours after an egg mass of this host laid in a sugar cane leaf, it was exposed to *Gonatocerus tuberculifemur* (Ogloblin). Then, the same egg mass was exposed to *Z. delicata* as explained above. Nine replications were carried out, with a total of 100 eggs exposed. Two egg masses (39 eggs) exposed to *Z. delicata* with no previous exposition to *G. tuberculifemur* served as control.

Adult longevity

Adult longetivy of *Z. delicata* was monitored twice a day using 84 individuals (57 females and 27 males) from a few h after emerging (within 12 h) until adult death. Observations were conducted on single wasps in individual vials without host material, but with honey as a food source. Differences between male and female longevity and duration of development were analyzed by a *t* test at the 0.05 level of significance (Statistica 5.0). The total time required for the development from egg to adult was based on 84 individuals.

Field Studies

To estimate parasitic activity a collection of egg masses was conducted in Tafi Viejo, Tucumán Province between 4 and 7 January 2001, when 68 egg masses (1018 eggs) were collected on Johnson grass. In addition, 12 samples were collected from September 2002 to April 2003 except in December (spring, summer and part of the fall) in San Miguel de Tucumán. We sampled a total of 6,253 eggs. For parasitoid emergence, each field-collected egg mass was placed in a Petri dish with the bottom filled with wet tissue paper. Each Petri dish was covered with food wrap to prevent eggs and leaves from dehydration and to keep the emerging wasps from escaping.

The survey with sentinel eggs was conducted from November 2000 to December 2002 in northwestern Argentina in areas that closely match the sub-climate types in the grape-growing regions of California. It also was conducted in the citrus-growing areas of eastern Argentina because it was suggested that the first generation of *H. coagulata* on citrus host plants is under poor natural control in California (Triapitsyn and Phillips 2000). Therefore sentinel egg masses in Argentina were produced on potted citrus plants. Field-collected females of *T. rubromarginata* were placed in sleeve cages on citrus plants. They were checked for presence of eggs daily, and when egg masses were detected, the sharpshooters were removed. Sleeves were kept on the plants to avoid uncontrolled parasitization from wild parasitoids. The sentinel eggs on the potted plants were placed in 41 selected sites in Tucumán, Jujuy, Misiones, Catamarca, La Rioja, Entre Ríos, Corrientes, San Juan, Salta, and Mendoza Provinces. Overall 8,000 eggs were exposed to parasitization.

Results

Laboratory Studies

Zagella delicata is a solitary wasp parasitoid producing only one adult per host egg. The overall results showed that out of 353 eggs exposed to *Z. delicata* females, 72.5 ± 28.3% (Mean ± SD) were parasitized, and wasps emerged from 43.8 ±

TABLE 1. PARASITIC ACTIVITY OF *ZAGELLA DELICATA* ON EGGS OF *TAPAJOSA RUBROMARGINATA* LAID ON THREE DIFFERENT PLANT SPECIES IN THE LABORATORY.

	Egg masses offered	Eggs offered	Egg masses attacked	*Z. delicata* emerged	Host nymphs emerged
Corn	5	10	5	57 (57.0 ± 14.5%)	29 (29.0 ± 17.9%)
Sugar cane	3	51	3	34 (66.7 ± 25.0%)	11 (21.6 ± 25.4%)
Citrus	12	148	1	7 (4.7 ± 2.0%)	69 (46.6 ± 4.7%)

41.4% of those. In the F1 progeny, 61.7 ± 40.3% of the exposed eggs were parasitized, and adult emergence was 26.9 ± 38.6%. In the F2 progeny, 94.5 ± 6.7% of the eggs were parasitized, and adult emergence was 85.0 ± 6.0%. Nymphs of *T. rubromarginata* emerged from 13.3 ± 28.7% of the exposed eggs.

In host plant searching preference test, *Z. delicata* females parasitized 34 host eggs (66.7 ± 25.0%) (3 egg masses) on sugar cane, 57 eggs (57.0 ± 14.5%) (5 egg masses) on corn and 7 (4.7 ± 2.2%) on citrus leaves (Table 1). In laboratory, *Z. delicata* females were able to find eggs of *T. rubromarginata* on citrus leaves. However, the wasps commonly failed to oviposit after several attempts. Citrus cuticle leaf is thicker than sugar cane and corn cuticle leaves, and probably cuticle thickness is a factor that interferes with oviposition of *Z. delicata*.

Developmental time (from oviposition to adult emergence) of *Z. delicata* averaged 23.5 ± 1.2 days (Fig. 1). Females and males did not show significant differences in developmental time (t = -1.1652, df = 1, $P > 0.05$) (females: 23.4 ± 1.2 days, males: 23.7 ± 1.1 days).

The average adult longevity was 10.3 ± 5.8 days. The analysis of adult longevity for females and males indicated significant differences, with females living longer than males ($t = 5.8717, P < 0.05$) (females: 12.2 ± 5.6 days, males: 6.2 ± 3.7 days).

The overall sex ratio of *Z. delicata* in the laboratory was 1: 2.1 (males/females). In F1 progenies it was 1: 3.2 and in F2 progenies 1: 1.9, respectively. In field-collected egg masses, the sex ratio was female biased, 1: 3.8 (27 males/103 females).

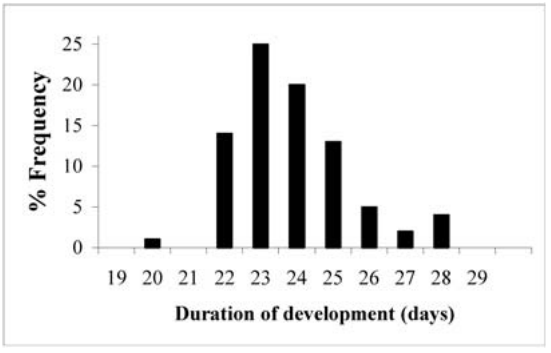

Fig. 1. Development time from egg to adult of *Zagella delicata* in the laboratory at room temperature.

Tests of Hyperparasitism

No adults of *Z. delicata* emerged from eggs previously exposed to *G. tuberculifemur*. Out of 100 exposed eggs, only 71 adults of *G. tuberculifemur*, and 2 nymphs of *T. rubromarginata* emerged. The remaining eggs yielded neither host nymphs nor parasitoids. Twenty seven adults of *Z. delicata* emerged from the 39 eggs used as control.

Field Studies

In the sample collected from Johnson grass in Tafi Viejo, 724 (71.1%) *T. rubromarginata* eggs were found to be attacked by a complex of egg parasitoids composed of several species of Trichogrammatidae and Mymaridae. *Zagella delicata* emerged from 626 eggs, or 86.5% of all parasitized eggs. Seasonal sampling carried out in San Miguel de Tucumán showed that *Z. delicata* occurred from September to March, with maximum abundance at the beginning of the season in October, where 57.2% out of the 1568 sampled eggs were parasitized (Table 2). From the 41 sites sampled in Argentina with sentinel eggs on citrus, *Z. delicata* adults were obtained in Santa Clara, Jujuy Province, and Tafi Viejo, Tucumán Province. In Santa Clara, 155 (61.7%) out of 251 exposed eggs were attacked by the parasitoid complex mentioned above. Sixty of these were parasitized by trichogrammatid species, including 38 (15.1%) by *Z. delicata*. In Tafí Viejo, 1,549 (47.0%) parasitoids emerged from 3,299 eggs exposed, but only 24 (1.5%) adults of *Z. delicata* emerged.

Distribution

In addition to Chacras de Coria in Mendoza Province (De Santis 1970), the locality where the type specimen was collected, *Z. delicata* also was collected in Tafi Viejo, Yerba Buena, and San Miguel de Tucumán in Tucumán Province as well as in Santa Clara, Jujuy Province, all in Argentina. Voucher specimens were deposited in Fundación e Instituto Miguel Lillo, San Miguel de Tucumán (IMLA), and Entomology Research Museum, University of California at Riverside (UCRC).

TABLE 2. OCCURRENCE OF *ZAGELLA DELICATA,* OTHER TRICHOGRAMMATIDAE, AND *GONATOCERUS* SPP. (MYMARIDAE) IN EGGS OF *TAPAJOSA RUBROMARGINATA* ON JOHNSON GRASS AT SAN MIGUEL DE TUCUMÁN, ARGENTINA.

	Sampled eggs	*Z. delicata*	Other Trichogrammatidae[1]	*Gonatocerus* spp.
September	2722	0.8[2]	0.0	4.4
October	1568	57.2	1.5	0.4
November	248	16.5	4.8	2.4
January	370	19.2	27.6	4.3
February	559	21.1	17.7	7.2
March	611	9.3	17.8	21.9
April	175	0.0	3.4	19.4
May[3]	0	0.0	0.0	0.0

[1]Includes at least 3 different species in 3 genera: *Oligosita* (1), *Paracentrobia* (1 or 2), and *Zagella* (1).
[2]Percent of sampled eggs.
[3]No eggs were found in the field.

DISCUSSION

Biological characteristics of *Z. delicata*, a primary egg parasitoid of *T. rubromarginata*, have allowed us to estimate its overall potential as a biological control agent of *H. coagulata*. This is the first study that provides biological information on a species belonging to the genus *Zagella*. *Zagella delicata* was successfully reared under laboratory conditions for two generations before the colony was discontinued. The low percentage of emergence (35.4%) of *Z. delicata* adults in the laboratory was due to the damage (rotting or drying of the leaves and the host eggs) during the long preimaginal period of this parasitoid, thus complicating colony management. Most of the unemerged host eggs were parasitized, and nymphs of *T. rubromarginata* emerged from 13.3% of the exposed eggs. In F2 progenies, the percentage of adult emergence was higher than in F1 progenies because humidity control in the Petri dishes was improved. Females showed a strong preference for parasitizing host eggs on monocotyledon plants such as corn, Johnson grass, and sugar cane, whereas they hardly parasitized eggs laid on citrus. The low preference of *Z. delicate* to attack eggs laid on citrus plants is not a desirable characteristic for the use of this natural enemy in the control of glassy-winged sharpshooter. Regulation strategy for *H. coagulata* in California calls for its control in the citrus orchards. Also, the results of this survey with sentinel eggs of *T. rubromarginata* on citrus plants should be considered with caution because absence of *Z. delicata* in most of the samples does not necessarily mean that this species is absent in the sampled area. For example, in January 2001 in Tafí Viejo, 61.6% of *T. rubromarginata* eggs on Johnson grass in the field were parasitized, whereas at the same time and 1 km from that site, only 4% out of 99 sentinel eggs of the same host on citrus leaves were parasitized by *Z. delicata* (E. G. V. unpubl. data).

Sex ratios observed in the field and under laboratory conditions were similar, being female biased. The sex ratio in another trichrogrammatid species studied in Argentina, *Paracentrobia* sp. (misidentified as *P. subflava* (Girault) by L. De Santis), showed a predominance of females over males (Virla 1999).

Females of *Z. delicata* were able to parasitize more than 60% of the eggs in the laboratory, and about 61.6% in the field, suggesting great potential of this species as a biological control agent in some crops. The importance of synchronization of the occurrence (in time and space) of a parasitoid with its target host is often emphasized in biological control. *Zagella delicata* occurs in the field from September to April, during the same period as its host, *T. rubromarginata*, and has two generations for every generation of the host. Most importantly, it is the predominant natural enemy of *T. rubromarginata* early in the season (Fig. 2). Overall, field and laboratory data suggest that *Z. delicata* could be a prospective biological control agent against other, exotic,proconiine sharpshooters including *H. coagulata*. However, the efficiency of *Z. delicata* appears to be restricted to habitats dominated by grasses.

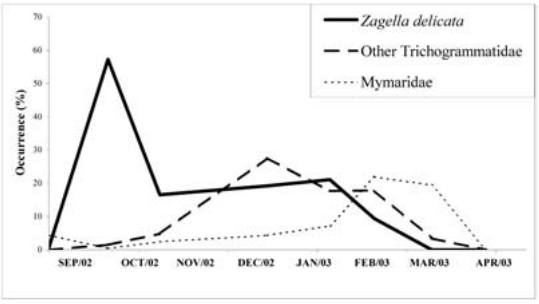

Fig. 2. Field occurrence of *Zagella delicata* and other egg parasitoids of *Tapajosa rubromarginata* on Johnson grass at San Miguel de Tucumán, Tucumán Province.

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NATIVE HYMENOPTERAN PARASITOIDS ASSOCIATED WITH FRUIT FLIES (DIPTERA: TEPHRITIDAE) IN SANTA CATARINA STATE, BRAZIL

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ABSTRACT

A survey of tephritid fruit fly species and their parasitoids was conducted in the western portion of Santa Catarina state, Brazil. A total of 9,197 fruits belonging to 46 plant species in 24 families was collected. The parasitoids recovered were *Aganaspis pelleranoi* (Brèthes), *Lopheucoila anastrephae* (Rhower) (Figitidae), *Doryctobracon areolatus* (Szèpligeti), *Doryctobracon brasiliensis* (Szèpligeti), *Odontosema anastrephae* Borgmeier, *Opius bellus* Gahan, *Opius* sp., *Utetes* (*Bracanastrepha*) *anastrephae* (Viereck) (Braconidae), and *Trichopria anastrephae* Lima (Diapriidae). *Agasnaspis pelleranoi* is the most frequent parasitoid species found in the west region of Santa Catarina. We recorded the first occurrence of *L. anastrephae* in Santa Catarina. Parasitism ranged from 1.2 to 46.9%.

Key Words: Insecta, *Anastrepha*, Braconidae, Figitidae, Diapriidae, parasitoids, fruit flies.

RESUMEN

Se realizo un estudio del reconocimiento de las especies tefrítidas de las moscas de las frutas y sus parasitoides en el área occidental del estado de Santa Catarina, Brasil. Un total de 9,197 frutas que pertenecen a 46 especies de plantas fueron recolectadas. Los parasitoides recolectados fueron: *Aganaspis pelleranoi* (Brèthes), *Lopheucoila anastrephae* (Rhower) (Figitidae), *Doryctobracon areolatus* (Szèpligeti); *Doryctobracon brasiliensis* (Szèpligeti), *Odontosema anastrephae* Borgmeier, *Opius bellus* Gahan, *Opius* sp., *Utetes* (*Bracanastrepha*) *anastrephae* (Viereck)(Braconidae) y *Trichopria anastrephae* Lima (Diapriidae). *Agasnaspis pelleranoi* es el parasitoide encontrado mas frecuentemente en la región oeste de Santa Catarina. El primer registro de la ocurrencia de *L. anastrephae* en Santa Catarina fue obtenido. El rango de parasitismo fue de 1.2 a 46.9%.

Surveys of tephritid fruit flies and their parasitoids are a first step to a better understand of the ecology of these economically important taxa (Zucchi 2000a). The major natural enemies of the fruit flies in Brazil belong to the families Braconidae, Figitidae, and Pteromalidae (Hymenoptera). Pteromalidae, mainly *Pachycrepoideus vindemiae* Rondani, are sporadically collected and specimens of Figitidae are collected in small numbers throughout the country. Typically the most frequent parasitoids collected in Brazil are members of the Braconidae family (Canal & Zucchi 2000).

In Santa Catarina state, Nora et al. (2000) previously found, in order of abundance, *Doryctobracon areolatus* (Szèpligeti), *Doryctobracon brasiliensis* (Szèpligeti), *Opius bellus* Gahan, and *Opius tomoplagiae* Lima. In addition, they obtained unidentified Diapriidae, Eulophidae, Figitidae, and Pteromalidae. Guimarães et al. (2000) added to this list the figitids *Aganaspis pelleranoi* (Brèthes) and *Odontosema anastrephae* Borgmeier, and Leonel, Jr. et al. (1995) and Canal & Zucchi (2000) obtained braconids *Microcrasis lonchaea* (Lima) and *Utetes* (*Bracanastrepha*) *anastrephae* (Viereck).

The present paper describes the parasitoids of fruit flies from the western portion of Santa Catarina state, an area which is a growing producer of citrus in the state (Koller et al. 1999) and which has not been surveyed thoroughly in the past. We collected 9,197 mature fruit from trees or on the soil comprising 46 species belonging to 25 families in the six towns Anchieta (26°53'S and 53°33'W), Chapecó (27°06'S and 53°16'W), Cunha Porã (26°07'S and 53°16'W), Palmitos (27°06'S and 53°16'W), São Carlos (27°07'S and 53°00'W), and Xanxerê (26°87'S and 52°40'W), Santa Catarina. Each fruit was weighed and put in a plastic container with about seven centimeters of sterilized sand, and covered with a net. The containers were kept in the entomology laboratory of the Agricultural and Environmental Science Center at the Universidade Comunitária Regional de Chapecó at 25 ± 3°C, 70 ± 10% and a 12-h photoperiod. After five days, the sand with pupae was transferred to lab Petri dishes containing filter

TABLE 1. PARASITOID SPECIES (HYMENOPTERA) FROM FRUIT FLIES COLLECTED IN SIX DIFFERENT LOCALITIES FROM THE WEST OF SANTA CATARINA, BRAZIL, DURING 1998-2000.

Vegetal specie	S[1]	N[2]	PaT[3]	Hymenoptera parasitoid species and relative frequency (%)		%P[4]	TIP[5]
				Braconidae	Figitidae and Diapriidae		
Fabaceae							
Inga sellowiana	5	246	2		*L. anastrephae* (100.0)	4.1	4.7
Myrtaceae							
Psidium cattleianum	11	635	42	*D. areolatus* (19.0) *D. brasiliensis* (33.3) *Opius* sp. (9.7) *U. anastrephae* (19.0)	*A. pelleranoi* (19.0)	1.9	2.1
Eugenia involucrata	3	446	46	*D. areolatus* (69.6) *Opius bellus* (8.7) *Opius* sp. (4.3) *U. anastrephae* (17.4)		46.9	47.9
Psidium guajava	17	190	147	*D. brasiliensis* (2.0) *Opius bellus* (1.1) *Opius* sp. (2.0)	*A. pelleranoi* (49.0) *T. anastrephae* (45.9)	20.1	20.3
Feijoa sellowiana	2	80	58	*D. areolatus* (48.3) *D. brasiliensis* (48.3) *Opius bellus* (3.4)		11.6	14.3
Myrcianthes pungens	2	52	6	*D. brasiliensis* (100.0)		28.5	46.1
Campomanesia xanthocarpa	4	702	4	*D. brasiliensis* (100.0)		7.7	12.5
Britoa guazumaefolia	6	255	48	*D. areolatus* (16.7) *D. brasiliensis* (37.5)	*A. pelleranoi* (3.5) *O. anastrephae* (8.3)	7.1	8.8
Eugenia pyriformis	5	264	4		*A. pelleranoi* (100.0)	3.0	4.0
Rosaceae							
Prunus domestica	5	109	26	*D. brasiliensis* (23.1) *U. anastrephae* (76.1)		16.1	16.8
Prunus avium	2	18	8	*D. areolatus* (75.0) *U. anastrephae* (25.0)		24.2	50.0

[1]S—sample, [2]n—number of fruit, [3]PaT—parasitoids total, [4]%P—Parasitism percentage, [5]TIP—total index Parasitism.

TABLE 1. (CONTINUED) PARASITOID SPECIES (HYMENOPTERA) FROM FRUIT FLIES COLLECTED IN SIX DIFFERENT LOCALITIES FROM THE WEST OF SANTA CATARINA, BRAZIL, DURING 1998-2000.

Vegetal specie	S[1]	N[2]	PaT[3]	Hymenoptera parasitoid species and relative frequency (%)		%P[4]	TIP[5]
				Braconidae	Figitidae and Diapriidae		
Eriobotrya japonica	9	1166	48	*D. areolatus* (8.3) *D. brasiliensis* (41.6) *Opius bellus* (4.3) *Opius* sp. (4.2) *U. anastrephae* (41.6)		4.3	5.9
Pyrus communis	2	62	4		*A. pelleranoi* (100.0)	14.8	33.3
Prunes persica	16	562	18	*D. brasiliensis* (22.2)	*A. pelleranoi* (66.7) *O. anatrephae* (11.1)	1.2	1.6
Total	89	4787	461				

[1]S—sample, [2]n—number of fruit, [3]PaT—parasitoids total, [4]%P—Parasitism percentage, [5]TIP—total index Parasitism.

paper dampened with distilled water. Flies and parasitoids were counted after seven days.

The relationship between a fly species and its parasitoids was determined only when a single species of fly was held in an emergence container (Canal et al. 1994).

The total index of parasitism (TIP) was calculated as the number of parasitoids emerged × 100/ number of flies emerged + number of parasitoids emerged. The relative frequency of fly species and parasitoids (RF) was defined as number of samples of a given species collected × 100/total number of collected species according to Matrangolo et al. (1998), and the parasitism percentage was calculated as %P = total parasitism × 100/total pupae, which was modified from Silveira Neto et al. (1997).

Species of *Anastrepha* were identified with Steyskal's key (1997) and Zucchi's key (2000b), which includes only Brazil species. The Braconidae were identified according to the key of Canal & Zucchi (2000). The flies and parasitoids belonging to other families were sent to Prof. Dr. Manoel Araécio Uchôa Fernandes, Biologist Jorge Anderson Guimarães, Dr. Allen Norrbom, and Prof. Dr. Roberto Antonio Zucchi for identification.

Of the 46 fruit species collected, 35 were infested by fruit flies, but only 14 of these fruit species contained parasitoids (Table 1). A total of 682 samples of parasitoids belonging to nine species and three families were obtained, as follows: *D. areolatus*, *D. brasiliensis*, *O. bellus*, *Opius* sp., *U. anastrephae* (Braconidae), *A. pelleranoi*, *O. anastrephae* (Figitidae), and *Trichopria anastrephae* Lima (Diapriidae).

Of the 461 hymenopterans associated with a particular fruit fly, *A. pelleranoi* was the most common and represented 25.6% of the total, followed by *D. brasiliensis* (21.1%) and *D. areolatus* (18.6%). These figures differ from those obtained by Salles (1996) for the state of Rio Grande do Sul and Leonel Jr. (1995, 1996) for the state of São Paulo. However, the relative abundance of parasitoids collected in the valley of Rio do Peixe, state of Santa Catarina by Nora et al. (2000) are similar to ours.

All the parasitoids developed in *A. fraterculus*, except for *L. anastrephae*, which was associated only with *Neosilba* sp. Also a lonchaeid was obtained in the samples (Table 2). *D. areolatus* and *U. anastrephae* also parasitized *Neosilba* sp. De Santis (1980) catalogued eleven species of *Trichopria* for Brazil, and believed that only *T. anastrephae* parasitized the genus *Anastrepha*. Aguiar-Menezes et al. (2001) previously reported *A. fraterculus* as the host of *T. anastrephae*. *L. anastrephae* has been recorded previously only in the southern part of the country, in the state of São Paulo and central Mato Grosso do Sul (De Santis 1980).

Parasitoids attacked fruit flies in 14 host-plant species, one Fabaceae, five Rosaceae and eight Myrtaceae (Table 1). Myrtaceae contained 79.6% of the parasitoids and Rosaceae had 21.2%. The total parasitism index and the parasitism percentage were greatest in *Eugenia involucrata*, followed by *Prunus avium* and *Myrcianthes pungens*. These indexes are higher than those obtained by Leonel, Jr. et al (1996), Salles (1996) and Matrangolo et al. (1998), but are similar to those of Guimarães et al. (1999).

TABLE 2. ASSOCIATION BETWEEN COLLECTED PARASITOIDS AND FRUIT FLIES IN SIX DIFFERENT LOCALITIES IN THE WEST OF SANTA CATARINA, BRAZIL, DURING 1998-2000.

Parasitoids	Fruit flies	
	A. fraterculus	*Neosilba* sp.
Braconidae		
D. areolatus	X	X
D. brasiliensis	X	
O. bellus	X	
Opius sp.	X	
U. anastrephae	X	X
Diapriidae		
T. anastrephae	X	
Figitidae		
A. pelleranoi	X	
L. anastrephae		X
O. anastrephae	X	

The high percent parasitism in *E. involucatra* was previously found by Salles (1996) in the Rio Grande do Sul state, and may be due, as he suggested, to the thin peel and small size of the fruit. Guimarães et al. (2000) previously observed affinity of *A. pelleranoi* to the Myrtaceae fruit, which we confirmed. Sivinski (1991), Sivinski et al. (1997, 2000) and Hickel (2002) found that braconid parasitism was negatively correlated to fruit pulp thickness, and we showed that weight data correlated to parasitism.

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MONITORING AND MANAGEMENT OF RED IMPORTED FIRE ANTS IN A TROPICAL FISH FARM

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ABSTRACT

Tropical fish farms provide a prime habitat for the red imported fire ant, *Solenopsis invicta* Buren, which is an invasive, stinging ant that has spread throughout the southern United States. Stings can be a serious health hazard to hypersensitive individuals, and the presence of large populations can interfere with operational activities. The most efficient method of controlling fire ants is the application of ant bait. However, most fish farmers are reluctant to use baits or other chemical methods of control because of the unknown risk to fish. Baited stations in combination with maps generated by geographical information system software were used to estimate locations of fire ant nests. Maps of estimated fire ant locations encompassed from 10 to 100% of actual fire ant nests surveyed when there was a minimum of a single fire ant within a station. Low percentages of overlap between mapped areas and fire ant nests were associated with low nest densities and when higher ant count thresholds were used to indicate positive stations. Ant bait containing the insect growth regulator methoprene was broadcast between ponds, with some unavoidable bait entry into ponds. Fire ant nest densities declined 57% within 4 months. In contrast, nest densities increased 86% in untreated areas. During the summer, fire ant populations declined an average of 68% and increased 110% for treated and untreated areas, respectively. Fire ant nest densities and populations began to increase by December in both treated and untreated areas. No obvious fish mortality related to the ant baiting was noted during the study.

Key Words: *Solenopsis invicta*, aquaculture, GIS application, pest detection, pest control, methoprene, bait, detection.

RESUMEN

Las fincas de peces trópicales proveen un perfecto hábitat para la hormiga de fuego roja importada, *Solenopsis invicta* Buren, la cual es una especie invasora que pica, la cual se ha espacido por todo el sur de los Estados Unidos. Las picaduras pueden ser de gran peligro para la salud de individuos hipersensibles, y la presencia de altas poblaciones pueden interferir con actividades de trabajo. El método mas eficáz para controlar la hormiga de fuego roja importada es la aplicación de cebos para hormigas. Sin embargo, la mayoria de los productores de peces son renuentes para usar los cebos u otros métodos químicos de control por el riesgo desconocido para los peces. Las estaciones de cebo en combinación con mapas generados con programas de computadores de sistemas de información geográficos fueron usados para estimar la localización de los nidos de la hormiga de fuego roja importada. Los mapas de las localidades de la hormiga de fuego roja importada estimadas abarcaron 10 a 100% de los nidos existentes en el muestreo cuando fue un minimo de una sola hormiga de fuego roja dentro de una estación. Los porcentajes bajos de traslapes entre las áreas en los mapas y los nidos de la hormiga de fuego roja fueron asociados con las densidades bajas de nidos y cuando se usaron umbrales de conteo altos de las hormigas para indicar estaciones positivas. El cebo para hormigas que cotiene el regulador de crecimiento de los insectos metoprina fue aplicado entre las lagunas, con alguna entrada inevitable de cebo en la laguna. Las densidades de los nidos de la hormiga de fuego roja bajaron 57% dentro de 4 meses. En contraste, las densidades de los nidos aumentaron 86% en áreas no tratadas. Durante el verano, las poblaciones de la hormiga de fuego roja bajaron un promedio de 68% en áreas tratadas y aumentaron 110% en áreas no tratadas. La densidad y la población de la hormiga de fuego roja empezaron a aumentar por diciembre en ambas áreas tratadas y no tratadas. No se observó mortalidad alguna sobre los peces relacionada con el cebo para las hormigas durante este estudio.

The production of tropical fish accounted for about 43% of the value of Florida aquaculture farm gate sales in 2001 from 160 active growers (Florida Agricultural Statistics Service 2002). Most farms are located in areas with sandy loam soils with a relatively high water table, allowing

for relatively inexpensive pond construction. Ponds are small (0.04 ha), and a typical 0.4 ha (1 acre) of "farm" will have up to eight ponds. Because of the soil type, open water, and high water table, these farms provide an ideal habitat for the red imported fire ant, *Solenopsis invicta* Buren. This ant is an invasive, exotic species from South America, which has spread throughout the southern United States since its inadvertent introduction in the 1930s (Callcott & Collins 1996). It has a painful sting and deaths have been reported in hypersensitive individuals (deShazo et al. 1990, 1999). Fish farmers typically work barefoot and in short pants along the pond banks, and because a fire ant colony can contain over 200,000 individuals (Markin et al. 1973; Tschinkel 1988; Macom & Porter 1996), the probability of being stung is very high. In addition, there are anecdotal reports of mass fish mortality due to the ingestion of fire ants, but studies have failed to confirm this phenomenon (Ferguson 1962; Crance 1965). On most tropical fish farms, fire ants are not controlled because of the fear that insecticides will detrimentally impact fish being raised in ponds (C. A. Watson, pers. obs.).

Effective control options for fire ants in areas where there is a high potential for people to be stung generally involve the use of chemical insecticides. The application of fire ant baits is one of the most efficient methods of control (Lofgren & Weidhass 1972). Baits utilize the natural behavior of ants to forage for food and then share it with colony members. In this manner, insecticides incorporated into baits are spread throughout the colony. Alternative methods of control are to treat individual colonies with an insecticide that must contact the majority of the members of the colony including the queen, or use of nonchemical alternatives such as steam or hot water (Drees et al. 1998; Tschinkel & Howard 1980). However, treating individual colonies is laborious and time consuming in contrast to broadcasting fire ant bait (Barr et al. 1999). Inherent to the integrated pest management approach of controlling insect pests is determining the location and population of the pest (Pedigo 1996). In this study our objectives were to assess the feasibility of (1) using baited monitoring stations to indicate locations of fire ant nests, and (2) broadcasting a fire ant bait to control fire ants on a tropical fish farming facility.

Materials and Methods

The study site was located at the University of Florida Tropical Aquaculture Laboratory in Ruskin, FL, which contains 51 ponds on approximately 2.63 ha (6.5 acres). Ponds contained swordtails, *Xiphophorus helleri* L. The banks and paths between ponds, as well as adjacent areas, were visually surveyed for red imported fire ant nests. Each nest was partially opened with a shovel to estimate the amount of adult ants and to search for immature worker caste ants, or brood. The presence of brood indicates that the colony is healthy. In contrast, a colony with no brood or a preponderance of immature reproductive caste ants indicates that the colony is abnormal and declining in vigor. To quantify fire ant populations, a standardized rating system, developed by the U.S. Department of Agriculture, provided a population index that incorporated the estimates of the number of adult ants and the presence or absence of worker caste brood in each nest (Lofgren & Williams 1982). The population indices of all nests in an area were then summed to provide a population index for the area. In addition, all nests containing live ants were counted to provide a density of active nests per area.

Baited monitoring stations also were utilized to determine if they could indicate areas containing active fire ant nests. Stations consisted of two plastic petri dishes (50 mm diameter × 9 mm depth, Gelman Sciences, Ann Arbor, MI). One dish was lined with a cotton cosmetic pad that was moistened with approximately 2 ml of sucrose-based ant attractant (Vail et al. 1999). The other dish was lined with filter paper, and held a toothpick dipped in peanut butter, which served as an oil-based attractant.

Baited monitoring stations were placed at the corners of each pond, and an additional station was placed in the middle of each long side for rectangular ponds or on all sides for square ponds. This resulted in stations being placed at intervals of 8.2 to 9.1 m (27-30 ft) along the upper banks of each pond. Distances between ponds were 3.0 to 6.1 m (10-20 ft). In an area where ponds were not present (control area on the west end), stations were placed at intervals of 7.6 m (25 ft) (Fig. 1). We utilized a total of 230 stations on each sampling date. Stations were set when air temperatures were conducive to ant foraging, i.e., 24.4 to 32.8°C (76-91°F). Fire ants inside stations were counted 30 to 45 min after placement.

Ponds, active nest locations, and monitoring stations were mapped with a geographical positioning system (Trimble Pathfinder Pro-XL, Sunnyvale, CA 94088) which was capable of less than 1 m accuracy. For each sampling date, an inverse distance weighted (IDW) interpolator provided in ArcView GIS software (version 3.2) was used to generate maps that displayed estimated areas of fire ant populations based on fire ant counts at each monitoring station. The IDW interpolator produced maps that were based on the assumption that there would be fewer fire ants in areas that were further away from positive stations. To determine if the maps could be used to indicate the presence of fire ant nests, we calculated the percentages of actual nest locations that were encompassed by these maps.

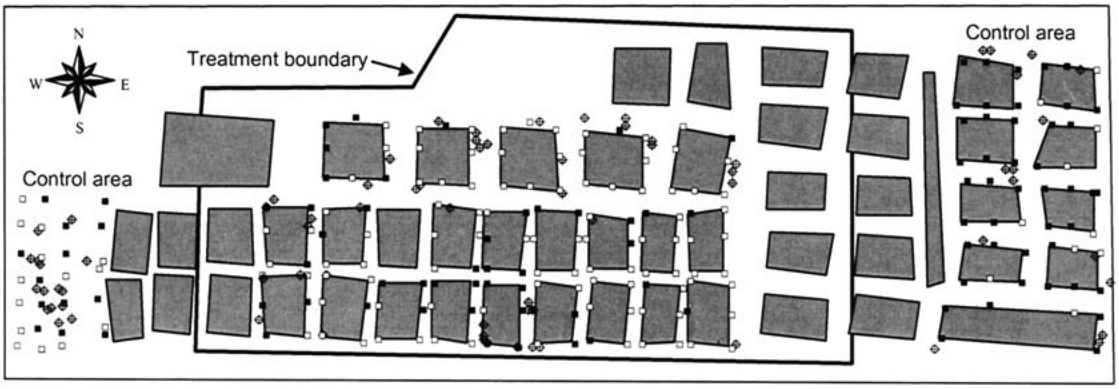

Fig. 1. Study site on March 13, 2000 with monitoring stations which contained fire ants (filled squares) and stations without fire ants (open squares). The lightly shaded area is the map generated from interpolating the ant counts from stations with at least a single fire ant. Actual fire ant nest locations are designated by circles containing a cross. Darkly shaded rectangular areas are the ponds.

The shapes of maps, or estimated areas with fire ants, are affected by the criteria used to define a positive station. Estimated areas with fire ants were mapped with three thresholds for positive stations: 1, 25, and 50 fire ants per station. We hypothesized that by increasing the threshold used to designate a positive station, the percentages of actual nest locations that were encompassed by these maps would decrease because higher thresholds would result in fewer positive stations, and subsequently smaller estimated areas with fire ants. We also hypothesized that the percentages of actual nest locations that were encompassed by maps would be less if fire ant population levels were low. A two-way analysis of variance and Tukey's HSD test (SAS Institute 2001) were used to compare the percentages of nest locations encompassed by maps generated from the three thresholds used to designate positive stations, and between control and treated areas which reflected areas of high and low fire ant populations.

The paths around 32 ponds were treated (Fig. 1) with fire ant bait that contained 0.5% of the insect growth regulator (*S*)-Methoprene (Extinguish™ Professional Fire Ant Bait, Wellmark International, Bensenville, IL 60106). This bait was selected because of the low toxicity of the active ingredient to fish and zooplankton (LC_{50} trout: 760 ppb, bluegill: >370 ppb, and Daphnia 360 ppb). Formulations that contain a much higher percentage of methoprene are used for mosquito control in aquatic ecosystems (e.g., 8.62% methoprene in Altosid®).

Fire ant bait was applied to the paths between ponds with a seed broadcaster (model GT-77 ATV, Herd Seeder Co., Logansport, IN 46947) mounted on an all-terrain vehicle. Bait was applied on March 14, 2000, at a rate of 0.454 kg (1 lb) bait per acre including ponds, which is within the label rate of 0.454 to 0.681 kg (1 to 1.5 lbs) per acre. However, because bait was broadcast onto paths between ponds, the majority of the bait landed on the paths and banks. This resulted in an application rate of approximately 10.4 kg (23 lbs) of bait per acre if only path surfaces are considered. Two areas encompassing eight ponds were used as untreated controls and were separated from the treated area by approximately 21.3 m (70 ft).

Fire ant populations were determined with the population index and the bait station methods the day before treatment (March 13, 2000) and on 15 (June 29), 22 (August 17), and 39 weeks (December 14) after treatment. Surveys were conducted within a week of moderate to heavy rains to ensure that soil was moist and allowed nest sites to be distinctly visible as mounds. Percent change in fire ant population indices and mound densities from the pretreatment surveys were compared between the treated and control areas.

Results and Discussion

As the thresholds for positive monitoring stations increased, the percentages of nests that were encompassed by maps that estimated fire ant populations decreased significantly ($F = 27.9$; $df = 2, 18$; $P < 0.001$) (Table 1). However, when fire ant populations were low (≤15 nests/ha) in treated areas, only 10-30% of the nest locations were encompassed by the maps based on the most stringent threshold of 1 ant. In contrast, when fire ant populations were above 35 nests per ha, 79 to 100% of the nests were included in the mapped areas based on the same threshold (Fig. 2). The higher percentages were associated with higher numbers of positive stations (Table 1). Stations with or without ants did not always reflect the presence or absence of nearby nests as there were several observations of fire ant nests adjacent to negative stations (Fig. 1). Perhaps when

TABLE 1. MEAN PERCENTAGES OF *S. invicta* NESTS THAT WERE ENCOMPASSED BY MAPS GENERATED BY INTERPOLATION OF POSITIVE MONITORING STATIONS AND THE MEAN NUMBER OF POSITIVE STATIONS (IN PARENTHESES), BASED ON MINIMUM THRESHOLDS OF 1, 25, OR 50 FIRE ANTS PER STATION IN METHOPRENE TREATED AND UNTREATED CONTROL AREAS AT THE UNIV. OF FLORIDA TROPICAL AQUACULTURE LABORATORY, RUSKIN, FLORIDA. MEANS WERE OVER SAMPLING DATES (n = 4).

	Threshold No. of *S. invicta* per station			
Area	1	25	50	Treated vs Control[a]
Treated	52.8 (25.5)	7.2 (10.5)	1.8 (5.0)	20.6a[b]
Control	92.5 (48.3)	43.7 (27.5)	16.6 (16.3)	51.0 b
Comparison among thresholds[c]	72.7 a[d]	25.5 b	9.2 b	

[a]Comparison over all sampling dates and thresholds (n =12).
[b]Means followed by a different letter within a column are significantly different by analysis of variance (F = 25.7; df = 1, 18; P < 0.001).
[c]Comparisons among thresholds over all sampling dates and areas (n = 8).
[d]Means followed by a different letter within a row are significantly different by Tukey's HSD test (P < 0.05).

fire ant nest densities are low, areas may be more accurately mapped by interpolating positive monitoring stations and nest locations that are encountered during the servicing of stations.

The time required to service stations during the March sampling, including the 30-45-min interval to allow ants to forage at stations, was 1.6 person-h. In comparison, a walking survey to flag nest locations required 2.8 person-h. Thus, servicing the bait stations was 43% faster than the walking survey when fire ant populations were relatively high. On the March sampling date when there were 35 and 52 nests per ha (14-21 nests/acre), the single fire ant threshold for a positive station resulted in maps that encompassed over 92% of the nests.

While fish mortality relative to the bait application was not formerly assessed, no obvious mortality was observed during the study (C. A. Watson, pers. obs.). The half-life of technical methoprene in pond water is less than 2 days, and approximately 10 days in soil (EXTOXNET 2001); thus, the accumulation of the active ingredient is probably minimal. The equipment used to apply the bait was calibrated to the recommended label rate. However, the amount of bait actually applied per area of dry land (i.e., excluding water surfaces) was well above the recommended rate. This reflected a typical reality of fire ant bait application, where calibrating commercially available broadcast bait applicators to the very low recommended application rates often is difficult. This difficulty was exacerbated at the fish farm because extremely slow speeds were required to navigate the application equipment through the numerous turns on narrow paths around ponds. Thus, the over-application of fire ant bait represents a problem with bait application that will require improvement.

Fire ant nests per hectare and population indices per hectare one day before bait application were 35 and 564 (14 and 228/acre), respectively,

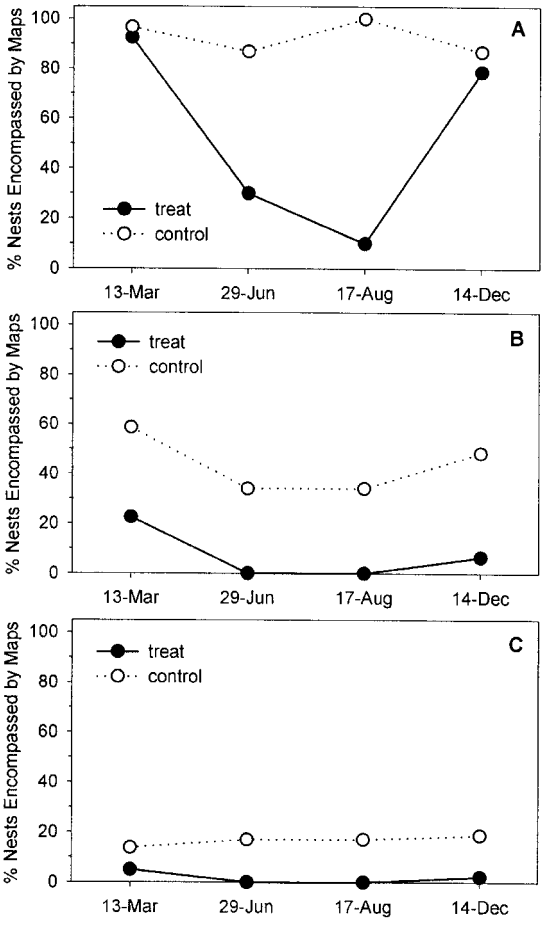

Fig. 2. Percentages of *S. invicta* nests encompassed by maps generated from the interpolation of positive bait stations based on thresholds of (A) 1, (B) 25, or (C) 50 ants between treated and untreated control areas among sampling dates. Treatment was applied on March 14, 2000.

for the treated area, and 52 and 629 (21 and 255/acre) for the control. Percent reductions of active fire ant nests per hectare in the treated area from the initial survey were 57 and 66% at 15 and 22 weeks after treatment. In contrast, the untreated control areas had 85 and 62% increases in the densities of active fire ant nests. These represent a 167 and 207% difference between the treatment and control for the June and August surveys. Similarly, there were 70 and 67% reductions in population indices in the treated area, and increases of 120 and 99% in the control for the same dates relative to pre-treatment populations. Employees at the facility also perceived a substantial reduction in fire ant stings in treated areas (C. A. Watson, pers. obs.). By the December survey, fire ants had re-infested the treated area beyond the pre-treatment number of active mounds by 83% and the pre-treatment population index by 135% (Table 2).

Methoprene is an insect growth regulator which results in an over production of the reproductive caste and little or no production of adult worker caste ants (Cupp & O'Neal 1973; Vinson & Robeau 1974). Without the worker caste, ant colonies eventually die because food foraging and other colony maintenance functions cease. Because the methoprene concentration in the fire ant bait does not affect adults, colonies will survive until there is sufficient natural attrition of existing adults. This process will generally take 8 to 10 weeks (Drees & Barr 1998) and is consistent with the reduction in fire ants obtained at 15 weeks in this study. Drees & Barr (1998) reported reductions of over 70% at application rates of 1.68 kg per ha, thus the large amount of bait applied in this study probably was not needed to obtain the level of control reported here. The estimated cost of the bait needed for application to the paths in the treatment area in this study was less than $3, based on a cost of $37 per ha at the maximum label rate (1.68 kg/ha).

Low precipitation during the study limited population surveys to irregular intervals dictated by episodes of enough rain to moisten soil and to permit mound building by the ants. When soil is dry, fire ant nests may not be visible, but populations can still increase. This was evident in the December survey where there was a large increase in fire ant mounds since the August survey. Drees & Barr (1998) reported an increase in fire ant mound densities between 6 and 12 months in methoprene treated field plots. We observed a greater concentration of large colonies in treated areas near a buffer area, which indicated that reinfestation was probably due to migration of mature colonies from the buffer and control areas. If all areas were treated, it would be reasonable to expect a slower reinfestation.

Surveying and mapping active mounds have aided in delimiting areas that require baiting and thus reduced the amount of bait that was applied per year in managed landscapes (Cobb & Cobb 1995). Bait stations could be used on tropical fish farms to delineate areas where fire ants are foraging and could indicate where bait treatments may be effectively applied. Using monitoring stations was faster for us than searching for individual nests, and may be advantageous during dry conditions or in areas with overgrown vegetation, where nests are difficult to see. Counting fire ants at monitoring stations required the ability to distinguish fire ants from other ants. Information on identifying common ants is available in several extension publications (Vail et al. 1994; Drees et al. 2000). Generating interpolated maps based on monitoring stations required investments in GPS equipment and GIS software. Continuing improvements in this technology have made it more affordable and usable. Our use of an IDW interpolator and a 1-ant threshold provided maps that estimated areas which contained over 78% of the fire ant nests when their densities were ≥35 nests per ha (14 nest/acre, Fig. 2a). Evaluations of other interpolators and thresholds may provide more accurate maps over a wider range of nest densities.

In summary, at low fire ant population levels, improvements were needed in estimating nest locations based on the baited monitoring stations. However, when populations were high, the methods utilized in this study detected the presence of most of the fire ant nests. Suppression of fire ants on a tropical fish farm was accomplished with the broad-

TABLE 2. FIRE ANT MOUND DENSITIES AND POPULATION INDICES OF METHOPRENE TREATED AND UNTREATED CONTROL AREAS AT THE UNIV. OF FLORIDA TROPICAL AQUACULTURE LABORATORY, RUSKIN, FLORIDA.

	Mounds/ha (% change)		Pop. Index[a]/ha (% change)	
Week[b]	Treated	Control	Treated	Control
0	35	52	564.1	629.1
15	15 (-57.1)	96 (+84.7)	169.7 (-69.9)	1383.2 (+220.0)
22	12 (-65.7)	84 (+61.5)	188.0 (-66.7)	1253.3 (+99.2)
39	64 (+82.9)	168 (+223.1)	1324.4 (+134.8)	3125.3 (+396.8)

[a]Population index.
[b]Week: 0 = March 13, 15 = June 29, 22 = August 17, 39 = December 14, 2000.

cast application of the methoprene fire ant bait. However, calibration and delivery of bait needs to be more accurate. With improvements, monitoring fire ants at stations and broadcasting fire ant bait has the potential to be an efficient approach of controlling fire ants on tropical fish farms.

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DYSTUS PUBERULUS STÅL (HETEROPTERA: SCUTELLERIDAE) A SHIELD BUG ASSOCIATED WITH FIGS IN MEXICO

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ABSTRACT

The life cycle of *Dystus puberulus* Stål, including all developmental stages, is described and notes on its biology are provided. The association of this rare species of Scutelleridae with several species of *Ficus* is reported for first time. Nymphs and adults of this species feed on the immature fruits, which resembles them in shape and color. Nymphs and adults are densely setose, with slightly flattened antenna, characteristics that are unusual in Scutelleridae.

Key Words: *Dystus*, Scutelleridae, *Ficus*, Mexico.

RESUMEN

El ciclo de vida de *Dystus puberulus* Stål, incluyendo todos los estadios de desarrollo, es descrito y se anexan notas acerca de su biología. La asociación de esta rara especie de Scutelleridae con varias especies de *Ficus* se reporta por primera vez. Ninfas y adultos de esta especie se alimentan de los frutos inmaduros, y se les asemejan en forma y color. Ninfas y adultos muestran una densa cubierta de sedas, con antenas ligeramente aplanadas, características inusuales en Scutelleridae.

Translation provided by the author.

The shield bug *Dystus puberulus* Stål is known only from a few individuals deposited in several collections. Eger & Lattin (1995) provided information on type specimens and synonymy for this species. A description and figure of the adult was published in Biologia Centrali Americana (Distant 1880). Immature stages and biology were previously unknown. In the present study, all developmental stages are described and illustrated and notes on the biology and distribution of *D. puberulus* are provided.

MATERIALS AND METHODS

Monthly collecting trips during 2001, 2002, and first half of 2003 were made to several localities in the Mexican states of Tamaulipas, Veracruz, Hidalgo, Puebla, and Campeche. The objective was to collect lygaeoids and other Hemiptera associated with fruiting fig trees. Around 30 fig species were sampled, and localities varied from sea level to an altitude of 1, 500 m. Several types of vegetation were sampled, including low tropical dry forest, medium tropical forest, high tropical rain forest, and cloud forest.

Adults and nymphs of *D. puberulus* were collected alive and put into plastic containers (9 × 8 cm) covered with muslin to avoid condensation. A small branch bearing immature fruits was put in each container as well as a small moist cotton ball. Containers were checked daily for the presence of eggs, and to record molting and mortality. Individuals were kept under laboratory conditions at 20°C and 70% RH. Individuals were fixed in 70% ethyl alcohol and used for illustrations and descriptions. Measurements are given in mm ±SD. Material studied were deposited in the Insect Collection of Instituto de Ecologia, A.C. (IEXA), the Insect Collection of Instituto de Biologia, U.N.A.M. (CNIN), the Florida State Collection of Arthropods (FSCA), Joe Eger Personal Collection (JEC), the Natural History Museum in London (BMNH), and the National Museum of Natural History in Washington (NMNH).

Dystus puberulus Stål
(Fig. 1A-H)

Description

Egg (Fig. 1A and B).—Oval, 1.34 ± 0.03 mm long, 1.09 ± 0.02 mm wide (n = 10). Eggs are laid in masses of 14 eggs (n = 3), arranged in three regular lines, two with five eggs and one with four eggs. Eggs were yellow-green when laid and turned yellow in approximately 3 days. Eight days later the eyes appeared as red spots, and the egg burster as a black triangle. Egg surface smooth, with 6-7 mycropilar process on anterior pole.

First Instar (Fig. 1C).—Body shape oval. Head declivent, pale yellow, tylus and base of head brown. Antennal segments and rostrum reddish. Pro-, meso-, and metanotum brown, punctures on these regions slightly paler than those on surrounding surfaces. Abdominal segments brown, except segments I and II which have some irregularly distributed reddish areas. Sternum and ventral abdominal area reddish orange, dorsal and

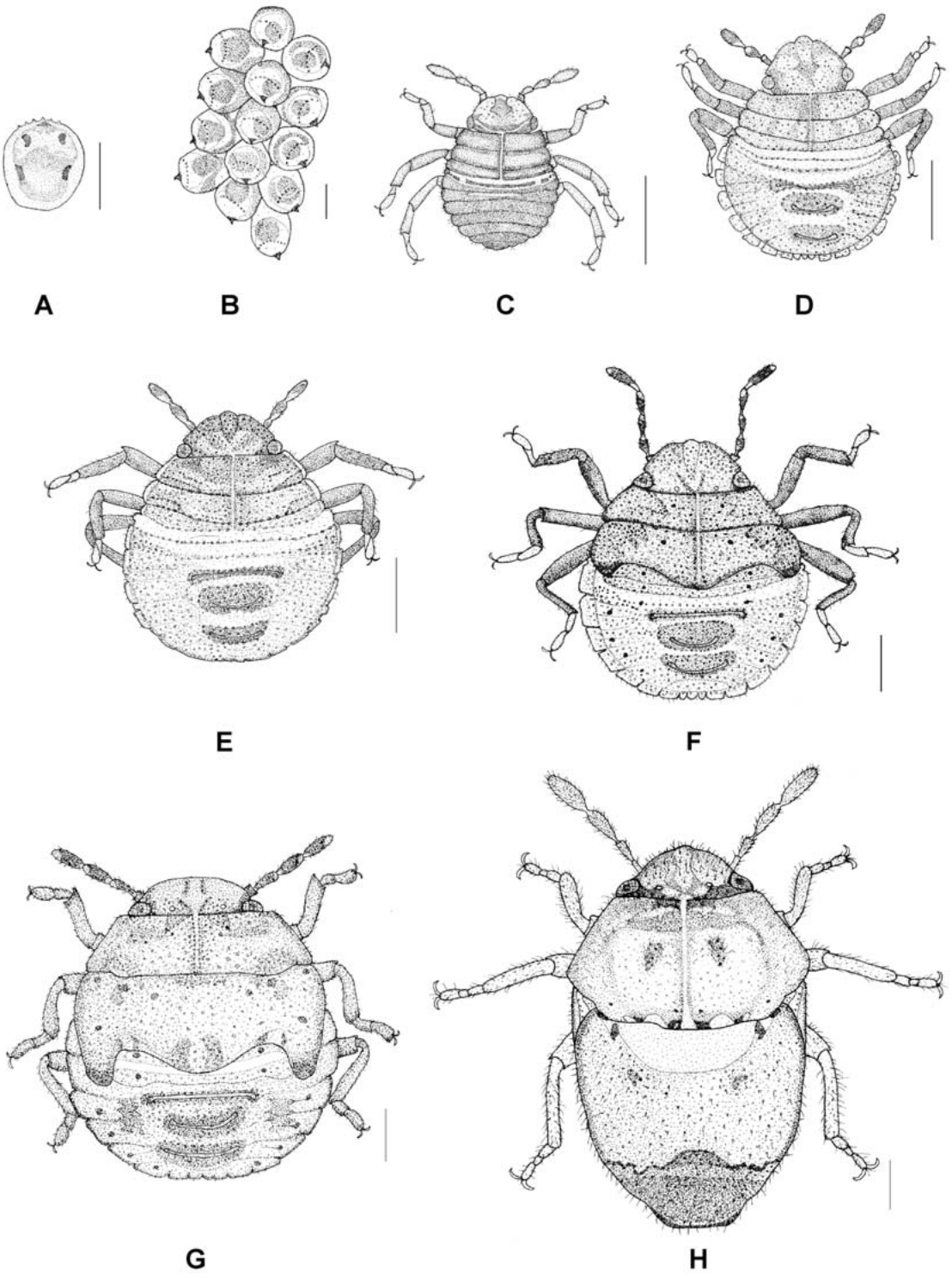

Fig. 1. Instars of *Dystus puberulus*. A, Egg. B, Egg mass. C, First Instar. D, Second Instar. E, Third Instar. F, Fourth Instar. G, Fifth Instar. H. Adult Male. (Scale = 1 mm).

ventral connexivum brown. Setae more abundant on head than on remainder of body. Eyes contiguous to anterior margin of pronotum. Rostrum reaching base of abdomen. Measurements (n = 2). Body length 1.7 ± 0; head length 0.44 ± 0.06; width across eyes 0.76 ± 0.01; interocular distance 0.59 ± 0.01; antennal segments: I 0.12 ± 0; II 0.14 ± 0.01, III 0.22 ± 0.05, IV 0.35 ± 0.04; rostral segments: I 0.2 ± 0, II 0.22 ± 0.03, III 0.19 ± 0.04, IV 0.26 ± 0.01; pronotum length 0.26 ± 0.01, width across humeral angles 1.0 ± 0, width across anterior margin 0.82 ± 0.04; length of hind leg: femur 0.41 ± 0.01, tibia 0.36 ± 0.01, tarsus: I 0.09 ± 0.01, II 0.2 ± 0.

Second Instar (Fig. 1D).—Oval, with maximum width across abdominal segment III. Head, pro-, meso- and metanotum pale brown with dark brown punctures. Antennal segments I to IV concolorous; ventral surface of head, rostrum and most of femora and tibiae brown. Abdominal segments pale yellow, with sparse brown punctures. Longitudinal brown bands present on segments II and III-IV, the last corresponding to the scent gland opening and slightly wider around the orifice. Scent gland openings present on segments IV-V and V-VI. Smaller brown plates present on connexivum of segments VI and VII. Abdominal venter reddish with pale brown rectangular macules on midline of sternite V to VIII. Margin of head, thorax and abdomen densely covered with setae. Rostrum reaching sternite VI. Measurements (n = 10). Body length 2.34 ± 0.14; head length 0.61 ± 0.07; width across eyes 1.04 ± 0.02; interocular distance 0.76 ± 0.04; antennal segments: I 0.16 ± 0.02, II 0.2 ± 0.01, III 0.25 ± 0.02, IV 0.41 ± 0.03; rostral segments: I 0.49 ± 0.04, II 0.53 ± 0.04, III 0.32 ± 0.03, IV 0.4 ± 0.03; pronotum: length 0.33 ± 0.03, width across humeral angles 1.4 ± 0.04, width across anterior margin 1.09 ± 0.04; length of hind leg: femur 0.54 ± 0.04, tibia 0.5 ± 0.02, tarsus: I 0.14 ± 0.02, II 0.23 ± 0.02.

Third Instar (Fig. 1E).—Oval, with maximum width across abdominal segment III. Very similar to third instar. Antennal segments I to III and base of IV dark brown to reddish; ventral surface of head, and rest of antennal segment IV brown. Measurements (n = 10). Body length 3.49 ± 0.11; head length 1.04 ± 0.07; width across eyes 1.45 ± 0.04; interocular distance 1.0 ± 0.04; antennal segments: I 0.21 ± 0.01, II 0.25 ± 0.02, III 0.3 ± 0.02, IV 0.51 ± 0.02; rostral segments: I 0.72 ± 0.05, II 0.79 ± 0.04, III 0.41 ± 0.02, IV 0.44 ± 0.03; pronotum: length 0.54 ± 0.06, width across humeral angles 2.07 ± 0.05, width across anterior margin 1.52 ± 0.04; length of hind leg: femur 0.78 ± 0.04, tibia 0.7 ± 0.03, tarsus: I 0.17 ± 0.02, II 0.35 ± 0.02.

Fourth Instar (Fig. 1F).—Oval, margin of body with numerous setae; body surface, sparsely setose abdomen pale yellow (individuals fixed in alcohol) pale green (living individuals) with numerous dark brown punctures. Eyes and ocelli reddish brown; antennae dark brown with pale yellow bases. Pro-, meso-, and metanotum and abdominal segments I to III each with a pair of brown mesial maculae; those on segment III-IV slightly reddish and corresponding to the scent gland openings on segments III-IV. Scent gland openings of segments IV-V and V-VI surrounded by red area. Femora dark brown with apices pale yellow, tibiae pale yellow with margins brown. Red spiracles visible ventrally on segments II to VIII. Rostrum reaching middle area of abdominal segment III. Measurements (n = 5). Body length 4.47 ± 0.41; head length 1.41 ± 0.02; width across eyes 1.92 ± 0.08; interocular distance 1.35 ± 0.05; antennal segments: I 0.29 ± 0.01, II 0.38 ± 0.02, III 0.45 ± 0.04, IV 0.63 ± 0.02; rostral segments: I 0.76 ± 0.03, II 0.83 ± 0.09, III 0.49 ± 0.06, IV 0.44 ± 0.04; pronotum: length 0.79 ± 0.03, width across humeral angles 3.09 ± 0.1, width across anterior margin 2.07 ± 0.04; scutellum: length 0.95 ± 0.07, width 2.29 ± 0.14; length of hind leg: femur 1.12 ± 0.12, tibia 1.02 ± 0.08, tarsus: I 0.25 ± 0.04, II 0.44 ± 0.02.

Fifth Instar (Fig. 1G).—Body round, margin of head, thorax and abdomen densely setose. Head, thorax and disc of abdomen pale yellow (individuals fixed in alcohol), pale green (living individuals) with dark brown punctures; lateral margins of head dark brown, particularly near eyes. Eyes and ocelli red, postero ventral area of eyes dark brown. Antennal segments reddish brown with base of each segment pale yellow. Anterior half of head brown ventrally with a few dark brown punctures; posterior half pale yellow, rostrum pale brown. Pro-, meso-, and metanotum with pairs of brown maculae as follows: a pair of subtriangular maculae on anterior margin, close to middle line; another pair slightly lighter and not always visible on discal area; mesonotum with four pairs of round maculae, two situated on anterior margin, one near its later margin and another one on the intersection of wing pad and scutellum; one pair on discal area of scutellum; another pair on discal area of wing pad; one pair of subtriangular maculae near apex of wing pad; and another pair on apex of scutellum. Exposed surface of metanotum with two pairs of irregular maculae. Femora and tibiae of all legs reddish to pale yellow with margins dark brown. Tarsi I pale brown and tarsi II reddish yellow. Abdominal segments I to III each with a pair of round brown maculae mesially, slightly closer to the middle line. The maculae of segment III corresponds to the scent gland openings of segments III - IV, scent gland openings of segments IV - V and V - VI well developed and surrounded by a brown area sometimes red margined. Lateral margins of segments I to VIII dark brown, segments IV to VI with a pair of red maculae mesially. Abdominal

punctation light red to pale brown, dark brown around scent gland openings.

Head declivent; eyes very close to anterior margin of pronotum. Antennal segments slightly flattened. Rostrum reaching metacoxae. Frontal angles of pronotum directed forward, lateral margins of pronotum slightly serrated. Mesonotal wing pads reaching base of abdominal segment III, scutellum reaching base of segment II. Scent gland openings of segment III-IV not very apparent, the ones of segments IV-V and V-VI slightly elevated; spiracles visible ventrally on segments II to VIII. Measurements (n = 10). Body length 5.8 ± 0.76; head length 1.52 ± 0.05; width across eyes 2.4 ± 0.15; interocular distance 1.68 ± 0.08; interocellar distance 0.86 ± 0.05; antennal segments: I 0.39 ± 0.04, II 0.54 ± 0.05, III 0.66 ± 0.06, IV 0.79 ± 0.04; rostral segments: I 0.94 ± 0.09, II 1.14 ± 0.11, III 0.53 ± 0.06, IV 0.53 ± 0.07; pronotum: length 1.24 ± 0.18, width across humeral angles 4.12 ± 0.23, width across anterior margin 2.58 ± 0.19; scutellum: length 1.78 ± 0.17, width 3.26 ± 0.22; length of hind leg: femur 1.51 ± 0.09, tibia 1.48 ± 0.08, tarsus: I 0.35 ± 0.05, II 0.55 ± 0.04.

Adult (Fig. 1H).—Body oval, convex; dorsal and ventral surface densely covered by long silvery setae, those on posterior of abdomen black. Specimens killed in alcohol are usually grayish yellow, but living individuals are greenish yellow. Head, anterior margin of pronotum near midline and apical third of scutellum usually dark brown, but varying from yellow to brown. Head, scattered areas of pronotum and apical third of scutellum with sparse dark brownish black punctures. Ventral surface of head brown, rest of body grayish yellow or greenish yellow. Eyes and ocelli red; antennal segments I to III dark brown, first two slightly darker, segments IV and V reddish-brown. Rostral segment I usually yellowish and the last three segments brown. Scutellum typically with only one discal brown macule on each side of midline, sometimes with additional paler maculae near posterior margin and a few irregular brown areas present on basal third near midline. Legs pale yellow, except apices of third tarsal segment and claws which are almost black; femora and tibia with a few dark brown punctures.

Head declivent, antennal segments I to III cylindrical, segments IV and V slightly flattened and longer than the first three segments. Labium laying in grove reaching posterior margin of metasternum, although labium does not reach metacoxae. Evaporative areas of metathoraxic scent glands are nearly glabrous and slightly elevated.

Male genitalia (Fig. 2A, B). Posterior margin of pygophore slightly sinuated, parameres sickle-shaped in lateral view.

Male. Measurements (n = 10). Body length 7.33 ± 0.26; head length 1.88 ± 0.07; width across eyes 2.64 ± 0.11; interocular distance 1.78 ± 0.13; interocellar distance 0.89 ± 0.06; antennal segments: I 0.42 ± 0.02, II 0.36 ± 0.02, III 0.41 ± 0.02, IV 0.82 ± 0.03, V 0.95 ± 0.05; rostral segments: I 1.03 ± 0.07, II 1.25 ± 0.07, III 0.48 ± 0.02, IV 0.58 ± 0.06; pronotum: length 2.37 ± 0.18, width across humeral angles 4.54 ± 0.15, width across anterior margin 2.67 ± 0.16; scutellum: length 3.93 ± 0.24, width 4.4 ± 0.17; length of hind leg: femur 1.8 ± 0.07, tibia 1.8 ± 0.06, tarsus: I 0.34 ± 0.04, II 0.16 ± 0.02; III 0.39 ± 0.01.

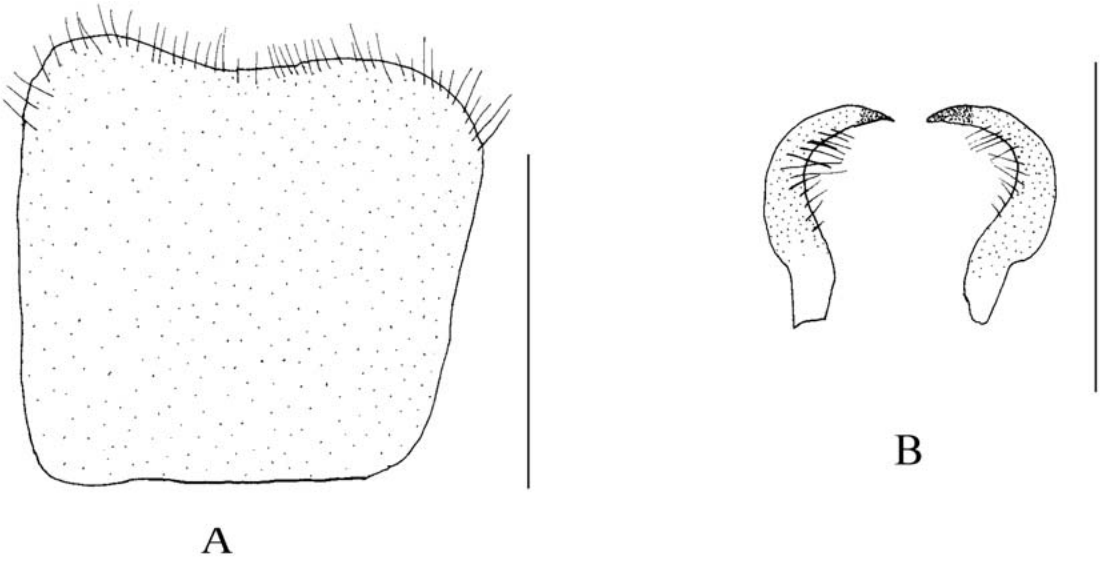

Fig. 2. (A) Pygophore of *Dystus puberulus* ventral view. (B) Parameres of *D. puberulus* lateral view. (Scale = 1 mm).

Female. Measurements (n = 10). Body length 8.09 ± 0.23; head length 2.05 ± 0.07; width across eyes 2.8 ± 0.07; interocular distance 1.83 ± 0.05; interocellar distance 0.94 ± 0.05; antennal segments: I 0.46 ± 0.05, II 0.41 ± 0.02, III 0.45 ± 0.04, IV 0.86 ± 0.06, V 0.99 ± 0.07; rostral segments: I 1.1 ± 0.08, II 1.26 ± 0.07, III 0.52 ± 0.04, IV 0.64 ± 0.04; pronotum: length 2.64 ± 0.25, width across humeral angles 4.92 ± 0.11, width across anterior margin 2.94 ± 0.1; scutellum: length 4.36 ± 0.39, width 4.79 ± 0.14; length of hind leg: femur 1.95 ± 0.08, tibia 1.91 ± 0.08, tarsi: I 0.36 ± 0.04, II 0.19 ± 0.02; III 0.39 ± 0.01.

Distribution: *Dystus puberulus* has been reported from Bolivia, Mexico, and Costa Rica. New Records.—MEXICO: Hidalgo, Tlachichilco, Km 5 Tlachichilco-Agua Blanca, 1238 m Veracruz, Naranjos, Km 10 Naranjos-Chontla, 118 m; Veracruz, Nautla, Km 10 Nautla-El Ciervo, 39 m;Veracruz, Vega de Alatorre, Colipa, 186 m; Veracruz, Cordoba, Km 11 Cordoba-Tezonapa; Veracruz, Tlapacoyan, Km 2 to Filobobos, 345 m; Veracruz, Tuxpan, Km 23 Tuxpan-Cazones, 13 m; Veracruz, Juchique, Km 2 Juchique-Xalapa, 364 m; San Luis Potosi, Ebano, Km 72 Ebano-Ciudad Mante, 33 m; Tamaulipas, Ciudad Mante, Grutas de Quintero, 33 m (IEXA); Tamaulipas, Gomez #Farias, Km 7 Gomez Farias-Ciudad Mante, 320 m (IEXA, BMNH, NMNH, FSCA).

Other material checked: MEXICO: Veracruz, Los Tuxtlas. SALVADOR: San Salvador. BRAZIL: Matto Grosso, Rio Caragusta (CNIN).

Biology

The bug *Dystus puberulus* was found associated with several species of *Ficus*. Nymphs and adults moved around the immature and ripe figs, which resemble the bugs in shape and color. Eggs were deposited on the under side of a fig leaf, the mass consisted on 14 eggs arranged in three lines, two of five eggs and one of four. Newly hatched nymphs stayed around the egg mass apparently without feeding until they molted to the second instar. Second to fifth instars fed on the fruit, and moved around them without moving onto leaves. Adults also move between fruits, and along branches and leaves. When disturbed, nymphs and adults usually stayed closely attached to their host plant.

D. puberulus was found associated with *Ficus calyculata* Miller, *F. cooki* Standl., *F. cotinifolia* (Kunth), *F. retusa* L., and *F. tecolutensis* (Liebm.) Miq., which are found from sea level up to an altitude of 1238 m. Two specimens deposited in CNIN and in NMNH from Salvador were found on *Ficus ovalis* (Liebm.) Miq. The types of vegetation in which the host figs grow varied from low tropical rain forest to cloud forest.

The entire life cycle took around 60 days, and due to the asynchronous fruiting of its host plants, it is possible that *D. puberulus* has several generations each year. No parasitoids or predators of *D. puberulus* were detected during the study. Other species of bugs found feeding on the same fruits were the rhyparochromid *Cholula bracteicola* Cervantes & Pacheco found on *Ficus cotinifolia* in Tamaulipas (Cervantes & Pacheco 2003) and *Ozophora baranowskii* Slater & O'Donnell in several localities (Cervantes et al. 2004).

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PATHOGENICITY OF *BEAUVERIA BASSIANA* (DEUTEROMYCOTA: HYPHOMYCETES) AGAINST THE CACTUS WEEVIL, *METAMASIUS SPINOLAE* (COLEOPTERA: CURCULIONIDAE) UNDER LABORATORY CONDITIONS

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ABSTRACT

Three strains of the entomopathogenic fungus *Beauveria bassiana* Vuill. were tested for pathogenicity against adults of *Metamasius* (=*Cactophagus) spinolae* Gyllenhal. *M. spinolae* is an important pest of cactus plants (*Opuntia ficus indica*), which are used as a food crop and to avoid erosion in Mexico. After inoculation in a spray tower, *M. spinolae* adults were susceptible to *B. bassiana* at concentrations of 1×10^8 conidia per milliliter. Female mortality was steadily higher than male mortality for all isolates. One of the three isolates caused significantly higher mortality (82%) in females, whereas male mortality was the same for all isolates. These results indicate for the first time the possible use of *B. bassiana* as biocontrol agent against this insect pest.

Key Words: *Beauveria bassiana*, *Cactophagus spinolae*, *Opuntia* spp., microbial control, Opuntia borer, cactus weevil, *Metamasius spinolae*.

RESUMEN

Tres aislamientos del hongo entomopatógeno *Beauveria bassiana* Vuill. fueron evaluados en su patogenicidad contra adultos de *Metamasius* (=*Cactophagus) spinolae,* Gyllenhal. *M. spinolae* es una importante plaga de plantas de nopal (*Opuntia ficus indica*), que son usadas como alimento y para evitar la erosión en México. Después de ser inoculados en una torre de aspersión, adultos de *M. spinolae* fueron susceptibles a concentraciones de *B. bassiana* de 1×10^8 conidios por mililitro. La mortalidad en hembras fue consistentemente mayor que la mortalidad de machos para todos los aislamientos. Uno de los tres aislamientos causó significativamente mayor mortalidad (82%) en hembras, mientras que la mortalidad en machos fue la misma para todos los aislamientos. Estos resultados muestran por vez primera el posible uso de *B. bassiana* como agente de control biológico en contra de este insecto plaga.

Translation provided by the authors.

The cactus (*Opuntia ficus indica*) is a very important plant in Mexico, especially in semi-arid regions where few crops can be cultivated (Vigueras & Portillo 2001). The cactus weevil *Metamasius* (= *Cactophagus*) *spinolae* Gyllenhal is a limiting factor for commercial production of *Opuntia* spp. (Baddi & Flores 2001; Flores-Valdez 2001). The larvae, 25-35 mm long, tunnel into apparently healthy cactus pads, from joint to joint, where they cause disintegration of the cactus tissues (Granados & Castañeda 1991). Pupation takes place in the hollowed stem of the plant, which provides a protected environment for pupal overwintering. Adults can be found from May through September; they are relatively large weevils at 23-36 mm in length. They feed on the margins of the young pads causing additional damage. Control strategies for *M. spinolae* rely to a large extent on the use of chemical insecticides (Borrego & Burgos 1986; Baddi & Flores 2001). However, a biologically based control strategy that can be used on *M. spinolae* would be highly desirable.

Microbial insect pathogens may offer a strategy for use as localized biopesticides, but little is known about natural microbial enemies of *M. spinolae*. Several products based on *Beauveria bassiana* Vuill. are available for managing adults of other pest insect species, such as *Hypothenemus hampei* Ferrary (coffee berry borer), and various species of Curculionidae (Adane et al. 1996;

de la Rosa et al. 1997; Rice & Cogburn 1999). So far, however, no entomopathogenic fungus has been evaluated for the control of *M. spinolae*. In this study we assess the susceptibility of adult *M. spinolae* to isolates of *B. bassiana* under laboratory conditions.

Materials and Methods

Biological Material

Isolates from the Insect Pathology Collection (Colegio Postgraduados Texcoco, Mexico) were used in bioassays with *M. spinolae* adults (Table 1). All isolates were cultured on Sabouraud dextrose agar (SDA) with yeast extract (2g/l) (SDA-Bioxon, Mexico) and incubated at 27°C, 70% RH for 2-3 weeks until conidia were produced. For bioassays, conidia were harvested into sterile 0.01% Tween 80 solutions to a final concentration of 1×10^8 conidia/ml. Conidia viability was determined by serial dilution plating onto SDA and colony forming units were counted six days after incubation at 27°C.

For bioassay experiments, *M. spinolae* adults were collected directly from cactus pads in Morelos, Mexico. They were sexed, held separately in screen cages ($32 \times 32 \times 32$ cm, 1.5 mm mesh size). Adults were stored in fresh cactus pads at 23 ± 1°C, 33 ± 5% RH, and a 12:12 (L:D) photoperiod, before use in bioassays.

Laboratory Test

For inoculation, *M. spinolae* adults were placed into 90-mm plastic Petri dishes. Groups of 10 adults of the same sex were stored at 4°C for 15 minutes to anesthetize them. The adults were exposed to a *B. bassiana* isolate by applying 10 ml of a 1×10^8 conidia/ml suspension in a spray tower (Altre et al. 1999) with constant pressure (0.7 kg/cm^2). On average, each insect received 1×10^8 conidia, while control adults received 0.1% Tween 80. The density of conidia on the insects was estimated by counting the number of conidia in a sample area on five agar disks (1 cm dia.) placed in the Petri dish during inoculation.

After application, the adults were placed in separate screen cages, fed with fresh untreated cactus pads and kept for 16 days at 23 ± 1°C, 33 ± 5% RH, and a photoperiod of 12:12 (L:D) h. The bioassays were repeated three times. The adults were examined for mortality every 48 h for 16 d. Dead insects were removed and incubated at 25°C and 90% relative humidity to check for *B. bassiana* infection by direct visual observation.

Statistical Analyses

Analysis was performed on mean cumulative percentage mortality data. After correction for control mortality (Abbott 1925), percentage mortality and average time to death were subjected to a one-way analysis of variance (SAS Institute 1999). Treatment effects were tested by the Fisher protected least significant difference (LSD) test (Sokal & Rohlf 1995).

Results and Discussion

B. bassiana isolates were pathogenic to *M. spinolae* exposed to 1×10^8 conidial suspensions (Table 2). However, the three *B. bassiana* isolates used for this report differed in their virulence to *M. spinolae* adults. For all three, the females were more susceptible to fungal infection than males ($F = 20.49$; $df = 1$; $P = 0.00005$). *B. bassiana* isolate Bb88 was more virulent against females than Bb4 or Bb113 isolate ($F = 5.55$; $df = 2$; $P = 0.04$). Fungal virulence of the three isolates were similar against males ($F = 1.18$; $df = 2$; $P = 0.36$;Table 2). All females exposed to the fungi died within 8.5 to 10.6 days ($F = 0.45$; $df = 2$; $P = 0.65$) and males died within 7.5 to 11 days ($F = 1.06$; $df = 2$; $P = 0.40$). There were no significant differences in mean time to death (Table 2). Most cadavers supported fungal sporulation, indicating successful infection and the ability of the isolates to sporulate under low humidity. An increase in female mortality was noted by day four after inoculation and female cumulative mortality was steadily higher than male mortality for all isolates (Fig. 1).

Table 1. *Beauveria bassiana* isolates[1] tested against *Metamasius spinolae*, their host insect, origin, and year isolated.

Fungus	Host insect	Country of origin	Year isolated
Bb4	*Hypothenemus hampeii* (Col.: Scolytidae)	Ecuador	1987
Bb88	*H. hampeii*	Mexico, Oaxaca	1994
Bb113	*Metamasius spinolae* (Col.: Curculionidae)	Mexico, Morelos	2003

[1]Deposited at the Colegio de Postgraduados (Texcoco, Mexico).

TABLE 2. MORTALITY OF *METAMASIUS SPINOLAE* ADULTS AFTER TREATMENT WITH THREE *BEAUVERIA BASSIANA* ISOLATES AT A CONCENTRATION OF 10^8 CONIDIA PER MILLILITER.

Treatment	Dosage[1]	Females		Males	
		% ±SE[2] mortality	Mean time ±SE to death (d)	% ±SE[2] mortality	Mean time ±SE to death (d)
Bb4	780 ± 43 a	46.4 ± 1.2 a	9.3 ± 1.4 a	10.0 ± 0.5 a	11.0 ± 2.8 a
Bb88	1187 b ± 51 b	82.1 ± 0.8 b	8.5 ± 0.2 a	20.0 ± 1.1 a	7.5 ± 0.2 a
Bb113	1341 b ± 86 b	42.8 ± 1.2 a	10.6 ± 2.3 a	26.6 ± 0.3 a	9.0 ± 0.4 a

Within columns, means followed by different letters are significantly different ($P \leq 0.05$; LSD test).
[1]Number ±SE of viable conidia per square millimeter. [2]Percentage of mortality corrected for control mortality of 0% in males and 6.6% in females (Abbott 1925).

Conidial dosages applied per mm^2 ranged from 780 ± 43 (Bb4) to 1341 ± 86 (Bb113) (Table 2). The Bb4 dosage was lower than Bb88 and Bb113 dosages ($F = 21.07$; $df = 2$; $P = 0.0003$). Thus, the low virulence of the Bb4 strain could be related to the lower conidial dosage applied. However, previous work showed that the same strain was highly virulent to its original host *H. hampei* (de la Rosa et al. 1997). Hence, these two species appear to have differing susceptibility to Bb4. Similar comparisons for the *M. spinolae* isolate (Bb113) are not available.

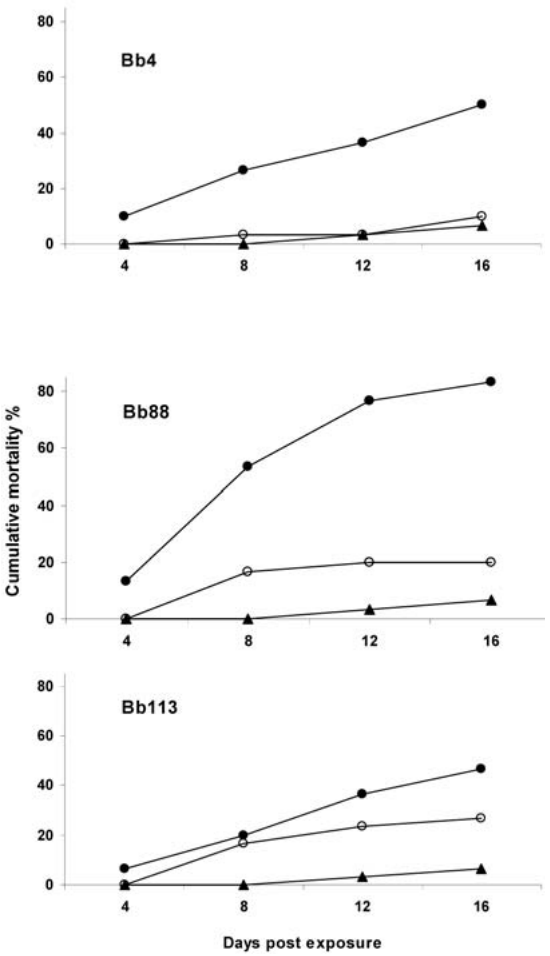

Fig. 1. Cumulative mortality % of adult *Metamasius spinolae* treated with three *Beauveria bassiana* strains under laboratory conditions. Females (○), males (●), and control (▲).

The data in this report also demonstrate that it is feasible to contaminate adults by spraying conidia, and that most cadavers supported fungal sporulation. This may be important for any control strategy aimed at attracting beetles to fungus contaminated traps, and subsequent transfer to adults or larvae in cactus pads and tunnels. Fortunately, there is evidence of an aggregation pheromone (Tafoya et al. 2003) which can be used to attract the males to a contaminated trap with fungi and possibly transfer the infective conidia to adults or larvae in tunnels. Similar strategies of autodissemination have been developed for other insects (Furlong et al. 1995).

The results presented in this study demonstrate a pathogenic effect of *B. bassiana* on *M. spinolae* adults under laboratory conditions. To our knowledge, this is the first report on infection of this pest insect with an entomopathogenic fungus. Further research is necessary to determine the effectiveness of *B. bassiana* under field conditions and to examine its potential impact on nontarget species.

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KEY TO THE GRASSHOPPERS (ORTHOPTERA: ACRIDIDAE) OF FLORIDA

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ABSTRACT

A dichotomous key is presented to aid in the identification of the adult stage of the 71 grasshopper species known to occur in Florida. Reflecting recent research one subspecies, *Schistocerca alutacea rubiginosa* (Scudder), has been elevated to species status *Schistocerca rubiginosa* (Harris) in this key.

Key Words: Acrididae, key, Orthoptera, morphology, systematics, taxonomy.

RESUMEN

Se presenta una clave dicótoma para ayudar en la identificación del estadio adulto de 71 especies de saltamontes conocidos que ocurren en Florida. Una subespecie, *Schistocerca alutacea rubiginosa* (Scudder), ha sido elevada al nivel de especie, *Schistocerca rubiginosa* (Harris) en esta clave.

Grasshoppers comprising the family Acrididae (Orthoptera) are easily identifiable and are quite common in Florida. Seventy-one species of grasshopper belonging to five subfamilies are known to exist in Florida (Table 1). While this group of insects contains some dramatic variation, there are a few morphological features that remain fairly consistent. All acridids have 3-segmented tarsi, short ovipositors, tympana found on the sides of the first abdominal segment, and the antennae are almost always shorter than the body. Adults of some species are winged, while other species are wingless or have extremely reduced wings. Eggs are usually deposited in soil and in clusters or pods with as many as 100 eggs. Grasshoppers are hemimetabolous insects, and therefore go through a gradual metamorphosis. Each instar looks like a smaller version of the adult, with wings not fully formed until adulthood (in the winged species). All grasshoppers are plant feeders, but will occasionally feed on dead insects, leaf litter, or even dung.

Because of their economic importance, grasshoppers have been the subject of thousands of publications, many with identification keys included. One of the most comprehensive of these was attempted by Otte (1981, 1984) in which he developed keys to all the species of North America within the subfamilies Acridinae, Gomphocerinae, and Oedipodinae. However, most identification keys are regional in nature (e.g., Blatchley 1920; Dakin & Hays 1970; Capinera & Sechrist 1982; McDaniel 1987; Richman et al. 1993; Pfadt 1994) and only one was dedicated solely to the grasshoppers of Florida (Capinera et al. 2001). A key to the grasshoppers of Florida is particularly useful considering that 18 species are endemic and six more are found almost exclusively in Florida, with a range also including extreme southern Georgia or Alabama. Thus, these species are absent from most other regional keys. While adequate for field identification, "Grasshoppers of Florida" (Capinera et al. 2001) does not contain a species-level key.

Schistocerca rubiginosa (Harris) has been an enigma in that it has been considered a species (Hubbell 1960; Helfer 1972), a subspecies of *Schistocerca alutacea* (Harris) (Morse 1904; Blatchley 1920), and a color phase of *S. alutacea* (Rehn 1901, 1902; Rehn & Hebard 1916). As mentioned by Blatchley (1920), the authors have found differences in the habitat preferences of *S. rubiginosa* and *S. alutacea* as well as significant morphological differences as mentioned in the key. *Schistocerca rubiginosa* is usually limited to dry sandy areas associated with scrub and turkey oaks whereas *S. alutacea* inhabits both xeric and mesic areas. Recently, a cladistic analysis of the *alutacea* group based on 22 morphological features found that *S. alutacea* and *S. rubiginosa* are, in fact, two separate species (Song 2004). This is a departure from the recent treatment of Florida grasshoppers by Capinera et al. (2001), so this change has been included.

Other species, such as *Melanoplus furcatus* and *M. symmetricus*, warrant additional study. They seem to represent different species based on the shape of the male cercus, normally a reliable character for differentiation of *Melanoplus* species. A related species or subspecies, designated as *M. clypeatus* (Blatchley 1920), has cerci intermediate in form, however, and because its status is uncertain, it is not recognized in this key. Blatchley differentiated *M. clypeatus* principally based on wing length, which often is a variable character in this genus. Suppression of *M. clypeatus* in this key follows Capinera et al. (2001). The other confusing

TABLE 1. GRASSHOPPERS (ORTHOPTERA: ACRIDIDAE) KNOWN TO OCCUR IN FLORIDA ARRANGED BY SUBFAMILY.

Subfamily	Genus	Species
Acridinae	*Metaleptea*	*brevicornis* (Johannson)
Cyrtacanthacridinae	Aptenopedes	*aptera* Scudder
		sphenarioides Scudder
	Eotettix	*palustris* Morse
		pusillus Morse
		signatus Scudder
	Gymnoscirtetes	*morsei* Hebard
		pusillus Scudder
	Hesperotettix	*floridensis* Morse
		osceola Hebard
		viridis (Thomas)
	Leptysma	*marginicollis* (Serville)
	Melanoplus	*adelogyrus* Hubbell
		apalachicolae Hubbell
		bispinosus Scudder
		davisi (Hebard)
		forcipatus Hubbell
		furcatus Scudder
		gurneyi Strohecker
		impudicus Scudder
		indicifer Hubbell
		keeleri (Thomas)
		nanciae Deyrup
		ordwayae Deyrup
		propinquus Scudder
		puer (Scudder)
		punctulatus Scudder
		pygmaeus Davis
		querneus Rehn and Hebard
		rotundipennis Scudder
		sanguinipes (Fabricius)
		scapularis Rehn and Hebard
		scudderi (Uhler)
		strumosus Morse
		symmetricus Morse
		tepidus Morse
		tequestae Hubbell
		withlacoocheensis Squitier and Deyrup
	Paroxya	*atlantica* Scudder
		clavuliger (Serville)
	Schistocerca	*alutacea* (Harris)
		americana (Drury)
		ceratiola Hubbell and Walker
		damnifica (Saussure)
		obscura (Fabricius)
		rubiginosa (Harris)
	Stenacris	*vitreipennis* (Marschall)
Gomphocerinae	*Achurum*	*carinatum* (F. Walker)
	Amblytropidia	*mysteca* (Saussure)
	Dichromorpha	*elegans* (Morse)
		viridis (Scudder)
	Eritettix	*obscurus* (Scudder)
	Mermiria	*bivittata* (Serville)
		intertexta Scudder
		picta (F. Walker)
	Orphulella	*pelidna* (Bermeister)

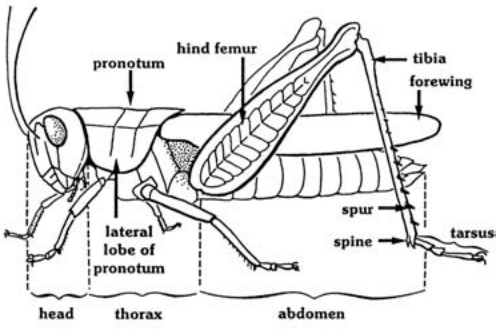
TABLE 1. (CONTINUED) GRASSHOPPERS (ORTHOPTERA: ACRIDIDAE) KNOWN TO OCCUR IN FLORIDA ARRANGED BY SUBFAM-

Subfamily	Genus	Species
	Syrbula	*admirabilis* (Uhler)
Oedipodinae	*Arphia*	*granulata* (Saussure) *sulphurea* (Fabricius) *xanthoptera* (Burmeister)
	Chortophaga	*australior* (Rehn and Hebard)
	Dissosteira	*carolina* (Linnaeus)
	Hippiscus	*ocelote* (Saussure)
	Pardalophora	*phoenicoptera* (Burmeister)
	Psinidia	*fenestralis* (Serville)
	Spharagemon	*bolli* Scudder *crepitans* (Saussure) *cristatum* (Scudder) *marmorata* (Scudder)
	Trimerotropis	*maritima* (Harris)
Romaleinae	*Romalea*	*microptera* (Beauvois)

species complex needing further study is *Gymnoscirtetes morsei* and *G. pusillus*. These relatively rare grasshoppers are difficult to distinguish.

Having fresh specimens is helpful because much of the key involves the color of grasshoppers. Grasshopper specimens tend to lose much of their color, with the green colors turning brown after drying and preservation. However, wing color remains fairly distinct, with only slight fading after preservation. In the Oedipodinae, the left wing should be spread immediately after capture; however, very old specimens can be relaxed and the wings spread. While occasionally the abdomen will shrivel, this is relatively unimportant because the abdomen usually is not an important taxonomic feature. The cerci, supra-anal plate, and the sub-genital plate, which are very important in identification, are usually unaffected by this shriveling. Many of the melanopline species, and some others, are only identifiable based on male genitalia. For this reason, it is very important that males be collected from each population to associate with the females of the same species.

The following key only treats adult acridids, but can be used to identify all species currently known to occur in the state of Florida. Females are not always indentifiable, so it is important to acquire males and identify females by association. In this key, length, when not specified otherwise, refers to the distance from the front of the head to the tip of the wings in long-winged species. In short-winged species, length refers to the distance from the front of the head to the tip of the abdomen. If the abdomen is shrunken or curved, the tips of the femora can be used instead, as this approximates the abdomen length.



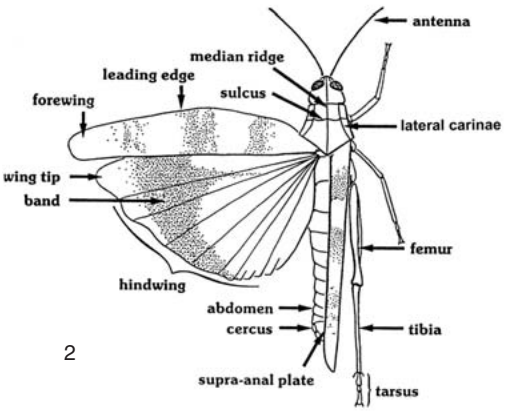


Fig. 1. Lateral view of a typical grasshopper.
Fig. 2. Dorsal view of a typical grasshopper.

KEY TO THE ADULT GRASSHOPPERS OF FLORIDA

1. Wings lacking, or apparently no wings 2

1'. Wings present 5

2(1). Small in size (12-22 mm in length); black stripe running along the sides of the body from the eyes to the tip of the abdomen; gold or brown in color; eyes round 3

2'. Medium in size (15-33 mm in length), green or brown in color, eyes oval 4

3(2). Males with dorsal edge of cerci strongly curved (Fig. 4B); tubercle at tip of subgenital plate about twice as high as wide (Fig. 4C) . . . . . *Gymnoscirtetes morsei*

3'. Males with dorsal edge of cerci not strongly curved (Fig. 5B); tubercle at tip of subgenital plate about as wide as high (Fig. 5C) . . . . . *Gymnoscirtetes pusillus*

4(2). No evidence of wings . . . . . *Aptenopedes aptera*

4'. Wings extremely reduced to small linear pads (If body is exceptionally long and narrow see *Achurum carinatum*) . . . . . *Aptenopedes sphenarioides*

5(1). Wing length short; wings distinct but less than, or about equal to, length of pronotum 6

5'. Wing length longer than length of pronotum. 32

6(5). Body form exceptionally long and narrow (Fig. 6) (If body is not long and narrow see *Aptenopedes sphenarioides*) . . . . . *Achurum carinatum*

6'. Body form not long and narrow 7

7(6). Body usually with a bold white stripe dorsally on pronotum or abdomen, or with distinct white lines running along the lateral ridges of pronotum 8

7'. Body does not have a bold white stripe on pronotum and abdomen 9

8(7). Body green in color; all antennal segments rounded; depression in the middle of vertex; small spine present ventrally between the forelegs (Fig. 7A) . . . . . *Hesperotettix osceola*

8'. Body color brown; first 9-10 segments of antennae flattened; vertex extending out beyond head to form a rounded point; spine not present between the forelegs. . . . . *Eritettix obscurus*

9(7). Body color uniformly bright green with, at most, a weak red stripe dorsally on pronotum 10

9'. Body color other than bright green. 11

10(9). Heavy-bodied species with large pronotum; texture of pronotum rough; no stripes on wings . . . . . *Hesperotettix floridensis*

10'. Body form normal; texture of pronotum smooth; bold stripe running down the center of each wing; white and red stripe running along dorsal portion of the abdomen. . . . . *Hesperotettix osceola*

11(9). Small spine not present ventrally between forelegs . . . . . *Eritettix obscurus*

11'. Small spine present ventrally between base of forelegs (Fig. 7A) 12

12(11). Body color iridescent yellowish, gold, or brown, sometimes with a black spot on pronotum; frontal costa raised and very pronounced all the way to the edge of the clypeus, frontal sutures also very pronounced (Fig. 8); uncommon. 13

12'. Body color indistinct brownish, reddish, or grayish, and with a black stripe or spot on side of pronotum; frontal costa not very pronounced and not running all the way to the edge of the clypeus, frontal sutures not pronounced 15

13(12). Forewings slightly longer than pronotum, tibiae bright red. . . . . *Eotettix signatus*

13'. Forewings shorter than pronotum, tibiae orange, yellow, or pinkish 14

14(13). Forewings nearly round. . . . . *Eotettix pusillus*

14'. Forewings oval. . . . . *Eotettix palustris*

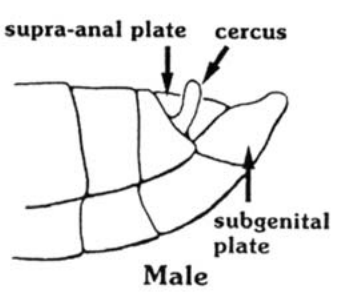
15(12). Male with distinct conical structure (pallium) pointing upward near tip of abdomen (Fig. 9C, 10C) 16

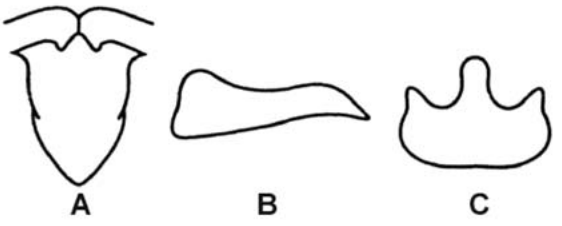
15'. Male without distinct conical structure at tip of abdomen 17

16(15). Cerci expanding at tip but flattened, lacking ventral point (Fig. 9B). . . . . *Melanoplus rotundipennis*

16'. Cerci expanded and swollen at tip, with small ventral point (Fig. 10B) . . . . . *Melanoplus withlacoocheensis*

17(15). Tip of cerci forked with at least one branch or with tooth. 18





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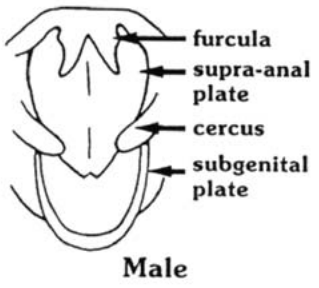

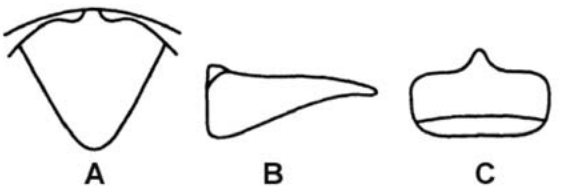

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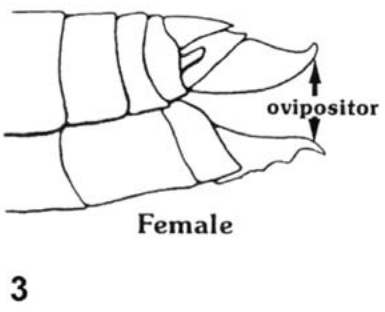

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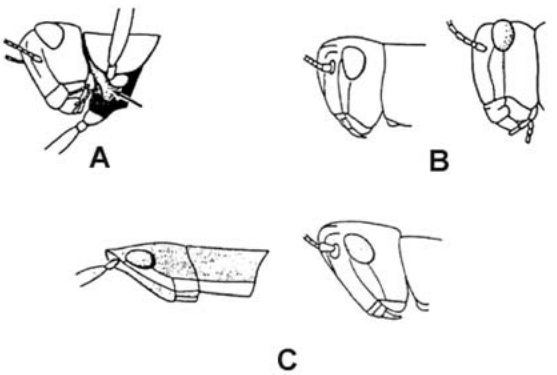

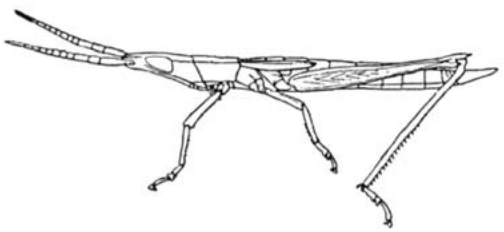

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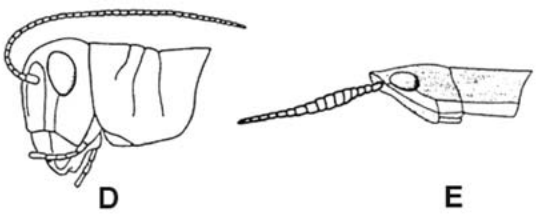

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Fig. 3. Tip of abdomen in adult male and female gr asshoppers.
Fig. 4. Male *G. morsei*; supra-anal plate and furcula (A), cercus (B) and subgenital plate (C).
Fig. 5. Male *G. pusillus*; supra-anal plate and furcula (A), cercus (B) and subgenital plate (C).
Fig. 6. *Achurum carinatum*.
Fig. 7. Ventral view of grasshopper showing spine between front legs (A), examples of grasshoppers with face not strongly slanted (B), examples of grasshoppers with a strongly slanted face (C), examples of threadlike antenna (D) and sword-shaped antenna (E).

17'. Tip of cerci not forked or branched 22

18(17). Cerci forked or split, with branches pointed dorsally and ventrally. 19

18'. Cerci not forked, tooth pointed ventrally 21

19(18). Cerci expanding from base before dividing into dorsal and ventral projections (Fig. 11B). . . . . *Melanoplus scapularis*

19'. Cerci tapering slightly before dividing into dorsal and ventral projections. 20

20(19). Cerci divided into dorsal and ventral projections near the tip (Fig. 12B) . . . . . *Melanoplus furcatus*

20'. Cerci divided in the center into a long spine dorsally and a rounded lobe ventrally (Fig. 13B) . . . . . *Melanoplus nanciae*

21(18). Cerci with large ventral tooth and small dorsal teeth on upper and inner surfaces (Fig. 14B); found in scrub habitats throughout central Florida. . . . . *Melanoplus forcipatus*

21'. Cerci with ventral tooth slender, lacking teeth on inner surface (Fig. 15B); found only in sandy habitats along the southeastern coast of Florida, north of West Palm Beach . . . . . *Melanoplus indicifer*

22(17). Cerci tapering to a point 23

22'. Cerci not tapering to a point. 28

23(22). Furcula visible (Fig. 3). 24

23'. Furcula not visible (Fig. 20A, 21A). 27

24(23). Cerci tapering rapidly, and triangular (Fig. 16B) . . . . . *Melanoplus davisi*

24'. Cerci very narrow and not triangular 25

25(24). Stripe on lateral lobe of pronotum narrows posteriorly; dorsal surface of subgenital plate with a triangular point (Fig. 17C) . . . . . *Melanoplus puer*

25'. Stripe on lateral lobe of pronotum expands posteriorly; dorsal surface of subgenital plate rounded 26

26(25). Cerci taper abruptly on dorsal margin (Fig. 18B) . . . . . *Melanoplus apalachicolae*

26'. Cerci taper equally on dorsal and ventral margins (Fig. 19B) . . . . . *Melanoplus gurneyi*

27(23). Found only in north-central Florida in Putnam and Clay counties; cerci longer than supra-anal plate (Fig. 20A,B). . . . . *Melanoplus ordwayae*

27'. Found only in south-central Florida, from Orlando south to Lake Okeechobee; cerci about as long as supra-anal plate (Fig. 21A,B). . . . . *Melanoplus tequestae*

28(22). Cerci broad, tapering only slightly (Fig. 22B) . . . . . *Melanoplus scudderi*

28'. Cerci expanding slightly beyond middle or spoon shaped (Fig. 23B) 29

29(28). Furcula short and rounded, or not visible 30

29'. Furcula evident and pointed. 31

30(29). Furcula very short (Fig. 24A) . . . . . *Melanoplus adelogyrus*

30'. Furcula not visible (Fig. 25A) . . . . . *Melanoplus pygmaeus*

31(29). Furcula large, about 1/2 the length of the supra-anal plate (Fig. 26A) . . . . . *Melanoplus strumosus*

31'. Furcula short, about 1/4 the length of the supra-anal plate or less (Fig. 23A) . . . . . *Melanoplus tepidus*

32(5). Wing length intermediate; wings appreciably longer than pronotum but not attaining tip of abdomen . . . 33

32'. Wing length long; wings nearly attaining tip of abdomen or extending beyond the tip 37

33(32). Size small (16-28 mm); color usually grass green 34

33'. Size medium to large (typically >28 mm); not green. 36

34(33). Purple or purple and white dorsal stripe present on pronotum . . . . . *Hesperotettix viridis*

34'. Dorsal stripe normally absent from pronotum; if present, stripe is brownish. 35

35(34). Lateral carinae on the pronotum cut by a single sulcus; head enlarged (Fig. 27B). . *Dichromorpha elegans*

35'. Lateral carinae on the pronotum cut by two sulci; head not enlarged (Fig. 27A) . . . . . *Dichromorpha viridis*

36(33). Size medium (22-40 mm); color usually grayish or brownish; hindwings transparent; subgenital plate deeply notched (Fig. 28C) . . . . . *Melanoplus querneus*

36'. Size large (43-70 mm); forewing color some combination of black, yellow, and reddish; hindwings brilliant red . . . . . *Romalea microptera*

37(32). Hindwings distinctly pigmented, usually brightly colored with transverse black band 38

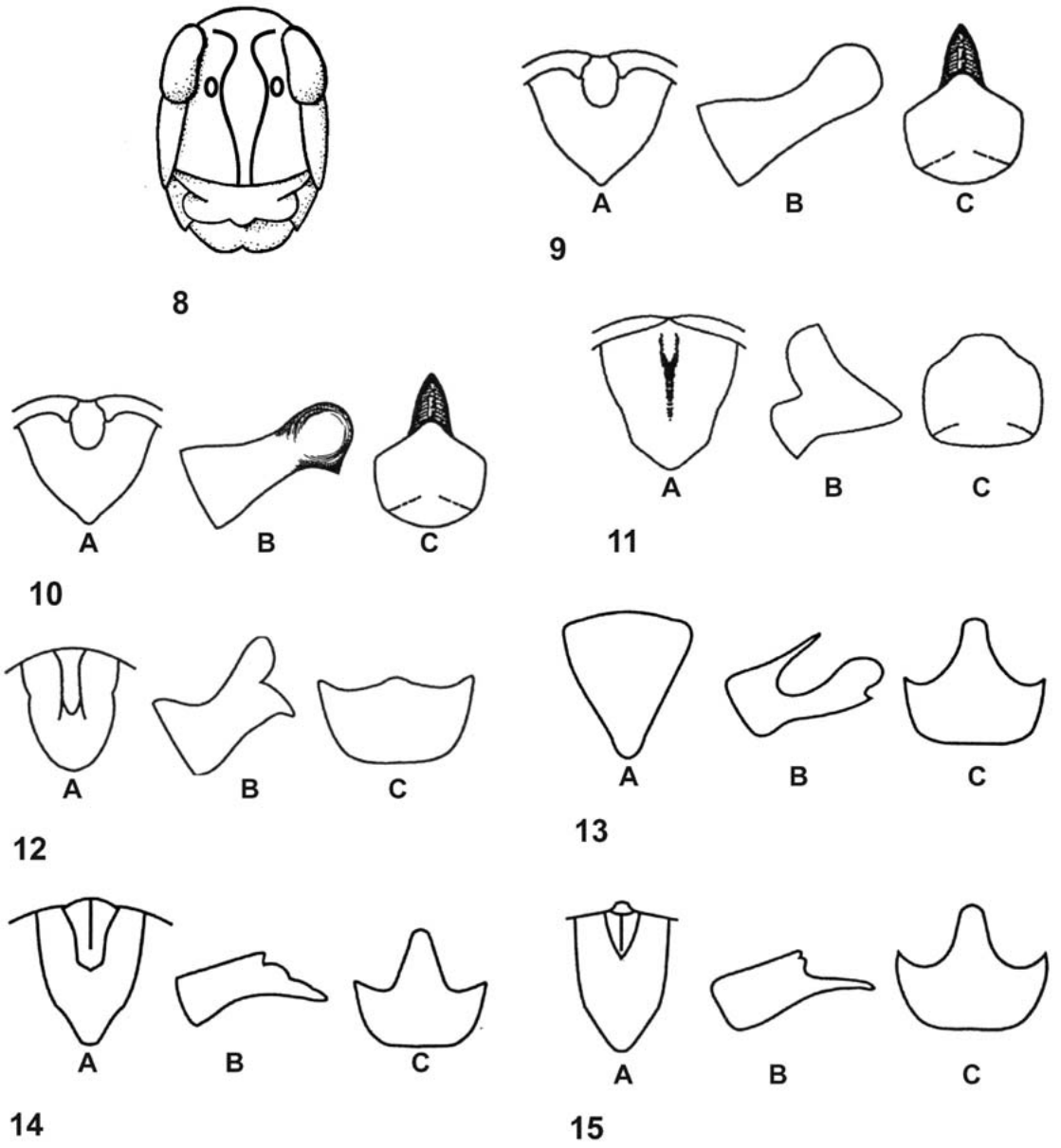

Fig. 8. Face of *Eotettix* spp.
Fig. 9. Male *M. rotundipennis*; supra-anal plate and furcula (A), cercus (B) and subgenital plate (C).
Fig. 10. Male *M. withlacoocheensis*; supra-anal plate and furcula (A), cercus (B) and subgenital plate (C).
Fig. 11. Male *M. scapularis*; supra-anal plate and furcula (A), cercus (B) and subgenital plate (C).
Fig. 12. Male *M. furcatus*; supra-anal plate and furcula (A), cercus (B) and subgenital plate (C).
Fig. 13. Male *M. nanciae*; supra-anal plate and furcula (A), cercus (B) and subgenital plate (C).
Fig. 14. Male *M. forcipatus*; supra-anal plate and furcula (A), cercus (B) and subgenital plate (C).
Fig. 15. Male *M. indicifer*; supra-anal plate and furcula (A), cercus (B) and subgenital plate (C).

37'. Hindwings not distinctly pigmented, usually transparent except for wing veins 53

38(37). Hindwings orange or pinkish 39

38'. Hindwings other than orange or pink 42

39(38). Transverse black band of hindwings wide, about 1/3 the width of the wing, and crossing near the center of the wing 40

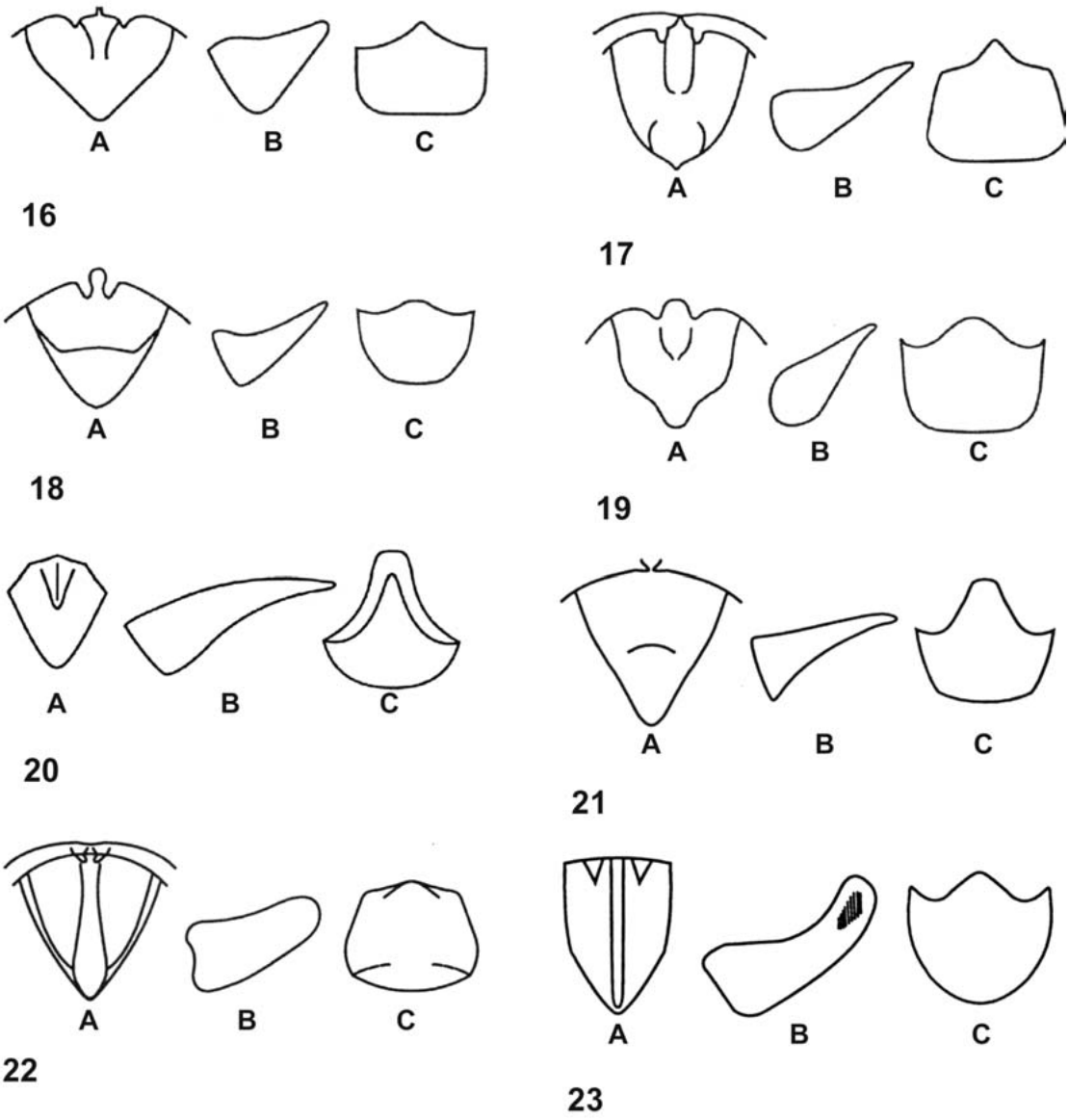

Fig. 16. Male *M. davisi*; supra-anal plate and furcula (A), cercus (B) and subgenital plate (C).
Fig. 17. Male *M. puer*; supra-anal plate and furcula (A), cercus (B) and subgenital plate (C).
Fig. 18. Male *M. apalachicolae*; supra-anal plate and furcula (A), cercus (B) and subgenital plate (C).
Fig. 19. Male *M. gurneyi*; supra-anal plate and furcula (A), cercus (B) and subgenital plate (C).
Fig. 20. Male *M. ordwayae*; supra-anal plate and furcula (A), cercus (B) and subgenital plate (C).
Fig. 21. Male *M. tequestae*; supra-anal plate and furcula (A), cercus (B) and subgenital plate (C).
Fig. 22. Male *M. scudderi*; supra-anal plate and furcula (A), cercus (B) and subgenital plate (C).
Fig. 23. Male *M. tepidus*; supra-anal plate and furcula (A), cercus (B) and subgenital plate (C).

39'. Transverse black band of hindwings not wide, about 1/4 the width of the wing or less, and not located centrally 41

40(39). Hind tibiae yellowish with black band; basal segments of antennae strongly flattened . . . . . *Psidinia fenestralis*

40'. Hind tibiae orange or red, yellow basally; basal segments of antennae weakly flattened . . . . . *Spharagemon marmorata*

41(39). Hind tibiae yellow; inner face of hind femora yellow and black . . . . . *Hippiscus ocelote*

41'. Hind tibiae orange; inner face of hind femora orange, blue, and black . . . . . *Pardalophora phoenicoptera*

42(38). Hindwings yellow. 43

42'. Hindwings black or largely transparent 52

43(42). Hindwings pale yellow basally, tips usually cloudy 44

43'. Hindwings pale yellow basally, tips usually transparent 48

44(43). Median pronotal ridge weak; hind tibiae orange or red; hindwing with dark band centrally . . . . . *Spharagemon marmorata*

44'. Median pronotal ridge pronounced; hind tibiae yellow, or yellow and black; hindwing with dark band near margin 45

45 (44). Forewings with large dark spots and transverse yellow stripe . . . . . *Hippiscus ocelote*

45'. Forewings without large dark spots; hind margin of front wings may be pale yellow, forming yellow line along back 46

46(45). Frontal costa not narrowed markedly above antennae (Fig. 29B) 47

46'. Frontal costa markedly narrowed above antennae; uncommon in Florida (Fig. 29A) . . . . . *Arphia sulphurea*

47(46). Forewings with pale yellow hind margin; median carina less elevated than in *A. xanthoptera*; common in Florida . . . . . *Arphia granulata*

47'. Forewings lacking yellow hind margin: median carina more elevated than *A. granulata*; uncommon in Florida . . . . . *Arphia xanthoptera*

48(43). Hind tibiae uniformly colored yellow to red 49

48'. Hind tibiae yellow basally and orange to red distally 50

49(48). Forewings with large dark spots and transverse yellow line . . . . . *Hippiscus ocelote*

49'. Forewings with small dark speckles, lacking transverse yellow line . . . . . *Trimerotropis maritima*

50(48). Short black band separating orange and yellow portions of hind tibiae . . . . . *Spharagemon bolli*

50'. Hind tibiae lacking black band, or with broad black band 51

51(50). Moderately elevated median carina; body usually lacking spotted or mottled pattern (if forewing heavily spotted and with transverse yellow line, see *Hippiscus ocelote*); hind tibiae subdued orange . . . . . *Spharagemon crepitans*

51'. Greatly elevated median carina; body spotted or mottled, hind tibiae bright red or orange . . . . . *Spharagemon cristatum*

52(42). Hindwings black, with yellow margin . . . . . *Dissosteira carolina*

52'. Hindwings largely transparent, with diffuse blackish area centrally . . . . . *Chortophaga australior*

53(37). Face strongly slanted (Fig. 7C); spine present or absent from between front legs 54

53'. Face not strongly slanted (Fig. 7B); spine present between front legs (Fig. 7A) 64

54(53). Tips of forewings sharply pointed; spine present between front legs 55

54'. Tips of forewings not pointed; spine absent from between front legs. 56

55(54). Head as long as pronotum, or longer; body brown, usually with a white stripe running along the base of the pronotal lateral lobe . . . . . *Leptysma marginicollis*

55'. Head shorter than pronotum; body green . . . . . *Stenacris vitreipennis*

56(54). Tips of forewings flattened, but forming sharp angle (Fig. 30) . . . . . *Metaleptea brevicornis*

56'. Tips of forewings rounded 57

57(56). Antennae clearly flattened and sword-shaped (Fig. 7E) 58

57'. Antennae not clearly flattened and sword-shaped (Fig. 7D). 60

58(57). Dorsal stripe absent from pronotum; lateral ridges absent from pronotum (Fig. 31B); white stripe may be on forewings. . . . . *Mermiria bivittata*

58'. Dorsal stripe usually present on pronotum 59

59(58). White stripe at base of forewings; lacking lateral carinae on pronotum (Fig. 31B). . . . *Mermiria intertexta*

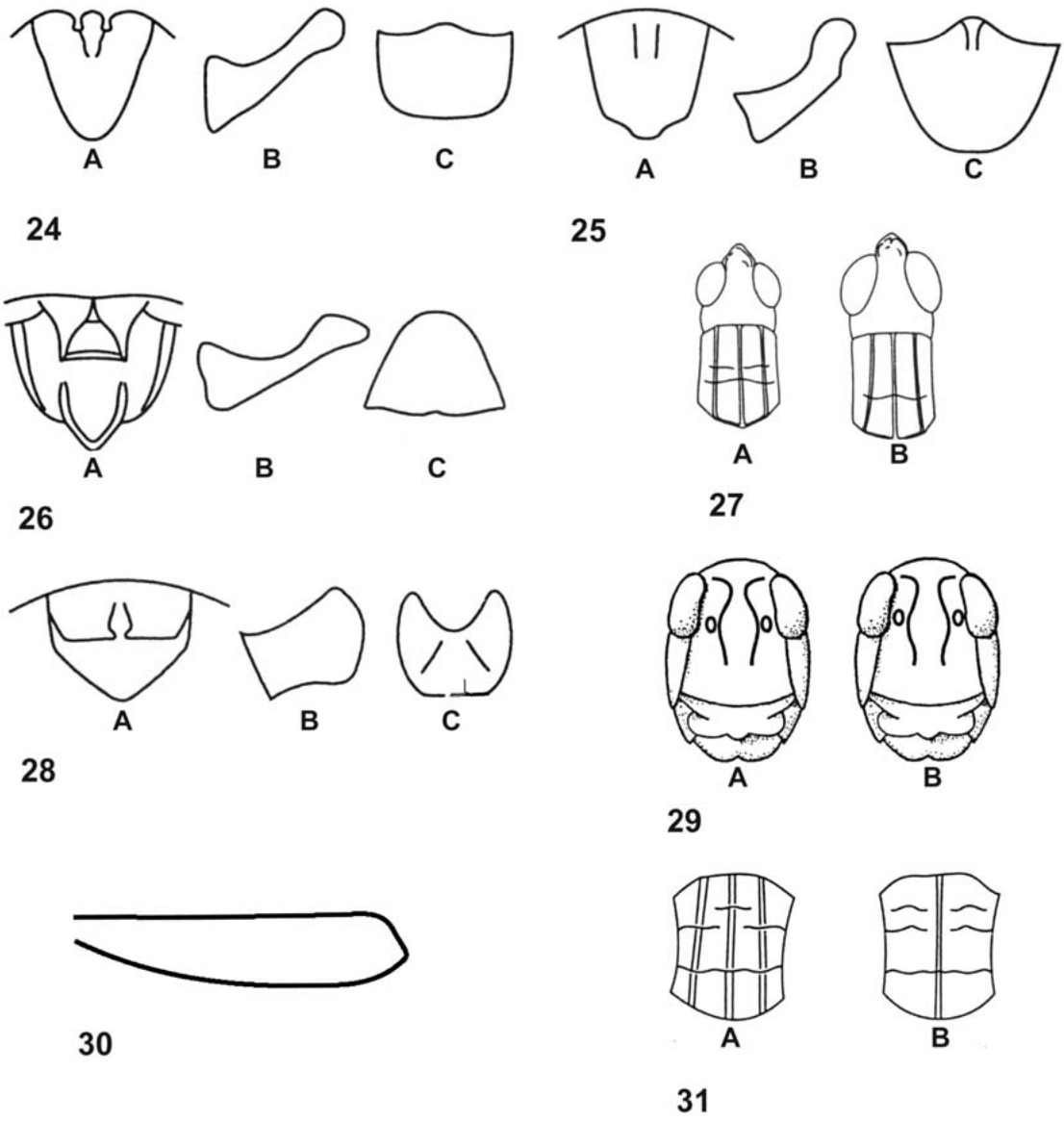

Fig. 24. Male *M. adelogyrus*; supra-anal plate and furcula (A), cercus (B) and subgenital plate (C).
Fig. 25. Male *M. pygmaeus*; supra-anal plate and furcula (A), cercus (B) and subgenital plate (C).
Fig. 26. Male *M. strumosus*; supra-anal plante and furcula (A), cercus (B) and subgenital plate (C).
Fig. 27. Two crevices or cuts in the lateral carinae on the pronotum present on *D. viridis* (A), and absent on *D. elegans* (B).
Fig. 28. Male *M. querneus*; supra-anal plate and furcula (A), cercus (B) and subgenital plate (C).
Fig. 29. Face of *A. sulphurea* (A) and *A. granulata* and *A. xanthoptera* (B).
Fig. 30. Lateral view of the forewing of *M. brevicornis*.
Fig. 31. Lateral carinae present on pronotum of *M. picta* (A), and absent on *M. intertexta* and *M. bivittata* (B).

59'. White stripe lacking from base of forewings; lateral carinae present on pronotum (Fig. 31A) . *Mermiria picta*

60(57). Lateral edge of dorsal surface of pronotum well marked with white lines 61

60'. Lateral edge of dorsal surface of pronotum not marked with white lines 62

61(60). Lateral pronotal ridges strongly compressed (Fig. 32); forewings spotted or speckled . . *Orphulella pelidna*

61'. Lateral pronotal ridges weakly compressed; forewings with a wavy pattern (Fig. 33); forewings may have distinct markings but not spotted or speckled . . . . . *Syrbula admirabilis*

62(60). Brownish, normally (in fresh specimens) with dorsal yellowish stripe on head and pronotum; males without enlarged front and middle femora; ventral surface of hind femora reddish . . . . . *Amblytropidia mysteca*

62'. Usually green, sometimes brown; lacking yellowish stripe on head and pronotum; males with enlarged front and middle femora; ventral surface of hind femora not reddish 63

63(62). Lateral carinae cut by single sulcus; head enlarged (Fig. 34B) . . . . . *Dichromorpha elegans*

63'. Lateral carinae cut by two sulci; head not enlarged (Fig. 34A) . . . . . *Dichromorpha viridis*

64(53). Male cerci broad, flat, with tip wider than base 65

64'. Male cerci with tip width about the same size or narrower than the base width 68

65(64). Male cerci with tip notched, one or both branches pointed 66

65'. Male cerci with tip not notched, bluntly rounded (Fig. 36B, 37B) 67

66(65). Male cerci with dorsal branch large and rounded, 3 times as wide as the small and pointed ventral branch (Fig. 35B); furcula visible . . . . . *Melanoplus keeleri*

66'. Male cerci with dorsal branch only slightly longer than ventral branch, and not 3 times as wide; furcula not visible (Fig. 12B). . . . . *Melanoplus furcata*

67(65). Body gray with numerous dark spots; inside of femur blood red . . . . . *Melanoplus punctulatus*

67'. Body brownish, lacking spots; inside of femur not red . . . . . *Melanoplus symmetricus*

68(64). Male cerci distinctly wider at base than at tip (e.g., Fig. 38B) 69

68'. Male cerci with width at tip about same as width at base (e.g., Fig. 42B, 44) 74

69(68). Cerci expanded at tip. 70

69'. Cerci with blunt or rounded tip, but not expanded 72

70(69). Cerci only slightly expanded at the tip (Fig 38B); black band usually indistinct; forewings with row of small spots . . . . . *Melanoplus impudicus*

70'. Cerci spoon-shaped; black band behind eye distinct on pronotum; forewings usually lacking spots 71

71(70). Black stripe normally fading on lobe of pronotum; size small: males 16-24 mm, females 22-28 mm, male cerci spoon-shaped (Fig. 39A) . . . . . *Paroxya atlantica*

71'. Black stripe normally crossing lobe pronotum, not fading; size moderate: males 20-30 mm, females 29-40 mm, male cerci spoon-shaped with small notch at tip forming an obscure lower lobe (Fig. 39B). . . . . *Paroxya clavuliger*

72(69). Body green, sometimes with purple . . . . . *Hesperotettix viridis*

72'. Body yellowish brown 73

73(72). Furcula at least 1/2 the length of supra-anal plate (Fig. 40A) . . . . . *Melanoplus propinquus*

73'. Furcula at least 1/4-1/3 the length of supra-anal plate (Fig. 41A) . . . . . *Melanoplus sanguinipes*

74(68). Cerci expanded, usually spoon-shaped at tip (Fig. 42B) 75

74'. Cerci about equal in width throughout (Fig. 44), and often flattened at tip 77

75(74). Forewings with row of small spots; dorsal surface of femur with 2-3 distinct transverse black bars . . . . . *Melanoplus bispinosus*

75'. Forewings lacking spots; femur lacking black bars 76

76(75). Black stripe normally fading on lobe of pronotum; size small: males 16-24 mm, females 22-28 mm; male cerci spoon-shaped (Fig. 39A) . . . . . *Paroxya atlantica*

76'. Black stripe normally crossing lobe of pronotum, not fading; size moderate: males 20-30 mm, females 29-40 mm; male cerci spoon-shaped but with small notch at tip forming an obscure lower lobe (Fig. 39B) . . . . . *Paroxya clavuliger*

77(74). Forewings with large dark spots. . . . . *Schistocerca americana*

77'. Forewings with small spots or lacking spots 78

78(77). Body size moderate: males 28-32 mm, females 36-40 mm; spots on forewings distinct; only found on or in close proximity to Florida Rosemary, *Ceratiola ericoides* Michx.; (if lacking spots on wings, or spots minute, see *S. damnifica*) . . . . . *Schistocerca ceratiola*

78'. Body size usually large: males often 30-40 mm, females often 42-67 mm; spots on forewings minute if present 79

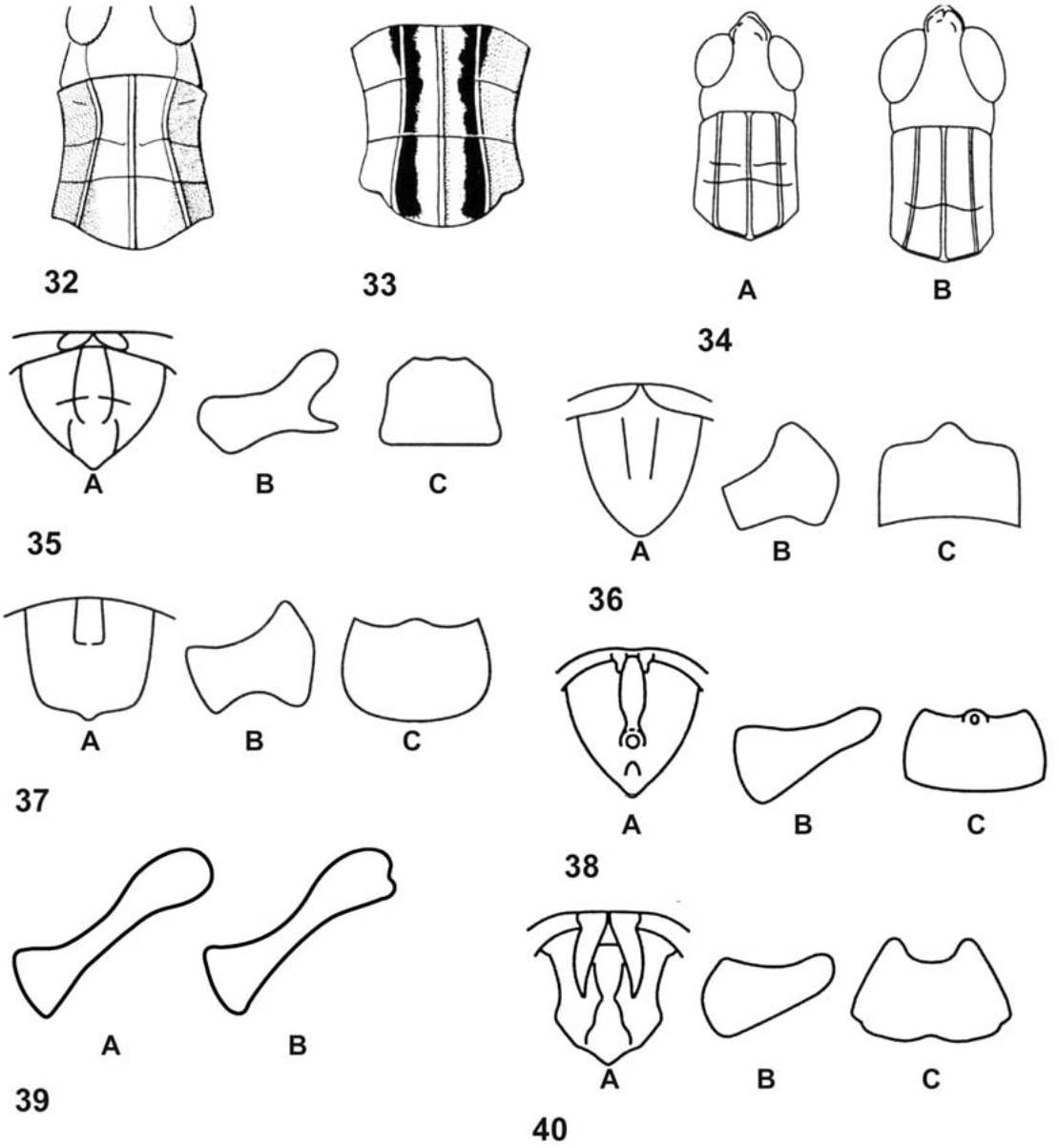

Fig. 32. Strongly compressed lateral carinae, *O. pelidna*.
Fig. 33. Weakly compressed lateral carinae, *S. admirabilis*.
Fig. 34. *D. viridis* (A) and *D. elegans* (B).
Fig. 35. Male *M. keeleri*; supra-anal plate and furcula (A), cercus (B) and subgenital plate (C).
Fig. 36. Male *M. punctulatus*; supra-anal plate and furcula (A), cercus (B) and subgenital plate (C).
Fig. 37. Male *M. symmetricus*; supra-anal plate and furcula (A), cercus (B) and subgenital plate (C).
Fig. 38. Male *M. impudicus*; supra-anal plate and furcula (A), cercus (B) and subgenital plate (C).
Fig. 39. Male cerci of *P. atlantica* (A) and *P. clavuliger* (B).
Fig. 40. Male *M. propinquus*; supra-anal plate and furcula (A), cercus (B) and subgenital plate (C).

79(78). Median ridge on pronotum elevated, often lacking dorsal yellowish line on head and pronotum; antennae shorter than head and pronotum; body size moderate: males 25-35 mm, females 28-52 mm . . . *Schistocerca damnifica*

79'. Pronotum lacking elevated medial ridge; antennae much longer than head and pronotum, especially in males; body size large; males 30-46 mm, females 42-67 mm 80

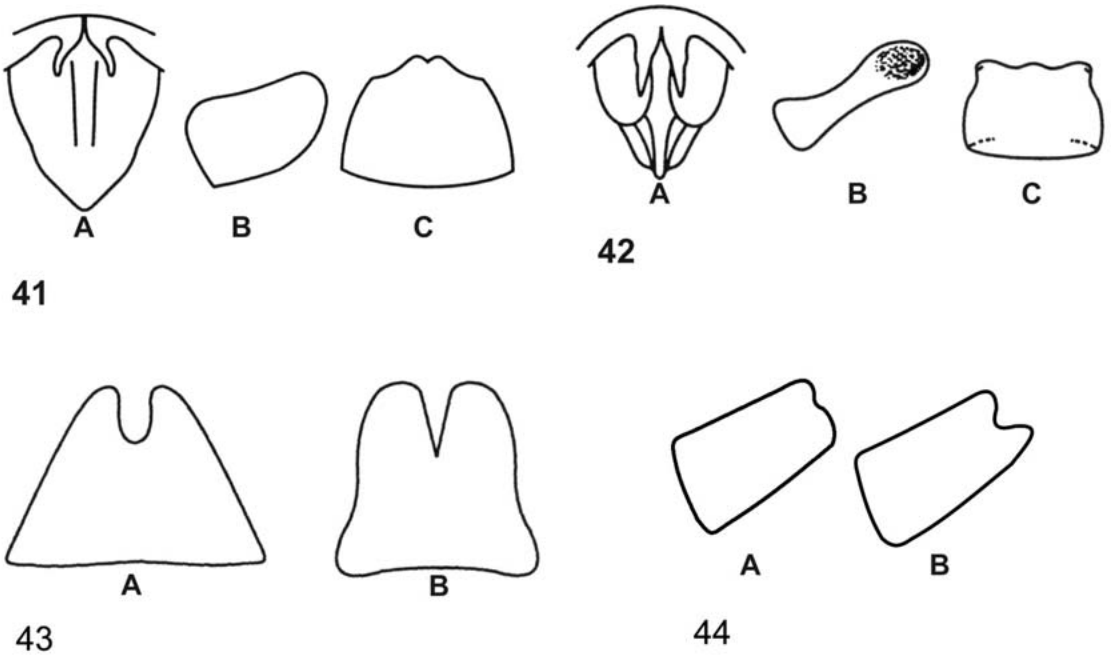

Fig. 41. Male *M. sanguinipes*; supra-anal plate and furcula (A), cercus (B) and subgenital plate (C).
Fig. 42. Male *M. bispinosus*; supra-anal plate and furcula (A), cercus (B) and subgenital plate (C).
Fig. 43. *S. alutacea* with a U-shaped notch at the tip of the males abdomen (A) and *S. obscura* with a V-shaped notch at the tip of the males abdomen (B).
Fig. 44. Male cerci of *S. rubiginosa* (A) and *S. alutacea* (B).

80(79). Tip of male abdomen, viewed from rear, with V-shaped notch in the subgenital plate (Fig. 43B); females usually over 55 mm in length . . . . . *Schistocerca obscura*

80'. Tip of male abdomen, viewed from rear, with U-shaped notch in the subgenital plate (Fig. 43A); females usually less than 55 mm in length. 81

81(80). Dorsal stripe always present; ventral lobe at tip of male cerci longer than dorsal lobe (Fig. 44B). . . . . *Schistocerca alutacea*

81'. Dorsal stripe usually absent; both lobes at tip of male cerci about equal in length (Fig. 44A). . . . . *Schistocerca rubiginosa*

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A NEW RECORD OF AN ENDEMIC CUBAN TIGER BEETLE, *CICINDELA* (*BRASIELLA*) *VIRIDICOLLIS* (COLEOPTERA: CARABIDAE: CICINDELINAE), FROM THE FLORIDA KEYS

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ABSTRACT

An endemic Cuban tiger beetle, *Cicindela* (*Brasiella*) *viridicollis* Dejean, is reported from Florida, based on a specimen in the Mississippi Entomological Museum. It is the first record of this species in the United States. A description and an illustration of the species are provided.

Key Words: Cicindelidae, Cuba, description, faunistics, immigrant insects.

RESUMEN

Se registra la presencia de un escarabajo tigre endémico de Cuba, *Cicindela* (*Brasiella*) *viridicollis* DeJean, en Florida, basado sobre un especimen del Museo de Entomologia de Mississippi. Este registro se representa el primer informe de esta especie para los Estados Unidos. Se provee la descripción y la ilustración de esta especie.

A number of years ago while studying the unidentified Cicindelinae in the Mississippi Entomological Museum at Mississippi State University, I discovered a specimen of *Cicindela* collected in Florida that was unfamiliar to me. After further study and examination of the literature, I identified it as a male *Cicindela viridicollis* Dejean, an endemic Cuban species not previously recorded from the United States. In order to verify my determination, I compared the Florida specimen with Cuban specimens of *C. viridicollis* borrowed from the American Museum of Natural History. The specimen from Florida is similar to those from Cuba in all essential characters. Choate (2003) reported the existence of this Florida specimen, but he did not examine it or report the specimen data.

The Florida specimen of *C. viridicollis* bears the following data: FLA., Monroe Co., Sugarloaf Key, 4 June 1983, W.H. Cross, Blacklight Trap. The late William H. Cross, who collected the specimen, was a U.S.D.A. research entomologist and an avid collector. Cross's field notes state that the specific location of his blacklight trap was at the Sugarloaf Lodge, which is located near mile marker 17 on U.S. Highway 1. According to James Robbins, a former graduate student of Cross who accompanied him on the collecting trip to Florida, the trap was actually located about 100 yards from the Sugarloaf Lodge in an open area with scattered shrubs (pers. comm.). It is unknown whether a population of *C. viridicollis* is or was established at this location, but it seems likely that only a very small or transient population would be overlooked by the numerous collectors that have conducted field work in the Florida Keys.

The possibility that the Florida specimen is actually a mislabeled Cuban specimen is very unlikely. Although Cross collected widely in both Central and South America, the only collecting he did in the West Indies was in the Bahamas. Also, the Mississippi Entomological Museum, which houses Cross's specimens and where the specimen was labeled, contains no contemporary Cuban insect specimens that could have served as a source for a mislabeled specimen.

Whether the occurrence of a specimen of *C. viridicollis* in the southern Florida Keys is the result of natural dispersal or accidental introduction through human activity is a matter of speculation that is not likely to be resolved. However, another Cuban species of tiger beetle, *Cicindela olivacea* Chaudoir, which is established in the Florida Keys, is hypothesized to have dispersed from Cuba by natural means (Woodruff & Graves 1963), and this may be the case for *C. viridicollis* as well. Peck & Thomas (1998) speculated that Cuba is close enough to southern Florida and the Keys (a distance of about 100 miles) that invasions of tropical species of Coleoptera by both active and passive dispersal are probably recurring phenomena. Supporting this idea is the presence in Florida of a number of Coleoptera species in several families (Cerambycidae, Coccinelidae, Scarabaeidae, and Staphylinidae) that are listed as recent immigrants from Cuba by Frank & McCoy (1992).

The habitat of *C. viridicollis* in Cuba, according to Leng & Mutchler (1916), is "along paths through grassy fields", and they provided a photo of the habitat where they collected it. They also stated that "It flies weakly and, while flying, the brilliant green head and thorax are so conspicuous as to suggest a small bee rather than a *Cicindela*." In Willis's (1968) key to *Cicindela* of North America North of Mexico, *C. viridicollis* will key to the group of three species that have the front trochanters with subapical setae and the middle

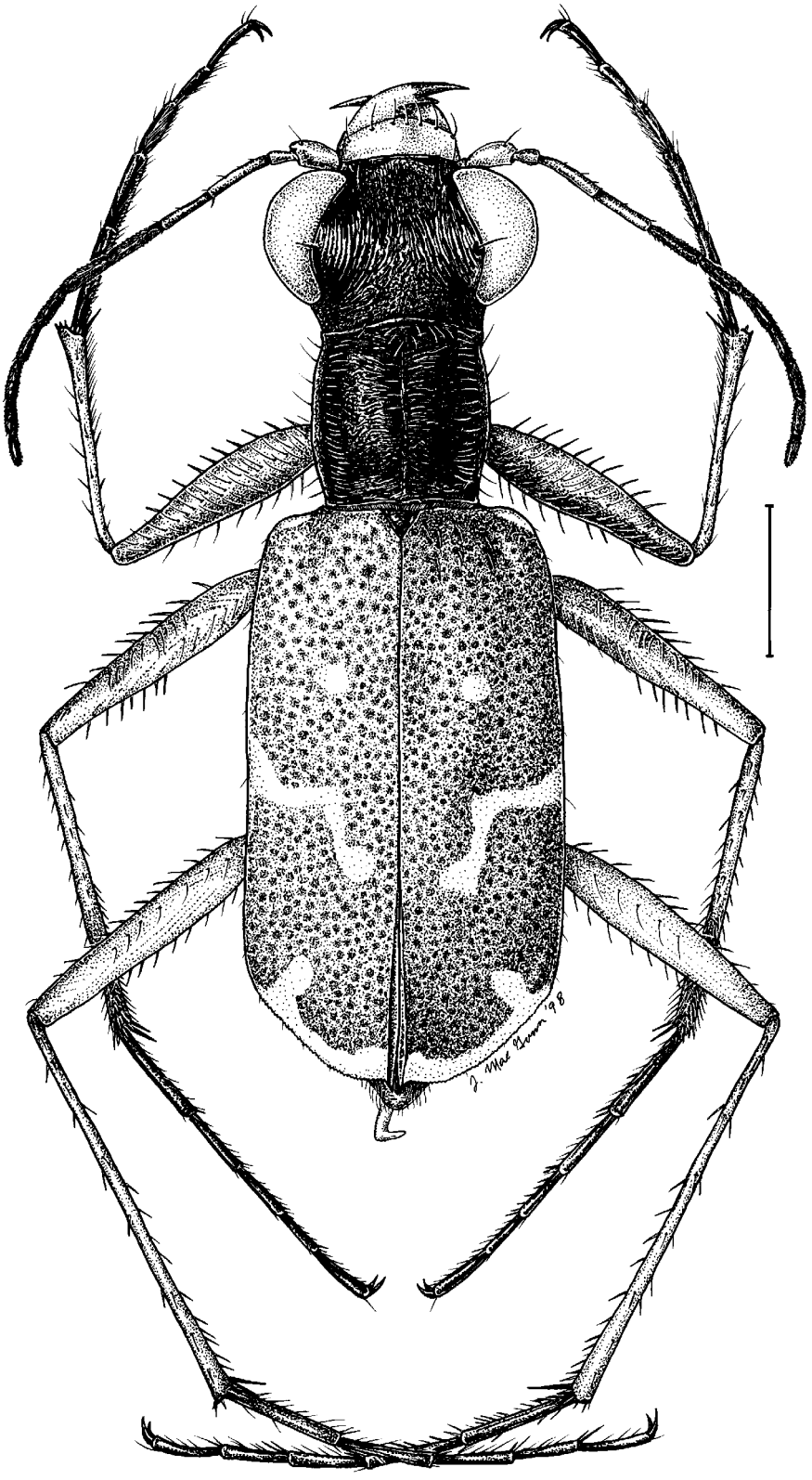

Fig. 1. Habitus of *Cicindela viridicollis* Dejean. Scale bar = 1 mm.

trochanters without subapical setae (couplets 12 and 13). These species, *C. lemniscata* LeConte, *C. wickhami* W. Horn, and *C. viridisticta* Bates, lack the brilliant green head and thorax and contrasting dull brownish-red elytra that characterize *C. viridicollis*. The general habitus of *C. viridicollis* is illustrated in Figure 1, and Choate (2003) provided color photographs of the species as well. The following description of *C. viridicollis* is given as an aid for identification and is based on six Cuban specimens in addition to the one from Florida.

Cicindela (*Brasiella*) *viridicollis* Dejean
Fig. 1

Head brilliant metallic green, glabrous except for two pairs of supraorbital setae; frons strongly longitudinally sulcate, sulci extending onto vertex, diverging posteriorly and extending laterally behind eyes; vertex strongly depressed between eyes, transversely rugose anteriorly; occiput transversely rugose medially, rugae extending anteriorly onto vertex; genae longitudinally sulcate; clypeus finely granulate to transversely sulcate; labrum brownish-yellow, subrectangular, about twice as wide as long, anterior margin slightly sinuate, small medial tooth variably present, with 5-8 submarginal setae; mouthparts brownish-yellow, except apex and teeth of mandibles and ultimate segment of both maxillary and labial palpi brown with metallic reflections; underside of head brownish-green, variably yellow-brown medially. Antennae with segment 1 yellowish, glabrous except for a single subapical setae; segments 2-4 yellowish-brown with metallic reflections, glabrous except for a few erect setae; segments 5-11 dark brownish, densely covered with short suberect setae in addition to a few longer erect setae.

Pronotum brilliant metallic green or blue-green; depressed subapically and basally; transversely sulcate becoming rugose laterally, sulci interrupted medially by fine longitudinal line; glabrous except for row of medially directed, short, white, depressed setae on lateral margins, a similar row of laterally directed setae on each side of median line anterior to middle, and a patch of similar anteriorly directed setae subapically. Proepisternum dull greenish-bronze or blackish-bronze with numerous erect white setae on lower half. Prosternum and underside of meso- and metathorax dull metallic brownish-green; glabrous except for white recumbent setae on mesepisternum, mesepimeron, metepisternum, metepimeron, and the lateral portion of the metasternum. Legs brownish-yellow except hind-coxae dull metallic brownish-green, tarsi and apex of tibia dark metallic brownish, and fore- and mid-coxae and femora with metallic reflections anteriorly; recumbent setae lacking except on lateral portion of hind-coxae; suberect, white setae present on fore- and mid-coxae and on all femora, tibia, and tarsi; males with dense pad of setae on underside of tarsal segments 1-3; subapical setae present on fore-trochanter, lacking on mid-trochanter. Scutellum triangular, brilliant metallic green. Elytra finely granulate, impunctate, dull brassy brownish-red with numerous small green or blue spots superficially resembling punctures, subsutural row of punctures lacking, extreme base metallic green; whitish markings consisting of humeral and post-humeral dots, a slightly sinuate middle band that is sometimes nearly broken, a short marginal band not connected with the apical lunule or humeral dot, and an apical lunule that is sometimes broken and isolating a subapical dot; surface slightly depressed around apical lunule; apices microserrulate and with a small sutural spine; a few scattered, transparent, erect setae present, especially basally.

Abdominal sternites dull metallic brownish-green; recumbent white setae present laterally on segments 1-6; fine, transparent setae present medially on segments 1-7. Segment 6 broadly, deeply emarginate in male, broadly subtruncate in female; segment 7 divided medially in male. Total body length (exclusive of mandibles) 6-7 mm.

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A FLORIDA CATERPILLAR AND OTHER ARTHROPODS INHABITING THE WEBS OF A SUBSOCIAL SPIDER (LEPIDOPTERA: PYRALIDAE; ARANEIDA: THERIDIIDAE)

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ABSTRACT

Caterpillars of *Tallula watsoni* Barnes & McDunnough regularly occur in the webs of the subsocial spider *Anelosimus studiosus* (Hentz) in south Florida. The caterpillars have not been found outside of the spider webs. Caterpillars feed on living and dead leaves that are on twigs incorporated into the webs of the spiders. A wide variety of trees and woody shrubs are accepted. In the laboratory caterpillars did not attack spiders or their prey. Spiders did not normally attack the caterpillars in the laboratory, but did so on two occasions. Pupation occurs in the spider web. We speculate that the spider web provides the caterpillars some protection from generalist predators and parasitoids. We suspect that *T. watsoni* is an obligate inquiline of *A. studiosus*. Other inquilines in the spider webs include 13 species of spiders and 10 species of insects. Two insects may have close or obligate relationships with *A. studiosus*: *Ranzovius clavicornis* (Knight) (Miridae), a scavenger, and *Zatypota crassipes* Townes (Ichneumonidae), a parasitoid of *A. studiosus*.

Key Words: *Tallula watsoni*, *Anelosimus studiosus*, inquilines, *Zatypota crassipes*.

RESUMEN

Los gusanos de *Tallula watsoni* Barnes & McDunnough a menudo se encuentran en las telas de la araña subsocial *Anelosimus studiosus* (Hentz) en el sur de Florida. Los gusanos no han sidos encontrados fuera de las telas de estas arañas. Los gusanos se alimentan sobre las hojas vivas y muertas sobre las ramas incorporadas dentro de las telas de las arañas. Una variedad amplia de árboles y arbustos estan asociados. En el laboratorio los gusanos no atacaron las arañas o sus presas. Normalmente, las arañas no atacaron los gusanos en el laboratorio, sin embargo paso en dos ocasiones. Los gusanos empupan en la tela de araña. Nosotros especulamos que la tela de araña provee a los gusanos con alguna protección de los depredadores y parasitoides generalistas. Nosotros sospechamos que *T. watsonie* es un inquilino obligado de *A. studiosus.* Otros inquilinos en las telas de estas arañas incluyen 13 especies de arañas y 10 especies de insectos. Dos insectos pueden tener una relación cercana u obligatoria con *A. studiosus*: *Ranzovius clavicornis* (Knight) (Miridae), un oportunista y *Zatypota crassipes* Townes (Ichneumonidae), un parasitoide de *A. studiosus*.

The insect world abounds with evolutionary opportunists stealing resources from fierce predatory arthropods. Camp-follower silverfish and wasps scurry beside raiding army ants (Hölldobler & Wilson 1990), milichiid flies land beside the fangs of spiders and sip the blood of crushed stink bugs (Eisner et al.1991), and satellite flies dart in to deposit larvae on the prey of fly-eating digger wasps (Evans 1966). This paper portrays another apparently risky relationship: a caterpillar that makes its home and finds its food in the communal webs of a subsocial spider.

Tallula watsoni Barnes & McDunnough (Fig. 1) is a moth in the subfamily Epipaschiinae of the family Pyralidae. It is known from Maryland through Florida (Adams 2003). To our knowledge, the only published host record is "larvae on oranges" (Kimball 1965), presumably referring to caterpillars feeding on orange tree foliage, not fruits. At the Archbold Biological Station (ABS) in south-central Florida we have repeatedly raised adult moths from caterpillars feeding on leaves incorporated into the webs of the spider *Anelosimus studiosus* (Hentz). This spider, whose range extends from New England to Argentina (Jones & Parker 2002), spins irregular, tangled webs in vegetation; at the ABS the webs are always on outer twigs of trees and shrubs. The web is begun by a single female, who is eventually accompanied by her offspring, including up to 50 individuals (Brach 1977, Furey 1998). The larger webs may be inhabited by inquilines, including a mirid bug, *Ranzovius clavicornis* (Knight), which scavenges on dead insects (Wheeler & McCaffrey

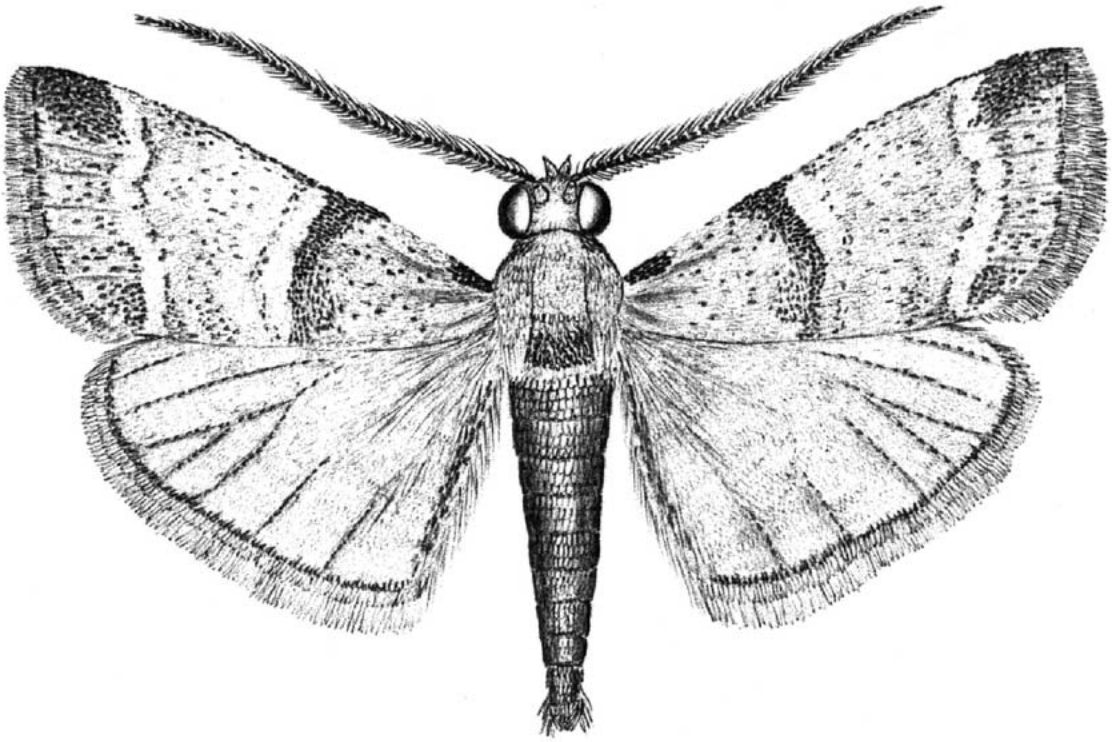

Fig. 1. *Tallula watsoni*, adult male; wingspread 16.0 mm.

1984). Vincent Brach, whose 1977 paper was based on work done at the ABS, mentions that the webs may be "shared by a host of other arthropods." The "pyralid webworms" that he found were almost certainly *T. watsoni*. In our paper we present some details of the natural history of *T. watsoni* and consider some implications of its inquiline life style.

Materials and Methods

The Archbold Biological Station, Highlands County, in south-central Florida has several plant associations where *A. studiosus* regularly occurs, including seasonal ponds with woody *Hypericum* species, Florida rosemary (*Ceratiola*) barrens, and oak scrub around the margin of a lake. For details of the vegetation of the ABS, see Abrahamson et al. (1984). Twenty-eight assemblages of spiders and caterpillars were kept in the laboratory by clipping the twigs in which the webs were constructed and placing the twigs in small jars of water. A plastic bag was placed over the twigs to maintain humidity and to retain the spiders' prey, wild-caught *Drosophila* sp. Caterpillars were also removed from the twigs and reared on foliage without spiders. Thirty-nine webs were removed from the field and completely dissected in the laboratory, with tabulation of the arthropod species found in the web system. Between February and April, 1997, we surveyed *A. studiosus* webs in the field for presence/absence of caterpillars. Parasitoids of the spiders also were surveyed.

Results and Discussion

Distribution of Caterpillars in the Field

At the ABS we disassembled and examined 503 webs of *A. studiosus*, of which 34 (6.8%) contained one or more caterpillars of *T. watsoni*; a total of 63 caterpillars were found. Up to four caterpillars occurred in a web system. Caterpillars fed on leaves from a wide variety of trees and shrubs whose leaves were incorporated into the spider webs. Plant hosts were: Empetraceae: *Ceratiola ericoides* Michaux; Fagaceae: *Quercus geminata* Small, *Q. chapmanii* Sargent, *Q. myrtifolia* Willdenow, *Q. virginiana* Miller; Myricaceae: *Myrica cerifera* L.; Asteraceae: *Baccharis halimifolia* L.; Ericaceae: *Lyonia fruticosa* (Michaux); Hypericaceae: *Hypericum edisonianum* (Small) Adams & Robson; Ulmaceae: *Celtis laevigata* Willdenow.

Behavior of Caterpillars and Spiders in Webs

The following observations on *T. watsoni* were made in the field and in the laboratory. Caterpil-

lars in the field were associated with gnawed leaves and with frass caught up in the webbing. In the laboratory, caterpillars fed on dead leaves as well as living leaves. In the field, dead leaves that fell from shrubs into the spider webs sometimes showed damage and associated frass, as if the caterpillars had fed on them. In the laboratory, caterpillars made a loose network of webbing, both when isolated from spiders and when kept together with spiders. We never found caterpillars in the field that had set up webs independently, without spiders. Spiders and caterpillars were often adjacent to each other in a web (Fig. 2D); we do not know whether the spiders move into, or utilize in any way, the webbing spun by the caterpillars. In the laboratory, caterpillars apparently did not respond to spiders or their prey. They spent most of the day suspended motionless in the web, with the head cocked back in a distinctive way (Fig. 2C). On two occasions, when a caterpillar was being introduced into a spider colony, the spiders attacked and ate the caterpillars. Pupation occurs in the web (Fig. 2B); we observed no interaction between spiders and pupae. Adults emerging in the laboratory always escaped from the spiders' web. During the day, adults assume a characteristic posture with the wings partially furled (Fig. 2A). We did not observe mating or oviposition. Caterpillars are occasionally (five instances out of 63 caterpillars) attacked by a parasitoid wasp, *Apanteles* sp. (Braconidae). One caterpillar produced a parasitoid wasp in the family Chalcididae: *Brachymeria hammari* (Crawford). At the ABS this wasp has also been reared from *Antaeotrichia vestalis* (Zeller) (Oecophoridae), whose caterpillars make retreats of leaves sewn together with silk. *Brachymeria hammari* is a widely distributed species known to be a primary parasitoid of caterpillars in the families Pyralidae, Gelechiidae, and Tortricidae (Burks 1960).

As far as we know, *T. watsoni* is an obligate inquiline of *A. studiosus*, at least at the ABS. We also suspect that larval *T. watsoni*, unlike the mirid *Ranzovius clavicornis* studied by Wheeler & McCaffrey (1984), is exclusively phytophagous. There is no evidence that it scavenges on dead insects in the manner of caterpillars of *Neopalthis madates* Druce (Noctuidae) in the webs of the tropical social spider *Anelosimus eximius* Simon (Robinson 1977).

There is no reason to suspect that *T. watsoni* has any positive or negative effect on *A. studiosus*. The caterpillars contribute webbing that might entangle passing insects that then fall into the spiders' web, but it seems unlikely that this occurs to any significant extent, or that the ability to produce webbing is limiting to the spiders. The spiders often shelter or hide below leaves in the web, but we have not seen cases in which the caterpillars removed all such leaves, depriving the spiders of shelter. We offer no hypothetical adaptive reason why the spiders should tolerate edible caterpillars in their webs. The simplest hypothesis is that the caterpillars avoid triggering predatory responses, possibly by remaining motionless when the spiders approach. It is reasonable to suppose that communal spiders are somewhat less reactive to movement in their web than are solitary spiders (Wheeler & McCaffrey 1984). This would help explain the variety of inquilines, including *T. watsoni*, that inhabit webs of *A. studiosus* at the ABS (see list below).

It is easier to speculate about possible advantages and disadvantages of its peculiar life style to *T. watsoni*. An obvious disadvantage is that the polyphagous caterpillars forego all but a tiny fraction of potential host material by confining its consumption to vegetation within the web of a particular spider. There is also the possibility that some caterpillars may fall prey to spiders, or that they may restrict their feeding and movements in order to prevent triggering a predatory response from the spiders. An advantage to living in a spider web might be protection by the spiders and their web from local predators that may be reluctant to enter spider webs, such as certain Formicidae, Vespidae, Tachinidae, Ichneumonidae, Braconidae, and Chalcidoidea. While this seems a likely benefit, it has not been tested.

We provisionally classify *T. watsoni* in a general category of inquilines associated with well-defended hosts that are presumed to be defended by those hosts, even though studying the inquiline in the absence of its host is not practical. There are familiar examples of this, such as the relationship between clown fish and stinging sea anemones. There are four additional species of *Tallula* in North America, and it is possible that one or more of these species is free-living and could be used in a comparative study of relative mortality.

Other Inquilines of *A. studiosus* at the Archbold Biological Station

As mentioned by Brach (1977), a wide variety of arthropods can be found in the webs of *A. studiosus* and on the vegetation incorporated into the webs. At the ABS 39 webs were removed from the field and fully dissected, with all species of arthropods noted from each web. In the following list, the number following the name of an arthropod indicates the number of samples (out of 39) that had the arthropod listed; numbers of individuals are not tallied. Araneida: Araneidae: *Metazygia* sp. (3), *Eustala* sp. (1); Linyphiidae: *Florinda coccinea* (Hentz) (2); Salticidae: *Hentzia palmarum* (Hentz) (1), *Hentzia* sp. (5), *Peckhamia* sp. (1); Anyphaenidae, unidentified to genus (8); Clubionidae: *Castianeira* sp. (1); Theridiidae: *Dipoena* sp. (1); *Argyrodes trigona* (Hentz) (4); Tetragnathidae: undetermined to genus (6); Mimetidae: *Mimetus* sp. (2); Oxyopidae: *Peucetia viridans* (Hentz) (2);

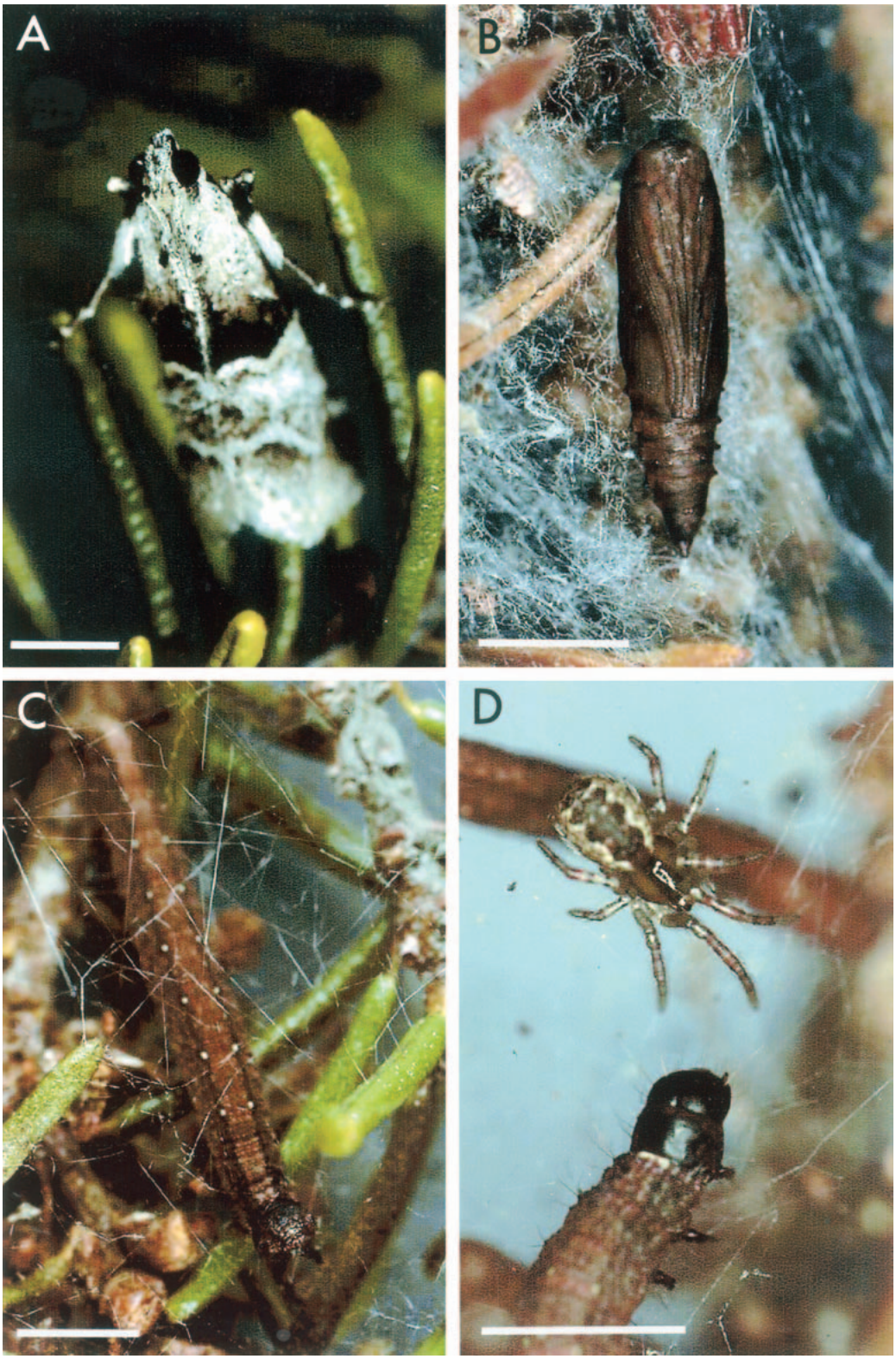

Fig. 2. *Tallula watsoni*: A: Adult; B: Pupa suspended in webbing; C: Larva in webbing; D: Larva in webbing with *Anelosimus studiosus*. Lines = 2 mm.

Heteroptera: Tingidae: *Corythuca floridana* Heidemann (1); Miridae: *Ranzovius clavicornis* (16); Diptera: Empididae: *Drapetis* sp. (2); Psocoptera: unidentified to family (12); Collembola: Entomobryiidae: unidentified to genus (15); Hymenoptera: Formicidae: *Monomorium viride* Brown (1), *Crematogaster ashmeadi* Mayr (1); Lepidoptera: Pyralidae: *Tallula watsoni* (8); Coleoptera: Tenebrionidae: *Epitragodes tomentosus* (LeConte) (1).

During the survey of 503 webs, wasp larvae (Ichneumonidae) were found feeding externally on five spiders. Four females and one male of *Zatypota crassipes* Townes were reared from these larvae. This appears to be the first record of *Z. crassipes* from any host spider. Members of the genus *Zatypota* are all presumed to be external parasitoids of spiders (Townes & Townes 1960). The cocoon of *Z. crassipes* is pale brown and covered with semi-erect loops of silk. Several other species of *Zaptypota* also have loops of silk covering their cocoons (Townes & Townes 1960); the function of these loops is unknown.

Aside from *T. watsoni*, the mirid *Ranzovius clavicornis* and the wasp *Z. crassicornis*, it is unlikely that many of the arthropods listed above have a close or obligate relationship with *A. studiosus*. Some may even be potential prey items with short persistence in the webs. Further studies at other sites might help distinguish between casual inquilines, habitual inquilines, and inquilines that are dependent on *A. studiosus*. There might also be oligolectic phytophagous inquilines that require plants that are absent from the ABS. The web inhabitants of *A. studiosus*, if studied throughout the range of the spider, could present an interesting model of the transitions between opportunism and specialization.

Acknowledgments

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EXTERNAL MORPHOLOGY AND DEVELOPMENT OF IMMATURE STAGES OF *ELACHERTUS SCUTELLATUS* (HYMENOPTERA: EULOPHIDAE) IN FLORIDA: THE FIRST NORTH AMERICAN RECORD

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ABSTRACT

The first North American record for *Elachertus scutellatus*, a parasitoid of *Calpodes ethlius* Stoll, occurred August 1999, in Florida. A simple rearing protocol was established to allow the morphology and development of this wasp to be examined. The egg and larval morphology and development of *E. scutellatus* resemble other *Elachertus* species. The freshly-ecdysed pupa, on the other hand, is rare among parasitoids in that it secretes a fluid from its anus which, when dry, fastens the pupa to its substrate. The death of the colony after eight months has many possible explanations including a laboratory-induced castration, inappropriate food source(s), and pathogenic infection.

Key Words: *Elachertus scutellatus*, *Calpodes ethlius*, koinobiont, ectoparasitoid, development, morphology.

RESUMEN

Le premier rapport nord-américain pour le *scutellatus d'Elachertus* s'est produit en août 1999, en Floride. Un protocole d'élevage a été établi pour permettre la morphologie et le développement de cette guêpe à examiner. L'oeuf morphologie et développement larvaires de *E. scutellatus* ressemblent à l'autre espèce *d'Elachertus*. Des chrysalides fraîchement "ecdysed", d'autre part, est rare parmi des parasitoids parce qu'il sécrète un fluide de son anus qui si sec attache les chrysalides à son substrat. La mort de la colonie après huit mois a beaucoup d'explications possibles comprenant une castration induite dans le laboratoire, source(s) inadequate de nourriture et infection pathogène.

Translation provided by the authors.

The names *Elachertus scutellatus* and *Ardalus scutellatus*, were used by Howard (1897) and Ashmead (1900) to describe what is now considered to be a single species of eulophid wasp (Boucek 1988). *Elachertus scutellatus* Howard, within the tribus Elachertini, is the name now used by convention (Boucek 1988). Boucek (1988) has provided a complete key to the genus *Elachertus*.

Although the genus *Elachertus* is cosmopolitan, it is especially well represented and diverse in Central America (Hanson & Gauld 1995). *E. scutellatus* has been recorded in the Caribbean (Howard 1897; Ashmead 1900). It was collected in southern Florida, the first North American record for this species, by the first author in August 1999 (identification confirmed by Dr. Michael Schauff, USDA, ARS, at the National Museum of Natural History, Washington, D.C.). With the exception of a few studies on the taxonomy of *E. scutellatus* and its distribution (Howard 1897; Ashmead 1900; Urlich 1932; Bennett & Hughes 1959; Boucek 1977; Cock 1985; Boucek 1988), very little is known of the biology of this wasp.

Elachertus scutellatus is an ectoparasitoid of the caterpillar of the Brazilian skipper, *Calpodes ethlius* Stoll (Lepidoptera: Hesperiidae) (Urlich 1932; Clausen 1978; Boucek 1977; Cock 1985). There are no recorded alternate hosts for *Elachertus scutellatus*. The Brazilian skipper is a serious pest of *Canna* species (Cannaceae) (Scudder 1889; Moore 1928; Young 1982; Reinert et al. 1983; Smith et al. 1994) and arrowroot, *Maranta arundinacea* (Marantaceae) (Cock 1985) in tropical and subtropical regions. Arrowroot is grown for the starch obtained from its rhizomes and is native to northern South America and the Lesser Antilles (Cock 1985). St. Vincent is the principal world producer, although it also has been grown in Barbados, Bermuda, Dominica, Jamaica, and St. Lucia (Cock 1985). The Brazilian skipper has been recorded in Bermuda since 1910 (Bennett & Hughes 1959; Cock 1985). Because of the severe damage caused to *Canna* by this caterpillar, ornamental *Canna* species are not planted extensively (Bennett & Hughes 1959; Cock 1985). In Florida, for instance, which is the northern-most permanent range of this skipper, the Miami Parks Authority discontinued the use of ornamental *Canna* plants because of the voracious feeding of skipper larvae (Smith et al. 1994).

Attempts have been made to introduce *E. scutellatus* into several Caribbean islands as a

biocontrol agent of *C. ethlius* caterpillars feeding on arrowroot. In 1951, *E. scutellatus* wasps were introduced from Trinidad to St. Vincent to control *C. ethlius* on commercial arrowroot crops (in Cock 1985). The Caribbean Agricultural Research and Development Institute (1982) made additional releases of *E. scutellatus* into St. Vincent and initiated introductions into Barbados. Biocontrol attempts in Bermuda began in 1953, when twenty adult *E. scutellatus* wasps were imported from Trinidad (Bennett & Hughes 1959). In 1960, more wasps were imported into Bermuda from Jamaica (Cock 1985).

In 1960 and 1962 the United States Department of Agriculture introduced *E. scutellatus* wasps (under the name *E. meridionalis*) into Bermuda in an attempt to control *C. ethlius* feeding on canna (Clausen 1978; Cock 1985). A successful rearing protocol was developed, but not published (F. D. Bennett, pers. comm.). Although an initial establishment of the parasitoid was confirmed, surveys in 1963 indicated that *E. scutellatus* was not maintained in Bermuda, likely due to the lower winter temperature and seasonal depletion of hosts, conditions not present at the collection sites (F. D. Bennett, pers. comm.). The Brazilian skipper is still a severe pest of canna and arrowroot plants in tropical areas (Cock 1985).

This paper provides a detailed description of the external morphology and development of *Elachertus scutellatus* with accompanying photographs.

Materials and Methods

A colony of *E. scutellatus* was established from parasitized *C. ethlius* caterpillars collected in residential gardens in southern Florida, where *Canna* is common as a perennial ornamental plant. Three main sites in the Fort Lauderdale and Pompano Beach area with persistent *E. scutellatus* wasp populations were identified between May 8 and June 30, 2000.

Elachertus scutellatus was reared in an incubator at 25°C (77°F), the average annual temperature for Fort Lauderdale, Florida as recorded by the National Oceanic and Atmospheric Administration (1998), and high RH under a L:D 14:10 regime.

Host *C. ethlius* caterpillars were reared in a greenhouse at 22°C, high RH and under long-day conditions. This caterpillar is a near-monophagous leaf-rolling species and was fed *ad libitum* on leaves of the *Canna* lily. Caterpillars were exposed to adult wasps early in the third larval stadium (MacDonald & Caveney 2004).

Pre-adult *E. scutellatus* Rearing

After exposure to wasps, the caterpillars were placed under a dissecting microscope to examine whether eggs had been laid on their integument. Parasitized caterpillars were placed individually in 100-mm diameter Petri dishes together with a damp piece of filter paper and a fresh piece of Canna leaf as food. Fresh leaf material was given daily as long as the hosts continued to feed. As a check for parasitism, apparently non-parasitized caterpillars were placed in 100-mm diameter Petri dishes with a damp filter paper and leaf material. These caterpillars were fed and observed until they completed the molt to the next instar. As molting confirmed the caterpillars were not parasitized, they were discarded.

Each Petri dish was sterilized daily with 70% ethanol, rinsed with distilled water and a new piece of damp filter paper was placed in the bottom. The host leafroll was kept folded to conserve moisture for parasitoid larvae and only opened briefly for observation each day. Upon pupation of the parasitoid, the leafroll was opened and stapled to filter paper to prevent curling of the leaf as it dried, and to allow easy observation. Observations of the immature wasp stages were made each day and the development of the parasitoid on the host was described.

Adult *E. scutellatus* Rearing

Adult wasp broods were held in Nalgene® transparent styrene-acrylonitrile utility boxes (7 × 6 × 12 cm). The wasps were provided with pure Billybee® clover honey brushed onto small yellow "Post-it"® notes (3.7 × 5 cm) stuck to the inside of the box (Hagley & Barber 1992; Pitcairn & Gutierrez 1992; Zaviezo & Mills 1999). Humidity was kept high in the container by placing moist filter paper in the bottom of the dishes. Moisture was not allowed to collect into droplets on the container walls, as the adults drowned easily within them. The wasps were removed daily with a mouth aspirator and the container was sterilized with 70% ethanol and rinsed with distilled water. The adults were sexed, mortality recorded and the healthy wasps then returned to the container with fresh honey.

One early third instar host caterpillar was provided per one or two adult female wasps. Host caterpillars were removed from the colony and exposed to adult wasps in the breeding population several times a week for an average of 24 h, although oviposition occasionally was completed within a few h.

Results

External Morphology and Development of *E. scutellatus*

Egg: The egg of *E. scutellatus* is a large, yolk-rich anhydropic structure with a thick, tough chorion (Fig. 1a). A simple anchor on the egg, possibly an extension of the chorion (Clausen 1940; Kasparyan 1981), penetrates the host cuticle and secures the egg to the host integument. The egg

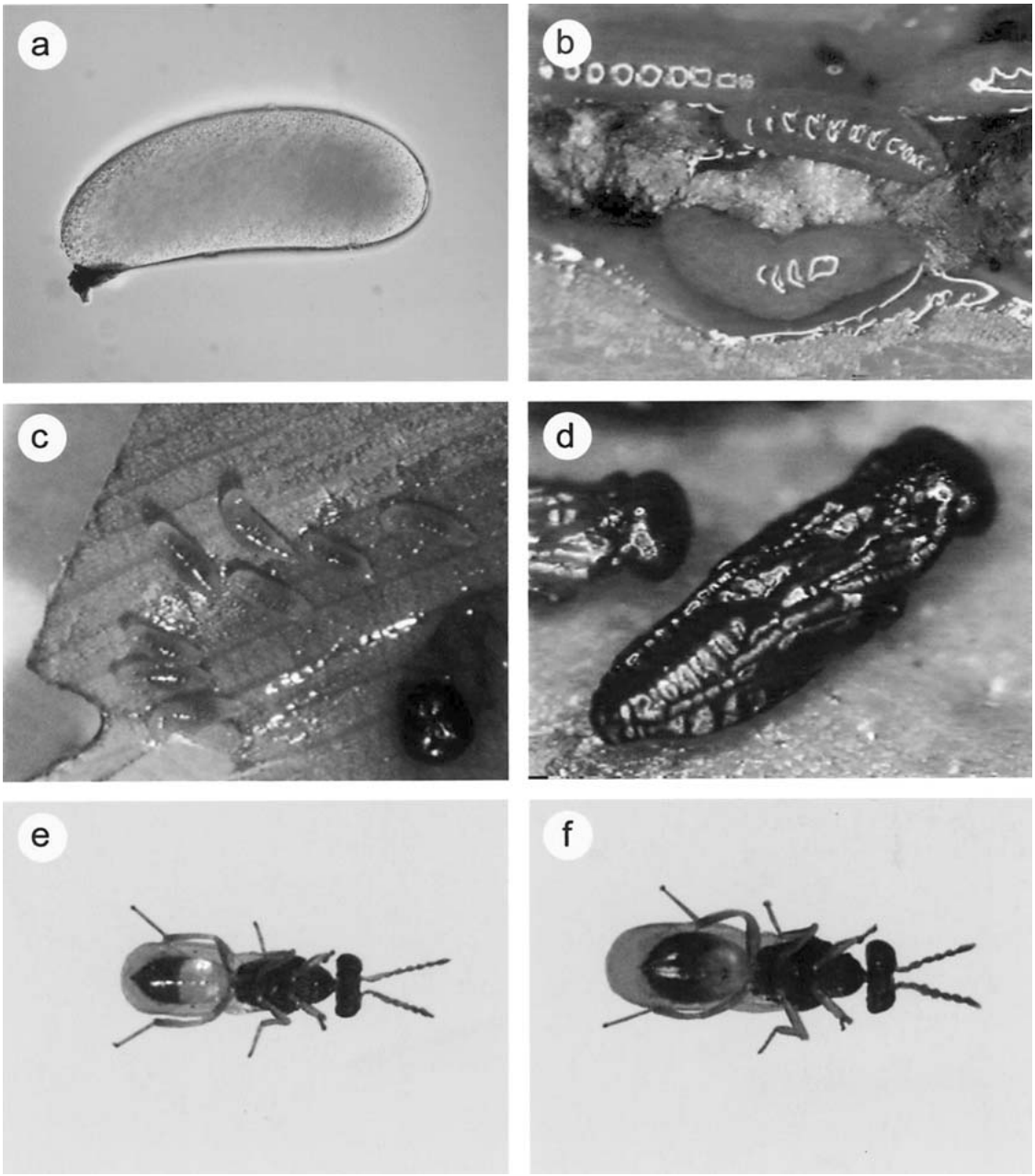

Fig. 1. Developmental stages of *Elachertus scutellatus*: a) large, proteinaceous, anhydropic egg with a portion of the egg stalk intact (magnification × 200); b) 4-day-old larvae feeding on dead, necrotic host (magnification × 20); c) 5-day-old larvae have completed feeding and wandered from host prior to pupation. They will expel their gut contents and pupate within 24 h (magnification × 10); d) 4-day-old pupae after hardening and tanning their cuticle (magnification × 30); e) adult male wasp (magnification × 20) f) adult female wasp (magnification × 20).

has the shortest developmental duration of the immature stages. Embryogenesis is completed in about two days (2.1 ± 0.2, n = 39).

Larva: *E. scutellatus* larvae are hymenopteriform, 13-segmented (Gauld & Bolton 1988) and lack any tubercles or spines (Fig. 1b). The number of instars was not determined. Following parasitoid hatching, the host caterpillar ceases to feed but continues to expel fecal pellets. The parasitoid larvae feed externally on the caterpillar for six days (6.0 ± 0.6, n = 13). During this time, the host turns from green to a dull yellow color, presumably

due to the absence of gut contents and hemolymph normally visible through its translucent cuticle. Initially, the gut contents of the wasp larvae appear white, but as feeding ensues the gut turns yellow or brown. The host caterpillar dies several days after the parasitoids begin feeding and is often entirely consumed before the wasps pupate. Frequently the hosts died prematurely, usually within two days of wasp feeding, from what appeared to be a pathogenic infection. This caused the death of the associated parasitoid larvae.

Pupa: When feeding is complete, approximately one day prior to pupation, *E. scutellatus* larvae leave the host carcass but remain within the leafroll (Fig. 1c). They then expel their gut contents and pupate. *E. scutellatus* does not spin a cocoon for pupation (Fig. 1d) but instead, as seen in most chalcidoid wasps, the pupa is obtect (appendages are held against the body by a secretion produced at the last larval molt and the exposed surfaces of the appendages are heavily cuticularized) (Richards & Davies 1964) and adecticous (has reduced mandibles not used in adult eclosion) (Richards & Davies 1964). The pupae actively pump a clear fluid from their anus (Fig. 2) prior to tanning and hardening of their exterior. When dry, this fluid becomes fibrous and attaches the pupae to the inner surface of their host's leafroll.

One to two days prior to eclosion in the female wasp, a dark ovipositor becomes visible through the pupal casing as it stands out against her yellow abdomen. Adult wasps emerge within one week of pupal ecdysis (6.5 ± 0.5, $n = 10$).

Adult: The adult wasp is black, approximately 1.5 mm long, with reddish eyes and ocelli (Howard 1897). The anterior portion of the ventral side of the adult male abdomen is translucent (Fig. 1e). The anterior portion of the ventral side of the female abdomen is honey-yellow (Howard 1897). The clearly visible ovipositor is positioned on the ventral midline of the female abdomen, but does not extend past its tip (Fig. 1f). Females were typically larger than males within a brood, although between broods the size range of the sexes overlaps considerably.

Discussion

As seen in many ectoparasitoids, *E. scutellatus* wasps lay anhydropic eggs that have a large enough quantity of yolk that the embryo can complete its development to the larval stage without the need to sequester protein and nutrients from the host (Quicke 1997). The yolk of anhydropic eggs is rich in protein, of which vitellin is the major component, and has numerous lipid droplets (Quicke 1997). A large amount of egg protein is associated with the consumption of host fluids by female wasps (Flanders 1950; Le Ralec 1995). The eggs of most parasitoids are roughly oval and many ectoparasitoid eggs have stalks or pedicels to anchor the egg to the host (Quicke 1997). The morphology of *E. scutellatus* eggs is much like the eggs of the ichneumonid subfamily Tryphoninae, another group of ectoparasitic koinobiont wasps (Mason 1967; Kasparyan 1981).

The egg of *E. scutellatus* is unusual in that it is very large with a tough chorion. This presumably protects against both desiccation (Mason 1967) and physical damage that may occur when the host is allowed to remain active and mobile after attack by a parasitoid wasp (Mason 1967). To protect against the dehydration of eggs, female wasps often will attack only hosts living in humid or partially concealed situations (Mason 1967). To protect the eggs from the active host, female *E. scutellatus* wasps most often place the eggs between the host prolegs where they are difficult

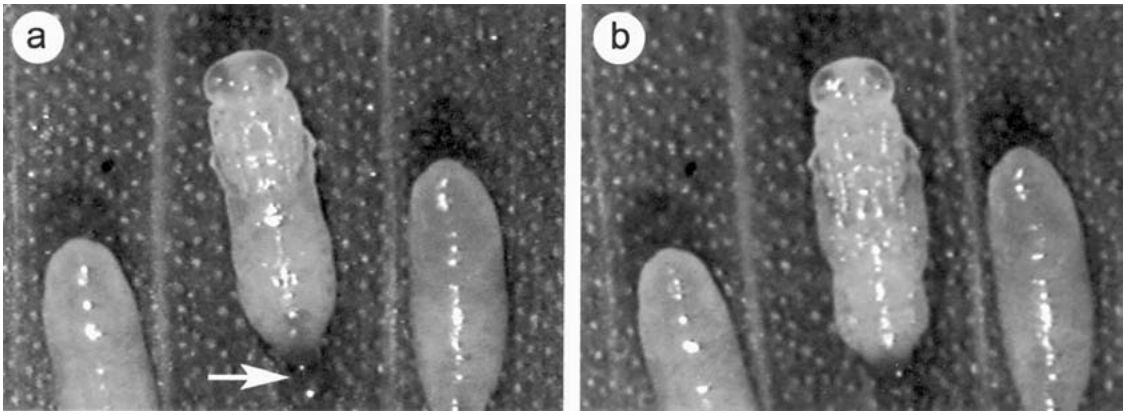

Fig. 2. Video images of a freshly-ecdysed *E. scutellatus* pupa secreting fluid from the anus. When dry, this fluid will form a fibrous attachment, fastening the pupa to the inside of the host leafroll: a) abdomen of pupa is relaxed, some fluid has been expelled from the anus (indicated by arrow); b) abdomen of pupa is contracted, actively expelling fluid from anus (magnification × 20).

for the host to detect and destroy. These constraints are claimed to account for the rarity of ectoparasitic koinobionts (Gauld 1988; Godfray 1994; Mayhew & Blackburn 1999).

The larval morphology of *E. scutellatus* resembles that of other eulophids (Gauld & Bolton 1988). The white appearance of first instars of the wasp probably is due to the prenatal yolk resorbed during embryogenesis (Anderson 1973) and as feeding ensues, the larvae take on the color of the consumed host fluids, which can be seen through the translucent cuticle. Although the number of instars for *E. scutellatus* was not determined, Hanson & Gauld (1995) determined that in other eulophids there are three to five instars.

Parasitoid larvae are capable of altering the physiology of their active hosts, increasing or arresting developmental rates (Harvey et al. 1998; Doury et al. 1995; Hemerik & Harvey 1999) and changing host feeding habits (Shaw 1981; Powell 1989). Oral secretions from larval *E. scutellatus* may be the cause of the observed cessation in caterpillar feeding (Godfray 1994; Morales-Ramos et al. 1995; Richards & Edwards 1999) as well as the lack of a host phenoloxidase reaction at larval feeding sites (Richards & Edwards 1999). Interestingly, the larvae of pimpline ichneumonids that parasitize lepidopteran pupae are capable of producing toxic anal secretions from modified Malpighian tubules that inhibit host phenoloxidase activity (Godfray 1994). Although the presence and targets of larval secretions have not been closely studied, it seems parasitoid larvae possess specific adaptations that may alter host physiology and result in an increase in their own chance of survival.

The Eulophidae are exceptional within the Chalcidoidea in that silk cocoon formation is rare (Quicke 1997). *Elachertus scutellatus* do not produce a cocoon but instead harden and tan their cuticle (Richards & Davies 1964) and fasten the pupa to the substrate with a clear fluid that becomes fibrous when dry. Fidgen and Eveleigh (1998) state that *Elachertus cacoeciae* pupae attach themselves by their meconium ["waste material excreted by an insect upon eclosion as an adult (a few parasitic wasps) or, in the case of the majority of parasitic wasps, just prior to pupation" (Quicke 1997)]. It seems unlikely that the fluid is simply a waste product in *E. scutellatus*, as the prepupae expel their gut contents far in advance of the active production of the clear fluid they use for attachment. It has been reported that many chalcidoids produce a cocoon-like structure from a liquid secreted by the anal end (and occasionally also the oral end) of the final instar (Flanders 1938; Quicke 1997). In several endoparasitoids, the fluid flows over the larvae under the pressure of the host hemolymph, then dries and hardens into an amorphous sheath often associated with host tracheae (Flanders 1938; Colazza & Bin 1992; Ceresa-Gastaldo & Chiappini 1994). In the Chalcididae, the origin of the liquid is the iliac glands, structures homologous to the specially modified Malpighian tubules found in many ichneumonoid larvae (Quicke 1997). Freshly-ecdysed *E. scutellatus* pupae may not need to produce a protective cocoon with this liquid (as they are ectoparasitic) and instead make use of the host shelter. In *E. scutellatus* the excreted fluid is restricted to the anal area and is used to fasten the pupa to its substrate. This behavior seems to be intermediate between the formation of endoparasitic or silk cocoon produced anally and a lack of anal silk excretion in most parasitoids. The hindgut of chalcid larvae is very muscular and adapted to active expulsion of cocoon-forming secretions (Flanders 1938).

Generally, adult male wasps emerge before females (Harvey et al. 1998; Hanson & Gauld 1995; Jyothi et al. 1999; Zaviezo & Mills 1999). This was also seen in *E. scutellatus*, and is likely an advantage to the male in securing a female for mating immediately upon her emergence. The observed size differences between broods is likely due to differences in available resources and competition for food as the size of parasitoids is often related positively to host size (Wen et al. 1995; Fidgen et al. 2000;) and negatively to brood size (Hardy et al. 1992; Harvey et al. 1998).

Population Mortality

The rearing of *E. scutellatus* in the laboratory ended eight months after the colony was first established. Despite close care, the size of the population declined slowly without an obvious cause until the entire population died out. The highest mortality of *E. scutellatus* occurred in the larval stage. In a closely related species, *Euplectrus plathypenae* (Eulophidae), a similar pattern of mortality was observed (Parkman et al. 1981).

The demise of the colony may have been a result of adult female behavior or physiology. According to Schneider (1941), ovarial regression (ovarialkrise) "is a tendency toward ecological castration and may so decrease the readiness to oviposit that under laboratory conditions egg deposition does not continue" (in Flanders 1950). Ovarialkrise is considered characteristic of tropical wasps (in Flanders 1950). Similarly, phasic castration (atrophy of ovaries resulting in sterility) was frequently observed in laboratory mating studies of *Chrysocharis laricinellae* (Eulophidae) (Quednau 1967).

Most laboratory-reared wasp populations are sustainable when the females are provided with a source of honey and hosts for host-feeding, and the males are provided with honey water (Hagley & Barber 1992; Pitcairn & Gutierrez 1992; Zaviezo & Mills 1999). The brand of honey routinely used to rear *E. scutellatus* was replaced for a

short duration of time with another brand, whereupon oviposition decreased. When the original brand of honey was restored, oviposition increased but failed to recover fully. It is possible the colony did not recuperate from the decline in female egg laying, but it seems unlikely that this was the cause of the death of the population. Synovigenic wasps such as *E. scutellatus* are capable of resorbing mature eggs (oösorption) should a food source become limited (Jervis & Kidd 1986).

Prior to the complete extirpation of the wasp colony, host caterpillars often turned black or bright red with yellow patches and sometimes developed a sweet odor, like that of citronella. Death of the host consequently resulted in the early death of the parasitoids. Several studies have concluded that the act of parasitoid oviposition can increase the host's susceptibility to viral infections resulting in the death of the host and associated parasitoids (Irabagon & Brooks 1974; Levin et al. 1981; Eller et al. 1988). Ectoparasitic larvae also have been shown to compromise host immune systems (Richards & Edwards 1999). The larvae of an ectoparasitic koinobiont eulophid wasp, *Eulophus pennicornis*, are known to produce factors that decrease the number of host hemocytes in order to reduce the host immune response (Richards & Edwards 1999). A reduction in host defences could result in pathogenic infection and death of both the host and the larval parasitoids. Although no direct evidence of infection was obtained, this is a possible explanation for the death of the parasitoid colony.

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NEW GENERA AND NEW SPECIES OF COLPURINI (HETEROPTERA: COREIDAE) FROM IRIAN JAYA AND PAPUA NEW GUINEA

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ABSTRACT

Two new genera and two new species from Irian Jaya and Papua New Guinea are described and illustrated. These taxa are discussed in relation to others in the tribe Colpurini.

Key Words: Insecta, Heteroptera, Coreidae, Colpurini, new genera, new species, New Guinea.

RESUMEN

Dos nuevos géneros y dos nuevas especies provenientes de Irian Jaya, y Papua Nueva Guinea son descritos e ilustrados. Cada taxa es discutido en relación con otros incluidos en la tribu Colpurini.

Translation provided by the author.

The tribe Colpurini is represented in the South Pacific Islands by a number of species that show various bizarre morphological specializations. The most striking features are the development of the head including the eyes, the remarkable reduction of the hemelytra, the great diversity in the male genital capsule and in the female genital plates, and the high degree of endemicity.

The knowledge of the tribe Colpurini, which includes 47 genera, has been summarized by Brailovsky (2003). Since completing that work, the author has accumulated additional material, including two new genera and two new species collected in Indonesia (Irian Jaya) and Papua New Guinea.

Based on the morphology of abdominal sternite VII in females, these genera are included in the group lacking a plica and fissura, and therefore abdominal sternite VII is entire (Brailovsky 2003). This paper is a contribution to the continuing effort to revise the tribe Colpurini to make identifications possible, and to stimulate the study of their biology in a wide sense.

All measurements are given in millimeters. Acronyms used are BMNH (The Natural History Museum, London, England); RNHL (Rijksmuseum van Natuurlijke Histoire, Leiden, Netherlands); SMTD (Staatliches Museum fur Tierkunde, Dresden, Germany); UNAM (Instituto de Biología, Universidad Nacional Autónoma de México); ZMHB (Zoologisches Museum, Humboldt Universitat, Berlin, Germany); ZSMC (Zoologische Staatsammlung Munchen, Germany).

Cephalohygia Brailovsky **NEW GENUS**

Type species: *Cephalohygia decorata* Brailovsky, monobasic.

Description. Head longer than wide, pentagonal, and dorsally flat; tylus unarmed, apically globose, extending anteriorly to and laterally higher than juga; juga unarmed, thickened, and apically rounded; antenniferous tubercles unarmed, with apices truncated; antennal segment I moderately robust, thickest, slightly curved outward, and shorter than maximal length of head; segments II and III cylindrical and slender, segment IV fusiform; antennal segment II longest, III shortest, and IV longer than I; ocelli well developed; preocellar pit deep; eyes spherical; postocular tubercles protuberant; buccula elongate, barely raised, short, not extending beyond antenniferous tubercles, without sharp spiny projection; rostrum extending beyond the apex of the last abdominal sternite; mandibular plate and genae unarmed (Fig. 1). Thorax: Pronotum wider than long, trapeziform, bilobed, and declivant; collar wide; frontal angles projected forward as tiny lobe; humeral angles rounded, not exposed; anterolateral borders sinuate, smooth; posterolateral and posterior borders straight, smooth; calli weakly convex; anterior lobe of metathoracic peritreme reniform, and posterior lobe sharp, small. Legs unarmed; tibiae sulcate. Scutellum triangular, longer than wide, with apex subacute to rounded, and weakly raised. Hemelytra macropterous, almost reaching the apex of the last abdominal segment; costal margin emarginate; apical margin obliquely straight; clavus and corium densely punctate; apical margin of endocorium almost impunctate. Abdomen: Connexivum higher than terga, with posterior angle of each segment entire, not projected into a spine. Male genitalia: Posteroventral edge of genital capsule with a pronounced U-shaped concavity expanded at middle third as small plate, laterally enclosed by two large and relatively slender arms (Figs. 2-3). Female genitalia: Abdominal sternite VII without plica or fissura, and with posterior margin of the plate projected as a triangular broad expansion; gono-

coxae I broad, enlarged antero-posteriorly, in caudal view with the concave mesial margins closed, in lateral view with upper third conspicuously convex and exposed, and lower third with apical angle protruding into small lobes; paratergite VIII short, square, with visible spiracle; paratergite IX squarish, longer than paratergite VIII (Figs. 7-8).

Integument. Body surface rather dull, almost glabrous.

Discussion. *Cephalohygia* New Genus, is similar to *Acarihygia* Brailovsky (1993) and *Monasavuhygia* Brailovsky (1996). The three genera share the following characters: head remarkably elongate, longer than wide, length of antennal segment I shorter than maximal length of head, tylus apically globose, antenniferous tubercles unarmed, buccula short without sharp spiny projection, legs unarmed, and abdominal sternite VII of female without plica or fissura.

Acarihygia and *Monasavuhygia*, described from the Fiji Islands, differ from *Cephalohygia* in possessing the following characters: micropterous, ocelli absent, abdominal segments IV to VI strongly convex, scutellum wider than long, antennal segment III the shortest or I, III and IV subequal, and rostrum reaching middle third of abdominal sternite V or apex of the last abdominal sternite. *Cephalohygia*, known from Irian Jaya (Indonesia), is macropterous, with ocelli well developed, scutellum longer than wide, antennal segment IV the shortest, rostrum remarkably elongate, extending beyond the apex of the last abdominal sternite, and abdominal segments IV to VI are not strongly convex.

Etymology. Referring to the appearance of the head.

Cephalohygia decorata Brailovsky, **NEW SPECIES**
Figs. 1-3, 7-8

Description. Holotype male. Dorsal coloration: head dark brown with longitudinal stripe adjacent to eyes, dorsal aspect of postocular tubercles, tylus and jugum yellow to pale brownish-orange; antennal segments I and II orange yellow, III brown with basal joint yellow, and IV pale yellow with basal joint brown; pronotum brownish-orange with irregular creamy white marks at middle third, and the area between calli and middle line of collar reddish brown; scutellum brownish-orange, with apex creamy yellow; clavus and corium brownish-orange with punctures reddish brown; hemelytral membrane dark ambarine with veins mostly brown; connexivum brown with posterior margin yellow; dorsal abdominal segments II to VI shiny orange, and VII shiny orange with posterior third reddish brown. Ventral coloration: ground color dark yellow with punctures shiny brownish-orange; rostral segments I to IV, posterior margin of pleural sterna III to VII, and legs ochre yellow; prosternum, mesosternum, and metasternum shiny orange; femora and tibiae with obscure subapical pale yellow ring; anterior lobe of metathoracic peritreme creamy yellow, and posterior lobe orange.

Measurements. ♂. Head length 1.82; width across eyes 1.36; interocular width 0.78; interocellar width 0.34; preocular distance 1.30. Length of antennal segments: I, 1.24; II, 1.84; III, 0.98; IV, 1.38. Pronotum: Total length 2.00; width across frontal angles 1.92; width across humeral angles 3.20. Scutellar length 1.68; width 1.52. Total body length 10.40.

Female. Coloration: similar to male; femora brownish-yellow with subapical yellow ring; tibiae brownish-yellow with two yellow rings, one subbasal, the other near middle third; connexival segments VIII and IX with upper margin yellow and inner margin reddish brown; dorsal abdominal segments III to IX shiny reddish orange; gonocoxae I with inner face ochraceus and punctures shiny brownish-orange, and outer face reddish brown; paratergite VIII and IX ochraceus.

Measurements. ♀. Head length 2.08; width across eyes 1.48; interocular width 0.86; interocellar width 0.40; preocular distance 1.38. Length of antennal segments: I, 1.28; II, 2.00; III, 1.04; IV, 1.32. Pronotum: Total length 2.16; width across frontal angles 2.00; width across humeral angles 3.52. Scutellar length 1.76; width 1.68. Total body length 11.70.

Holotype: ♂, PAPUA, NORTH NEW GUINEA, Toricelli (Gebirge), Kais Wilhelmsland, II-1910, Dr. Schlaginhaufen (SMTD).

Paratype: 1 ♀ INDONESIA, Irian Jaya, Humboldt Bay District, Bewani Mts., IX-1937 (BMNH).

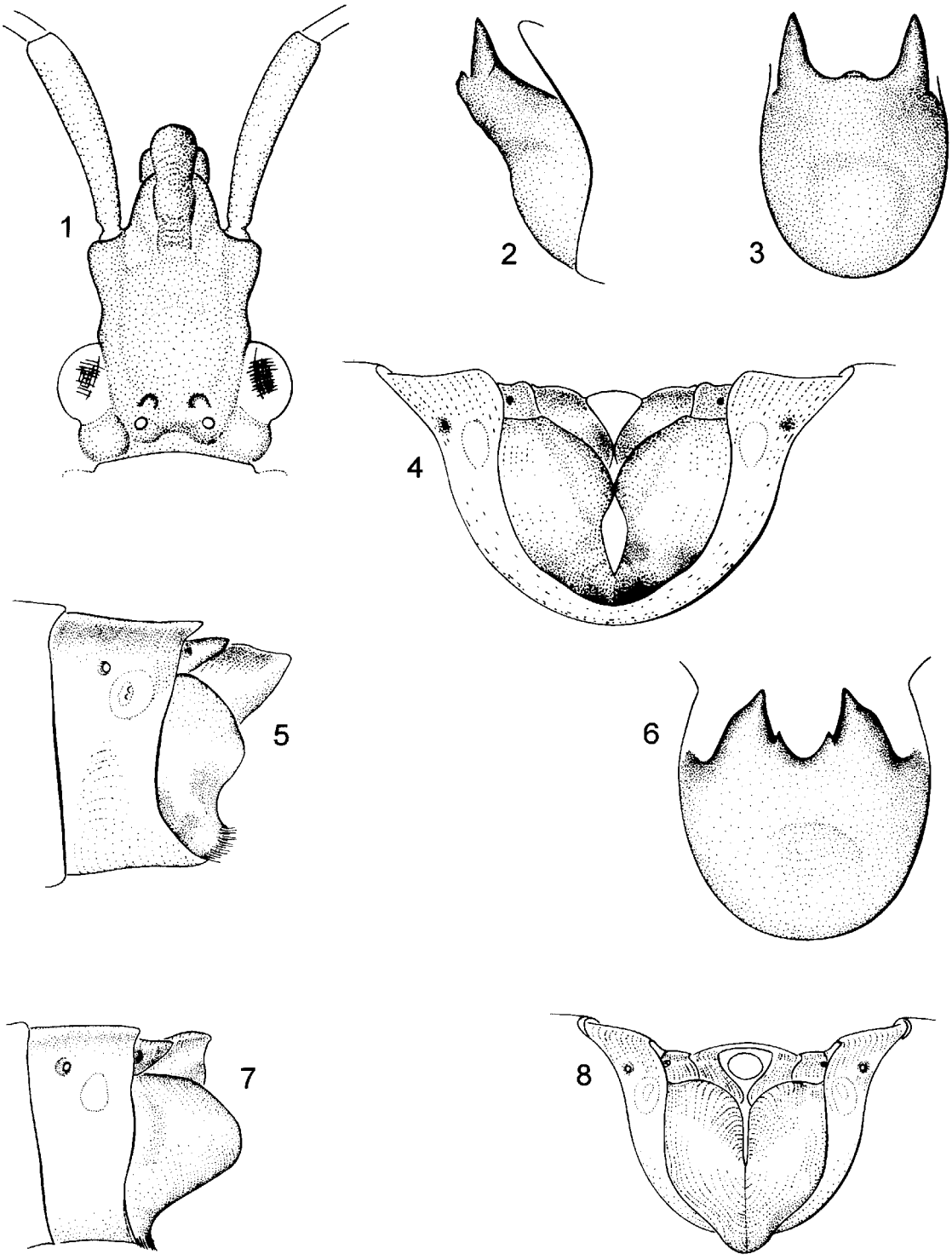
Etymology. The specific name refers to the fine color and proportions of this species.

Distribution. Known only from Irian Jaya (Indonesia) and northern Papua New Guinea (Toricelli).

Scioriedeli Brailovsky **NEW GENUS**

Type species: *Scioriedeli mandibularis* Brailovsky, monobasic.

Description. Body medium size, and relatively elongate. Head longer than wide or as long as wide across eyes, pentagonal, and dorsally flat; tylus unarmed, with the apex barely bifid, extending anteriorly to and laterally higher than juga; juga unarmed, apically truncate; antennal segment I moderately robust, thickest, slightly curved outward and longer than maximal length of head; segments II and III cylindrical and slender, and segment IV fusiform; antennal segment II longest, IV shortest, and I longer than III; ocelli well developed; preocellar pit deep; eyes spherical; postocular tubercles protuberant; buccula rounded, short, not extending beyond antenniferous tubercles, with sharp spiny middle projec-



Figs. 1-3. *Cephalohygia decorata* Brailovsky. 1. Head in dorsal view. 2-3. Male genital capsule. 2. Lateral view. 3. Caudal view. Figs. 4-6. *Scioriedeli mandibularis* Brailovsky. 4-5. Female genital plates. 4. Caudal view. 5. Lateral view. 6. Male genital capsule in caudal view. Figs. 7-8. Female genital plates of *Cephalohygia decorata* Brailovsky. 7. Lateral view. 8. Caudal view.

tions; rostrum reaching anterior or middle third of abdominal sternite V; mandibular plate armed with short tubercle; genae unarmed. Thorax: Pronotum wider than long, trapeziform, bilobed, and declivant; collar wide; frontal angles projected forward as tiny lobe; humeral angles rounded, not exposed; anterolateral borders sinuate, smooth; posterolateral and posterior borders straight, smooth; calli barely convex; anterior lobe of metathoracic peritreme reniform, posterior lobe sharp, small. Legs unarmed; tibiae sulcate. Scutellum triangular, longer than wide, apex subacute, and weakly raised. Hemelytra macropterous, reaching the apex of the last abdominal segment; costal margin emarginate; apical margin obliquely straight; clavus and corium including the apical margin of endocorium punctate. Abdomen. Connexivum higher than terga, with posterior angle of each segment entire, and not projected into a spine. Male genitalia: Posteroventral edge of genital capsule with pronounced U-shaped concavity, enclosed by two large, robust arms; inner face of each arm at middle third with small denticle (Fig. 6). Female genitalia: Abdominal sternite VII entire, without plica or fissura, and with posterior margin of the plate projected as a large and broad triangular expansion; gonocoxae I enlarged dorso-ventrally, in caudal view opened, with the space relatively broad, in lateral view with upper third broadly convex and exposed, and lower third with apical angle blade-shaped, noticeably protruding into large and broad lobes, directed upward; paratergite VIII short, square, with visible spiracle; paratergite IX squarish, longer than paratergite VIII (Figs. 4-5).

Integument. Body surface rather dull, almost glabrous.

Etymology. Named for Alexander Riedel, distinguished German entomologist.

Discussion. *Scioriedeli* New Genus, with abdominal sternite VII of female without plica or fissura belongs to the "*Sciophyrus*" complex, recently revised by Brailovsky and Barrera (1996). In that contribution a key to closely related genera was given, as well as a full description of each genus. In *Scioriedeli*, *Sciophyrus* Stål, *Sciophyropsis* Brailovsky and Barrera, *Sciophyrella* Brailovsky and Barrera, and *Schaeferhygia* Brailovsky and Ortega (1994), the fore femora are unarmed, while in *Sciophyritides* Brailovsky and Barrera, and *Sciophyroides* Brailovsky and Barrera they are armed.

Scioriedeli, *Sciophyrus*, *Sciophyrella*, and *Schaeferhygia* share the body surface rather dull, the pronotum weakly bilobed, and the hemelytra clearly macropterous. In *Sciophyropsis* the body surface is shiny, the pronotum clearly bilobed, and hemelytra are submacropterous.

In *Sciophyrus* the antennal segment III is the shortest, in the other three closest genera the antennal segment IV is the shortest. *Schaeferhygia* has the genae armed, the external face of gonocoxae I rounded without lobulations, and in caudal view the concave mesial margins of the first gonocoxae are closed. In *Sciophyrella* and *Scioriedeli* the genae are unarmed, and the external face of gonocoxae I basally and apically lobulate.

Sciophyrella has the apical margin of endocorium smooth, without punctures, the gonocoxae I in caudal view with the concave mesial margins closed, the antennal segment III longer than I, and the mandibular plate unarmed. The apical margin of endocorium in *Scioriedeli* is clearly punctate, the gonocoxae I in caudal view with the mesial margins opened, antennal segment III shorter than I, and mandibular plate armed with short tubercle.

Scioriedeli mandibularis Brailovsky **NEW SPECIES**
(Figs. 4-6)

Description. Holotype male. Dorsal coloration: head shiny brownish-orange with longitudinal stripe adjacent to eyes, dorsal aspect of postocular tubercle, jugum, and apex of tylus yellow; antennal segments I and II brownish-orange, III brown with basal joint yellow, and IV yellow with basal joint brown; pronotum shiny brownish-orange with anterolateral margins and irregular creamy white marks at middle third; scutellum dark yellow with punctures shiny brownish-orange, and apex pale yellow; clavus and corium dark brownish-yellow with punctures shiny brownish-orange; hemelytral membrane dark yellow with veins pale brown; connexivum reddish brown with posterior margin yellow; dorsal abdominal segments shiny orange with black marks at posterior margin. Ventral coloration: Ground color dark yellow with punctures shiny brownish-orange; rostral segments I to IV, and posterior margin of pleural sterna III to VII yellow; prosternum, mesosternum, and metasternum shiny orange; metathoracic peritreme with anterior lobe creamy white, and posterior lobe orange; coxae and trochanters yellow; fore and middle femora yellow with subapical third brown; hind femur and tibiae pale brown with two yellow rings one subbasal, the other near middle third; tarsi pale brown with yellow marks.

Measurements. ♂. Head length 1.70; width across eyes 1.70; interocular width 0.90; interocellar width 0.38; preocular distance 1.12. Length of antennal segments: I, 2.14; II, 3.52; III, 1.92; IV, 1.64. Pronotum: Total length 2.20; width across frontal angles 2.04; width across humeral angles 3.80. Scutellar length 1.92; width 1.68. Total body length 11.50.

Female. Coloration: similar to male. Connexival segments III to V reddish brown with posterior margin yellow; segments VI to VIII reddish brown with anterior and posterior margin yellow, and IX reddish brown with anterior margin yel-

low; dorsal abdominal segments shiny orange yellow; gonocoxae I yellow with punctures shiny brownish-orange; paratergite VIII and IX yellow.

Measurements. ♀. Head length 1.94; width across eyes 1.84; interocular width 0.98; interocellar width 0.48; preocular distance 1.32. Length of antennal segments: I, 2.24; II, 3.64; III, 2.06; IV, 1.76. Pronotum: Total length 2.60; width across frontal angles 2.54; width across humeral angles 4.64. Scutellar length 2.26; width 2.04. Total body length 13.95.

Holotype: ♂ INDONESIA, Irian Jaya, mountain slope above Bernhard Camp, 750 m, 19-III-1939, L. J. Toxopeus (Neth. Ind. Amer. New Guinea Exped.) (RNHL).

Paratypes. INDONESIA: 1 ♀ Irian Jaya, mountain slope above Bernhard Camp, 750 m, 25-III-1939, L. J. Toxopeus (Neth. Ind. Amer. New Guinea Exped.) (UNAM). 1 ♀, Irian Jaya, Jayawijaya Prov., Bime, 1600-2000 m, 10-IX-1993, A. Riedel (ZSMC). 1 ♀, Irian Jaya, Lordberg, 7-XII-1912, S. G. Burgers (Kais Augustfl Exp.) (ZMHB). 1 ♀, Irian Jaya, Etappenberg, 850 m, 10-12-XI-1912, S. G. Burgers (Kais Augustfl Exp.) (ZMHB).

Etymology. Named for the appearance of the mandibular plate.

Distribution. Only known from Irian Jaya (Indonesia).

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TANYTARSUS (DIPTERA: CHIRONOMIDAE) FROM EGYPT WITH DESCRIPTION OF A NEW SPECIES

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ABSTRACT

Three species of the genus *Tanytarsus* van der Wulp were captured during a survey of chironomid midges (Diptera: Chironomidae), conducted from September 1997 to October 1999, of all major geographical zones in Egypt. *Tanytarsus spadiceonotatus* Freeman is recorded from Egypt for the first time, whereas *T. itsae* sp. nov. is described for the first time from male imagines. *Tanytarsus nocticola* Kieffer, which had been recorded by Kieffer from Egypt (Aswan) in 1911 (Freeman 1958), was not found at the sampled localities. A key to the male imagines of *Tanytarsus* in Egypt is presented. New distributional data for the three collected species are provided.

Key Words: Taxonomy, distribution, keys, *Tanytarsus itsae* sp. nov., *Tanytarsus formosanus*, *Tanytarsus spadiceonotatus*.

RESUMEN

Tres especies del género *Tanytarsus* van der Wulp fueron capturadas durante un muestreo de los mosquitos chironomidos (Diptera: Chironomidae) realizado desde septiembre 1997 hasta octubre 1999 en todas las regiones geográficas de Egipto. Se registra *Tanytarsus spadiceonotatus* Freeman por primera vez en Egipto, también por primera vez se describe el adulto del macho (imago) de *T. itsae* spec nov. No se encontró ejemplares de *Tanytarsus nocticola* Keiffer en las localidades muestreadas, el cual fue registrado en Egipto (Aswan) por Keiffer en 1911. Se presenta una clave de los imagos de los machos de *Tanytarsus* en Egipto. Se provee información sobre la distribución de las tres especies informadas en Egipto.

Tanytarsus van der Wulp, is one of the most species rich genera in the Holarctic region, and the immature stages inhabit many different aquatic habitats. Thus, many species are potential indicators for short and long term environmental change (Ekrem 2004). In the Afrotropical region, 26 species of this genus are hitherto recognized (Ekrem 2001).

The present work is based on material collected during a survey conducted from September 1997 to October 1999, covering major geographical zones of Egypt (28 locations). *Tanytarsus* spp. were collected from 9 locations (Fig. 1); *T. itsae* Ghonaim sp. nov. is described for the first time. Additional distributional data for a new record, *T. spadiceonotatus* Freeman and a previous record, *T. formosanus* Kieffer are also given.

MATERIALS AND METHODS

The description of the new species is based on specimens that were mounted on slides. The preparation of slides followed the method of Pinder (1989). Identifications were based on adult males. The morphological terms, ratios, and abbreviations used are those recommended by Sæther (1980), and adopted by Pinder (1989), with the additions and corrections of Sæther (1990). Slide-mounted specimens were photographed at 100-1000× magnifications with a microscope-mounted camera. The resulting 35 mm positive slides (Kodak, USA100 Tungsten, positive exposures) were digitally scanned (Polaroid, *Sprintscan* 4000 plus) and processed for the best available resolution, printed on a glossy paper, and subsequently traced with ink pens.

All specimens used in this study were collected by the first author and preserved in 70% alcohol. Authorship of the new species is attributed to the first author. The holotype of the new species is deposited at the Museum of Entomology, Florida State Collection of Arthropods, Gainesville, Florida, USA.

RESULTS

Tanytarsus formosanus Kieffer, 1912

Distribution. Lower Egypt: Memphis, numerous imagines, 17-IV-1954 (Fittkau) (Reiss & Fittkau 1971). ASWAN: El-Mahareef, 4 ♂♂, 2 ♀♀, 8-IX-1999; Toshka, 12 ♂♂, 3-XI-1997. BEHEIRA:

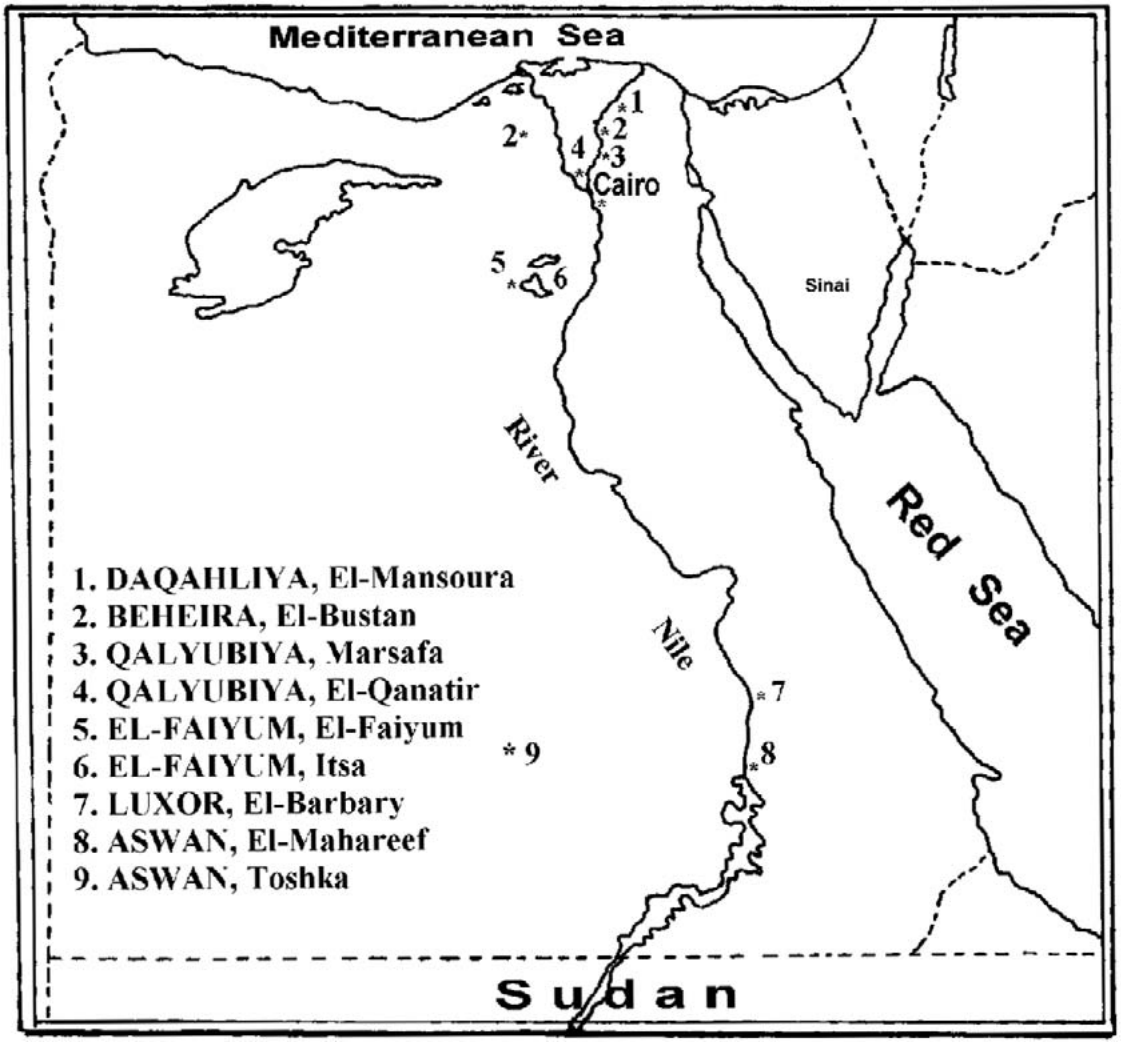


Fig. 1. Map of Egypt showing collection locations of *Tanytarsus*.

El-Bustan, 1 ♂, 21-IX-1999. DAQAHLIYA: El-Mansoura, 1 ♂, 15-IX-1999. EL-FAIYÛM: Itsa, 4 ♂♂, 123 ♀♀, 28-IX-1999. LUXOR: El-Barbary, 6 ♂♂, 9-IX-1999. QALYUBIYA: El-Qanatir, 4 ♂♂, 9-X-1998; Marsafa, 2 ♂♂, 24-X-1998; 1 ♀, 10-IV-1999; 87 ♂♂ (swarm), 27-VII-1999.

Tanytarsus itsae Ghonaim, **sp. nov.**
(Figs. 2-7)

Material Examined. Holotype, male: EGYPT, El-Faiyûm, Itsa, 28-IX-1999, slide mounted in Euparal. Paratype 1 male as holotype.

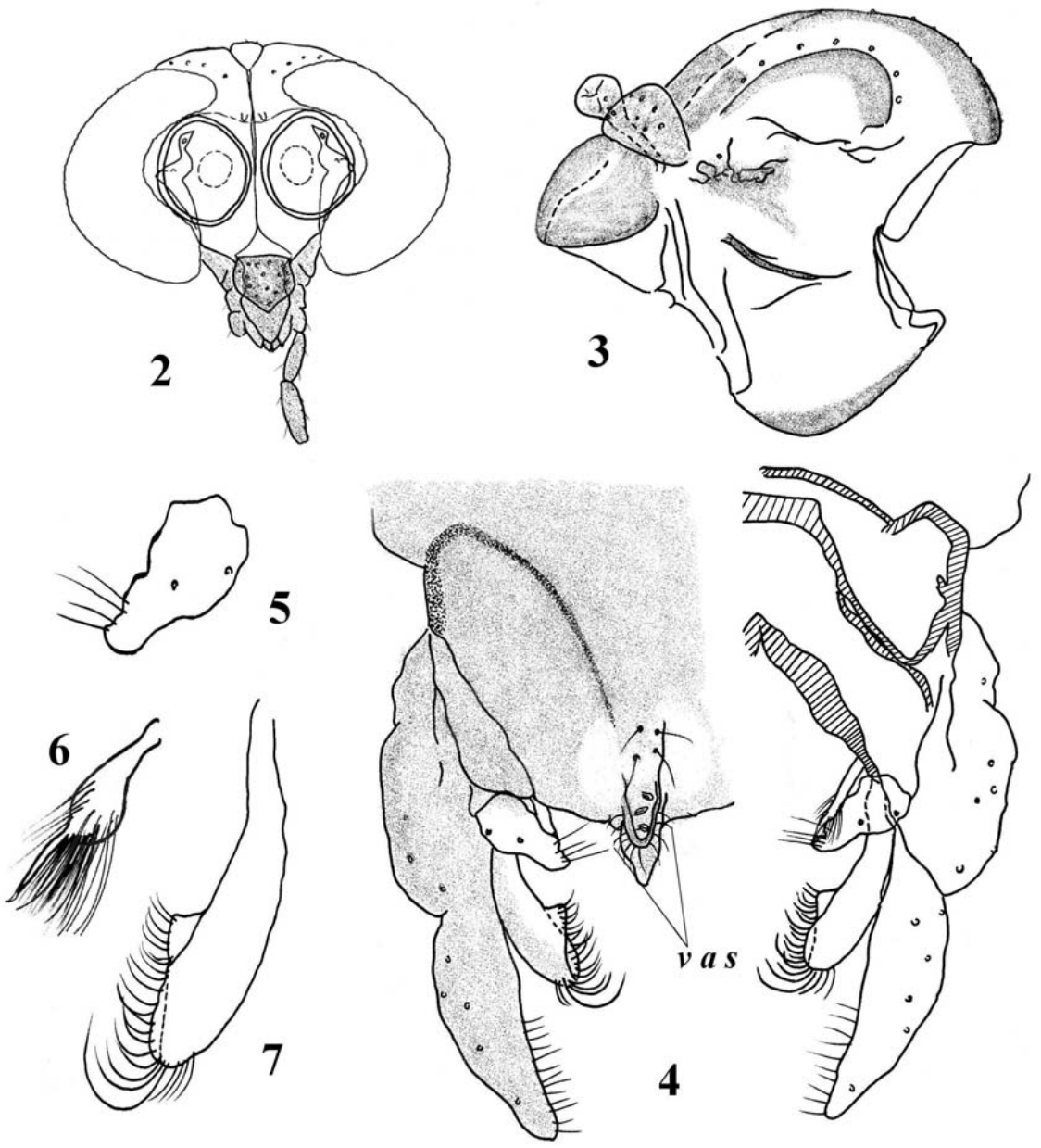
Diagnostic characters. *Tanytarsus itsae* sp. nov. is yellowish to dark brown species with distinct color pattern of thorax and abdominal tergites; it is separable from other species of *Tanytarsus* by its relatively smaller size, short anal point, with lateral and ventroapical setae, short median volsella, and relatively large inferior volsella with apicomedial projection. Superior volsella without digitus.

Etymology. *Tanytarsus itsae* is named after the type locality.

Description. MALE (Figs. 2-7, $n = 2$ unless otherwise stated). Total length 1.75-1.77 mm. Wing length 1.16-1.19 mm. Total length/wing length 1.49-1.51. Wing length/length of profemur 2.03-2.09.

Coloration. Yellowish to dark brown species, head brown, thorax yellowish brown with darker stripes, legs faint brown, abdominal tergites with dark brown incisures.

Head (Fig. 2). AR 1.09. Thirteenth flagellomere 410-435 µm long. Longest antennal seta 410-420 µm long. Eyes with moderate dorsomedial extension; distance between eyes 125-182 µm. Frontal



Figs. 2-7. *Tanytarsus itsae* Ghonaim, sp. nov., male imagine. 2, Head; 3, Thorax; 4, Hypopygium; 5, Superior volsella; 6, Median volsella; 7, Inferior volsella (*v a s*: Ventroapical and lateral setae).

tubercles 27 µm long. Temporal bristles 7-12; 5-7 inner verticals, 2-3 outer verticals, 0-2 postorbitals. Clypeus 53-62 µm long, 74 µm wide, with 14-17 setae. Tentorium 75-95 µm long, 30-45 µm wide at sieve plate, 7 µm wide at posterior pore. Stipes indistinct. Lengths of palpomeres (in µm): 25-26, 31-32, 68-70, 79(1), last palpomere lost.

Thorax (Fig. 3). Dorsocentrals 6, acrostichals 7-9, prealars 0-3, scutellars 5. Halteres with 3-4 fine setae.

Wing. VR 1.11-1.15. Brachiolum with 1 seta, Sc bare, R with 9-12 setae, R_1 18-24, R_{4+5} 26-30, M bare, M_{1+2} 24-39, M_{3+4} 18-20, Cu 6-8, Cu_1 16-19, An 4-14. Cells r_{4+5} with about 64-77 setae, m_{1+2} 35-42, m_{3+4} 15-16, an 3-4. Setae denser towards wing tip.

Legs. Spur of front tibiae 17-19 µm long, of mid tibiae 15-17 µm and 23-24 µm long, of hind tibia 29-32 µm and 30-31 µm long. Combs of mid tibia 23-24 µm and 30-31 µm long, of hind tibia 23 µm and 35-38 µm long. Apical width of fore tibia 40-

41 µm, of mid tibia 41 µm, of hind tibia 47-49 µm. Pulvilli present, subequal to claws, of front legs lost, of mid legs 15-17 µm long, of hind legs 17-18 µm. Lengths (in µm) and ratios of legs:

	fe	ti	ta_1	ta_2	ta_3	ta_4
P_1	555-587	305-325	—	—	—	—
P_2	560-570	475-510	490 (1)	170 (1)	145 (1)	105 (1)
P_3	610-655	595-675	478 (1)	319 (1)	268 (1)	165 (1)

	ta_5	LR	BV	SV	BR
P_1	—	—	—	—	—
P_2	80 (1)	0.96 (1)	3.07 (1)	2.20 (1)	2.45 (1)
P_3	103 (1)	0.71 (1)	2.11 (1)	2.78 (1)	3.08 (1)

Hypopygium (Figs. 4-7). Anal tergite 83-112 µm long with 2-5 median setae. Apical margin with one shoulder. Anal tergite bands smoothly curved, separated posteromedially, not connected to anal crests. Anal point very short, 10-35 µm long, 15-16 µm wide at base, 4-8 µm wide at tip, with about 7-12 ventroapical and lateral, curved setae, and 3 wide spinules between anal crests (Fig. 4). Fine microtrichiae scattered between anal crests. Microtrichiae present on anal tergite, with a microtrichia free area around the base of anal point. Transverse sternapodeme 63-65 µm long, phallapodeme 36-42 µm long. Gonocoxite 67-68 µm long. Gonostylus 90-112 µm long, with fine medial setae. Superior volsella (Fig. 5) bare, 39-40 µm long, 22-23 µm wide, with 3 apicomedian and 3 lateral setae; digitus absent. Median volsella (Fig. 6) 17-28 µm long, 5-6 µm wide, with one foliate lamella and about 12 simple lamellae, 15-26 µm long. Inferior volsella (Fig. 7) relatively large, 58-86 µm long, 14-30 µm wide at apex, with apicomedial projection and approximately 20-24 apical long setae. HR 0.61-0.74; HV 1.56-1.97.

Tanytarsus nocticola Kieffer, 1911

Distribution. SUEZ: Suez, 2 ♀♀, 1911 (Kieffer); ASWAN: Aswan, 1 ♂, I-1923 (Hirst) [Freeman 1958].

Tanytarsus spadiceonotatus Freeman, 1958

Distribution. ASWAN: Toshka, 8 ♂♂, 3-XI-1997. EL-FAIYÛM: El-Faiyûm, 6 ♂♂, 24-VIII-1998. DAQAHLIYA: El-Mansoura, 23 ♂♂, 15-IX-1999. LUXOR: El-Barbary, 6 ♂♂, 9-IX-1999. QALYUBIYA: El-Qanatir, 2 ♂♂, 9-X-1998; Marsafa, 3 ♂♂, 24-X-1998; 76 ♂♂ (swarm), 27-VII-1999; 6 ♂♂, 37 ♀♀, 18-IX-1999; 9 ♂♂, 19-IX-1999; 2 ♂♂, 4-X-1999.

Key to Male Imagines of the Genus *Tanytarsus* from Egypt

1. Anal point without spines nor anal crests . . . . . *T. nocticola* Kieffer
— Anal point with stout spines between anal crests 2

2. Anal point fairly short (not exceeding 35 µm in length). Digitus of superior volsella absent (Figs. 4 & 5) . . . . . *T. itsae* Ghonaim, **sp. nov.**
— Anal point longer (at least 40 µm long). Digitus small but distinct 3

3. Large microtrichiae-free area at base of anal point. Apical margin of anal tergite with two shoulders on each side of anal point. Superior volsella elongated, tapered towards apex . . . . . *T. formosanus* Kieffer
— Microtrichiae present around anal point base. Apical margin of anal tergite without lateral shoulders. Superior volsella almost quadrate . . . . . *T. spadiceonotatus* Freeman

Discussion and Conclusion

Tanytarsus itsae Ghonaim, sp. nov. fits well in the *gregarius* group of species by the well developed pulvilli, the presence of a large michrotrichia-free area around the base of the anal point, and the absence of the digitus of superior volsella. *T. itsae* Ghonaim, sp. nov. does not fit any species in the key provided in Ekrem's reviews (2001, 2002) of Afrtropical *Tanytarsus* and South and East Asian *Tanytarsus*, or the key provided by Reiss & Fittkau (1971) for the European *Tanytarsus*. Its small size (about 1.77 mm) and noticeably short anal point, with ventroapical and lateral setae (Fig. 4) make it a distinct species, easily separated from other Afrotropical and Palaearctic *Tanytarsus* species. *T. itsae* Ghonaim, sp. nov. is close to *T. formosanus* Kieffer in the shape and setation of volsellae. However, *T. itsae* is much smaller in size, its wing length does not exceed 1.20 mm (in *T. formosanus* Kieffer, not less than 1.35 mm long), the puvilli of legs are well developed, the digitus of superior volsella is absent, and the cubitus is setose.

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Egypt) completed at the Department of Entomology and Nematology/Mid-Florida Research and Education Center/IFAS, University of Florida. This is Florida Agricultural Experiment station Journal R-10254.

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CLADISTIC ANALYSIS OF PALEO-ISLAND POPULATIONS OF THE FLORIDA HARVESTER ANT (HYMENOPTERA: FORMICIDAE) BASED UPON DIVERGENCE OF MITOCHONDRIAL DNA SEQUENCES

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ABSTRACT

To examine the relationships of geographically isolated paleo-island populations of *Pogonomyrmex badius* (Latreille 1802) in Florida we generated a phylogeographic hypothesis based on mitochondrial DNA (mtDNA) sequences. We found at least three distinct mtDNA lineages and a positive correlation between genetic and geographic distances. The relationships between nowadays isolated *P. badius* populations might resemble a long lasting separation due to either restricted gene flow caused by inbreeding, paleo-climatic events or the impact of novel invasive species. The current depletion of the only representative of the ant genus *Pogonomyrmex* in the south-eastern USA makes a more fine-scaled mapping of the remaining, small *P. badius* populations necessary to identify evolutionary distinct units for conservation purposes.

Key Words: *Pogonomyrmex badius*, Cytochrome c Oxidase I, Cytochrome b, genetic distance, geographic distance, restricted gene flow, phylogeny.

RESUMEN

Para examinar las relaciones de poblaciones de *Pogonomyrmex badius* (Latreille 1802) de paleo-islas geograficamente aisladas en Florida nosotros generamos una hipótesis filogeografica basada sobre las secuencias de ADN mitocondrial (mtDNA). Nosotros encontramos por lo menos tres linajes distintos de mtDNA y una correlación positive entre las distancias genéticas y geográficas. Las relaciones entre las poblaciones de *P. badius* aisladas de hoy dia puede representar una separación de largo plazo debido al flujo de genes restringidos causado por la reproducción entre individuos de la misma familia, los eventos paleo-climáticos o el impacto de nuevas especies invasoras. La reducción actual de la unica hormiga representativa del género *Pogonomyrmex* en el sureste de los Estados Unidos hace necesario que se traze un mapa de una escala mas precisa para las poblaciones pequeñas de *P. badius* restantes para identificar las distintas unidades evolucionarias para propósitos de conservación.

The paleogeographic history of Florida and its islands during Pleistocene with regular flooding of major parts of the Florida peninsula is well documented (Faught & Carter 1998; Froede 2002; Cunningham et al. 2003; Portell et al. 2003). The general pattern is that animals expanded their range from their refuges with the end of the last glaciation period. This historical isolation of different populations of the same species could have created distinct strains (Ribera & Vogler 2004) with their own history and genetic composition. The genetic distinctiveness of those "paleo-island" populations might be further increased if the population structure is highly viscose.

Demographic responses to climate change and resulting range changes usually result in genetic manifestation, making them genetically traceable with adaptively neutral genetic markers (Hewitt 2000; Lessa et al. 2003). Therefore, the genetic analysis of current Florida paleo-island populations might provide us with insights into historical events. In between numerous exotic ant species inhabiting the Florida peninsula, *Pogonomyrmex badius* (Latreille 1802) is considered "the closest approach to an endemic genus" (Deyrup & Trager 1986; Deyrup et al. 1988). *Pogonomyrmex badius* was isolated in the xeric uplands of central Florida during Pleistocene (Deyrup & Trager 1986). These isolated populations came again into contact after climatic changes at the end of the Pleistocene. Recently, colonization of Florida by aggressive, invasive species like *Solenopsis invicta* (Whitcomb et al. 1972; Deyrup et al. 2000; Cherry 2001) and abundant agricultural land use resulted again in a restriction of *P. badius* to more or less isolated island populations in remnant sand hill and scrub habitats. Therefore, the current populations of *P. badius* are the remains of formerly larger populations with a very restricted gene flow between them.

Retained ancestral polymorphisms can yield distinct phylogenetic relationships (Bulgin et al. 2003). Therefore, assuming restricted gene flow between isolated "island" populations of *P. badius* even since Pleistocene, a DNA based cladogram should resemble the historical events of the withdrawal of *P. badius* into ice-age refuges before and after introduction of invasive species. Genetic distances should increase with geographic distances if the phylogenetic pattern between island populations is based on ancient colonization events rather than splitting of a wide population zone and subsequently random, but incoherent mutation events. To examine the relationships of geographically isolated populations of *Pogonomyrmex badius* in Florida, we analyzed population samples phylogenetically using mitochondrial DNA sequences.

Materials and Methods

Collection of Specimens

Specimens of *Pogonomyrmex* (*sensu stricto*) *badius* (Table 1) were collected from six populations throughout Florida (USA) and preserved in 70-100% Ethanol for later DNA analyses. Similarly, *Pogonomyrmex* (*Ephebomyrmex*) *imberbiculus* and four additional *Pogonomyrmex* (*sensu stricto*) species (Table 1) were collected as outgroup-specimens for later phylogenetic analyses. Subgenus-classification followed Bolton (1995), whereas determination of species followed the keys of Taber (1998) and Cole (1968), and was confirmed by independent researchers where possible (Table 1). Nestmates from the analyzed workers are deposited as pinned voucher specimens in the collections of Harvard University (Cambridge, MA, USA) and collections of P.S. Ward at University of California in Davis (USA) and preserved in 100% Ethanol (stored at -70°C) at Museum Koenig (Bonn, Germany).

DNA-Isolation

DNA was extracted from workers with their gasters removed by phenol/chloroform extraction (Gadau et al. 1996), with the DNeasy Kit (Quiagen; following manufacturers tissue-protocol A for insects), or the Puregene Kit (Biozym/Gentra Systems, following the protocol of Gadau et al. 2003). The latter two methods worked well for specimens conserved in 70% Ethanol, which was problematic for the phenol/chloroform extraction method. Genomic and mitochondrial DNA was not separated by this method. DNA was dissolved in low TE-buffer and the success of DNA-isolation was tested on agarose gels. Good samples were diluted 1:10 with HPLC-water to a final concentration of approximately 5-10 µM.

PCR

We amplified fragments of the Cytochrome c Oxidase I (COI, 1054 bp) and Cytochrome b (CytB, 439 bp) mitochondrial genes (Table 2). PCR reactions were performed on a Biometra thermocycler (heating rate 5°C/s) with the following primer pairs (degenerate positions of primer-sequences are placed within brackets; numbers in

Table 1. Specimens of *Pogonomyrmex* used for MTDNA sequence analyses (cytochrome c oxidase I; cytochrome oxidase b); Acc.-No. = accession numbers (numbers indicate collectors of specimen/determinators of species names: 1 = Annett Endler, 2 = Alexander Mikheyev; 3 = Christoph-P. Strehl (CPS); 4 = Juergen Gadau (JG); 5 = Juergen Liebig; 6 = Phil S. Ward; 7 = Robert A. Johnson (RAJ); 8 = Susanne Hoyer; 9 = Stefan P. Cover; 10 = Z. Punsak); Location = place of collection, connected to GPS-data; COI/CytB = genebank accession numbers for Cytochrome C Oxidase I sequences/Cytochrome B sequences corresponding to samples.

Species	Acc.-No.	Location	Sequence name [COI/CytB]
P. badius	CPS125 [10/7]	Titusville, FL (N28°32' W80°50')	TIV [AY510637/-]
P. badius	CPS199 [3,5/3,5]	Lake Placid, FL (N27°11' 37.7" W81°20' 42.1")	ABS [AY510636/AY538614]
P. badius	CPS200 [1,3/1,3]	Withlacoochee, FL (N 28°48' 42.4" W 82°29' 6.3")	WIT [AY510633/AY538616]
P. badius	CPS201 [1,3/1,3]	Ocala Natl. Park, FL (N29°16' 26.5" W81°49' 5.4")	OCA [AY510635/AY538619]
P. badius	CPS203 [3/3]	Fort Pierce, FL (N27°28' 26.5" W80°17' 32.0")	FTP [AY510638/AY538621]
P. badius	CPS204 [1,3/1,3]	Lake Placid, FL(N27°13' 11.8" W81°22' 48.5")	LKP [AY510634/AY538615]
P. badius	CPS230 [2/2]	Tallahassee, FL (N30°27' W83°20')	TA1 [AY510631/AY538618]
P. badius	CPS234 [2/2]	Tallahassee, FL (N30°27' W83°20')	TA2 [AY510632/AY538617]
P. barbatus	CPS56-30 [3,4,8/3,7]	Phoenix, AZ (N33°32' 40.7" W111°38' 3.6")	BRB [AY510639/AY538620]
P. californicus	RAJ2269 [7/6]	La Chocera, Mexico (N30°30.96' W116°2.46')	CAL [AY510649/AY538625]
P. huachucanus	CPS123 [4,3/6,9]	Portal, AZ (N31°55' 56.1" W109°12' 26.2")	HUA [AY510657/AY538623]
P. occidentalis	JG27 [3,4/3,5]	Seligman, AZ (N35°19' 37.5" W112°52' 35.7")	OCC [AY510667/AY538622]
P. (*Ephebomyrmex*) *imberbiculus*	CPS268 [3/3]	Portal, AZ (N31°55' 49.7" W109°7' 59.4")	IMB [AY510614/AY538624]

TABLE 2. PAIRWISE DISTANCE BETWEEN EACH SEQUENCE (SEQU.), CALCULATED USING PAUP 4.0B10 (SWOFFORD 1998); BELOW DIAGONAL: TOTAL CHARACTER DIFFERENCES; ABOVE DIAGONAL: *P*-DISTANCE MATRIX; SEQUENCE NAMES: COMPARE TABLE 1.

Sequ.	IMB	TA1	TA2	WIT	LKP	OCA	ABS	FTP	BRB	CAL	HUA	OCC	TIV
IMB	—	0.18201	0.18137	0.18880	0.18953	0.18620	0.18942	0.18823	0.18510	0.19351	0.19411	0.20116	0.15907
TA1	252	—	0.00000	0.02617	0.04885	0.02602	0.05106	0.04973	0.10452	0.09134	0.14300	0.11041	0.03139
TA2	253	0	—	0.02601	0.04792	0.02585	0.04989	0.04893	0.10242	0.09122	0.14113	0.10949	0.03136
WIT	268	37	37	—	0.04096	0.00480	0.04457	0.04048	0.10688	0.09232	0.14897	0.11422	0.01766
LKP	267	69	68	60	—	0.04108	0.00140	0.00348	0.10624	0.09520	0.14732	0.11279	0.00314
OCA	258	36	36	7	59	—	0.04396	0.04120	0.10786	0.09608	0.15098	0.11011	0.01555
ABS	260	70	69	64	2	63	—	0.00285	0.10552	0.09749	0.14736	0.11312	0.00311
FTP	260	69	68	58	5	58	4	—	0.10587	0.09886	0.14342	0.11299	0.00314
BRB	258	145	143	155	153	154	150	150	—	0.11654	0.14747	0.12224	0.09953
CAL	254	121	121	126	130	129	130	135	158	—	0.16228	0.11672	0.09293
HUA	275	201	200	219	215	217	210	205	213	221	—	0.16021	0.13272
OCC	273	150	149	161	159	154	157	158	171	156	225	—	0.10316
TIV	50	10	10	7	1	6	1	1	34	32	46	36	—

brackets following the 3' end of each primer refer to the next nucleotide positions relative to the sequence of the *Apis mellifera* mitochondrial genome published by Crozier & Crozier 1993, GeneBank-accession number NC_001566.1): LCO (sense) 5'-GGTCAACAAATCATAAAGATATTGG-3' [1835] and HCO (anti-sense) 5'-TAAACTTC-AGGGTGACCAAAAAATCA-3' [2492] (Folmer et al. 1994), Jerry (sense) 5'-CAACATTTATTTTGA-TTTTTT-3' [2502] (modified bee-primer Ca-J-2183 of Simon et al. 1994) and Ben3R (anti-sense) 5'-GC(AT)AC(AT)AC(AG)TAATA(GT)GTATCATG-3' [2888] (Brady et al. 2000) for CoxI; CB1 (sense) 5'-TATGTACTACCATGAGGACAAATATC-3' [11426] and CB2 (anti-sense) 5'-ATTACACCTC-CTAATTTATTAGGAAT-3' [11858] (bee-primers CP-J-10933 and CB-N-11367 of Simon et al. 1994) for CytB. The reaction volume was 25 µl, containing 2 µl of 1:10 diluted DNA-extraction, 2.5 µl of 10× PCR Buffer (750 mM Tris-HCl, 200 mM $(NH_4)_2SO_4$, 0.01% Tween 20), 0.2 mM of each dNTP, 2.0 mM $MgCl_2$, 0.52 µM of each primer, and 1.0U Taq DNA polymerase (MBI Fermentas, Lithuania). Cycling parameters were 3 min at 95°C for initial denaturation, followed by 33 cycles of denaturing 30 sec at 95°C, annealing 60 sec at 45°C, and elongation 30 sec at 72°C; two final steps of elongation 90 sec at 72°C and cooling down to 4°C were added. Amplicons were purified by ammonium acetate-precipitation (Sambrook et al. 1989) or with the Quiaquick purification kit (Quiagen). Sequencing reactions were performed by SeqLab (Göttingen, Germany).

Sequence Analysis

Obtained sequences were analyzed on Personal Computers. Proof reading was accomplished by comparing the forward and reverse amplicons and aligning them in a text-program with subsequent use of ClustalX (Thompson et al. 1997). Statistical analysis was performed with the programs PAUP 4.0b10 (Swofford 1998) and Mega 2.1 (Kumar et al. 2001). All sequences are deposited in GenBank (Table 1). For comparing population pairings we analyzed both types of sequences (COI, CytB) together or separately. Gene-trees were constructed in PAUP 4.0b10 using the Neighbor-Joining method (uncorr. *p*-distance; Kimura 2-parameter; HKY85; 100,000 bootstrap replicates), or Maximum Parsimony method (branch and bound search, 1000 bootstrap replicates).

We tested for a correlation of genetic distances with geographic distances between the analyzed populations. Geographic distances between samples were calculated with GPS data of Table 1 transformed into UTM-data (metric) and plotted against the genetic distances (uncorrected "*p*") calculated in PAUP 4.0b10 with the set of sequences used for constructing the gene-trees (Table 2). Between the Tallahassee samples, in which no detailed GPS-data were available, a geographic distance of 4 m was assumed.

RESULTS

Sequencing of both gene fragments resulted in general in 1493 base pairs used as characters in the subsequent phylogenetic analysis. Among the variable characters, 213 were parsimony-uninformative and 249 were parsimony-informative. Table 2 shows the absolute and *p*-distance between each sequence. Among the *Pogonomyrmex badius* samples there were 14 variable amino acids found among a total of 497.

Population pairings were identical with both sequences (COI, CytB) separately or together and either Neighbor-Joining (Fig. 1) or Maximum Parsimony analysis. We therefore show a tree based on both genes (Fig. 1). The populations of Fort

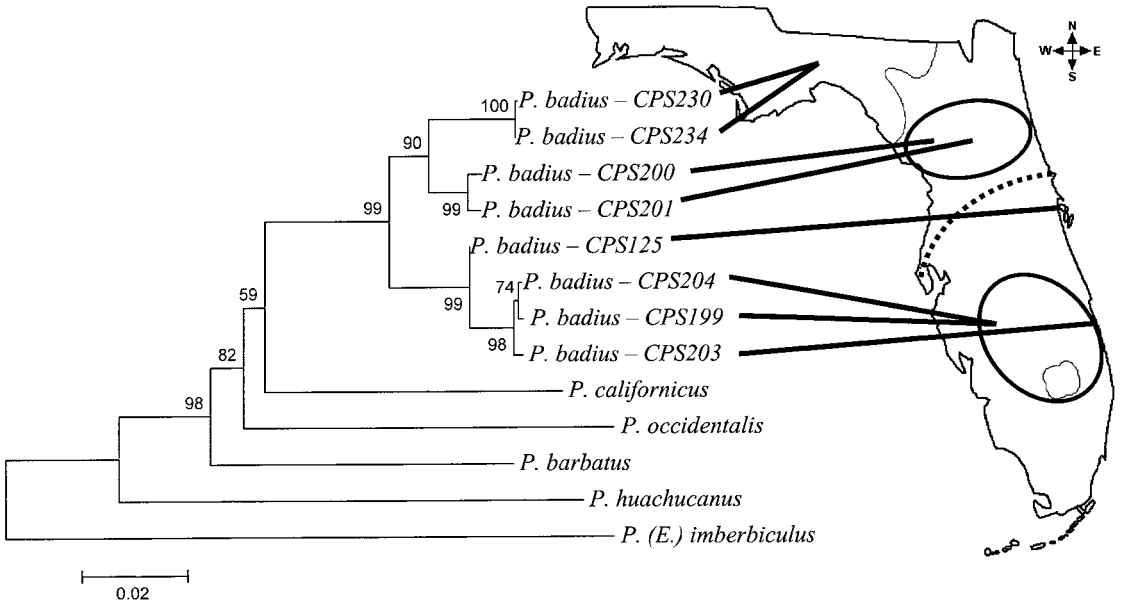

Fig. 1. Unrooted neighbor-joining tree of 1493-bp sequences (Cytochrome c Oxidase I and Cytochrome Oxidase b) of *Pogonomyrmex* spec., created with MEGA 2.1 [Distance method: Nucleotide: Kimura 2-parameter (Pairwise distance); Gaps/Missing Data: pairwise deletion; No. of bootstrap Reps: 100,000; SBL = 0.52206366] connected to the collection places of *Pogonomyrmex badius* on a contour of Florida, with circles/bows indicating hypothetical boundaries of genetically separated lineages (see text); numbers at branches indicate bootstrap replicates over 50%.

Pierce and Lake Placid grouped together, as did those from Withlacoochee and Ocala. The separation of the Fort Pierce/Lake Placid populations from the Titusville population (CPS125) was not supported in the MP analysis, and we considered them as one mitochondrial lineage. Moreover, the characters used for the Titusville population are based on the shortest of all sequences, as only one of the primer pairs yielded a sequence out of the single worker available. We justify the inclusion of this sample into the data because omitting it did not change the pattern shown in Fig. 1, and it provided additional information about the putative range of the southern mtDNA-lineage. The grouping of the Withlacoochee/Ocala population lineage together with the Tallahassee population was well supported by high bootstrap values. Because our sampling was very limited, however, the dotted line in Fig. 1 should be seen as preliminary.

Genetic distance showed a positive linear correlation with geographic distances between all population samples (Fig. 2; $n = 28$, $R^2 = 0.259$, $t = 3.011$, $P = 0.00573$). By excluding the Titusville population because of their limited genetic information, this correlation became even stronger ($n = 21$, $R^2 = 0.498$, $t = 4.341$, $P = 0.00035$). To prevent a bias of those populations where two samples were available (Tallahassee, Lake Placid) compared to those with only one, we included only one of them (CPS204 and CPS234) and reanalyzed the data. This procedure did not increase the significance of correlation, but increased the R^2 value ($n = 10$, $R^2 = 0.793$, $t = 5.540$, $P = 0.000547$). This effect is mainly due to an exponential increase in genetic variability with distances over 100 km (62.14 mi).

Discussion

Our analysis of mitochondrial DNA of *Pogonomyrmex badius* populations in Florida yielded three distinct lineages: (1) a southern lineage including samples from Lake Placid to Titusville, (2) a middle lineage including the Withlacoochee and Ocala populations, and (3) the northern lineage of the Tallahassee populations. The Withlacoochee and Ocala populations probably are more closely related to the Tallahassee populations. This view is further supported by a positive correlation of genetic with geographic distances.

The gene-tree and significant correlation of genetic and geographic distances suggest an ancient North to South movement of *P. badius* during its colonization of Florida with either very limited gene-flow back north, or less likely, higher mutation/fixation rates at the border of the expanding populations (Edmonds et al. 2004). Over all, the genetic relationships between the different population samples of *P. badius* might reflect past paleo-climatic events with a long lasting separation of populations withdrawn to protected "island" areas, like those found in the Lake Wales

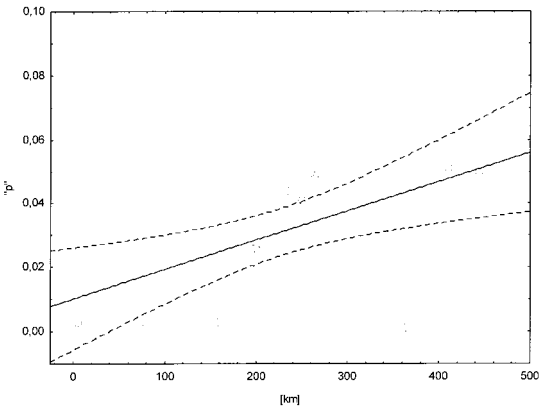

Fig. 2. Plot showing the positive correlation for calculated geographic [km] and genetic ("*p*") distances between *Pogonomyrmex badius* population samples sequenced for this study ($P < 0.006$, see text). The linear regression line is defined as "*p*" = 0.0102 + 9.176-5 [km], and accompanied by the corresponding 95% confidence interval (dotted line).

Ridge (Deyrup & Trager 1986). After the Pleistocene these populations might have come into secondary contact. This contact, however, did not lead to a sufficient flow and intermixing of the maternally inherited mitochondrial DNA before the *P. badius* populations were once again separated by anthropogenic devastation of their habitats, which was re-enforced by new and more competitive invasive species (Whitcomb et al. 1972; Deyrup et al. 2000; Cherry 2001).

This might be explained by the unique mating behavior of *P. badius* sexuals, and therefore dispersal of the gene carrying 'units' of this species. Mating behavior of *P. badius* is highly promiscuous (Page 1986; Crozier & Pamilo 1996; Rheindt et al. 2004) and makes gene flow between populations probable. However, there is also some indication for inbreeding, as females were reported to mate on their native nests, probably even with their brothers (Van Pelt 1953; M. Deyrup, Archbold Biological Field Station, Lake Placid/FL, pers. comm.). Additionally, no data are available on the dispersal of *P. badius* females, e.g., no reports on huge mating swarms similar to other *Pogonomyrmex* species (Hölldobler 1976). Rheindt (2003) showed that differences exist in allele frequencies of microsatellites (nuclear DNA) between three of the *P. badius* populations analyzed in this study (Withlacoochee, Ocala, Lake Placid). This was a first indication of either restricted gene flow between populations or rapid diversification in these distinct populations. As *P. badius* populations seem to show significant inbreeding (Rheindt 2003), restricted gene flow is likely between them. This is further corroborated by our analysis of mitochondrial DNA variations obtained from six different localities in Florida. Our analyses show clearly separated lineages and an increase of genetic distances with geographic distance.

A more fine-scaled mapping of *P. badius* populations will be needed to separate exactly the geographic range of the different mtDNA-lineages. However, we found at least three lineages which showed inter-population sequence variations that are normally found between *Pogonomyrmex* species (Strehl, unpublished data). Such phylogenetically distinct lineages, which are restricted in their geographical distributions might be characterized as Evolutionary Stable Units (ESUs), warranting protection because they may contain significant components of the evolutionary history of a species (Moritz 1994; Bulgin et al. 2003). This is of special interest because *Pogonomyrmex badius* is considered to represent an endemic ant genus of Florida (Deyrup & Trager 1986). It is also the only representative of the genus *Pogonomyrmex* east of Mississippi and the only North American *Pogonomyrmex* species with a substantial worker polymorphism (Taber 1990). Therefore, to protect the genetic diversity of the probably endangered ant species *P. badius* it is important to clarify the population structure and determine the range and extent of ESUs in Florida.

Acknowledgments

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A MODIFIED POOL DESIGN FOR COLLECTING ADULT MOLE CRICKETS (ORTHOPTERA: GRYLLOTALPIDAE)

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Mole crickets are one of the most damaging groups of turf and pasture grass pests found in the southeastern U.S. The need to collect mole crickets for use in laboratory studies and the advantages of monitoring adult flight for the timing of insecticide applications initiated the search for effective methods for monitoring flight activity. Ulagaraj and Walker (1973) determined that mole crickets would recognize and fly to stations that utilized electronic reproductions of the male calling song. Basic requirements for developing mole cricket calling traps were outlined by Ulagaraj (1975) and Ulagaraj and Walker (1973, 1975). The three main components of these early traps included a sound source, catching device, and power controller (Walker 1982).

The sound sources, which were once tape-recorded songs of the crickets, now consist of an electronic caller that synthesizes the mole cricket song, similar to that developed by Walker (1982). Over time, the electronic callers have improved so that an external controller is no longer necessary to establish the on/off periods. Since the late 1980s, photocells that detect darkness and automatically turn on the callers at sundown have been in use rather than manual controllers (Walker 1996). In this design, originally developed by Bernie Mans for the University of Florida, the callers are also outfitted with a timer that resets the photocell after a specified time (Walker 1996), in our case two h. This allows for the production of sound during the first couple of h after sunset, a time period when most female mole crickets fly (Ulagaraj 1976). We, too, use the Mans design, and emitters were built for us by Precision Technologies Co. (Raleigh, NC).

Various designs for the catching devices have been utilized including funnels or pans constructed from galvanized sheet metal that direct captured mole crickets into buckets of moist sand, and also into water-filled wading pools covered with coarse netting to prevent predation (Walker 1982). Although some of these earlier sheet metal traps have now been in use at some Florida locations for over 20 years (Frank 2001), they are expensive and not easily transported. A similar funnel design that uses lightweight fiberglass instead of sheet metal was first constructed in 1989 by Parkman and Frank (1993) to inoculate adult mole crickets with *Steinernema scapterisci* Nguyen Smart. This modification is less expensive to construct than the sheet metal design, but still has some disadvantages.

For our laboratory and greenhouse studies at North Carolina State University, it has been necessary to collect large numbers of adult mole crickets. Unfortunately, frequent collection of crickets from calling traps is problematic, if not impossible, due to the long distances between sites. Funnel traps have been used successfully in the past, but require semi-permanent establishment at a site, something that is often difficult to accomplish on golf courses (which constitute the majority of our research sites). The funnels are also difficult to handle and transport, subject to damage during coastal storms, time-consuming to assemble, and expose the crickets to overcrowded conditions in the collection buckets. Wading pools filled with water were tested in the spring of 2002 and found to be ineffective because the crickets are only able to float for 12-24 h (Walker 1982), and frequent checking of the traps was not possible. For our research purposes, we needed a design that was inexpensive, quick and easy to assemble, temporary at each site, and able to maintain the live crickets for up to a week between visits.

A modified design of the wading pools that met all of our requirements was developed in 2003. Instead of one wading pool (General Foam Plastics Corp., Norfolk, VA), two are used, one suspended above the other by four wooden, evenly distributed spacers that prevent excessive sagging of the top pool. The two pools are secured to one another by inserting a bolt (with washer) through the top pool, wooden spacer, bottom pool and then fastening all components with a nut (Fig. 1A). All metal pieces were sprayed with WD-40® spray (San Diego, CA) to prevent rusting and allow for easy disassembly. The top wading pool has ten to twelve holes that are 135 mm in diameter cut into it, which allows the crickets to fall through into the bottom pool as they land and walk in the pool (Fig. 1B). Instead of filling the bottom pool with water, it is filled with moist sand (Fig. 1C). Because sand is used, the mole crickets are in their natural habitat when they fall through the hole in the top pool and stay healthy until retrieved. The bottom of the top pool does not touch the sand layer so it is not possible for many crickets to fly back out through the holes. The cut out holes allow for rain to moisten the sand layer, and drainage holes drilled in the bottom pool prevent flooding. The electronic caller speakers are placed on wooden boards that are centered over the top pool (Fig. 1D).

Fig. 1A

Fig. 1B

Fig. 1C

Fig. 1D

Fig. 1. (A), Bolts fasten two wading pools and wooden spacers; (B), holes cut into top pool for mole cricket entrance into trap; (C), bottom pool filled with moist sand from collection site; (D), speakers for electronic caller are centered above top pool on wooden board.

To retrieve the live mole crickets, it is necessary to unbolt the two pools from one another and remove the top pool. Because the sand is typically less than 5 cm deep, the tunnels can easily be observed and the crickets found quickly. Reassembly of the pools takes approximately five minutes. The time to break down and reassemble the pools is the only disadvantage we have noted compared to the fiberglass funnels which only require a quick emptying of the bucket, but the time to check the traps is still rather minimal and acceptable to us. The soil layer in the bottom pool provides enough surface area for the crickets to move around freely and avoid overcrowding, which is one problem associated with the smaller collection containers (i.e., buckets) used with the funnel traps. Overcrowding is especially important for the southern mole crickets, since they are cannibalistic. Water is added to the sand when the traps are serviced to keep the soil at optimal moisture levels. If a particular collection site is found to be unproductive in terms of number of collected crickets, the pools can be transported easily to a new location. Also, at the end of the flight season, the pools can be removed from the site, an important consideration for many golf course superintendents.

Summary

A modification of the wading pool mole cricket catching devices was designed to allow for the collection of live adult mole crickets. This new design takes less construction than the funnels built by Parkman and Frank (1993), and is inexpensive, minimizes overcrowding (and subsequent canni-

balism), and only requires temporary establishment at collection sites. The use of moist sand instead of water keeps the mole crickets healthy if daily collections cannot be made and the two-pool design prevents flight escape.

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FIRST RECORDS FOR *ISCHNODEMUS VARIEGATUS* (HEMIPTERA: BLISSIDAE) IN NORTH AMERICA

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In this paper we give the first U.S. records of *Ischnodemus variegatus* Signoret from the United States, review the literature, provide a short diagnosis of the adult, and discuss the possible impact of this species in Florida.

Ischnodemus variegatus was described from Colombia and is known to occur throughout South America north to Belize, and in Trinidad (Slater 1987). Baranowski (1979), who first reported *I. variegatus* from Trinidad, provided information on its biology and described the egg and five instars. The nomenclatural history of this species was reviewed by Slater (1987), who raised *I. variegatus* from synonymy with another blissid, *Ischnodemus oblongus* Fabricius, with which it had been considered a junior synonym. Slater (1987) explained that although the adults of these two blissids are similar, they feed on different hosts and the nymphs are very distinct. He produced illustrations of the adults, fifth-instars, parameres, sperm reservoirs, and male genital capsules of both species. He stated that *I. variegatus* may be sympatric in some areas with *I. oblongus*, which is restricted to Central America.

Nine species of *Ischnodemus* are known from Florida, according to Slater and Baranowski (1990). When one uses the identification key to species of *Ischnodemus* in Florida (Slater and Baranowski 1990), *I. variegatus* keys to couplet #4, containing *I. rufipes* and *I. praecultus*. *Ischnodemus variegatus* adults strongly differ from these and other *Ischnodemus* in Florida primarily by wing coloration. It may be distinguished by a large black marking that covers most of the membrane of each front wing (Fig. 1) and by the mostly black femora.

Ischnodemus variegatus was first collected in the United States in Florida, Sarasota Co., Myakka River State Park, on 21-IX-2000, by Belinda Perry, from *Hymenachne amplexicaulis* (Rudge) Nees (Poaceae, Gramineae) leaves. Vouchers were deposited at the FSCA (#E2000-4134). Additional specimens, collected by others, have been deposited since then. This blissid has been found in Myakka River State Park, the Carlton Reserve, and the Crowley Museum and Nature Center, all in Sarasota County.

Baranowski (1979) found that *I. variegatus* in Trinidad feeds and breeds on the grass *Hymenachne amplexicaulis*. He found eggs in older parts of the grass, near the ground, and under the tightly appressed leaf sheaths; first instars in the same location as the eggs and also under older and looser leaf sheaths, along with older nymphs; and adults in the terminal whorl of the plants as well as on the ground. He stated that the population density of *I. variegatus* was lower where the grass was partially submerged or subject to frequent flooding.

Fig. 1. *Ischnodemus variegatus*, adult, feeding. Photo by F. Santana.

Hymenachne amplexicaulis, also known as West Indian marsh grass or trompetilla, is a tall (up to 2.5 meters), perennial aquatic grass that occurs in tropical America and the West Indies (Wunderlin & Hansen 2003). It was introduced into Florida more than 30 years ago and has been found in at least twelve counties (Langeland & Burks 1998). *Hymenachne amplexicaulis* grows in or near shallow ponds, along or in streams, in ditches, swamps, marshes, wet disturbed sites including wet pastures, drainage canals, river banks, cypress swamps, and may be found floating (Baranowski 1979; FLEPPC 20003; Keith Bradley 2000, pers. comm.).

West Indian marsh grass is adapted to fluctuating water levels and is difficult to control (Florida Exotic Plant Pest Control—FLEPPC 2003). It is considered a Category I invasive plant, which is capable of disrupting native plant communities in wetlands (FLEPPC 2003). Feeding by large numbers of *I. variegatus* seems to cause death of the grass (it dies back at least to the soil level) (P.

Benshoff 2001, pers. comm.). However, when stress factors such as cold, flood, or drought reduce the number of bugs, the grass grows rapidly and continues to spread. In Myakka River State Park, where *I. variegatus* was first detected, herbicides have been applied to reduce the area covered by the grass that is not within the immediate river corridor (i.e., isolated marshes) (P. Benshoff 2001, pers. comm.).

Hymenachne amplexicaulis originally might have arrived in Florida in the form of seeds carried by migratory birds (FLEPPC 2003). It also might have been introduced intentionally to Florida, as it has been into many other countries because of its high nutritional value for cattle. For example, it was purposely introduced into Australia to provide a high quality forage for cattle in the winter (Inglis et al. 1996). Because *I. variegatus* is a recent introduction and because it lays its eggs on live plants, not on the ground or on the plant's seeds, it is possible that this insect was introduced into Florida with cuttings of fresh plant material, the primary way West Indian marsh grass is spread according to Inglis et al. (1996).

It appears that *Ischnodemus variegatus* feeds only on *Hymenachne amplexicaulis*. A single record of a specimen collected in Surinam on *Thalia geniculata* (Slater and Wilcox 1969) was considered a "sitting" or non-feeding record by Baranowski (1979).

The effect of *I. variegatus* in Florida is uncertain. Host-range testing is required to determine whether *I. variegatus* may be a threat to native or agriculturally important plants. If *I. variegatus* proves to feed only on *Hymenachne amplexicaulis*, however, then its introduction can be viewed as fortuitous biological control.

We extend appreciation to Belinda Perry (Natural Resources, Sarasota County Government), who collected the first specimens; Paula Benshoff (Myakka River State Park, Sarasota), who led us in the field to observe the insects and provided field observations; Keith Bradley (The Institute for Regional Conservation, Miami) for sharing his observations; James A. Slater (University of Connecticut, Storrs) for verification of the species identification; Nancy Coile, Mark Garland, and Richard Weaver (DOACS/DPI) for botanical information; Beverly Pope (DOACS/DPI) for assistance in library research; Oscar Perez (University of Florida, Gainesville), Susan Halbert (DOACS/DPI), William Overholt (University of Florida, Ft. Pierce) and Thomas Henry (USDA/SEL, Washington, D.C.) for reviewing the manuscript; and Paul Pratt (USDA/Ft. Pierce), William Overholt (University of Florida, Ft. Pierce), and Rodrigo Diaz (University of Florida, Gainesville) for interest in continuing research on this blissid. This is Entomology Contribution No. 966, Bureau of Entomology, Nematology, and Plant Pathology, Florida Department of Consumer Services—Division of Plant Industry.

Summary

Ischnodemus variegatus Signoret, a grass-feeding Neotropical blissid, has recently become established in Florida. It feeds and breeds on West Indian marsh grass, *Hymenachne amplexicaulis* (Rudge) Nees, an invasive exotic grass that grows in wetlands. This lygaeoid can be separated from all of the other *Ischnodemus* species that occur in Florida by the distinctive black and white color pattern on the wings. A photograph of *I. variegatus* is included.

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TEMPORAL OCCURRENCE OF *PODISUS MACULIVENTRIS* (HEMIPTERA: HETEROPTERA: PENTATOMIDAE) IN NORTH FLORIDA

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Podisus maculiventris (Say) is a generalist predator of over one hundred species of insects, including approximately fifty different crop pests. Its primary prey items are larval Coleoptera and Lepidoptera (Warren & Wallis 1971; McPherson 1980; Wiedenmann et al. 1994), and it can have a significant impact on pest populations in these orders (DeClerq et al. 1998). In greenhouse studies, DeClerq et al. (1998) report a reduction of tomato looper *Chrysodeixis chalcites* (Esper) populations of 40% in 48 h and a 65% decrease in leaf-feeding damage after 1 week when fourth instar *P. maculiventris* were released at a predator:prey ratio of 1:3.3. Adults and nymphs of *P. maculiventris* can be found in a variety of agroecosystems such as soybean, alfalfa, corn, potato, apple, grape, and brassica, and in other plants and plant communities such as goldenrod, shrubland, deciduous forest, hemlock, and pine (Culliney 1986; Wiedenmann et al. 1994). Adults overwinter under ground debris or under the bark of trees. *Podisus maculiventris* occurs from Quebec to British Columbia and south to Florida and Arizona (McPherson 1982). However, the occurrence and activity of this species may vary throughout its distribution.

The objective of this study was to determine the temporal occurrence of *P. maculiventris* at a site in northern Florida. This type of information can be valuable when assessing the potential predator guild of an insect pest throughout the growing season of a crop in a specific area. Moreover, understanding the occurrence of a predator species can be useful when integrating pest management tactics in agricultural systems.

The study took place at the Florida Agricultural and Mechanical University, Viticulture and Small Fruit Research Center, Tallahassee, Florida. The site is located in north Florida at a latitude of approximately 30°28'70"N and longitude of 84°10'31"W, 62 m above sea level. From west to east the site consisted of grape *Vitis rotundifolia* Michx, mowed grass, golden rod *Solidago* L. spp., small trees and shrubs, and large trees.

Six mesh screen funnel traps contained within a plastic container (19.5 cm in height, with a 14-cm diameter funnel ending in a 4-cm diameter entrance hole 8 cm deep, and a removable sealed bottom) (Fig. 1) were hung on small trees between the grape vineyard and large trees. The traps were 45 m apart and placed 1-1.5 m above ground level. Traps were baited every two weeks with a synthetic male sex pheromone formulation of 3478 µl (*E*)-2-hexenal, 193.6 µl benzyl alcohol, and 4246 l (±)-α-terpineol (Aldrich et al. 1984; Aldrich 1988). This pheromone blend attracts males and females of *P. maculiventris* (Aldrich et al. 1984; Aldrich 1988). Baiting the traps every two weeks allowed the majority of the pheromone to evaporate and allowed some pheromone to remain in the trap to maintain its attractiveness. Each trap contained a 10-ml glass vial with a cotton wick and approximately 7 ml of pheromone. For each season, traps were set or inspected at approximately 11:00 AM EDT. In 2001, 2002, and 2003 traps were set on 7 June 2001, 1 March 2002, and 28 February 2003. Sampling began three days after trap placement. Traps were inspected twice a week from 10 June 2001-26 October 2001, 4 March 2002-17 June 2002, and 3 March 2003-16 June 2003. Data are plotted with moving average trend lines for visual interpretation (Fig. 2).

In the 2001 season, adults of *P. maculiventris* were captured within the first three days after trap placement, indicating that adults were already active. Adults were most abundant in June and peaked on 22 June 2001 (Fig. 2A). Captures remained low (<2.0 ± 0.3 adults/trap) from 4 July 2001-26 October 2001 (Fig. 2A). In the 2002 and 2003 seasons, sampling began in early March, before *P. maculiventris* were active. During both seasons, overwintering adult *P. maculiventris* became active in March, with populations peaking in May. Adult populations peaked on 13 May 2002 and 23 May 2003. Populations decreased in late-May during the 2002 and 2003 seasons and increased, to a lesser extent, in mid-June (Figs. 2B and 2C). Large decreases in adult captures in May likely indicate the end of the first generation offspring from the progeny of overwintering adults. The presence of all life stages explains the delay in adult populations during the second generation. Reduced activity in adult populations during the summer months also could be caused from decreased survival of various developmental stages at higher temperatures (DeClercq & Degheele 1992). Adult activity from March through September and peaks of adult activity in May and June are similar to results found by Aldrich et al. (1984) at a site in Geneva, NY.

The overall sex ratio of adults captured was 1:2 males:females (n = 297). Using a similar pheromone formulation, Aldrich (1988) reports a ratio of approximately 2:1 males:females. This difference could be due to differences in trap type, frequency of rebaiting the traps, location, and/or pheromone blend (Aldrich et al. 1984; Aldrich

Fig. 1. Funnel trap.

1988). Overwintering adult captures from the first trap catch in 2002 through 29 April 2002 revealed a male:female of 1:1. Similarly, overwintering adult captures from the first trap catch in 2003 through 28 April 2003 revealed a male:female of 1:1. Adult tachinids, most likely the endoparasitoid *Hemyda aurata* Robineau-Desvoidy, were only captured on three occasions.

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Summary

In northern Florida, *P. maculiventris* emerge in March-April and begin overwintering in October-November. The data indicate that *P. maculiventris* has a bimodal phenology, with peak abundance in May and a second less prominent peak in June. Comparisons of trap collections in northern Florida and central New York suggest little deviation in adult seasonal activity of *P. maculiventris* between the northeastern and southeastern USA.

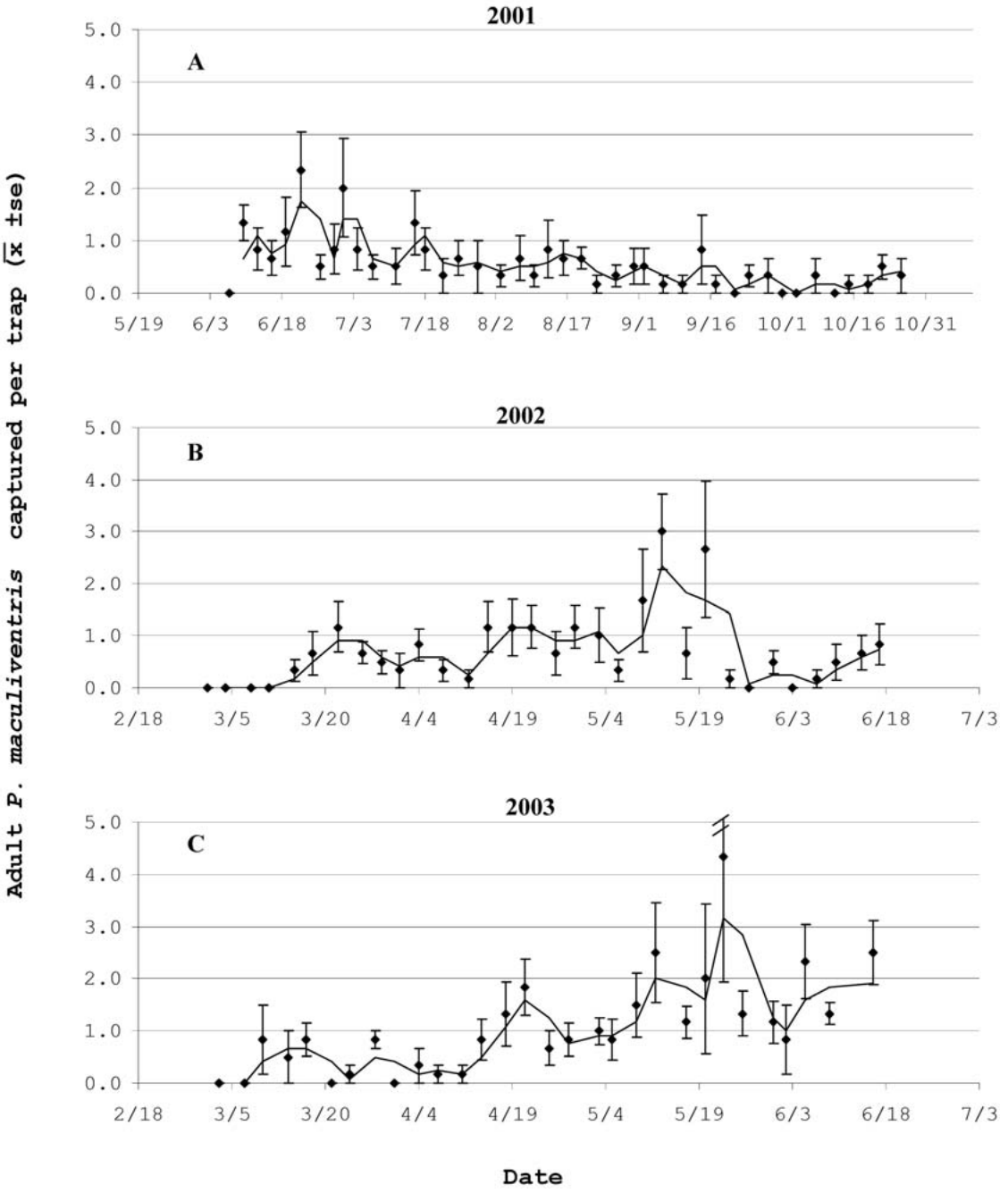

Fig. 2. Adult *Podisus maculiventris* captured per trap (mean ± se) during the 2001, 2002, and 2003 sampling seasons. Lines are moving average trendlines.

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PARAGONATAS DIVERGENS (HEMIPTERA: RHYPAROCHROMIDAE): FIRST CONFIRMED RECORD FOR FLORIDA AND THE UNITED STATES

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A specimen of *Paragonatas divergens* (Distant) (Hemiptera: Heteroptera: Rhyparochromidae: Lethaeini) was collected in northeastern Lee County, Florida, May 16, 2003, by the senior author during a routine survey. The specimen was taken by general sweep-net collecting along a roadside at the intersection of North River Road (Hwy. 78) and Parkinson Road. A variety of herbaceous plants at the site was sampled but ragweed, *Ambrosia artemisiifolia* L., was particularly abundant.

In general, members of the Lethaeini are poorly represented in Florida. Slater and Baranowski (1990) noted the presence of only three genera: *Cistalia* Stål, *Cryphula* Stål, and *Paragonatas* Barber, each with a single species reported from Florida. One additional genus, *Valtissius* Barber, has been reported from Florida as *Petissius* Distant (Van Duzee 1917) but without specific locality data.

Paragonatas divergens occurs throughout tropical America and the West Indies (O'Donnell 1986; Slater 1964; Slater and Baranowski 1990). This species has been referred to as the most common lethaeine in the New World (O'Donnell 1986), yet it was not previously known to occur in the United States. Palmer and Bennett (1988) reported this bug (as *Palagonatas* [sic]) on *Baccharis halimifolia* L. in Florida but without a specific locality. The current location of the specimen upon which their record was based is unknown, resulting in Slater and Baranowski (1990) considering this a questionable Florida record.

The single female was initially identified by the senior author. The identification was later confirmed by Thomas J. Henry (USDA-ARS-SEL, Washington, D.C.) on July 7, 2003, and the specimen is currently housed at the National Museum of Natural History, Smithsonian Institution, Washington, D.C.

Paragonatas contains two known species, *P. costaricensis* (Distant) and the type species, *P. divergens* (Distant). The former has been known to occur in the South Florida counties of Miami-Dade and Monroe for some time, but *P. divergens* has not been reliably reported from the United States until now (Slater and Baranowski 1990). *Paragonatas divergens*, however, has been intercepted on imported commodities numerous times at several of Florida's ports of entry (Fort Lauderdale, Jacksonville, Miami, and West Palm Beach) as well as other ports of entry in at least seven other states, including Texas and California.

The generic limits for *Paragonatas* need to be redefined. O'Donnell (1986) stated that she could find no common diagnostic characters that united the two species. Slater and Baranowski (1990) questioned whether the two species are congeneric, observing that *P. divergens* more closely resembles species of *Cistalia*. In addition, we have noted substantial differences in the general appearance of some *P. divergens* specimens from the West Indies compared with those from Central America. In our opinion, at least one additional species occurs in the West Indies. The specimen captured in Florida in May, 2003, more closely resembles those we have seen from Central America.

Paragonatas divergens is a ground-dwelling species that apparently feeds on fallen seeds of a variety of plants characteristic of old field habitats (Slater and Baranowski 1990). *Paragonatas divergens* can be easily distinguished from *P. costaricensis* by the following characters: dorsal surface dull, or at most sub-shining, pubescent, reddish-brown, usually with an obvious comma-shaped, pale macula distally on the corium; forefemora possessing 2-3 acute spines distally; scent gland auricle slender, not strongly curved posteriorly. *P. costaricensis*: dorsal surface strongly polished, brown, lacking a distinct comma-shaped pale macula distally on the corium; forefemora lacking acute spines distally, scent gland auricle broad and distinctly curved posteriorly.

The authors express appreciation to Thomas J. Henry for verifying the identification and for reviewing an earlier version of this manuscript. In addition, we thank Charles F. Brodel (USDA-APHIS-PPQ, Miami, FL) and James A. Slater (University of Connecticut, Storrs, CT) for critically reviewing the manuscript. We thank T. J. Henry and A. G. Wheeler (Clemson University, Clemson, SC) for companionship during the trip in which the specimen was taken.

SUMMARY

The establishment of *Paragonatas divergens* (Distant) in Florida and the United States is confirmed for the first time. A female was captured

on May 16, 2003, by general sweep-net collecting in Lee County, located in southwestern Florida.

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FIRST RECORDS FOR *DIEUCHES ARMATIPES* (HETEROPTERA: RHYPAROCHROMIDAE) IN NORTH AMERICA

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Dieuches armatipes (Walker), a moderately large lygaeoid that occurs throughout Africa, has been discovered recently in several Florida counties. Previously, it had been intercepted by the U.S. Department of Agriculture, APHIS/PPQ, at several Florida ports-of-entry. In Africa, *D. armatipes* feeds on peanuts (*Arachis hypogaea* L.) during the harvesting process; thus, this species has the potential to become a serious pest in northern Florida. In this paper, we give the first United States records of *D. armatipes*, review the literature, provide a diagnosis, and discuss its pest potential.

Henry and Froeschner (1993) gave the first New World records of *D. armatipes* from the West Indies, based on collections from Dominican Republic, Grand Cayman, Jamaica, and St. Kitts. It also has been collected in St. Croix and Cayman Brac (R. M. Baranowski 2003, pers. comm.). In the Old World, *D. armatipes* is distributed throughout Africa and as far north as Spain (Andalucia) (Eyles 1973).

In his world review, Eyles (1973) redescribed *D. armatipes* and included a key to all species of *Dieuches*, photographs of adults, and illustrations of genital capsules, parameres, and spermathecae. At present, 132 species of *Dieuches* are known (Eyles 1995). Henry and Froeschner (1993) redescribed the adult of *D. armatipes* and included dorsal and lateral photographs of an adult female to help distinguish it from other rhyparochromid species in the United States.

Diagnosis

Dieuches armatipes (Fig. 1) is distinguished from other rhyparochromids in Florida primarily by its large size (up to 11.5 mm long). Additionally, it may be recognized by the following characters: dark brown to nearly black, antennal segment IV dark brown with a wide subbasal white band, corium with a large isolated subapical white marking, relatively large eyes, labium ending between midcoxae, lateral pronotal margins lamellate, and forelegs incrassate and armed with two rows of spines.

Collection Records

The following acronyms and abbreviations are used: RMBC—Richard M. Baranowski collection, Homestead, Florida; FSCA—Florida State Collection of Arthropods, Gainesville, Florida; VGC—Vince Golia collection, Boynton Beach, Florida; JECC—J. Eric Cronin collection, Gainesville, Florida; ABSC—Archbold Biological Station collection, Lake Placid, Florida; MV—mercury vapor; BL—black light.

In Florida, *Dieuches armatipes* has been intercepted with various commodities imported through Ft. Lauderdale, Miami, and West Palm Beach; it also has been intercepted in Puerto Rico and Texas (T. Dobbs 2003, pers. comm.). The first specimen (female) collected in the United States has the following label data: Florida, Palm Beach Co., Delray Beach, Country Lake, 2-VII-1992, dead in pool, Vince Golia (RMBC). The following are label data from other material collected in Florida (Fig. 2): PALM BEACH COUNTY. 1 (sex unknown), Delray Beach, 9-VIII-1994, MV light, V. Golia (RMBC); 1 same but ♂ (FSCA, #E2002-5964); 1♂, Delray Beach, Country Lake, 28-VI-1995, MV light, V. Golia (VGC); 1♂, same but 26-VIII-1995 (VGC); 1♂, same but 9-IX-1995 (FSCA); 1♀, same but 23-V-1996 (FSCA); 1♀, same but 12-VIII-1997, BL (VGC); 2♂ 1♀, Boynton Beach, Nautica Sound, 26-V-2001, V. Golia, MV Light (FSCA). ALACHUA CO. 1♀, Gainesville, 27-V-1999, BL trap, J. E. Cronin (JECC, FSCA #E1999-1561); 1♀, Gainesville, 10014 SW 87 Terrace, 18-IX-2002, BL, Lyle J. Buss (at Univ. of Florida); 1 same but ♂ (FSCA). POLK CO. 1♂, Winter Haven, 5-XII-2002, in a citrus tree, in a Jackson trap with trimedlure bait, Martha A. Simpson (RMBC, FSCA #E2002-5918); 19 adults and nymphs, Winter Haven, 23-XII-2002, on ground under and between citrus trees, J. Brambila and S. E. Halbert (FSCA, #E2002-6120 through 6122). ST. LUCIE CO. 1♂, Ft. Pierce, 30-V-2003, on ground under fallen sabal palm frond, Ken Hibbard (FSCA, #2003-2291). HENRY CO. 1♂, LaBelle, Duda Farms, 25-VIII-1-IX-2000, aphid suction trap, M. Terrell (FSCA); 1♀, same but 13-19-X-2000 (FSCA). HIGHLANDS CO. 2♂, Lake Placid, Archbold Biological Station, 10-X-1997, MV light, Mark Deyrup (ABSC); 1 (abdomen missing), Lake Placid, ABS, 24-VI-2001, MV, V. Golia, (VGC). LEVY CO. 1♂, 1♀, Williston, 25-VIII-2003, in a peanut field, A. Drew, S. Krantz, and S. E. Halbert (FSCA, #E2003-3803).

Biology

In Africa, *D. armatipes* has been collected under *Mimosa*, a legume that could be its native host, although direct observation of feeding on its

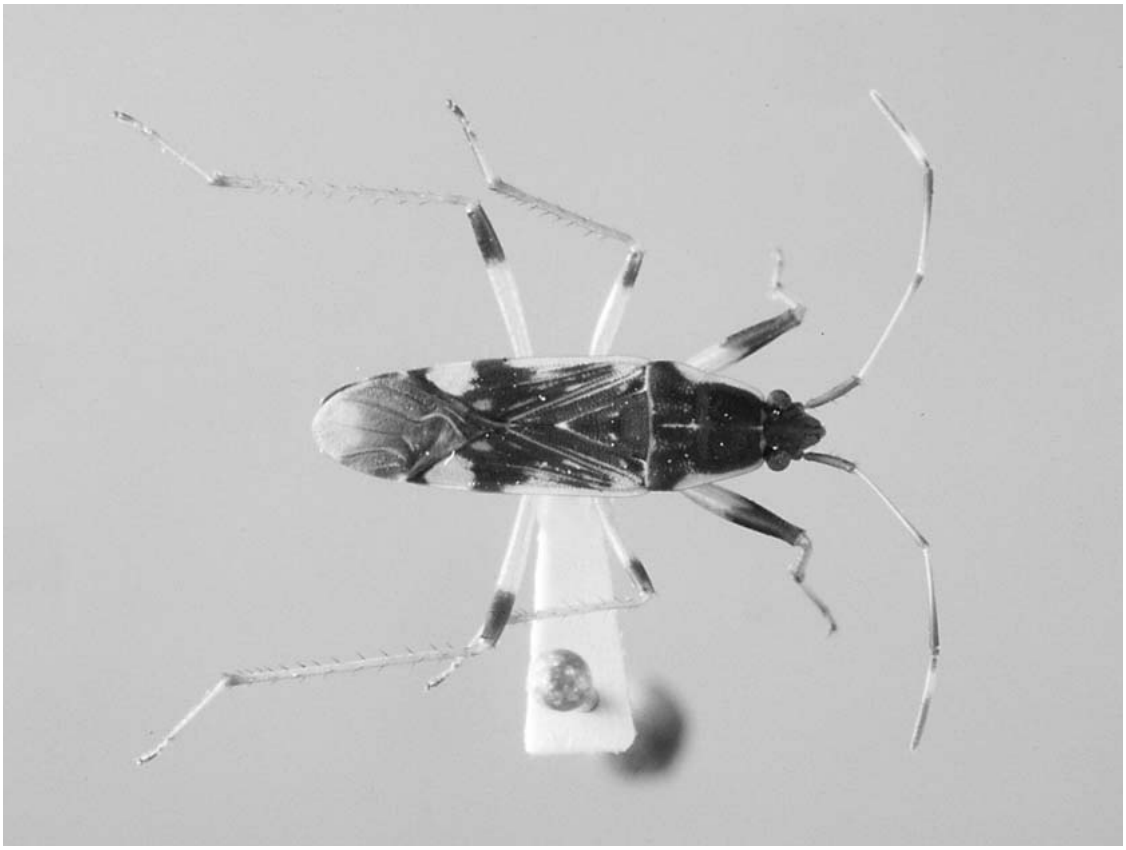

Fig. 1. Dorsal view of an adult *Dieuches armatipes* (Walker).

seeds was not reported; it also has been collected under stones and along the roadside at 1,200 meters above sea level in mixed grasses and herbs (Eyles 1973). This species reportedly feeds on harvested peanuts, a legume introduced from South America into Africa in the 16th century (Hill 1975). In Grand Cayman, West Indies, *D. armatipes* has been collected under coastal plants (R. M. Baranowski 2003, pers. comm.).

In Florida, *D. armatipes* has been found on dry sandy soil in leaf litter under and between citrus trees and under weeds between rows of trees. The only seeds apparently available at the collection site were those of native sandspurs, *Cenchrus brownii* Roemer & J. A. Schultes (Poaceae); puncture vine, *Tribulus terrestris* L. (Zygophyllaceae); and *Citrus* sp. (Rutaceae). We observed *D. armatipes* feeding on sandspur and puncture vine seeds in captivity. The bugs were maintained in the laboratory in plastic Petri dishes with dry sand, green and mature seeds, a vial with cotton and water, and dry, curled citrus leaves from the ground. Adults lived up to 4 weeks and were observed drinking, feeding, mating, and molting. A male and a female were collected in a peanut field in Levy Co. In captivity, they fed on shelled and unshelled peanuts. Eggs were deposited in a moist cotton ball, as well as on the peanuts, peanut stems, and on dry leaves. Nymphs fed on shelled and unshelled peanuts.

Pest Potential

According to Eyles (1973), *D. armatipes* is a serious pest of harvested peanuts in Africa. When the plants are inverted to expose the peanuts to the air for drying, or when stored outdoors in heaps, these bugs pierce the pods and suck the oil from the nuts, causing them to shrivel and to become rancid and bitter, and sometimes reducing the percentage of germination by one-half (Risbec 1941). Currently, there is no record for *D. armatipes* occurring in the panhandle of Florida, the major peanut production area. However, it has been found in a peanut field in Levy Co., in north central Florida. Harvested peanuts in the drying stage have received little scrutiny for pests as a result of mechanical harvesting and threshing practices. In Florida, the peanut plants are turned over in the field and left to dry for 3 to 4

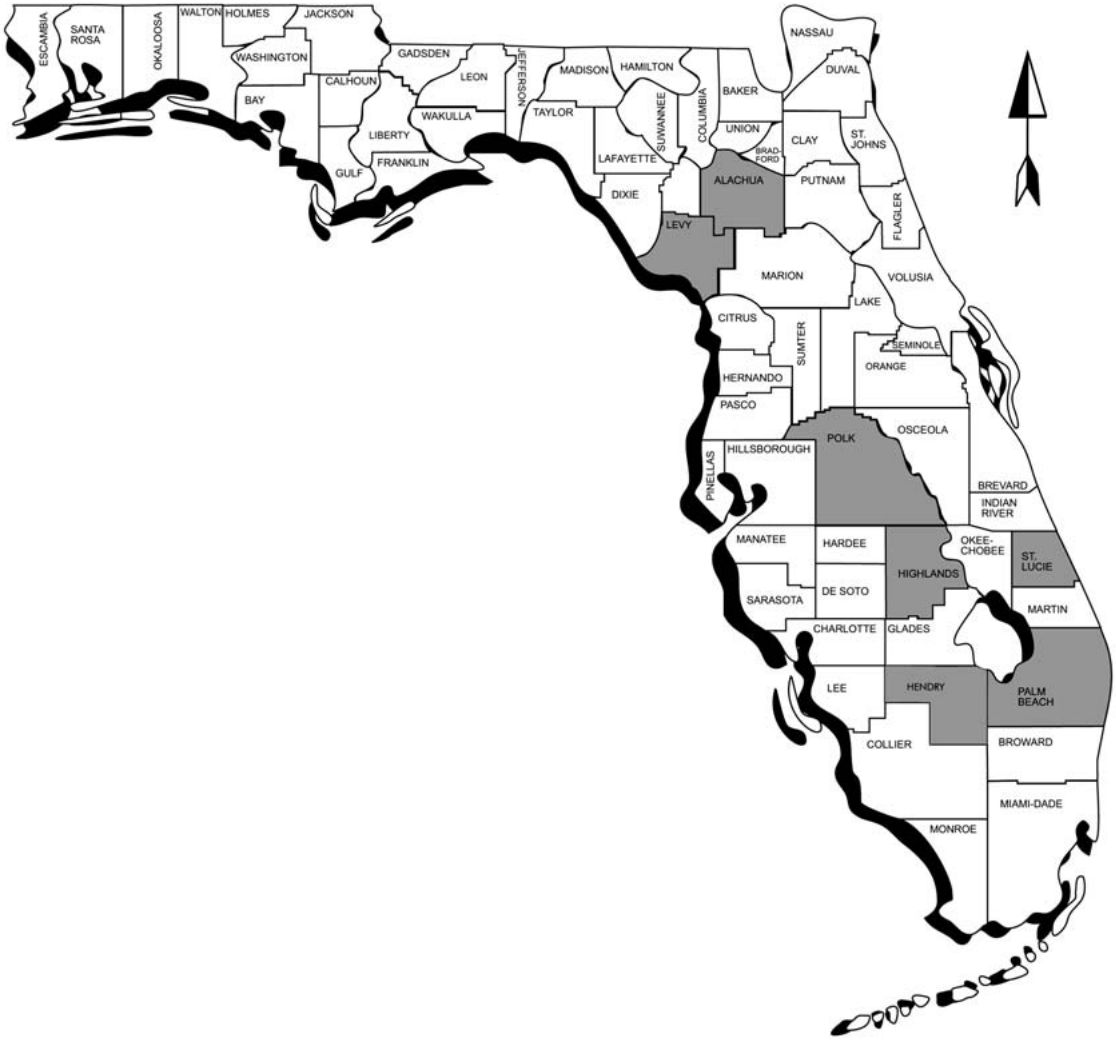

Fig. 2. Florida distribution of *D. armatipes*.

days (or longer if it rains); then, after threshing, they are placed in a wagon with a false bottom and dried in hot air (approx. 15-20°F above ambient) either on the farm or at the peanut buying stations (R. K. Sprenkel 2003, pers. comm.).

Peanuts are grown in at least 17 Florida counties. In year 2000, 94,000 acres of peanuts were planted in Florida, 86,000 acres of which were planted for dry peanuts, yielding 2,485 pounds per acre (FDACS 2002). Cash receipts of $53.64 million for 213 million pounds of peanuts made Florida the sixth largest producer in 2000. Jackson and Santa Rosa are the top two peanut-producing counties in the state. More surveys and inspections are planned for the coming harvest season by FDACS department surveyors, especially for these two counties.

Management Options

Management options include chemical treatment, weed control, and biological control. Risbec (1941) recommended not storing peanuts in previously infested premises without first spraying with an emulsion of oil and soap. Weed control might help prevent the build-up of *D. armatipes* population prior to harvest.

Several natural enemies are associated with *D. armatipes* in Africa. Eyles (1973) listed lizards, reduviids, *Nabis* spp., carabid beetles, *Anystis* and *Treatia* mites, *Pholcus* spiders, and jumping and lycosid spiders as predators on adult and immature *Dieuches*. Although the bethylid parasitoid *Cephalonomia* sp. (Hymenoptera) was listed as an egg parasitoid of *D. armatipes* by Eyles

(1973) quoting Risbec (1941), the latter author indicated that it was only reared in association with this species. Indeed, *Cephalonomia* wasps are known only to parasitize pupae or larvae of small Coleoptera in cryptic situations (Krombein 1979). The effectiveness of natural enemies in mitigating the pest status of *D. armatipes* is not known since most, if not all, of its known natural enemies are generalist predators. Classical biological control should be pursued with great caution. Modification of harvesting, drying, storing, and shipping methods could minimize losses in the event that this species becomes a pest in Florida.

We thank Martha Simpson (USDA/PPQ, Winter Haven) for guiding us to the collecting site in Polk Co., Vince Golia (Boynton) for sharing his records and specimens, Thomas Dobbs (USDA/PPQ, Miami) for interception data, Thomas J. Henry for improving this manuscript, Greg Hodges and Greg Evans for reviewing early drafts of this work, Michael Thomas (DOACS/DPI) for the photograph, Beverly Pope (DOACS/DPI) for assistance in library research, Katrina Vitkus (DOACS/DPI) for producing the map, Joseph Funderburk (University of Florida, IFAS, Quincy) for assistance in the survey in north Florida, Richard K. Sprenkel (University of Florida, IFAS, Quincy) for sharing information on Florida peanut harvesting and processing practices, and Stefanie Krantz (DOACS/CAPS, Gainesville) for her interest and assistance in this project. We are grateful to Dr. Richard M. Baranowski (University of Florida, TREC, Homestead) for sharing specimen data and for verifying the identification of this interesting species. This is Entomology Contribution #965, Bureau of Entomology, Nematology, and Plant Pathology, FDACS-DPI.

Summary

Dieuches armatipes (Walker), previously known to occur only in Africa and the West Indies, has become established in Florida. At present, the northernmost occurrence of *D. armatipes* is Gainesville. This species feeds on a variety of seeds on the ground. It has serious pest potential in northern Florida because it has been observed to feed on peanuts.

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FIRST REPORT OF *JUGLANS AUSTRALIS* (JUGLANDACEAE) AS A NATURAL HOST PLANT FOR *ANASTREPHA SCHULTZI* (DIPTERA: TEPHRITIDAE) WITH NOTES ON PROBABLE PARASITISM BY *DORYCTOBRACON AREOLATUS*, *D. BRASILIENSIS*, *OPIUS BELLUS* (BRACONIDAE) AND *AGANASPIS PELLERANOI* (FIGITIDAE)

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Anastrepha schultzi Blanchard (1938) (Diptera: Tephritidae) has so far only been found in Peru (province of Cuzco) and Argentina (Norrbom et al. 1999a). In Argentina, its presence was reported in the NW provinces of Salta (localities of La Caldera, Cerrillos, Campo Santo and Rosario de Lerma; Rosillo 1953; Blanchard 1961), Jujuy (localities of León, Tumbaya, Maimará and Jueya; Blanchard 1961; Manero et al. 1989), Tucumán, Catamarca and in the NE province of Misiones (Blanchard 1961). With the possible exception of Blanchard (1961), the latter reports stem from adults collected in liquid-based traps placed in *Psidium guajava* L. (Myrtaceae), *Prunus persica* (L.) Batch, *Prunus domestica* L. (Rosaceae) and *Citrus* spp. (Rutaceae) trees. *Anastrepha schultzi* belongs to the *fraterculus* species group (Norrbom et al. 1999b) and differs from *A. fraterculus* (Wiedemann) and also from *A. distincta* (Greene) by its longer, broader, and less serrate aculeus tip (length 0.43-0.59 mm vs. 0.34-0.43 mm in *A. distincta* and 0.21-0.30 mm in *A. fraterculus*; width 0.16-0.18 mm vs. 0.12-0.16 mm in *A. distincta* and 0.12-0.15 mm in *A. fraterculus*; and non-serrate or at most with distal 0.25 finely serrate, vs. distal 0.41-0.56 serrate in *A. distincta* and distal 0.50-0.67 serrate in *A. fraterculus*). *Anastrepha schultzi* further differs from *A. fraterculus* by its longer oviscape and aculeus (2.44-3.30 mm and 2.28-3.00 mm vs. 1.65-2.15 mm and 1.50-1.95 mm in *A. fraterculus*), and from *A. distincta* in consistently having the sides of the subscutellum dark brown (*A. distincta* is variable for this character, but more commonly has the subscutellum entirely orange). Blanchard (1961) reported that the host plants of *A. schultzi* were purportedly *Citrus* spp., *P. guajava*, and "peaches" ("duraznos").

Here we report for the first time field infestations by *A. schultzi* in *Juglans australis* Grisebach (locally known as "nogal criollo") (Juglandaceae) and confirm field infestations in *P. guajava* (locally known as "guayaba"). *Juglans australis* is a tree that reaches 10-20 m in height with the tree trunk measuring 40-50 cm in diameter when fully grown. The fruit is a subglobose drupe, with a fleshy mesocarp, measuring 3-4 cm when fully ripe (Digilio & Legname 1966). In Argentina, it is found at altitudes of 500-1500 meters above sea level and is distributed in the NW provinces of Jujuy, Salta, and Tucumán, where it forms part of the "Yungas" forests (also known as "Nuboselva" or Montane Cloud Forest). According to Digilio & Legname (1966) the fruiting period starts in February, but here we found that it spanned from mid December until mid February (details in Ovruski et al. 2003). *Psidium guajava* grows wild in perturbed patches of "Yungas" forests and it can be commonly found growing next to or near *J. australis* trees. In the study area, its fruiting period spans from February to April (Ovruski et al. 2003).

Our study site covered an area of 12 km^2 in the locality of Horco Molle, province of Tucumán located between 500 and 800 meters above sea level at 26°45' to 26°49'S latitude and 65°20' to 65°18'W longitude. The selected area belongs to what is locally known as "primer piso de la Selva Montana Basal de Las Yungas en el Distrito Pedemontano" (Brown 1995). We collected fallen fruit from the ground between December 27, 2001 and February 13, 2002 under the canopies of 15 *J. australis* trees and between March 7, 2001 and April 28, 2001 under the canopies of 20 *P. guajava* trees on a weekly basis. Between 8 and 10 *J. australis* fruit were collected and transported to the laboratory where they were individually measured, weighed and placed in ½-liter plastic containers covered with Organdi cloth. Each container had a 5-cm layer of moistened sand as a pupating medium. Fruit of *J. australis* weighed (mean ± SE) 27.7 ± 9.2 g and measured 12.0 ± 1.3 cm in diameter. Some late second and early third instars stemming from *J. australis* were separated to measure and weigh them. Mean ± SE size and weight were 8.0 ± 0.7 mm and 20 ± 5 mg, respec-

tively (n = 21). All pupae stemming from *J. australis* also were weighed and measured and then individually placed in 30-ml plastic containers covered with Organdi cloth and with moistened vermiculite on the bottom. Mean ± SE pupal weight was 13.0 ± 0.3 mg (n = 58). Once adults emerged, they were fed with a mixture of sugar and hydrolyzed protein plus water *ad libitum* to allow for full wing and body coloration.

We collected a total of 98 *J. australis* and 1,854 *P. guajava* fruit, weighing 2.7 and 75.1 kg, respectively, from which 854 and 36,919 *Anastrepha* spp. pupae, respectively, were recovered. Infestation rates were 318.5 and 492.6 larvae per kg of fruit in *J. australis* and *P. guajava*, respectively. Of the 430 *Anastrepha* adults that emerged from *J. australis* (50.4% emergence rate), 332 (142 ♀ and 190 ♂) were *A. schultzi*, and 98 (46 ♀ and 52 ♂), *A. fraterculus*. From 389 (45.6%) of the remaining 424 *Anastrepha* pupae nothing emerged, and from the remainder (n = 35) we recovered 4 (3 ♀ and 1 ♂) *Aganaspis pelleranoi* (Bréthes) (Hymenoptera: Figitidae), 2 (both ♀) *Doryctobracon areolatus* (Szépligeti) (Hymenoptera: Braconidae), 27 (15 ♀ and 12 ♂) *D. brasiliensis* (Szépligeti) (Hymenoptera: Braconidae), and 2 (1 ♀ and 1 ♂) *Opius bellus* Gahan (Hymenoptera: Braconidae) adults. Given that we were unable to distinguish *A. schultzi* and *A. fraterculus* at the pupal stage, we are not certain if the above mentioned parasitoids attack both *Anastrepha* species in our study area. We also recovered 5 *Ceratitis capitata* (Wiedemann) (Diptera: Tephritidae) pupae, from which 2 (both ♀) adults emerged.

Of the 18,901 *Anastrepha* adults that emerged from *P. guajava* (51.2% emergence rate), 152 (128 ♀ and 24 ♂) were *A. schultzi* and 18,749 (10,123 ♀ and 8,626 ♂), *A. fraterculus*. From 16,103 (43.6%) of the remaining 18,018 *Anastrepha* pupae nothing emerged, and from the remainder (N = 1,915) we recovered 705 (351 ♀ and 354 ♂) *A. pelleranoi*, 713 (368 ♀ and 346 ♂) *D. areolatus*, 226 (97 ♀ and 128 ♂) *D. brasiliensis*, 246 (141 ♀ and 106 ♂) *Utetes anastrephae* (Viereck) (Braconidae), and 25 (19 ♀ and 6 ♂) *O. bellus* adults. We also recovered 669 *C. capitata* pupae, from which 379 (222 ♀ and 157 ♂) adults emerged. As was the case with *J. australis*, we do not know which of the latter parasitoid species attack *A. schultzi* and *A. fraterculus*. Nevertheless, we were able to ascertain that only *A. pelleranoi* (none of the braconid parasitoids did so) attacked *C. capitata* given that the pupae of this species are clearly distinguishable from the pupae of both *Anastrepha schultzi* and *A. fraterculus*.

Our finding that *A. schultzi* infests *J. australis* and that it shares this resource with *A. fraterculus* is interesting from an ecological perspective given the fact that fruit within the Juglandaceae are usually infested by flies in the genus *Rhagoletis* (Smith & Bush 1999). Thus, this apparently represents a novel host shift that probably required adaptation to a unique chemical environment. We are in the process of studying the interaction of *A. schultzi* and *A. fraterculus* in *J. australis* in nature and also are trying to determine to what extent fruit from this tree differs chemically from *P. guajava*. Our finding of infestations of *P. guajava* by *A. schultzi* under natural conditions confirms the early report by Blanchard (1961). Elsewhere (Ovruski et al. 2003, 2004), we report on extensive collections (>20,000 fruit) of *Citrus aurantium* L., *C. paradisi* Macfad., *C. sinensis* (L.) Osbeck, *Prunus armeniaca* L., *P. domestica* and *P. persica* over a 5-year period (1991-1995) in NW Argentina that did not yield a single *A. schultzi* individual. Furthermore, between September 1999 and August 2002, we collected 5,665 *C. aurantium* (= 737.9 kg) and 5,974 *P. persica* fruit (= 209.9 kg) in the same study area, from which no *A. schultzi* emerged. We therefore believe that the *Citrus* spp. and "peach" host records by Blanchard (1961) need to be handled judiciously until confirmed. We consider them doubtful.

Anastrepha schultzi and *A. fraterculus* adults were identified by A. L. Norrbom using morphological characters. Voucher specimens were placed in the National Museum of Natural History, Washington, DC, USA, and Fundación Miguel Lillo (Tucumán, Argentina) entomological collections. *Juglans australis* and *P. guajava* were identified by the expert plant taxonomist Cristina Martín (Cátedra de Fanerógamas, Facultad de Ciencias Naturales e Instituto Miguel Lillo, Universidad Nacional de Tucumán). Voucher specimens of *J. australis* were placed in the herbarium of Fundación Miguel Lillo, San Miguel de Tucumán, Argentina. *Aganaspis pelleranoi*, *Doryctobracon areolatus*, *D. brasiliensis* and *Opius bellus* adults were identified by S. Ovruski using morphological characters. Voucher specimens were placed in the entomological collection of the Fundación Miguel Lillo (Tucumán, Argentina). This work was financed by the Agencia Nacional de Promoción Científica y Tecnológica de Argentina through the Fondo Nacional de Ciencia y Tecnología (FONCyT), Consejo Nacional de Investigaciones Científicas y Técnicas de Argentina (CONICET, grant PIP No. 4973/97), and the Instituto Superior de Entomología "Dr. Abraham Willink" (INSUE)—Facultad de Ciencias Naturales e Instituto Miguel Lillo—Universidad Nacional de Tucumán. Martín Aluja acknowledges financial support by the Mexican Campaña Nacional contra Moscas de la Fruta (Dirección General de Sanidad Vegetal—Secretaria de Agricultura, Ganadería, Desarrollo Rural, Pesca y Alimentación).

Summary

We document for the first time that *J. australis* is a natural host plant of *Anastrepha schultzi*

Blanchard, a species belonging to the *fraterculus* group. We also confirm earlier reports indicating that *A. schultzi* infests *P. guajava*. Fruit were collected in the locality of Horco Molle, province of Tucumán in NW Argentina, in a perturbed "Yungas" forest (Montane Cloud Forest). Infestation rates were 318.5 and 492.6 larvae per kg of fruit in *J. australis* and *P. guajava*, respectively. We also report that *A. schultzi* and *A. fraterculus* are attacked by the larval-pupal hymenopterous parasitoids *Aganaspis pelleranoi* (Figitidae), *Doryctobracon areolatus*, *D. brasiliensis* and *Opius bellus* (all Braconidae) in the study region.

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DESCRIPTION OF *ALLOTROPA ORACELLAE* (HYMENOPTERA: PLATYGASTRIDAE), A PARASITOID OF *ORACELLA ACUTA* (HETEROPTERA: PSEUDOCOCCIDAE)

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The genus *Allotropa* is in the subfamily Sceliotrachelinae of the family Platygastridae (Masner & Huggert 1989). The members of *Allotropa* are known as primary endoparasitoids of various mealybugs (Masner & Huggert 1989; Vlug 1995). Twenty-one species are described from all major biogeographic regions of the world, with five species described from the Nearctic region (Muesebeck 1979).

The mealybug *Oracella acuta* (Lobdell) was accidentally introduced into Guangdong Province, China in 1988 (Sun et al. 1996). Due to a lack of natural enemies, the mealybug spread rapidly through stands of slash pine, *Pinus elliotti* Englm. (Zhou et al. 1994), causing severe growth loss (Ren et al. 2000). After failed attempts to find native natural enemies in China, a Sino-U.S. forestry cooperative project was initiated to study the parasitoid complex of *O. acuta* in the U.S. and to evaluate the potential for a classical biological control program against the mealybug in China. Three primary endoparasitoids of *O. acuta* were identified: *Zarhopalus debarri* Sun (Encyrtidae), *Acerophagus coccois* E. Smith (Encyrtidae), and one in the genus *Allotropa* (Clarke et al. 1990, 1992; Sun et al. 1998). This plastygastrid was identified by one of the authors (L.M.) as a species new to science. Due to its potential inclusion in a biological control program, a description of this species is undertaken.

New character states were used in this description because those traditionally used in taxonomy of *Allotropa* offered only limited value in species discrimination. Among the new character states used were the morphology of the mesopleuron, the pilosity of the metapleuron, and the microsculpture of the mesoscutum. The mesopleuron offered excellent distinguishing character states, such as the shape of the mesopleural depression, and the presence or absence of a sternaulus or of deep pits. In contrast to the glabrous mesopleuron, the metapleuron is typically hairy in most, but not all species. The microsculpture of the mesoscutum offered useful diagnostic character states in most species. Since the character states above were not considered in previous descriptions, types of all species were re-examined for confirmation.

Allotropa oracellae Masner **sp. nov.**

Diagnosis

Body black, antennae and legs predominantly dark brown; wings clear; mesopleuron posteriorly with coriaceous microsculpture refined to form nearly smooth area; scutellum rather flattened, predominantly smooth; mesopleuron with complete horse-shoe shaped depression and no pits, sternaulus well developed; metapleuron entirely covered with silvery pilosity.

Description, Holotype (female)

Body length 0.86 mm; body black, nucha of propodeum and T1 brownish, legs predominantly brownish, with trochanters, basal part of tibiae and all tarsi yellowish brown. A1 and clava (A7-A9) brown, A2-A6 light brown; wings clear. Head in dorsal view (Fig. 1C) subellipsoidal, transverse (27:13); hyperoccipital carina only weakly indicated, almost effaced; vertex and occiput with coriaceous microsculpture; lateral ocellus contiguous with inner orbit of eye; eye with short fine pilosity; head in front view with frons coriaceous, also inner orbit but with smooth central part below anterior ocellus; sculptured part of frons with scattered apressed silvery hairs; inter-orbital space distinctly larger than eye height (17:11); antennomeres (A1-A9) in relative proportions (length:width) 15:5, 5.5:3, 5:1.5, 2.5:1.5, 2.5:1.7, 3:3, 4:3, 5:3, A7 and A8 strongly extended-pointed outwardly (Fig. 1A).

Mesosoma in dorsal view (Fig. 1D) slightly narrower than head (25:27); mesoscutum with dense coriaceous microsculpture; microsculpture refined to gradually form an almost smooth area posteromedially (better appreciated if viewed from behind); series of anterior scutellar pits (bellow transcutal articulation) strongly developed;

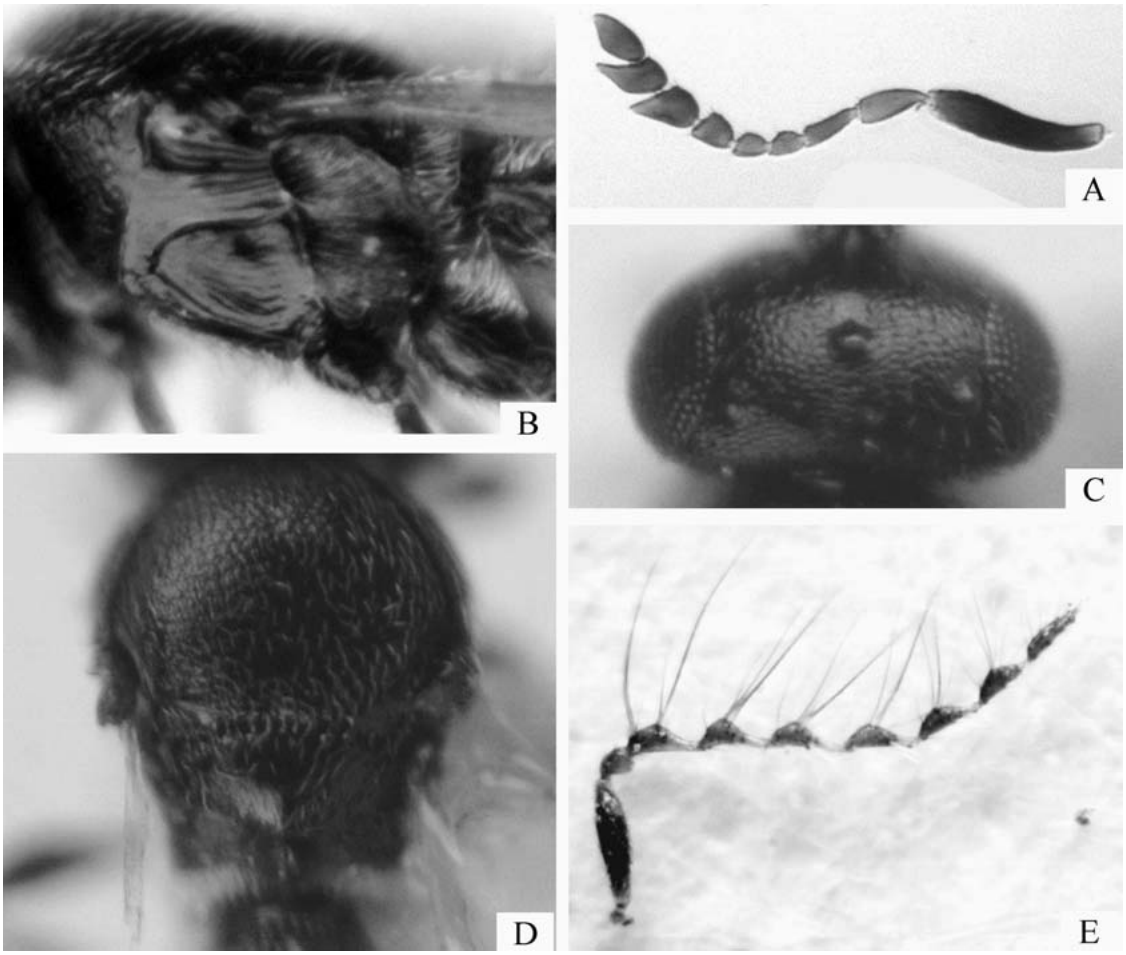

Fig. 1. *Allotropa oracellae* Masner **sp. nov**. A-D female; A: antenna (outer aspect), B: thorax in lateral view showing mesopleurum, C: head in dorsal view, D: thorax in dorsal view, E: male antenna (inner aspect).

scutellum predominantly smooth; mesosoma in lateral view (Fig. 1B) with mesoscutum and scutellum almost flat, non convex; side of pronotum with fine irregular coriaceous sculpture and with scattered apressed pilosity; mesopleuron predominantly glabrous, with several horizontal wrinkles near upper margin; mesopleural depression with distinct horse-shoe shaped declivity sharply margined dorsally by a deep sulcus and a complete sternaulus ventrally, deep pits not developed; anteroventral part of mesopleuron with patch of coriaceous microsculpture and sparse silvery pilosity; entire metapleuron with coriaceous microsculpture and side of propodeum with dense long, non-apressed silvery hairs.

Metasoma distinctly elongate (45:22); T1 with strong longitudinal costae not surpassing basal half of tergite; T2 basally with short costae not exceeding T1 length, rest of T2 glabrous and smooth; T3-T5 short, each with sparse hairs; T6 broadly triangular, wider than long (10:6), sharply pointed apically.

Allotype (male)

The male differs from female principally in structure of the antenna (Fig. 1E); antennomeres (A1-A9) in relative proportions (length:width) 15:5, 4:3, 10:3, 9:3, 9:3, 8:3, 8:3, 11:2.5, A3-A9 with long upright bristles, bristles on A3-A6 about 2.5 times as long as antenomeres; A3-A8 distally with long neck-like constriction, constricted part nearly as long as basal unconstricted part, especially in A3-A5; A9 lancetoid, not differentiated in broader basal and distal neck-like parts.

Etymology

The specific epithet is derived (in genitive form) from *Oracella*, the generic name of the host mealybug; the gender is feminine.

Material Examined

Holotype, female, United States, Georgia, Toombs Co., Lyons, 30 September 1996, ex. *Oracella acuta* Lobdell on loblolly pine (*Pinus taeda* L.), emerged in laboratory, Jianghua Sun, Hopkins # 67519; Allotype, male, with same data as holotype; Paratypes, 11 females and 21 males, with same data as holotype. The holotype and some paratypes are deposited in the United States Natural History Museum, Washington, D.C.; other paratypes are in the Canadian National Insect Collection (CNIC) and the Natural History Museum of the University of Georgia.

Recognition and Relationships

Allotropa oracellae Masner sp. nov. belongs to the larger group of *Allotropa* species characterized by distinct horse-shoe shaped mesopleural depression. In this group, *Allotropa oracellae* is further distinguished by gradually effaced microsculpture on postero-median part of mesoscutum and also by distinctly neck-like constricted distal parts of the male antennomeres A3-A5. The degree of refinement of the microsculpture on the posterior mesoscutum may vary from weak, especially in females, to almost completely smooth, as in most males. The coloration of the antennae and legs appear constant, at least in material examined.

Host and Distribution

Allotropa oracellae was reared from the mealybug, *O. acuta* infesting loblolly pine in the southeastern United States. No additional specimens were discovered in the rich Nearctic material in CNIC. Additional specimens have been collected in Arkansas, South Carolina, North Carolina, Louisiana, and Texas. *Allotropa oracellae* appears to occur throughout the range of *O. acuta* in the southeastern United States.

This work was supported by grants from the USDA Forest Service, the National Center of Forest Health, and the USDA Foreign Agricultural Service, International Cooperation and Development. A visit to the U.S. Natural History Museum, Washington DC by Dr. Masner to study type material of *Allotropa* species was supported by a USDA Forest Service grant. We gratefully acknowledge the leading role played by Dr. Gary DeBarr in this project. Thanks are extended to Dr. Yanzhou Zhang, Institute of Zoology, Chinese Academy of Sciences for taking the photographs. Publication of this paper is partially supported by the CAS Innovation Program (KSCX1-SW-13).

Summary

The parasitoid *Allotropa oracellae* Masner sp. nov. (Platygastridae) is described. This species was collected in Georgia (United States) and is an endoparasitoid of the mealybug, *Oracella acuta* (Lobdell). New character states for the genus were used, including the pilosity of metapleuron and the microsculpture of mesoscutum.

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HOT PEPPERS AS A HOST FOR THE MEXICAN FRUIT FLY *ANASTREPHA LUDENS* (DIPTERA: TEPHRITIDAE)

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On the 28th of April, 2003, a shipment of manzano chile peppers (*Capsicum pubescens* Ruis & Pavon cv Rocoto) entering the United States at Pharr, Texas, was found to be infested with insect larvae. USDA inspectors first noted maggots crawling in the bed of the truck underneath the 16 cardboard boxes (240 Kg) containing the chile peppers. Further inspection confirmed that the larvae were in, and emerging from, the fleshy pods. Two of the larvae were immediately preserved in alcohol while 50 more larvae were kept alive. All specimens were hand carried to the nearby USDA-ARS laboratory in Weslaco, Texas for identification. Microscopic examination established that the larvae had the morphological characteristics of the Mexican fruit fly, *Anastrepha ludens* (Loew), as described by Steck et al. (1990). However, this identification was tentative because there are approximately 200 described species in this genus (Norrbom et al. 1999) and the larval stages are known for only thirteen. Several kinds of maggots will breed in rotting vegetable matter including chile peppers, but these are non-pest species, and this incident involved sound fruit (Fig. 1). No dipterans are listed as economic pests of chile peppers by English & Lewis (2004). Baker et al. (1944) cited incidents of *A. ludens* in "bell peppers and chili peppers" and there are equally ambiguous reports of another tephritid, *Zonosemata vittigera* (Coquillet), taken in "peppers" (Cole 1969). *Zonosemata electa* (Say) is known as the "pepper maggot" (Peterson 1960) and has been reared from "*Capsicum annuum* L." (Smith & Bush 1999). The latter solanaceous plant species includes both hot and sweet peppers. The usual host plants for *Zonosemata* spp. are members of the genus *Solanum* (Norrbom 2002). To confirm the specific identity of the larvae infesting the manzano peppers, the available live larvae were placed in culture and maintained in the laboratory to obtain adults. Larval specimens that died before puparation were preserved in alcohol and sent to Bruce

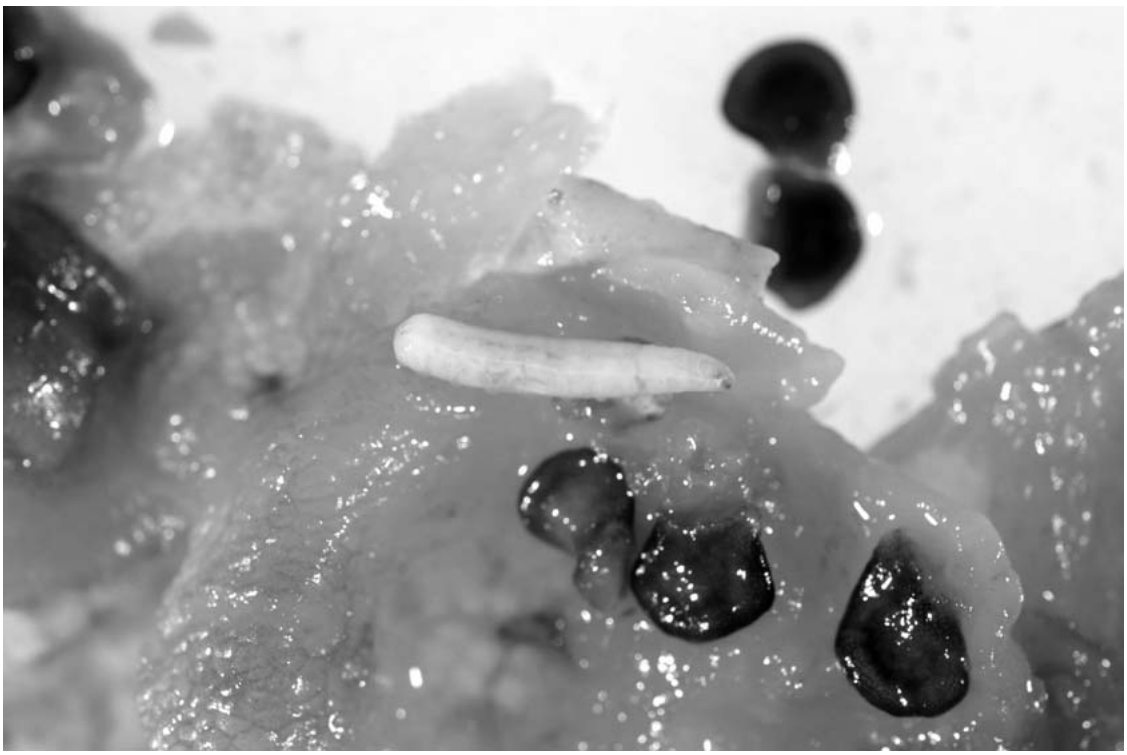

Fig. 1. Larvae of *Anastrepha ludens* infesting a manzano pepper intercepted at the U.S.-Mexico border. Note the black seeds characteristic of *Capsicum pubescens*.

A. McPheron of Pennsylvania State University for genetic fingerprinting. Based on sequencing of a fragment of the mitochondrial 16S ribosomal RNA gene, the specimens were indistinguishable from sampled populations of *A. ludens* (Silva et al. 2001). This gene has been studied and is diagnostic for 40 of the most important species of *Anastrepha* (McPheron et al. 1999).

A total of 42 larvae pupariated and of these eleven eclosed as adults. All were *A. ludens*, a determination confirmed by Allen L. Norrbom of the USDA-ARS Systematic Entomology Laboratory in Washington D.C. At Weslaco, all non-eclosed puparia were examined and the number of tubules on the anterior spiracles did not differ from those in puparia of *A. ludens*. On the 2nd of May, the 16 boxes of embargoed manzano peppers were taken to a disposal site for burial. At that time additional larvae were seen egressing the fruit and some of these were collected as voucher specimens by USDA-APHIS personnel.

Because records indicated that shipments of manzano peppers had cleared customs in the days immediately previous to the discovered infestation, an effort was made to track these shipments to their destinations. Manzano peppers infested with larvae were recovered from Chicago, IL; Detroit, MI; Atlanta, GA; Richmond, VA; and at two retail outlets in Pinellas County, FL. Two weeks later, on 16 May 2003, an adult *A. ludens* was found in a fruit fly trap in Orlando, FL. Because the previous detection of this species in Florida was in Sarasota in 1972 (Steck 1998) the new detection was presumed to have originated with the infested manzano pepper shipments.

Anastrepha ludens is a major pest of citrus and mangoes with a wide host range known to include at least 60 varieties of fruit (Norrbom & Kim 1988). Sweet peppers, cultivars of *Capsicum annuum* that lack the alkaloid capcaicin, are occasionally infested by *A. ludens*, but confirmed records of hot peppers (cultivars containing capcaicin) as larval hosts have not been reported. According to the inspectors who first discovered the infested shipment, just standing next to the open truck with the manzano peppers caused their eyes to water. On the Scoville scale manzano peppers (also marketed as "rocoto" or "perón" peppers) are rated at 12-30K (by comparison, jalapeños are rated 2.5-8K Scoville Units) (DeWitt & Gerlach 1990). Although manzano peppers are a low volume specialty item, accounting for much less than 1% of all peppers exported by Mexico (McClure 2003), chile pepper species in aggregate are a major commodity imported to the United States. Because of its non-host status, chile pepper importations had not required a disinfestation treatment or more than cursory inspection. In response to this incident, higher than normal inspection rates were implemented on all peppers, and a stricter protocol established for shipments destined to citrus producing states. Nonetheless, the more intense inspections failed to result in further interceptions of infested chile peppers of any species.

In order to further our understanding of hot peppers as potential hosts of *A. ludens*, a series of experiments were conducted. To provide material for these tests, arrangements were made with the International Services branch of USDA-APHIS to provide fresh manzano peppers from Mexico, inasmuch as these peppers are not commercially cultivated in the United States. One box (15 kilos) of manzano peppers was acquired at a market in Mexico City and shipped by air to our satellite laboratory in General Teran, Nuevo Leon, Mexico. On arrival, technicians discovered that these peppers also were heavily infested with *A. ludens* larvae.

Questions raised by these incidents include whether other species of hot peppers are susceptible hosts for oviposition by *A. ludens*; whether the host status of chile peppers is determined primarily by physiological or ecological factors; whether the flies infesting the manzano peppers were adaptively different from other populations of *A. ludens* (host-races); and are flies reared on chile peppers reproductively competent.

The flies used in these experiments were from two sources. One line was established from adults reared from the initial interception of manzano peppers at Pharr, Texas in April 2003. The second source was the research colony of *A. ludens* maintained at the USDA-ARS laboratory in Weslaco, Texas. This laboratory colony originated with specimens collected from yellow chapote, *Casimiroa greggii* (S. Wats.) Chiang, in Nuevo Leon, Mexico in 1994. Yellow chapote is a wild Rutaceae and the primary native host of *A. ludens* in Mexico (Plummer et al. 1941).

Two sets of experiments were conducted. In the first set of tests the flies were offered fresh fruits in both choice and non-choice configurations under laboratory conditions. In the second set of experiments the flies were released into a green house within the Weslaco quarantine facility with potted pepper plants to determine the acceptability of the living, undehisced pods as oviposition sites.

The Weslaco colony flies were reared on an artificial larval media described by Spishakoff & Hernandez-Davila (1968). Because wild flies are reticent to lay eggs in the artificial substrate used in mass-rearing colonization, the flies bred from the manzano peppers were offered fresh fruit for oviposition. Placed in the cage with these adults were manzano peppers, bell peppers, grapefruit and mangoes. Although the flies were observed "stinging" all of these fruits with the aculeus, only the mangoes became infested.

Flies and fruit were distributed among separate fine mesh screen cages, $30 \times 30 \times 30$ cm in dimension. The cages were maintained in an environmental chamber at 24°C, 12:12 DL. Each

cage contained a glass vial filled with distilled water plugged by a cotton wick and an open petri dish with granulated sugar and torula yeast. All flies were females of 15 d age that had been caged with males up until the time of the experiment. Females are capable of laying eggs at age 11 d when maintained at 24°C (Liedo et al. 1993). The test fruits were set in the cage on short wooden pegs so that flies could access the bottom side of the fruit. Aluja et al. (1999) cite field observations that *A. ludens* always "sting" oranges on the bottom side.

Test 1: This test used the Nuevo Leon strain from the USDA colony. Ten female flies were released into each cage. In order to approximately equalize surface area of fruit, one sour orange (*Citrus aurantium* L.) was considered equivalent to four peppers or four chapotes. The sour oranges were picked fresh from trees on the day previous to testing. Habanero peppers (*Capsicum chinense* Jacq.) were purchased from a local grocer. Because yellow chapote does not grow in the U.S., fruits were collected in Nuevo Leon, Mexico and transported in coolers to our laboratory, under permit, the week prior to the test.

The fruits were distributed in six cages as follows: habanero peppers only, sour orange only, yellow chapote only, one sour orange combined with habanero peppers, four yellow chapote combined with four habanero peppers, and four yellow chapote combined with one sour orange.

At the end of 24 h the fruit was removed from the cages and placed individually in cups containing moistened vermiculite for pupariation and held in the chambers at 24°C. In the three cages where only one choice was provided, new fruits replaced those exposed, but a sour orange was placed in the cages that held the habanero peppers and habanero peppers placed in the cages provided orange or chapotes. The rationale of this design was to demonstrate that any failure to oviposit was due to choice and not due to reproductive incapacity. After 24 h these fruits were removed and maintained in separate cups as those previously exposed. After ten days (normal larval development time in the laboratory) the fruits were cut open to determine degree of infestation, if any.

Test 2: Because the only fruits infested in the first test were those offered on the second day, it was reasoned that oviposition response required more than a 24 h entrainment. For the second test, no rotation of fruit was included, but instead, exposure time was increased to 48 h. Also, manzano peppers acquired from Mexico City replaced the yellow chapote in this test. This test was conducted with the USDA colony flies with the same numbers and conditions as the previous test.

Test 3: For this test, mango (*Mangifera indica* L. cv Tommy Atkins) was substituted for the sour orange and only manzano peppers were offered, alone or in combination with the mango. This test was also conducted with the Nuevo Leon strain with the same numbers and conditions as the previous test.

This test was conducted with progeny of the larvae found in the intercepted manzano chile peppers at Pharr in April 2003. These were reared in mangoes maintained under a constant temperature and light regime. Both sexes of adult flies were maintained together until the flies were 11 d old. The test was conducted in the ARS quarantine security green house with naturally cycling temperatures and light regime. Ten females were released into a large screened cage ($78 \times 48 \times 32$ cm) containing three potted chile pepper plants with mature fruit. A bell pepper plant (*Capsicum annuum*) with two mature (red) pods; a habanero pepper plant with seven mature (orange) pods; and an Anaheim pepper plant (*Capsicum annuum*) with three mature (red) pods. All potted pepper plants had been grown together in the greenhouse from seedlings. These particular plants were selected on the basis that the surface area of the pods on each plant was approximately the same, although the number of pods differed. In the cage, the flies were provided with wicked water and open petri dishes with sugar and yeast as before. The flies were exposed to the potted plants for 48 h. After that time, the potted plants were removed to an environmental chamber and held for three weeks. At that time, the pods were cut open to determine infestation. The test was then repeated by installing new plants and replacing the females that had died during the first test with flies from the same cohort.

The majority of the live larvae that were recovered from the intercepted manzano peppers (42 of 50) successfully pupariated. From these 42 puparia only 11 adults successfully developed and eclosed, about 25%. However, survival of larvae collected from citrus and yellow chapote fruit in nature and brought into the laboratory is typically less than 50%. Because wild flies do not readily accept the artificial oviposition medium used in mass rearing, these adults were offered a variety of fruits including sweet and hot peppers, oranges and mangoes. The F_1 generation (4 females and 3 males surviving to maturity) successfully infested and produced progeny only in the mangoes, with over 50 larvae developing. The larger F_2 generation oviposited in both peppers and mangoes. We recovered 27 pupariating larvae from the manzano peppers, 21 larvae from red Tommy Atkins mango and 36 from yellow Manila mango.

In the first test with the Nuevo Leon strain, none of the offered fruit became infested during the 24 h exposure although "stinging" was observed. Evidently, oviposition had not become entrained because after the subsequent 24 h exposure, third instars were found in both the sour orange (2 larvae) and the habanero peppers

(4 larvae). A typical clutch oviposited by a female *A. ludens* is 5-6 eggs according to Berrigan et al. (1988). Habanero peppers are the most pungent of the hot chile pepper species with a rating of 100-500K Scoville Units. Evidently, capcaicinoids do not inhibit oviposition or larval development. It is interesting that the yellow chapotes did not become infested even though it is the native, and therefore presumably, the preferred host fruit for this fruit fly species.

In the second test, manzano peppers were substituted for the yellow chapote and the test extended to 48 h to allow entrainment of oviposition. In subsequent dissection, one larva developed in the manzano pepper and pupariated. However, no adult emerged. In the habanero peppers, one fruit was infested with nine larvae. All pupariated and five adults emerged. The sour orange was not infested.

In the third test, mango was substituted for the sour orange and tested against the manzano peppers. None of the manzano peppers became infested. The mango combined with the peppers produced two larvae which pupariated. The mango by itself produced 72 pupariating larvae. The results of these tests suggest that under laboratory conditions mango, though a non-native host, is preferred by *A. ludens* as an oviposition site compared both to the citrus and the chile peppers. But the results also suggest that chile peppers are as acceptable as citrus, which is the normal host.

A factor which can influence the acceptability of a fruit for oviposition is its ripeness and its status pre- and post-dehiscence. Perhaps this is why the yellow chapotes were not infested. The laboratory tests established that hot peppers are physiologically acceptable as breeding hosts for *A. ludens*. Greenhouse tests were conducted to test if fruits on the bush were similarly acceptable. Bell peppers (n = 2) were not infested. Anaheim peppers (0.5-2.5 Scoville Units) were infested, with five larvae found in one pod and six in another. The larvae were placed in vermiculite for pupariation with five of the larvae pupariating and four eclosing as adults. Of the seven habanero peppers, one was found infested with 11 larvae. Three pupariated and two eclosed as adults. In the second replicate only the Anaheim peppers became infested. Of the six larvae recovered, four pupariated and three eclosed as adults.

The results of these tests demonstrate that even the hottest chile peppers are adequate hosts for development of *A. ludens* larvae and that *A. ludens* females are not deterred from oviposition by the capcaicin alkaloids. Such being the case, the important question is why then is the incidence of infestation in commercially grown chile peppers so infrequent? Manzano pepper is unlike other commercial cultivars of peppers in both its growth habit and habitat. The three most commonly cultivated species, *Capsicum frutescens* L. *C. annuum* and *C. chinense* are low (up to 2 m), herbaceous perennials, grown in commercial plantings as a row crop. *Capsicum pubescens* is native to the high elevations of the Andes Mountains of Peru and Bolivia (Eshbaugh 1979) where its growth is reportedly bushy and reaches considerable size, up to 2 m according to Rick (1950). However, the commercial cultivars grown in Mexico are viney in habit, much like tomato plants. In April 2004, the author visited the manzano pepper growing areas in Mexico. In the region around Patzcuaro, Michoacan, cited by Andrews (1984) as the primary commercial production area, the crop is known locally as "chile perón." This region is better known for its commercial production of avocados, *Persea americana* (Mill.). The manzano pepper plants are rooted in the shade of the avocado trees and grow as a vine using the branches of the avocado tree for support. *A. ludens* is trapped in this area but is not considered to be a pest of avocados (Aluja et al. 2004), and local growers were unaware of infestations in the manzano peppers. At the time of the visit in April the peak harvest season was well past, with only late season fruit remaining. We examined mature pods in the avocado groves at Tacambaro and in the local markets of Patzcuaro and none were infested with fruit fly larvae. The habitat at Tacambaro is cool and dry with an elevation of ca. 2200 m. The native vegetation in the area is pine and oak forest.

The infested manzano peppers intercepted at Pharr, TX, were in boxes labeled "Ixhuatlan de Cafe, Veracruz." At Ixhuatlan the product is known locally as "Chile de Arbol." In this region the peppers are grown as an understory plant intercropped with coffee plants. The pepper plants are staked to provide support. The elevation at Ixhuatlan is ca. 1,400 m and the climate is cool but humid. The overstory trees are those indigenous to the tropical montane forest on the eastern slope of the Sierra Madre Oriental (*cf.* Rzedowski 1983). The phenology is apparently variable because this April the plants were in flower with only a few having green immature pods (Fig. 2). Moreover, we found no manzano peppers for sale in the local markets. According to the growers, the manzano pepper is a relatively new crop to this area, introduced only in recent years. They were aware of the fruit fly problem but were also experiencing serious problems with plant diseases, especially fungal pathogens. The high humidity in this region may be less favorable for manzano pepper production compared to the traditional growing area in Michoacan.

Aluja et al. (1999) cite observations in citrus that *A. ludens* females shun exposed fruit in favor of those in the well-shaded parts of the tree. It may be that *A. ludens* avoids most peppers because it avoids open exposed habitats in favor of groves, forests, and shaded urban settings. If the primary factors influencing oviposition are behavioral and

Fig. 2. Manzano pepper plant in a coffee finca at Ixhuatlan de Cafe, Veracruz where the infested peppers originated.

ecological, then manzano peppers may be more susceptible to infestations than other commercial pepper varieties. If so, this information is relevant to quarantine and import inspection protocols.

Summary

Hot chile peppers were not previously considered to be hosts for the Mexican fruit fly. Laboratory tests demonstrate that cultivars with high levels of capcaicinoids are acceptable to ovipositing females, even when given a choice between peppers and citrus, and are adequate for larval development. Recent intercepts of manzano peppers infested with larvae are the first indication that such infestations occur in nature and their import is a potential risk for entry by this invasive tephritid species. Reasons for the low incidence of natural infestations in hot peppers are discussed.

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PHYTOSEIIDAE INCREASE WITH POLLEN DEPOSITION ON CITRUS LEAVES

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The Phytoseiidae can be classified into four categories based on feeding habits (McMurtry & Croft 1997). Type I phytoseiids are specialized predators of *Tetranychus* species (e.g., *Phytoseiulus* species). Type II phytoseiids are selective predators of tetranychid mites, *Galendromus*, some *Neoseiulus* species, and a few *Typhlodromus* species. Type III phytoseiids are generalist predators consisting mostly of *Typhlodromus* and *Amblyseius* species. Type IV phytoseiids are specialized pollen feeders/generalist predators such as *Euseius* species. The three most prevalent phytoseiid species on Florida citrus are *Euseius mesembrinus* (Dean), *Typhlodromalus peregrinus* (Muma) (Childers 1994), and *Iphiseiodes quadripilis* (Banks) (Villanueva & Childers, unpubl. data; Childers, unpubl. data). All three species can complete their life cycle on an exclusive pollen diet (Abou-Setta & Childers 1987; Peña 1992; Villanueva & Childers, unpubl. data). These studies demonstrated that the most abundant phytoseiids in Florida citrus are either type III or IV species. Furthermore, one peak of abundance in Florida coincides with flowering in *Citrus*, *Pinus* sp., and *Quercus* sp. Members of the genera *Pinus* and *Quercus* are commonly found around citrus orchards in uncultivated areas such as windbreaks or in densely planted stands for use as pulp or lumber. Pollen from these plants and species of weeds and shrubs accumulate on the adaxial surfaces of citrus leaves. These pollens can provide important food sources for phytoseiid mites. Studies on citrus in South Africa demonstrated a high correlation between early pollen availability and abundance of *Euseius addoensis addoensis* (van der Merwe and Ryke) (Grout & Richards 1992a,b). The objective of this study was to examine the relationship between pollen on grapefruit leaves and the number of phytoseiids present on these leaves during the period of citrus flowering.

Weekly leaf samples were taken from 10 (11-yr-old) 'Ruby red' grapefruit trees at the Citrus Research and Education Center in Lake Alfred between 2 February and 16 March 2001. The orchard had not received a pesticide application since September 2000. All 10 sampled trees were selected at random within one row and were spaced at least 25 m apart. Samples consisted of one terminal with 5 leaves damaged by citrus leaf miner [*Phyllocnistis citrella* Stainton (CLM) (Lepidoptera: Gracillariidae)] and one terminal with 5 leaves without damage per sample tree. Leaf terminals were taken to the laboratory and both phytophagous and predatory mite eggs and motiles were counted with a stereomicroscope.

One healthy leaf from each of the same trees was collected for pollen counts. A 5- to 7-cm long strip of transparent adhesive tape was placed along the middle vein on the adaxial surface of each leaf for 3 to 5 min and then removed. Each leaf was placed on a sheet of wax paper, labeled, and stored in the refrigerator until processed. Slides were prepared by placing 1-cm^2 pieces of tape individually on a slide with the adhesive part up. Prior to placement of the cover slip, a drop of dye was added consisting of 0.2 g of Trypan Blue in 200 ml of 50% glycerol (Addison et al. 2000), a formulation typically used for staining ascospores (Skaria & Tao 1996). The glycerol causes the pollen grains to swell slightly (Addison et al. 2000). A phase contrast microscope was used to count pollen grains at a magnification of 400×. The area for counting pollen grains was the field of view, approximately 1.13 mm^2 as calculated with a Hemacytometer Bright Line® (Reichert, Buffalo, NY). Five fields of view were counted for each 1 cm^2 of prepared area, yielding an estimate of the mean number of pollen grains per 1.13 mm^2. The first grain of pollen found while searching the slide was centered in the middle of the field of view and then all pollen grains in that field of view were counted. Pollen counts and the total number of phytoseiids present from leaf samples on the indicated dates were evaluated to determine correlations between the two factors.

Phytophagous mite species observed in this study included *Eutetranychus banksi* (McGregor), *Eotetranychus sexmaculatus* (Riley), *Phyllocoptruta oleivora* (Ashmead), and *Aculops pelekassi* (Keifer). The numbers of the two eriophyid species are combined as *P. oleivora* in Fig. 1. Known predacious species included *T. peregrinus* and *I. quadripilis* (two phytoseiids), and *Agistemus* sp.(Stigmaeidae). Phytoseiid numbers increased from 8 February to 16 March with significantly larger numbers of phytoseiids occurring on the mined leaves (Villanueva & Childers, unpubl. data). Most phytoseiid mites were found on 16 March, and included 55 *I. quadripilis* and 38 *T. peregrinus* (Fig. 1).

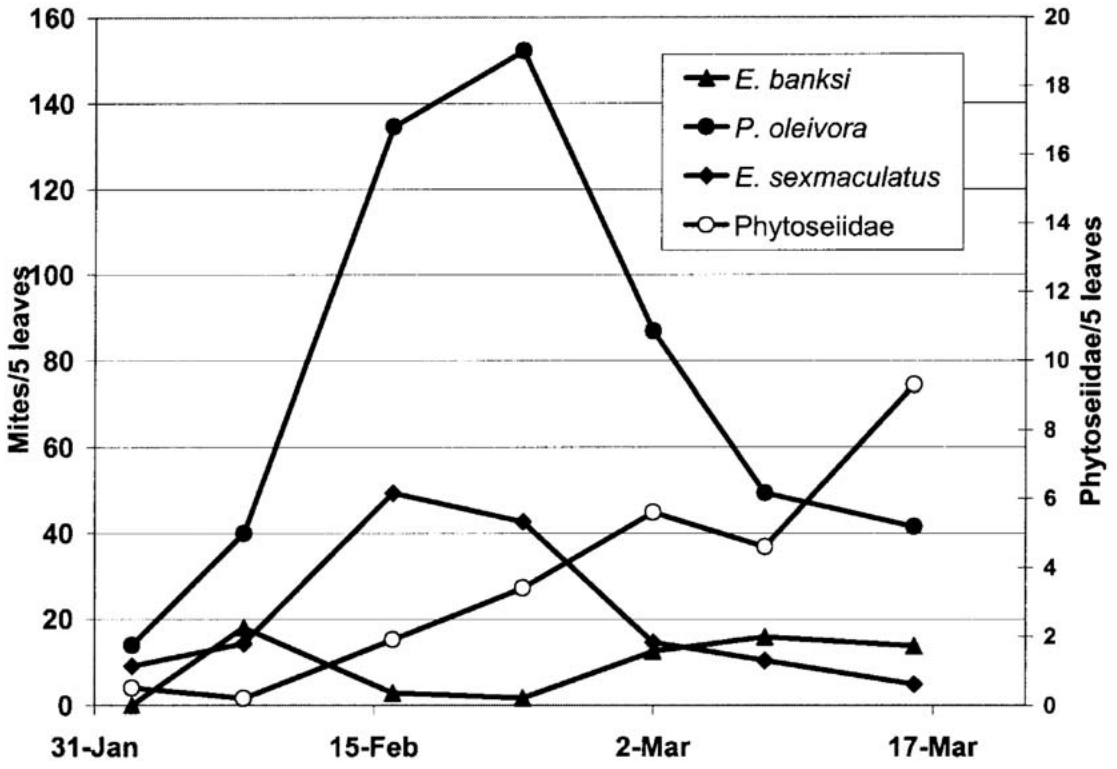

Fig. 1. Population densities of phytophagous mites in a grapefruit orchard in Lake Alfred.

Eggs and motiles of *E. sexmaculatus* were abundant during February and the phytoseiids likely preyed on them in addition to the pollen. The identified pollen types found on the grapefruit leave included *Quercus* sp., *Pinus* sp., *Citrus*, and other unidentified types. The highest counts of pollen observed on 8 March were for *Quercus* (6.7 ± 2.0, n = 50), on 16 February for *Pinus* sp., (6.9 ± 1.4, n = 50), on 16 March for *Citrus* (5.5 ± 0.9, n = 50), and on 8 March for unidentified pollen types (10.7 ± 1.6, n = 50). Grapefruit trees in this orchard bloomed between 2 and 16 March with most of the flowers completing anthesis by 16 March. The correlation between the number of pollen grains and phytoseiid mites was positive and highly significant (P = 0.004), yielding a Pearson correlation coefficient r^2 = 0.83, n = 50 (Statsoft, Inc. 2000). Both phytoseiid numbers (8.5 ± 0.5, n = 50) and pollen grains (22.0 ± 9.3, n = 50) increased between 2 February and 16 March (Fig. 2).

Our data show that the eriophyid population reached its peak by the end of February, whereas the phytoseiid population showed only a small incremental increase around the same time but reached its peak by mid March (Fig. 1). This is approximately one week after pollen grain counts were the highest. Others have reported increases in phytoseiid populations with pollen availability. Addison et al. (2000) observed that *T. pyri* abundance had a better correlation with early season pollen density in apple than with the abundance of its eriophyid mite prey, *Aculus schlechtendali* Nalepa. Similarly, when *Euseius tularensis* Congdon was released into navel orange orchards in California, the mite exhibited a greater population increase in orchards with a ground cover crop of mixed leguminous plants than in orchards without ground cover to serve as a pollen source (Grafton-Cardwell et al. 1999). The results shown here demonstrate the potential importance of citrus and non-citrus pollens in phytoseiid increase. The effect of pollen on the reduction of predation during prey abundance and/or as a food source for survival during times of prey scarcity remains to be studied. Further studies are needed to identify possible use of supplemental pollens either introduced into citrus orchard sites or grown as cover crop plants to sustain higher phytoseiid populations during April-May when eriophyoid and tetranychid mite populations often begin to increase.

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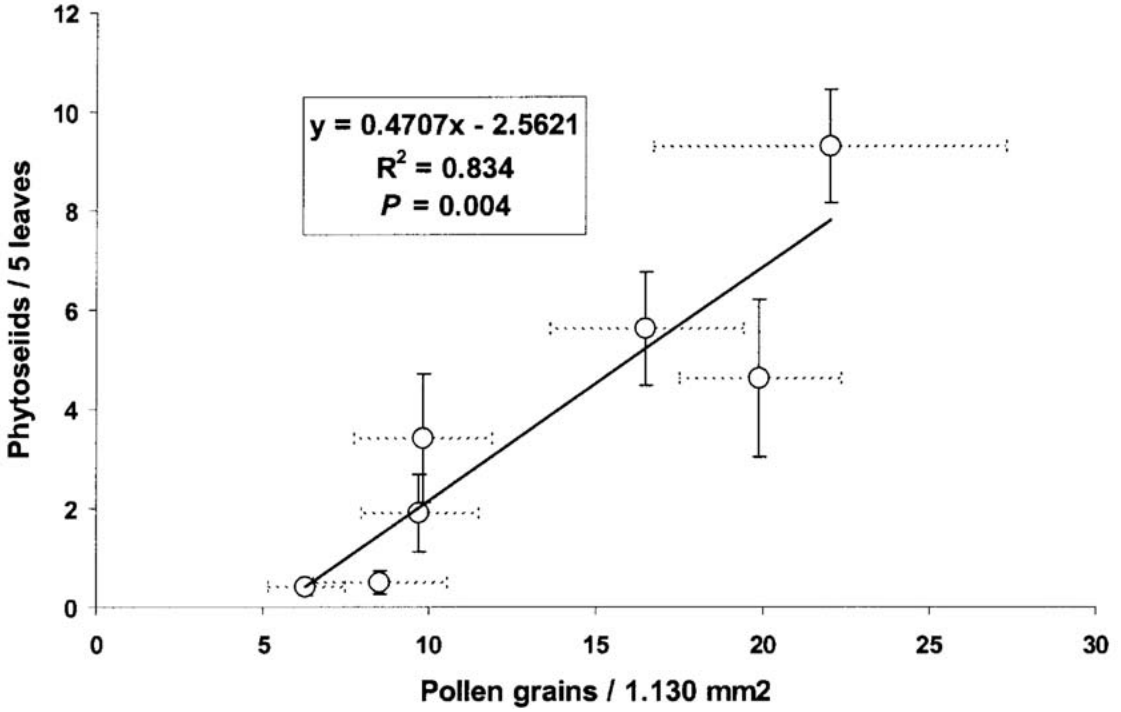

Fig. 2. Relationship between number of Phytoseiidae motiles (± SEM) per 5 leaves per tree and number of pollen grains (± SEM) per 1.13 mm^2 per 5 units per leaf per sample tree on grapefruit between 2 February and 16 March 2001.

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Summary

A positive correlation was found between numbers of phytoseiids and numbers of pollen grains on grapefruit leaves in this study. One or more pollens are important food sources for many phytoseiid species. Pollens of *Citrus* sp., *Pinus* sp., *Quercus* sp., and other plants coincided with increases in phytoseiid numbers in the field. The dominant phytoseiid species, *I. quadripilis* and *T. peregrinus*, are generalists that can be reared in the laboratory on exclusive diets of pollen from the ice plant, *Malephora crocea* (Jacquin).

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CHAETOSIPHON FRAGAEFOLII (HOMOPTERA: APHIDIDAE): A POTENTIAL NEW PEST IN FLORIDA?

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During the spring of 2003-2004, the strawberry aphid, *Chaetosiphon fragaefolii* (Cockerell) (Homoptera: Aphididae), was found infesting ten different strawberry cultivars, *Fragaria ananassa* Duchesne, grown under protected culture in a greenhouse in Marion County, FL. The cultivars were 'Treasure', 'Earlibrite', 'Strawberry Festival', 'Sweet Charlie', FL 97-39, 'Camarosa', 'Carmine', 'Camino Real', 'Diamante', and 'Ventana'. This is the first report of the presence of *C. fragaefolii* in cultivated strawberry in Florida (Division of Plant Industry, DPI E2004-278-201).

In December 2003, the strawberry aphid was observed on a strawberry cultivar trial at the University of Florida Plant and Science Research Unit at Citra. The susceptibility of ten strawberry cultivars to natural infestations to the cotton aphid, *Aphis gossypii* Glover, was being evaluated. 'Treasure', 'Earlibrite', 'Strawberry Festival', 'Sweet Charlie', FL 97-39, 'Camarosa', and 'Carmine' plugs were grown at UF facilities as described by Paranjpe et al. (2003); 'Camino Real', 'Diamante', and 'Ventana' plugs came from a Canadian nursery. Two months after the beginning of the trial, samples of an "unknown" aphid in the cotton aphid trial were taken to the Division of Plant Industry in Gainesville, FL, for identification. On 22 January, samples were identified as *C. fragaefolii*, the true strawberry aphid. Since the strawberry aphid was detected relatively early in the season, two applications of insecticidal soap (10%) (28 January and 12 February) were required to effect control. Two days after the second soap application, 10 *Chrysoperla rufilabris* L. and *Aphidoletes aphidimyza* L. per m^2 were released. Both methods were relatively successful; however, resurgence of pests was heavy by mid March causing the early termination of the crop. No insecticides were used to control the aphid because the strawberry trial and other crop trials in the greenhouse depended on bumblebees (highly susceptible to pesticides) for pollination. At the end of the trial, the strawberry aphid was eliminated from the greenhouse and hopefully eradicated from Florida. A heavy application of soap and oil (40%) was made before removing the plant material from the greenhouse. All material was buried and burned. The strawberry aphid has not been detected in strawberry crops on other outdoor locations of the farms or in other production areas in Florida.

Based on a sample of the aphid population, the following observations and conclusions were made: the life cycle of *C. fragaefolii* includes overwintering eggs, nymphs, adult apterae (wingless) and alatae, and parthenogenetic females. Eggs are white yellowish when deposited, but soon after, become shiny and black. Nymphs are small (0.8-1.1 mm) (n = 20) and morphologically similar to the adults. They vary in color from light green to pale yellow. Adults are 1.3-1.5 mm long, pale to yellowish green with short knobbed setae over the body; the antennae are as long as, or longer, than the length of the body; siphunculi are long, pale and slender, about ¼ body length, and legs are pale green and almost translucent. According to Heinz (1998), sexual forms are quite rare because aphids reproduce parthenogenetically throughout the winter if the temperature remains above 4.5°C; however, *C. fragaefolii* was found in a greenhouse (average day temperature 21°C), and sexual forms were observed at the cultivar trial (DPI E2004-278-202). Blackman & Eastop (2000) suspected that day length rather than temperature trigger the formation of sexual forms. The large number of exuviae on the leaves indicates proliferation of the aphid in the crop. As in other species of aphids, the strawberry aphid feeds on the underside of leaves close to veins. The insect also was observed feeding on tips, petioles, small fruits, calyxes, and young flowers. Leaf curling was not observed but foliage turned chlorotic, which probably diminished the photosynthetic capability of the plant. Aphids were more abundant on certain cultivars. 'Carmine' (4.3 ± 1.8 aphids/leaflet), 'Strawberry Festival' (3.7 ± 0.8 aphids/leaflet), FL 97-39 (2.9 ± 1.3 aphids/leaflet), and 'Diamante' (2.1 ± 1.1 aphids/leaflet) were the most affected (alpha = 0.05, confidence interval 95%).

The strawberry aphid is considered an important pest of strawberries in open fields worldwide, including the U.S. (California, Michigan, Minnesota, South Carolina, and Washington), Canada, northern Mexico, Europe, Great Britain, South Africa, New Zealand, and Australia (Dixon et al. 1987; Blackman & Eastop 2000). *Chaetosiphon fragaefolii* is well known in most of North America where strawberries are grown but not in Florida. Whether *C. fragaefolii* might have been in Florida prior to its recent discovery or may have come from transplants from Canada is uncertain. The taxonomy of the genus is difficult. In 1938-1939, specimens taken from *Rose* spp. in Florida were identified as *C. fragaefolii* (DPI records); however, it was recently determined that they actually correspond to *C. thomasi* Hille Ris Lambers. It was established that *C. fragaefolii* will not colonized rose, and that records of *C. fragaefolii*

on roses should be referred to *C. thomasi* (Blackmand & Eastop 2000). *Chaetosiphon fragaefolii* has been reported on wild strawberry, especially *F. chiloensis* in North America, *F. vesca*, *F. virginianana*, and *Ponsetia anserine* L. (Frazier 1974; Blackman & Eastop 2000). We report *C. fragaefolii* on cultivated strawberry in greenhouses.

Chaetosiphon fragaefolii transmits viruses that can cause strawberry yellow edge virus (SYEV), strawberry crinkle virus (SCV), and strawberry mottle virus (SMV) (Krczal 1979, 1982; Blackman & Eastop 2000; Converse 2002; Posthuma et al. 2002). For instance, symptoms of SCV are necrotic lesions with irregular spots on veins, epiniasty, crinkling, distortion, and uneven expansion of leaflets. Lesions on petioles and stolons produce angularity, streaking and deformation of petals (Frazier & Mellow 1970; Frazier 1974). These symptoms were not observed on any of the cultivars in our trial. *Chaetosiphon fragaefolii* can acquire the viruses within 24 h of birth. After a latent period of 10-19 days, the infected aphid can transmit virus for up to 2 weeks (Mellow & Frazier 1970).

In Florida, strawberries are an annual crop grown on approximately 7,100 acres; 95% of the acreage is located in the Plant City area of west central Florida (NASS-USDA 2003). Production costs average more than $23,000 per acre, making strawberry one of the most expensive crops in Florida to produce (FAFD 2002). The area of strawberry grown under protected cultivation in Florida is less than 1 ha (NASS-USDA 2003). The overall industry produces 15% of the total U.S. crop and accounts for more than 17% of the total dollar value generated from sales of fresh berries in the U.S. (NASS-USDA 2003). If the strawberry aphid were to spread into commercial production areas of Florida, it could cause severe damage to the strawberry industry, especially if viruses were present. Strawberries require a highly integrated management system to control pests to insure profitability. In addition to *C. fragaefolii* (the strawberry aphid), *C. jacobi*, *C. minor* (Forbes), *A. gossypii* (the cotton aphid), *A. forbesi* Weed (the strawberry root aphid also sometimes known as "the strawberry aphid"), *Macrosiphon euphorbiae* (Thomas) (the potato aphid), and *Myzus persicae* (Sulzer) (the green peach aphid) infest strawberries (Table 1) (Blackman & Eastop 2000). *Chaetosiphon fragaefolii* and *C. jacobi* have not been reported before in Florida strawberries.

Should the strawberry aphid become established in Florida, effective and timely control is essential in strawberry production due to the aphid's ability to develop large populations in a short period. Cultural and mechanical control of the strawberry aphid in the greenhouse or open field should include inspecting incoming transplants and eliminating infested crop material from the production site. Plant monitoring should begin early in the season and continue throughout the duration of the crop. Leaflets and shoots must be visually inspected from random locations throughout the field. After identification of the pest, yellow sticky cards can be used to detect the winged form; however, if alates are found, a well-established aphid population is already in the crop. The presence of ants, which feed on sugar produced by the aphids, may also be a sign of a heavy infestation.

No extended information is available regarding the effect of natural enemies on *C. fragaefolii*. In general, augmentation of biological control suffers from a lack of basic and well-designed strategies for release on a large scale, especially in open field conditions (Heinz 1998). Although parasitic wasps are species specific, *Aphelinus* species which are commercially available, may have an effect on strawberry aphid populations (Biobest Aphelinus-system http://www.biobest.be, http://bugssandbees.com; Koppert, http://www.koppert.nl; Syngenta, http://syngenta.com). The parasitic wasp *Aphidius colemani* L, was used successfully in the greenhouse to control the cotton aphid in the cultivar trial; however, *A. colemani* was not observed parasitizing *C. fragaefolii*. The numerous capitate setae of *C. fragaefolii* may protect the aphid from parasitic wasps. Several biological control options are available for aphid control. Lady beetles, such as *Hippodamia convergens* Guérin-Méneville (Rodriguez-Saona & Miller 1999) and *Coleomegilla maculata* DeGeer (Rondon et al. 2004) are important aphid feeders. Lacewings such as *Chysoperla rufilabris* and *C. carnea* Say, and the predatory midge *A. aphidimyza* are voracious predators of aphids (Heinz 1998). *Aphidoletes aphidimyza* attacks many species of aphids; it can act alone or in combination with a parasite for rapid knockdown of aphid infestations. This predator is most effective on aphid "hot spots."

If biological control is used, one should reduce or limit the use of broad-spectrum pesticides; preventive releases are recommended; monitor weekly to detect first sign of pest. If honey dew is present, it may interfere with the search capability of the parasitoid, and the use of light soap is suggested. If insecticides are used to suppress the strawberry aphid, a full coverage is recommended. For greenhouse aphid control, Tanigoshi & Bergen (2003) recommend Acatara® (thiomethoxam), Admire® (imidacloprid), Assail® (acetar) and Fulfill® (pymetorzine). Insecticidal soap (2.5 oz/gal water) can effectively reduce strawberry aphid population but may harm the crop (personal observation); in addition, botanical insecticides such as neem (Azatin™, Neemazad™, and Nemix™) may be effective. Strains of the fungus *Beauveria bassiana*, commercially available as Naturalis-O™ and BotaniGard™, may provide good control of aphids. Insect growth regulators

TABLE 1. THE STRAWBERRY, *Fragaria ananassa* DUCHESNE, APHID COMPLEX (MODIFIED FROM BLACKMAN & EASTOP 2000).

Scientific name	Frequency in the strawberry crop in the U.S.*
Acyrthosiphon rogersii (Theobald)	2
Aphis forbesi Weed	1
Aphis gossypii Glover	1
Aphis ruborum (Börner)	2
Aulacorthum solani (Kaltenbach)	2
Chaetosiphon fragaefolii (Cockerell)	1
Chaetosiphon jacobi Hille Ris Lambers	2
Chaetosiphon minor (Forbes)	2
Ericaphis fimbriata (Richards)	2
Ericaphis wakibae (Hottes)	2
Macrosiphum euphorbiae (Thomas)	2
Macrosiphum rosae (Linnaeus)	2
Myzus ascalonicus (Doncaster)	2
Myzus ornatus Lains	2
Rhodobium porosum (Sanderson)	2
Sitobion fragariae (Walker)	2
Aphis ichigocola Shinji	3
Aphis maidiradicis Forbes	3
Aphis nasturtii Kaltenbach	3
Aulacorthum circumflexum (Buckton)	3
Hyalomyzus fragaricola L.K. Ghosh	3
Hyperomyzus rhinanthi (Schouteden)	3
Macrosiphum pallidum (Oestlund)	3
Myzaphis rosarum (Kaltenbach)	3
Myzus cymbalariae Stroyan	3
Myzus persicae (Sulzer)	3
Abstrusomyzus valuliae (Robinson)	3
Paramyzus longirostris Miyazaki	3
Pemphigus bursarius (Linnaeus)	3
Rophalosiphoninus latysiphon (Davidson)	3

*1 Frequent, 2 Occasional, 3 Rare.

(IGR) are another least toxic insecticide that can be used. IGR kills insects by disrupting their development, stopping the molting process (interfere with chitin production), and mimicking juvenile hormone (insects never get into a reproductive stage) (Sunderland 1992).

Future information regarding biological and ecological aspects of the strawberry aphid will set the basis of an effective integrated pest management program for the aphid if it becomes established in Florida.

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SUMMARY

During the spring 2003-2004 growing season, the strawberry aphid, *Chaetosiphon fragaefolii* (Cockerell) (Homoptera: Aphididae), appeared for the first time in damaging numbers on the strawberry, *Fragaria ananassa* Duchesne, a high value commodity in Florida. Nymphs and adults of the strawberry aphid were found infesting ten different strawberry cultivars: 'Treasure', 'Earlibrite', 'Strawberry Festival', 'Sweet Charlie', FL 97-39, 'Camarosa', 'Carmine', 'Camino Real', 'Diamante', and 'Ventana'. A brief description of the morphology, biology, damage, and ecology of the pest is presented. Correct identification, early detection of the strawberry aphid, and adequate timely control can prevent the spread of this pest.

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RESPONSE OF *FOPIUS ARISANUS* (HYMENOPTERA: BRACONIDAE) TO FRUIT VOLATILES IN A WIND TUNNEL

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Fopius (*Biosteres*) *arisanus* (Sonan) is a solitary endoparasitoid that oviposits primarily in fruit fly eggs and completes its development within the host. Adults emerge from the host puparium 18-20 d after oviposition (Bess et al. 1961). *Fopius arisanus* was originally collected from *Bactrocera dorsalis* (Hendel), but it is able to parasitize other fruit flies, including *Carpomyia vesuviana* Costa, *Ceratitis capitata* (Weidemann), three species of *Anastrepha*, and 11 other species of *Bactrocera* (Quimio & Walter 2001; Zenil et al. 2004, and references therein). Because of its potential as a biocontrol agent for fruit flies, several aspects of the biology of *F. arisanus* have been studied in detail (e.g., Bautista et al. 1998, 1999; Calvitti et al. 2002; Zenil et al. 2004), although the host location behavior of this parasitoid has been scarcely investigated. Field studies suggest that *F. arisanus* females use visual (Vargas et al. 1991; Liquido 1991) and chemical (Liquido 1991) cues to orient toward eggs of *B. dorsalis*, but the identity of attracting odors is still unknown. Here we conducted a study to determine whether *F. arisanus* are attracted to uninfested fruits in a wind tunnel as a first step to future investigations aimed to identify the compounds responsible for parasitoid attraction.

We used naïve female *F. arisanus*, 10-15 d old obtained from Moscamed facilities in Metapa de Dominguez, Chiapas, Mexico. Parasitoids used for experiments have been reared from eggs of *C. capitata* placed in pieces of papaya at 24 ± 2°C and 60-80% RH, and 12:12 L:D photoperiod as described previously (Zenil et al. 2004). Guavas, *Psidium guava* L. (dessert type), and oranges, *Citrus sinensis* L. (Valencia variety) were obtained from vendors for experiments. These fruits were chosen because they have different chemical and physical characteristics and it has been shown that eggs of *C. capitata* inoculated in common guava were parasitized by *F. arisanus* more heavily than those in citrus fruits (Bautista & Harris 1996).

Two experiments were conducted. In the first one, the response of female parasitoids to uninfested guava fruit was compared to a polystyrene ball (5 cm diameter) painted with vinyl acrylic water-based paints mixed to match (as detected by the human eye) the yellow of guava fruit. In this experiment, treatments were replicated 30 times. In the second experiment, the response of parasitoid females to volatiles of uninfested guava and orange was evaluated. Treatments were replicated 50 times. Both experiments were performed as non-choice tests and treatments were tested in a random order. Observations were carried out in a flight wind tunnel, 120 cm long and 30 cm high and wide. A fan was used to pull air through the tunnel with a velocity of 0.2 m/s and activated charcoal was used to filter intake air. Illumination was provided by two fluorescent bulbs mounted 60 cm above the wind tunnel giving a light intensity of 230 lux. The wasps were individually placed in a 5-cm high plastic container (4 cm internal diameter) (release cylinder) and they were allowed to acclimatize to the wind tunnel room conditions (25 ± 1°C, 70-80% RH) for at least one h before being observed. A fruit or yellow sphere was placed in the center of the wind tunnel, 10 cm from the upwind end. Each observation began by placing the release cylinder on a 15-cm high platform at the downwind end of the tunnel and one insect was released. Individual parasitoids were observed for a maximum time of 5 min. Specific response, including taking off, flight, landing on source, and duration of each response were recorded. Each female had only one flight opportunity within a test and was then discarded. A flight response was considered oriented towards the experimental target when the female flew directly upwind and landed on or not further than 5 cm away from the target. Data of responding *F. arisanus* females were compared between paired treatments by independent samples *t*-test (SPSS version 10.0). In all cases, $P < 0.05$ was used to detect significant differences.

In the first experiment, the number of wasps taking off was not significantly affected by the presence of guava fruit (90%) or yellow sphere (93%) ($t = 0.378$; $df = 4$; $P = 0.362$). The time before flight initiation of the wasp that were exposed to yellow sphere ($n = 30$) and guava fruits ($n = 27$) was of 39.03 ± 8.65 and 51.44 ± 8.89 sec, respectively. Oriented flights only were observed when guava fruit (40%) was present, but not with the yellow sphere. Only 30% of females landed on guava fruits, whereas no females landed on spheres. Landing time of females exposed to guava volatiles was 153.87 ± 29.99 sec ($n = 8$).

In the second experiment, significantly more females left the release cylinder when they were

exposed to guava than to orange fruits (t = -2.88 df = 6, P = 0.02, Fig. 1). However, the time before taking off between females of both groups was similar (guava = 69.9 ± 46.5 sec, n = 48; orange = 62.5 ± 40.6 sec, n = 43). Significantly more females flew upwind to guava than orange (t = -3.28, df = 8, P = 0.01, Fig. 1). Also, females landed more frequently on guava than orange (t = -5.16, df = 8, P = 0.001, Fig. 1). Landing time of females exposed to guava volatiles was 154.76 ± 51.05 sec (n = 30), while females landing on orange took 237.66 ± 61.04 sec (n = 3).

Even though the yellow sphere did not attract or stimulate female landing under the conditions of the present study, a possible role for visual cues during the host-finding behavior of *F. arisanus* can not be ruled out because a previous study has shown that this species was captured on 4-cm yellow and white spheres in field conditions (Vargas et al. 1991). One possibility is that the light conditions used in the present study could have affected the visual response of *F. arisanus*. Also it is possible that with a bigger sample size some response to the yellow sphere would have been obtained.

The results of the present report agree with those obtained by Liquido (1991), who suggested that *F. arisanus* females may use fruit volatiles during the host location process. The use of fruit volatiles during host location behavior has been demonstrated in other parasitoid species that attack late instars of several species of tephritid flies (Greany et al. 1977; Eben et al. 2000; Jang et al. 2000; Henneman et al. 2002). Females of these species are attracted to odor emanating from healthy and infested fruits, but wasps visited more frequently infested fruits over healthy ones. We have shown that females are attracted to volatiles from uninfested fruits, but still we do not know whether *F. arisanus* females orient to infested fruits and this will be investigated in future studies.

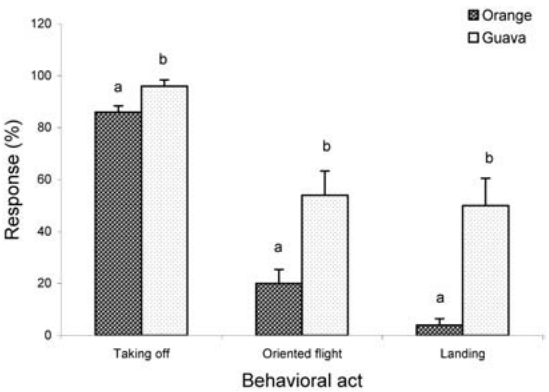

Fig. 1. Mean (±SEM) responses of *F. arisanus* females to orange and guava fruits offered in non-choice situations in a wind tunnel. Significant differences are indicated by different letters over the bars (t-test, $P < 0.05$).

We found that there was a significant difference in take offs with guava vs. orange, but not for guava vs. yellow sphere. Also, the time before flight initiation of females exposed to orange was longer than that of females exposed to guava. These results suggest that volatiles emanating from oranges may affect wasp behavior. Bautista & Harris (1996) suggested that chemicals in citrus peel are toxic to fruit fly eggs and larvae of tephritid fruit flies and this factor may explain low host parasitization by *F. arisanus* in citrus fruits.

We do not know if the strategy of use of fruit volatiles during host location by *F. arisanus* is common to other egg and young larval parasitoids of tephritid fruit flies. Nevertheless, it is well known that three parasitoid species attacking the eggs and young larvae of tephritid fruit flies use the fly's marking pheromone as kairomone for host location by searching longer on pheromone marked than on unmarked fruits (Prokopy & Webster 1978; Roitberg & Lalonde 1991; Hoffmeister & Gienapp 1999). Also, *Halticoptera rosae* Burks, an egg-larval parasitoid of *Rhagoletis basiola* Osten-Sachen, uses the marking pheromone as a guide to the fly's oviposition site and thus the host egg (Hoffmeister et al. 2000). In contrast, a parasitoid attacking late instars of *Rhagoletis pomonella* (Walsh) does not respond to the marking pheromone (Prokopy & Webster 1978). Whether *F. arisanus* females use host marking pheromone during host location behavior remains unknown.

In conclusion, this study confirms that *F. arisanus* females may use fruit volatiles during host location process as previously suggested by Liquido (1991). Also, it shows that females orientate to volatiles emanating from healthy fruits. The chemical identity of the attractants is currently unknown, but the bioassay used here can be useful during the identification of behaviorally active compounds. Semiochemicals offer good prospects as a tool for managing parasitoid behavior, particularly in a view of possible application to enhance the efficacy of parasitoids in biological control programs (Vet & Dicke 1992).

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Summary

The response of *Fopius arisanus* (Sonan) females to fruit volatiles was evaluated in a wind tunnel. In one experiment, the response of female parasitoids to uninfested guava fruit was compared to a yellow sphere. We found that females flew upwind and landed only on guava fruit but

not on the yellow sphere. In the second experiment, the response of parasitoid females to volatiles from uninfested guava and orange was evaluated. In this experiment, significantly more females flew upwind to guava fruit than orange fruit, and females landed more frequently on guava than orange fruits.

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SPECIES OF *MELITTOBIA* (HYMENOPTERA: EULOPHIDAE) ESTABLISHED IN BAHAMAS, COSTA RICA, CUBA, HISPANIOLA, PUERTO RICO, AND TRINIDAD

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Melittobia is a cosmopolitan genus with about 13 species. They are gregarious ectoparasitoids primarily on prepupae of aculeate hymenopterans, but their wide host range includes species in orders as diverse as Coleoptera and Diptera, especially under laboratory conditions (Dahms 1984b). They are normally associated with mud dauber wasps of the genera *Trypoxylon* and *Sceliphron* (Hymenoptera: Sphecidae) and some species serve as model organisms to demonstrate biological, ecological, and behavioral concepts to students from elementary to college level (Matthews 1997, 2000; Matthews et al. 1996). Due to confusion surrounding species identity in this genus, this report is part of efforts to promote nomenclatural stability and facilitate future work on *Melittobia* (González & Matthews 2002; González et al. 2004a, b). Our findings suggest that *Melittobia* are more widely distributed in the Caribbean region than previously thought, although probably not native to this region.

Melittobia was previously reported from Cuba as parasites of the sphecid wasps *Sceliphron assimile* (Dahlbom), *S. jamaicense* (Fabricius), *Trypoxylon succintum* Cresson and *T. subimpressum* (Smith), and the solitary vespids *Pachodynerus nasidens* (Latreille), *P. cubensis* (Saussure), and *Ancistrocerus cingulatus* (Cresson) (Alayo & Hernández 1978; Fernández et al. 2002; Genaro 1994, 1996). While examining specimens of *Melittobia* deposited in the Museum of Comparative Zoology at Harvard, we found 5 females of *Melittobia acasta* (Walker) labeled: Baraguá, Cuba. VI-9-29, T.P.R.F. Ent No. 3541, taken on a mud cell, L.C. Scaramuzza, Col., *Melittobia acasta* (Walker), det. E. Dahms 1985.

Melittobia acasta is regarded as the only species of this genus native to Europe (Bouček 1977) but it also has been reported from Argentina, Canada, India, Japan, New Zealand, USA, and Venezuela (Dahms 1984a; De Santis 1983; Husain & Khan 1986; Hobbs 1968; Hobbs & Krunic 1971; González 1994; González & Terán 1996; González et al. 2004b; La Salle 1993; MacFarlane & Donovan 1989) where it presumably has been accidentally introduced.

Melittobia australica (Girault) was originally described from Australia (Girault 1912), but it has been found in Jamaica, Japan, New Zealand, Trinidad, South Africa, USA, and Venezuela (Dahms 1984a; MacFarlane & Palma 1987; La Salle 1993; González 1994; González & Terán 1996). One of us (JAG) has collected specimens of *Melittobia australica* from two locations in Cuba comprising eight females from Playa Vitoria, Yaguajay, Sancti Spiritus, Cuba, VII-1996, from nests of *Sceliphron jamaicense* (Fabricius); and 23 females from Cienfuegos, Cuba, XI-1988 from *Sceliphron* sp.

Melittobia was reported from Costa Rica by Hanson & Gauld (1995). However, an earlier report (Hunt 1993) mentions wasps identified by one of us (RWM) as *Melittobia australica* from Guanacaste Province attacking *Sceliphron assimile* (Sphecidae). We also keep a culture in our laboratory obtained originally from a species of *Centris* (Anthophoridae) collected at Lomas Barbudal Reserve, 8.5 km NW of Bagaces, Guanacaste Province (S.B. Vinson, March 2001) in Costa Rica.

Melittobia sp. was also reported to occur in Puerto Rico (Maldonado C. & Navarro 1967), and we thought it most likely to be *M. australica*. This was confirmed when we discovered many specimens of *M. australica* from various Puerto Rico localities in the collection of the National Museum of Natural History (Smithsonian Institution).

As far as we know, *Melittobia* parasitoid wasps had not been reported previously from the Bahamas, Trinidad, and Hispaniola. However, in material borrowed from the Entomology Research Center—Agriculture, Canada C.E.F., we found a female of *Melittobia australica* collected at San Salvador, Bahamas (8-13/XII/80, sweeping pool, B. Brown), and from 2 localities in Trinidad (Carapichaima and Curepe) parasitizing Eumeninae wasps (Vespidae) as well as *Trypoxylon* sp. (Sphecidae). Finally, during an expedition by one of us (JAG) to Dominican Republic, *Melittobia australica* was found parasitizing *Sceliphron* sp. at Oviedo Province, Pedernales (20-XI-2003).

We thank Philip D. Perkins (Museum of Comparative Zoology, Harvard University), Michael Gates (Smithsonian institution), and John Huber (C.E.F. Agriculture, Canada) for allowing us to study the *Melittobia* specimens under their care. S.B. Vinson and F. Cônsoli sent us the Costa Rican *M. australica*. Ivon Arias (Grupo Jaragua) and Kelvin Guerrero (Parque Nacional del Este)

helped J.A.G. with expedition logistics in the Dominican Republic. Part of this work was supported by NSF Grant 0088021 to RWM.

Summary

The presence of *Melittobia australica* (Hymenoptera: Eulophidae), a cosmopolitan ectoparasitoid, is confirmed from Bahamas, Costa Rica, Cuba, Hispaniola, Puerto Rico, and Trinidad. Also *M. acasta*, the only known European species in the genus, is reported for the first time from Cuba. Some hosts of the genus and for both species in these Latin American countries are reported.

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LEPTODEUTEROCOPUS NEALES: A NEW RECORD FOR FLORIDA AND THE UNITED STATES (LEPIDOPTERA: PTEROPHORIDAE: DEUTEROCOPINAE)

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The genus *Leptodeuterocopus* Fletcher consists of 9 species worldwide, one species from Indonesia, the rest Neotropical in distribution (Gielis 2003). *Leptodeuterocopus neales* (Walsingham) is distributed widely in the Neotropics. Previously published records are from Mexico and Ecuador. Walsingham's (1915) description of *Oxyptilus neales* is based on 2 specimens from Mexico. The type, listed by Walsingham as "♀ (67135, Teapa) Mus. Wlsm. (Godm-Salv. Coll.) BM," is from Teapa, Tabasco. The other specimen is from Atoyac, Vera Cruz. These specimens were collected in March and April respectively by H. H. Smith. The day and year is not specified in the description.

Two specimens have been identified from Florida. The first is a male collected at UV blacklight by J. B. Heppner, 29 April 1978, Dade Co.: Chekika State Recreation Area (Grossman Hammock) [FSCA] (Fig. 1). We have compared the male genitalia of this specimen (Slide DM 334) (Fig. 2) with a male from Mexico: Vera Cruz: Atoyac IV.18?? H. H. Smith, Slide CG 5059 [BMNH] as basis for the determination. The second specimen was retrieved from a CO_2 mosquito trap by R. A. Belmont, 10-25 June 1992, Collier Co.: Naples [DMC]. The second specimen is missing the lower abdominal segments but is recognizable by wing maculation. On first examination, specimens of *L. neales* may be mistaken for a common South Florida species, *Sphenarches anisodactylus* (Walker). The wingspan of *S. anisodactylus* is 12.0-18.0 mm (Matthews 1989), while that of *L. neales* is about 9.0-11.0 mm. The forewing of *L. neales* has a distinct pale band across the basal third of each lobe, contrasting the coppery base color. In *S. anisodactylus*, this pale band is narrower and does not extend to the cleft base. Unlike the type species, *Leptodeuterocopus citrogaster* Fletcher, there is no second cleft apparent on the second forewing lobe of *L. neales*.

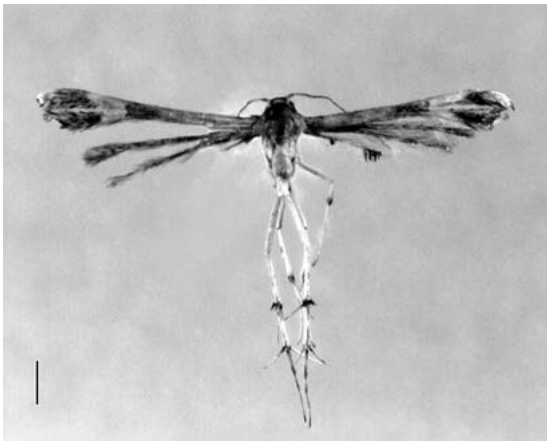

Fig. 1. *Leptodeuterocopus neales*, male; Florida, Dade Co., Chekika State Rec. Area (Grossman Hammock), 29 Apr 1978 J.B. Heppner. Scale line = 1 mm.

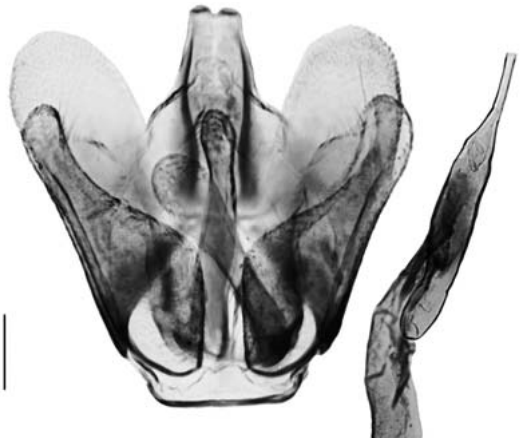

Fig. 2. Male genitalia of *Leptodeuterocopus neales*; (same data as Fig. 1), slide DM 334. Scale line = 0.1 mm.

The life history of *L. neales* is unknown. The subfamily Deuterocopinae includes 27 species worldwide. Of the 4 genera, *Leptodeuterocopus*, *Hexadactilia*, *Heptaloba*, and *Deuterocopus*, larval hosts are only known for 6 species of *Deuterocopus*, all feeding on species of Vitaceae. The localities for both Florida specimens are well within the subtropical zone. The location of the first specimen, Chekika State Recreation Area (Grossman Hammock), is now part of Everglades National Park. This hammock is situated on a slightly elevated rocky ridge in an area known as "rocky glades" or "East Everglades" (Snyder et al. 1990). The second specimen was from a mosquito control monitoring trap set up next to a mangrove swamp in Naples.

We thank Dr. John B. Heppner and Robert A. Belmont for making the specimens available for study. Material examined is from the British Museum of Natural History (BMNH), Florida State Collection of Arthropods (FSCA), and the first author's collection.

SUMMARY

Leptodeuterocopus neales is reported from Florida and the United States for the first time. Florida Experiment Station Journal Series No. R-10481.

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IMPACT OF AUXILIARY STATIONS IN A BAITING PROGRAM FOR SUBTERRANEAN TERMITES (ISOPTERA: RHINOTERMITIDAE)

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Termite baiting programs rely on proper placement of monitoring stations to establish feeding sites for termite workers. To successfully suppress or eliminate a colony, termites must remain at baited sites long enough to transfer the toxicant from the station to cohorts. Other studies have reported on proper placement of stations to increase the probability of termite attacks and to reduce the time required for termites to initially locate monitoring stations (Henderson et al. 1998; Jones 2003). Henderson et al. (1998) reported that termites attack monitors twice as often when placed in conducive locations rather than in a random pattern. Jones (2003) reported an average 38-day decrease in time to termite attack when monitoring stations were targeted rather than random.

To increase the probability of termites continuing to feed at a baited site and deliver more bait to the colony, the label recommends the installation of one or more auxiliary stations 30 cm from the original baited station when the Sentricon® *Termite Colony Elimination System* and Recruit™ III bait (Dow AgroSciences, Indianapolis, IN) is used. While the strategy appears reasonable, no research has been published to confirm the merit of the assumption. The purpose of our study was to investigate the benefits of auxiliary stations in a *Reticulitermes* spp. termite baiting program. Specifically, we wanted to determine whether (1) auxiliary stations are more likely to be found by termites than stand-alone stations, (2) the use of auxiliary stations improves our ability to retain termite foraging in active stations over time, and (3) the use of auxiliary stations increases the total consumption of bait matrix.

We monitored eight homes in upstate South Carolina with newly-installed Sentricon Systems for approximately one year. Stations were placed around the perimeter of buildings three meters apart. A total of 183 stations were installed initially. Monthly monitoring and baiting was conducted during the peak termite foraging season of 2002, from April to October. Additionally, a final inspection was completed in March 2003 to assess the impact of auxiliary stations on post-winter termite activity.

During each station inspection, termite activity was recorded as present or absent and those with new termite feeding were randomly divided into two treatments, auxiliary and non-auxiliary control. In the auxiliary treatment, termites were placed into a Baitube™ device and three auxiliary stations containing wooden monitors were installed with a power auger, 25-30 cm from the active station and evenly spaced at 120-degree intervals. During subsequent monitoring visits, auxiliary stations with active termite feeding in the wooden monitors were switched to a Baitube. In the non-auxiliary control, termites also were placed into a Baitube, but no auxiliary stations were applied. Cellulose Baitube devices without active ingredient were used to avoid impacting foraging. The amount of cellulose matrix left in the Baitube was visually estimated during each monthly inspection. Tubes were replaced when consumption was greater than 50 percent. To compare consumption between treatments, we combined bait matrix consumption estimates of the parent station and surrounding auxiliaries in the auxiliary treatment.

A *z*-test for comparison of proportions (Sigmastat, SPSS, Inc., Chicago, IL) was used to compare the rate at which termites found auxiliary stations to that of all stand-alone stations. The number of active station locations that remained active over time in the two treatments was also compared with the *z*-test. A *t*-test (Sigmastat, SPSS, Inc., Chicago, IL) was used to compare the mean consumption per month between the treatments.

After monthly monitoring for six months, 16 of the 183 original, stand-alone stations were found by *Reticulitermes* spp. termites at six of the eight homes. Thus, eight stations were randomly assigned to the auxiliary treatment and 24 auxiliary stations were placed. Fourteen of those were found by termites. The total percent of auxiliary stations that termites found (58%) was much higher than that of stand-alone stations (9%). The difference between the treatments was highly significant at $\alpha = 0.05$ ($z = 6.071$, $P < 0.001$).

After six months of monitoring, we were able to maintain feeding in only two of eight active station locations in the non-auxiliary treatment. In our auxiliary treatment, four out of eight had activity in the parent station or in one or more of the auxiliary stations associated with it. After 11 months, only one of the non-auxiliary stations was still active, while four of the auxiliary treatment locations were still active. Although differences were not statistically significant at $\alpha = 0.05$ (6 months $z = 0.516$, $P = 0.606$; 11 months, $z = 1.055$, $P = 0.291$), a four-fold increase in feeding stability was observed.

During the six-month baiting period 31.5 baittubes were consumed by termites with 9.7 in the non-auxiliary treatment and 21.8 in the auxiliary treatment. Comparing mean percent Baitube device consumption/month between treatments, the non-auxiliary mean was 49.5% versus 90.6% in the auxiliary treatment. This difference in consumption was not statistically significant at $\alpha = 0.05$ ($t = -1.834$, $df = 14$, $P = 0.088$).

Based on a statistically significant six-fold increase in feeding after six months alone, we recommend using auxiliary stations in termite baiting programs. This recommendation is further supported in that both long-term feeding and bait-tube consumption were substantially, but not significantly higher with the auxiliary treatment.

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Summary

Eight homes with a newly installed Sentricon System were studied for 11 months to assess the benefits of placing auxiliary stations around stations with termite activity. Auxiliary stations developed active termite foraging at a significant rate over six times greater than that seen in stand-alone stations. Although results were not statistically significant at $\alpha = 0.05$, use of auxiliary stations improved our ability to maintain termite foraging in active stations over time by 36% and increased overall consumption of bait matrix by 41%.

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PRESENCE OF *THELOHANIA SOLENOPSAE* AND *VAIRIMORPHA INVICTAE* IN SOUTH AMERICAN POPULATIONS OF *SOLENOPSIS INVICTA*

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The microsporidia, *Thelohania solenopsae* (Knell et al. 1977) and *Vairimorpha invictae* (Jouvenaz & Ellis 1986) have been reported to be effective self-sustaining biological control agents against the fire ant, *Solenopsis invicta* (Williams et al. 1999; Briano & Williams 2002; Briano et al. 2002). *Thelohania solenopsae* is well established among North and South American *S. invicta* populations and causes declines in queen egg production, queen weight, and worker and queen survivorship (Williams et al. 1999; Oi & Williams 2002). *Solenopsis invicta* is found in 2 distinct social forms, polygyne and monogyne; polygyne colonies have multiple fertile queens, while monogyne colonies have only a single fertile queen. Recently, North American *T. solenopsae* infections were shown to be restricted to the polygynous social form of *S. invicta* (Oi et al. 2004). Despite sympatry and sampling in areas with a high incidence of *T. solenopsae* infection (up to 78%), no monogyne fire ant colonies were found to be infected. Would this social form-specific *T. solenopsae* infection be similarly restricted to polygynous *S. invicta* in South America? To address this question, we determined the social form of archived *T. solenopsae*- and *V. invictae*-infected *S. invicta* samples from Argentina and Paraguay.

Samples of *T. solenopsae*- (n = 20) and *V. invictae*-infected (n = 15) nests of *S. invicta* were collected from the provinces of Santa Fe and Corrientes in Argentina and from Paraguay from 1999 to 2003. Infections for each microsporidian parasite were determined in each sample by the observation of spores in wet mount preparations of macerated adult ants under a phase-contrast microscope (400×, Briano & Williams 2002). Genomic DNA was extracted from 20 to 30 adult ants as described by Valles et al. (2002).

Social form was determined with PCR by exploiting nucleotide differences between the 3 *Gp-9* alleles ($Gp\text{-}9^{B}$, $Gp\text{-}9^{b}$, $Gp\text{-}9^{b'}$) found in South American *S. invicta* (Krieger and Ross 2002) by the method described by Valles & Porter (2003). Briefly, monogyne individuals are homozygous $Gp\text{-}9^{BB}$, whereas polygyne individuals are heterozygous (either $Gp\text{-}9^{Bb}$ or $Gp\text{-}9^{Bb'}$). $Gp\text{-}9^{B}$-specific oligonucleotide primers corresponded to positions 1683-1703 (primer 26: 5'CTCGCCGATTCTAACGAAGGA) and 2167-2199 (primer 16: 5'ATGTATACTTTAAAGCATTCCTAATATTTTGTC). Oligonucleotide primers designed to amplify either $Gp\text{-}9^{b}$ or $Gp\text{-}9^{b'}$ corresponded to positions 1307-1334 (primer 24: 5'TGGAGCTGATTATGATGAAGAGAAAATA) and 1702-1729 (primer 25: 5'GCTGTTTTTAATTGCATTTCTTATGCAG).

Multiplex PCR was conducted by the hot start method in a PTC 100 thermal cycler (MJ Research, Waltham, MA) under the following optimized temperature regime: 1 cycle at 94°C for 2 min, then 35 cycles at 94°C for 15 sec, 55°C for 15 sec, and 68°C for 30 sec, followed by a final elongation step of 5 min at 68°C. The reaction was conducted in a 50 µl volume containing 2 mM $MgCl_2$, 200 µM dNTP mix, 1 unit of Platinum *Taq* DNA polymerase (Invitrogen, Carlsbad, CA), 0.4 µM of primers p24, p25, p26, and p16, and 1 µl of the genomic DNA preparation (50 to 500 ng). PCR products (12 µl) were separated on a 1% agarose gel and visualized by ethidium bromide staining. For all experiments, positive and negative controls were run alongside treatments.

Among the 20 *T. solenopsae*-infected nests evaluated by PCR, 45% were polygyne and 55% monogyne (Table 1). Similarly, 46% and 54% of *V. invictae*-infected nests were polygyne and monogyne, respectively (Table 2). Therefore, *T. solenopsae* is not restricted to the polygyne social form as in North American *S. invicta* sampled in Florida. Despite failing to detect the *T. solenopsae*-infection in established monogyne colonies in North America, Oi et al. (2004) did find the infection in newly-mated monogyne queens (hypothesized to originate from *T. solenopsae*-infected polygyne queens). Thus, they concluded that the monogyne genotype ($Gp\text{-}9^{BB}$) did not preclude infection by *T. solenopsae*. In light of our results, their conclusion is validated. However, the question remains, why is *T. solenopsae* infection not observed in field populations of monogynous *S. invicta* in North America?

It is well documented that the population bottleneck during founding resulted in significant intrinsic differences between North and South American *S. invicta* (Ross et al. 1993). For example, there are differences in the number of alleles at the *Gp-9* locus (Krieger & Ross 2002), loss of variation at the major sex-determining locus resulting in greater male sterility (Ross et al. 1993), differences in queen relatedness among polygyne colonies (Ross et al. 1996), and differences in the proportion of permanently unmated queens (Ross

TABLE 1. *T. solenopsae*-INFECTED *S. invicta* EVALUATED FOR SOCIAL FORM.

Collection date	Collection site	Social form
27 April 1999	Santa Fe, Argentina, Route 11, 490.8 km	Polygyne
27 April 1999	Santa Fe, Argentina, Route 11, 490.8 km	Polygyne
27 April 1999	Santa Fe, Argentina, Route 11, 490.8 km	Polygyne
27 April 1999	Santa Fe, Argentina, Route 11, 505.4 km	Monogyne
27 April 1999	Santa Fe, Argentina, Route 11, 624.8 km	Polygyne
27 April 1999	Santa Fe, Argentina, Route 11, 624.8 km	Polygyne
27 April 1999	Santa Fe, Argentina, Route 11, 624.8 km	Polygyne
27 April 1999	Santa Fe, Argentina, Route 11, 600 km	Monogyne
27 April 1999	Santa Fe, Argentina, Route 11, 649.9 km	Monogyne
5 July 1999	Santa Fe, Argentina, Route 11, 490.8 km	Polygyne
5 July 1999	Santa Fe, Argentina, Route 11, 490.8 km	Monogyne
5 July 1999	Santa Fe, Argentina, Route 11, 490.8 km	Polygyne
5 July 1999	Santa Fe, Argentina, Route 11, 490.8 km	Polygyne
24 April 2001	Santa Fe, Argentina, Route 11, 560 km	Monogyne
26 April 2001	Santa Fe, Argentina, Route 11, 560 km	Monogyne
19 January 2003	Paraguay, Route 5, 368 km	Monogyne
24 January 2003	Misiones, Argentina, Iguazu Airport	Monogyne
24 January 2003	Misiones, Argentina, Iguazu, Airport	Monogyne
10 April 2003	Misiones, Argentina, Route 12, 1445 km	Monogyne
10 April 2003	Misiones, Argentina, Route 12, 1445 km	Monogyne

et al. 1996). Therefore, there may be a genetic basis for the differences in *T. solenopsae* infection among North and South American monogyne *S. invicta*. However, it would seem equally plausible that an extrinsic factor was responsible for the observed difference. Specifically, an intermediate host for *T. solenopsae* may be required for infection of monogyne *S. invicta*. Only a fraction of the known natural enemies of *S. invicta* are present in its North American range (Porter et al. 1997). Furthermore, perhaps the intermediate host would not be required for transmissibility in the polygyne social form because of their unique behavioral characteristics (less aggressive and more accepting of conspecific queens); colony organization can influence pathogen transmission in social insects (Naug & Camazine 2002).

Now that we know *T. solenopsae* infects field monogyne colonies in Argentina, investigations to elucidate the life cycle of this pathogen should continue with the hope of discovering a method to initiate a self-sustaining infection in monogyne *S. invicta* in the United States. *Vairimorph invictae* was included in this study because its suitability for release as a natural enemy of *S. invicta* in North America currently is being evaluated in

TABLE 2. *V. invictae*-INFECTED *S. invicta* EVALUATED FOR SOCIAL FORM.

Collection date	Collection site	Social form
27 April 1999	Santa Fe, Argentina, Route 11, 490.8 km	Monogyne
27 April 1999	Santa Fe, Argentina, Route 11, 490.8 km	Polygyne
27 April 1999	Santa Fe, Argentina, Route 11, 490.8 km	Polygyne
27 April 1999	Santa Fe, Argentina, Route 11, 490.8 km	Monogyne
27 April 1999	Santa Fe, Argentina, Route 11, 560 km	Polygyne
27 April 1999	Santa Fe, Argentina, Route 11, 560 km	Polygyne
27 April 1999	Santa Fe, Argentina, Route 11, 560 km	Polygyne
27 April 1999	Santa Fe, Argentina, Route 11, 560 km	Monogyne
27 April 1999	Santa Fe, Argentina, Route 11, 560 km	Monogyne
5 July 1999	Santa Fe, Argentina, Route 11, 560 km	Monogyne
5 July 1999	Santa Fe, Argentina, Route 11, 578.2 km	Monogyne
5 July 1999	Santa Fe, Argentina, Route 11, 600 km	Monogyne
5 July 1999	Santa Fe, Argentina, Route 11, 600 km	Monogyne
24 April 2001	Santa Fe, Argentina, Route 11, 729 km	Polygyne
28 February 2003	Santa Fe, Correientes, Route 123, 205 km	Polygyne

quarantine at the USDA-ARS facility in Gainesville, Florida, and we wanted to determine whether both social forms were capable of being infected.

We thank Chuck Strong for technical assistance. We thank D. H. Oi (USDA) and S. J. Yu (University of Florida), who provided helpful reviews of a previous version of the manuscript. The use of trade, firm, or corporation names in this publication are for the information and convenience of the reader. Such use does not constitute an official endorsement or approval by the United States Department of Agriculture or the Agricultural Research Service of any product or service to the exclusion of others that may be suitable.

Summary

Thelohania solenopsae- and *Vairimorpha invictae*-infected *Solenopsis invicta* from South America were genotyped at the *Gp-9* locus to determine their social form. Unlike counterparts in the United States, monogyne nests are infected with both microsporidia species in South America.

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STERILE MALES OF THE MEDITERRANEAN FRUIT FLY EXPOSED TO GINGER ROOT OIL INDUCE FEMALE REMATING: IMPLICATIONS FOR THE STERILE INSECT TECHNIQUE (DIPTERA: TEPHRITIDAE)

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The Sterile Insect Technique (SIT) is used worldwide to control infestations of the Mediterranean fruit fly, *Ceratitis capitata* (Wiedemann) (Hendrichs et al. 2002). The effectiveness of the SIT is constrained, in part, by artificial selection inherent in the mass rearing process, which leads to changes in male courtship behavior. This, in turn, reduces the sexual competitiveness of mass-reared males (Cayol 2000). There is also evidence (Bloem et al. 1993) that sterile males may not fully inhibit female remating, presumably owing to the reduced transfer of sperm and accessory gland products during copulation. This has serious implications for SIT programs because in *C. capitata* the final male partner fertilizes the majority of the eggs in multiply mated females (Saul & McCombs 1993). If an initial mating with a sterile male does not dramatically inhibit female receptivity, the mated female may remate with a wild male, whose sperm then fertilizes most of the eggs. Conversely, if a female mates initially with a wild male, her receptivity may be so dramatically reduced that any remating (possibly with a sterile male) will be unlikely.

Several recent studies (e.g., Shelly et al. 2004) have shown that the aroma of ginger root oil (GRO) enhances the mating success of mass-reared, sterile males of *C. capitata*. However, these studies did not consider possible effects of male GRO exposure on female remating. Here, we present data addressing two questions: (1) Are females that initially mated with wild males more likely to remate with GRO-exposed, sterile males than GRO-deprived, sterile males? (2) Do initial matings with GRO-exposed, sterile males reduce the likelihood of female remating with wild males?

Methods used to maintain flies and run remating trials closely follow earlier studies (Shelly & Kennelly 2002; Shelly et al. 2004), consequently only an abbreviated description of the experimental protocol is provided here. Owing to the limited availability of wild flies, we used flies from a laboratory colony started with >500 adults reared from field-collected coffee berries (these are termed "laboratory" flies hereafter). Adults were held in screen cages and provided a sugar-protein mixture, water, and an oviposition substrate. Eggs were placed on larval medium over vermiculite for pupation. Adults used in the experiments were separated by sex before reaching sexual maturity and kept in screen-covered buckets with food and water. When used, laboratory flies were 10-12 generations removed from the wild.

Mass-reared flies were from a temperature sensitive lethal (*tsl*) strain produced by the California Department of Food and Agriculture Hawaii Fruit Fly Rearing Facility, Waimanalo, Oahu. Larvae of the *tsl* mass-reared strain were reared on standard diet, and males were irradiated as pupae 2 d before eclosion. Irradiated pupae were placed in screen-covered plastic buckets, and the adult males were maintained on sugar agar gel under the same conditions as laboratory flies.

For GRO exposure, 100 *tsl* males were placed in a plastic bucket, and 80 µl of GRO were applied to a filter paper disk, which was then placed on the bottom of the bucket. This dose was similar (in terms of ml GRO/m^3 of the holding container) to a 1 ml application on a PARC box (the standard type used in SIT programs), found previously to enhance the mating success of *tsl* males (Shelly et al. 2004). For all tests, treated *tsl* males were exposed to GRO in an isolated room for 3 h when 4-5 d old and were used 2 d after exposure; control *tsl* and laboratory males were not exposed to GRO.

Four experiments were performed. Laboratory females were mated first to laboratory males and 2 d later were given the opportunity to remate with control (Experiment A) or treated (Experiment B) *tsl* males. In the second pair of experiments, laboratory females were mated first to control (Experiment C) or treated (Experiment D) *tsl* males and 2 d later were given the opportunity to remate with laboratory males. In all cases, laboratory females were 7-9 d old when first mated, and laboratory and *tsl* males were 7-11 and 5-8 d old, respectively, when used for mating.

Initial matings by females were obtained in the laboratory in plexiglass cages (30 × 30 × 40 cm). Approximately 200 laboratory females and 200 males of a given type were used per cage, and mating pairs were collected continuously for 2-3 h. Only females from pairs coupled for >90 min were tested for remating; males were discarded. Prior to the remating trials, mated females were provided with the sugar-protein mixture and water but no oviposition substrate. A complete set of remating experiments (A-D) was run indoors using the plexiglass cages and outdoors in field tents each containing 2 artificial trees. Fifty mated females and 50 males of a given type were placed in either cages or tents at 0800 hours, and mating pairs were collected over the next 4 h. The experiments were conducted in a randomized order.

In both the laboratory and field cages, females initially mated to laboratory males were more

TABLE 1. FREQUENCY OF FEMALE REMATING. VALUES REPRESENT MEAN NUMBER (± 1 SD) OF FEMALES REMATING PER REPLICATE. SEVEN AND 14 REPLICATES WERE CONDUCTED PER EXPERIMENT FOR THE LABORATORY AND FIELD TENT TRIALS, RESPECTIVELY. VALUES NOT SHARING ANY LETTER ARE SIGNIFICANTLY DIFFERENT (TUKEY'S TEST FOLLOWING DETECTION OF SIGNIFICANT VARIATION IN 1-WAY ANOVA WITH RAW DATA; α = 0.05 IN ALL CASES).

	Male Type		
Experiment	First Mating	Second Mating	Female Rematings
Laboratory cages			
A	Laboratory	Control *tsl*	8.6 (3.4)[a]
B	Laboratory	Treated *tsl*	16.4 (3.3)[b]
C	Control *tsl*	Laboratory	12.6 (5.1)[ab]
D	Treated *tsl*	Laboratory	7.8 (2.8)[a]
Field tents			
A	Laboratory	Control *tsl*	2.5 (3.0)[a]
B	Laboratory	Treated *tsl*	4.9 (2.1)[b]
C	Control *tsl*	Laboratory	4.2 (2.0)[ab]
D	Treated *tsl*	Laboratory	3.7 (1.6)[ab]

likely to remate with treated *tsl* males than control *tsl* males (Table 1). However, in both indoor and outdoor tests, the probability of female remating with a laboratory male was independent of the treatment status of the *tsl* male from the initial mating. There was no difference (indoor or outdoor) in remating between females first mated to a laboratory male and then offered control *tsl* males and females tested in the reverse sequence. Overall experiments, the incidence of female remating was much greater in the laboratory cages than the field tents, presumably reflecting the higher fly density in the smaller, laboratory cages.

Prior studies (e.g., Shelly et al. 2004) have demonstrated GRO exposure may improve SIT effectiveness by increasing the mating competitiveness of *tsl* males. The present findings indicate another potential benefit of GRO exposure: inducement of female remating with *tsl* males, which may, given the last male advantage in sperm competition, lower the reproductive potential of wild medfly populations.

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EGG DEVELOPMENT MAY REQUIRE MULTIPLE BLOODMEALS AMONG SMALL *AEDES AEGYPTI* (DIPTERA: CULICIDAE) FIELD COLLECTED IN NORTHEASTERN MEXICO

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Aedes aegypti imbibes multiple blood meals in each gonotrophic cycle (Scott et al. 1993). It prefers human blood, and rarely feeds on sugar (Edman et al. 1992; Van Handel et al. 1994). Host-seeking *Ae. aegypti* females have bodies of widely differing sizes, and exhibit variable blood feeding frequencies (Nasci 1986). Smallest females often engage in more multiple feeding than largest ones (Nayar & Sauerman 1975). This metabolic adjustment is compensated by remaining previtellogenic in Christopher's stage I-IIb (Roubaud 1923; MacDonald 1956), hence they will require a subsequent blood meal to become vitellogenic beyond stage IIIa and produce mature eggs (Clements 1992).

Both wing-length and ovarian development of engorged mosquitoes have been examined only for laboratory *Ae. aegypti*, where the previtellogenic phase was found only in females ≤2.9 mm (Feinsod & Spielman 1982). Relationships between wing-length and egg maturation for neotropical *Anopheles* species was explored by using the logistic regression model (Lounibos 1994; Lounibos et al. 1998). This paper describes the wing-length distribution of wild *Ae. aegypti* host-seeking females, and a logistic regression model to estimate the probability for the previtellogenic phase occurrence in engorged females.

The study site was a dengue endemic neighborhood in Monterrey, Mexico. Climate in Monterrey is arid with an annual mean rainfall and temperature of 450 mm (range = 270 mm-620 mm), and 23°C (range = -2°C-44°C), respectively. The two rainy months are May and October, and the highest population densities of *Ae. aegypti* occur during these months (Salas-Luevano & Reyes-Villanueva 1994). Six human-biting collections (one in October 1994, May 1995, October 1995, and three in October 1996) were gathered from five houses between 17:00 to 20:00 h. Each collection consisted of 60 host-seeking female *Ae. aegypti*. Each collection was made within a 5-day period by a two-person team (the human bait and the collector catching mosquitoes on the human bait). Mosquitoes landing (i.e., posed) on the legs and arms of the human bait were immediately captured with a mouth aspirator by the collector. They were held in cardboard cages (25 × 15 cm) and transported to the laboratory immediately after their capture. Females were anesthetized by exposure to -2°C for 10 minutes and those with abdomen completely empty were separated for blood feeding, whereas those with blood vestiges in their stomach and gravid females were removed. They had access to water and fed to repletion without interruption on the hand of a human volunteer 24 h after capture. Fed females were held 48 h before dissection to determine ovarian development under insectary conditions of 28°C and R. H. 80%. They were immobilized again by freezing, and ovaries removed and placed into a 0.5% saline solution. Christopher's stage of oocyte development was registered (Clements and Boocock 1984), and adult body size was estimated by measuring the left wing-length (Kelly & Edman 1992). If ovarian follicles had developed beyond stage IIIa, the mosquito was considered positive for egg development, otherwise, if the ovarian follicles were in stage I-IIb, the mosquito was previtellogenic (Clements 1992).

Mean ± SE wing length (mm) were calculated for all females. Wing-length of previtellogenic, and vitellogenic females among collections were analyzed by ANOVA (SAS 1995). The data set consisted of 360 scores made up of two variables: The dependent "previtellogenic" (a binary variable), and the covariate "wing-length" (WL). Dependent variable, which is the probability to be a previtellogenic female, was equal to 1 if the mosquito was previtellogenic and it was equal to 0 if the mosquito had follicles in stage IIIa. The logistic regression model was used (SAS 1995) to estimate the covariate WL influence on the probability to pass from previtellogenic to vitellogenic phase after engorgement.

One hundred and twenty three *Ae. aegypti* host-seeking females out of 360 (34%), remained previtellogenic in stage I-IIb. Of these, 58% were in Christophers stage IIb, while 48% exhibited previtellogenic stage I. Previtellogenic females exhibited a wing-length range from 1.8 to 3.1 mm, which was similar to the range observed for all mosquitoes in this survey (1.7-3.2 mm). There were 13 previtellogenic mosquitoes (2.92-3.15 mm range) with wings longer than 2.9 mm, which was 11% of total previtellogenic (13/123), and 4% of all captured individuals (13/360). Mean wing-length for all females was 2.52 ± 0.03 mm. A t test for unpaired means (t = 3.20, df = 5, $P < 0.001$) showed that previtellogenic females (2.45-0.02 mm, n = 123) were smaller than vitellogenic ones (2.56 ± 0.01 mm, n = 237). The biggest previtello-

TABLE 1. MEAN WING-LENGTH ± SE (STANDARD ERROR) (MM) FOR PREVITELLOGENIC AND VITELLOGENIC FEMALES OBSERVED IN SIX HOST-SEEKING *Aedes aegypti* COLLECTIONS FROM MONTERREY, MEXICO.

Collection	No. previtellogenic females	Mean ± SE[1]	No. vitellogenic females	Mean ± SE[2]
Biting (Oct. 1994)	17	2.76 ± 0.05 a	43	2.69 ± 0.04 a
Biting (May 1995)	12	2.66 ± 0.08 ab	48	2.66 ± 0.04 a
Biting (Oct. 1995)	15	2.49 ± 0.05 bc	45	2.57 ± 0.03 ab
Biting (Oct. 1996)	23	2.37 ± 0.06 c	37	2.43 ± 0.04 b
Biting (Oct. 1996)	30	2.34 ± 0.04 c	30	2.45 ± 0.04 b
Biting (Oct. 1996)	26	2.32 ± 0.06 c	34	2.45 ± 0.04 b
Total	123	2.45 ± 0.06	237	2.56 ± 0.03

[1]Means for previtellotenic females with different letters were significantly different ($F = 7.94$, $df = 5$, $P < 0.0001$) among collections according to a REGWQ multiple comparison test.

[2]Means for vitellogenic females with different letters were significantly different ($F = 7.04$, $df = 5$, $P < 0.0001$) among collections according to a REGWQ multiple comparison test.

genic mosquitoes were collected in October 1994 (2.76 ± 0.05 mm) with a wing-length significantly higher ($F = 7.94$, $df = 5$, $P < 0.001$) than that of all *Ae. aegypti* mosquitoes collected in 1996 (Table 1).

The overall logistic model to calculate the occurrence probability for a previtellogenic female was significant ($P = 0.01$) according to a χ^2 distribution. The likelihood-ratio was -2 LOG L = 10.63 with 1 *df*, versus a $\chi^2 = 6.63$ from tables at $P = 0.01$. The model predicted 58.2% of the responses correctly. In addition, the Wald statistics $(\beta/S.E._{\beta})^2$ which also has a χ^2 distribution with 1 *df*, was also significant ($P = 0.001$) with a value of 10.25. The coefficient for the "wing-length" covariate was negative and highly significant ($\beta = -1.2080$, $P = 0.001$), demonstrating an inverse relationship between wing-length and the likelihood for the previtellogenic female occurrence. The ratio $\pi/(1 - \pi)$ in the logit transformation is referred to as the Odds, and an important way of interpreting the logistic regression coefficient β is its effect on the Odds (Agresti & Finlay 1986). Particularly, when the antilog of β as e^{β} was calculated, an output of 0.2987 was obtained of which the reciprocal was 3.34. This means that after engorgement, smallest mosquitoes are 3.34 times more likely to remain as previtellogenic than largest mosquitoes.

In this survey, the previtellogenic phase was observed in small *Ae. aegypti* females as well as in large biting females (>2.9 mm). Herein, the results showed that 34% of the host-seeking females will need to have at least two blood meals to mature eggs, and three to complete a full gonotrophic cycle. Moreover, it is highly plausible that a human could be the host when a mosquito is imbibing the 2nd or 3rd blood meal, because this vector has acquired a noticeable synanthropic feeding habit to survive in domestic settings (Chow et al. 1993; Day et al. 1994).

Our results are not in line with one report, in which, *Ae. aegypti* exhibited a size-dependent divergence in host-seeking responses in favor of large individuals (Klowden et al. 1988). However, when previtellogenic mosquitoes were sorted by their wing-length in ascending order, the small ones located in the 2.2-2.8 mm classes were clearly predominant (64% = 79/123) in the collections. The prediction power of the logistic regression exploited here, to estimate the probability of being a previtellogenic *Ae. aegypti* mosquito, is acceptable according to the χ^2 distribution (Agresti & Finlay 1986). The model should be validated in further field surveys with the samplings including blood-deprived, and host-seeking females from human-landing collections. The high anthropophilism of *Ae. aegypti*, and the likely influence on oogenesis by the low isoleucine in human blood (Day et al. 1994) would need to be considered when constructing a more powerful model.

SUMMARY

Samplings of *Aedes aegypti* host-seeking females in Monterrey, Mexico, exhibited a 34% of previtellogenic phase in stage I-IIb after engorgement. Failure to reach the vitellogenic phase was present in females with a wing-length range from 1.8 to 3.1 mm. Logistic regression for a binary response (where Y = 1, previtellogenic, and Y = 0, non-previtellogenic), in function of wing-length as covariate, demonstrated that the likelihood of previtellogenic phase increases 3.34 times in smallest mosquitoes compared to the largest.

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851 NE Jensen Beach Blvd.
Jensen Beach, FL 34957

Terminix International
Attn: Norman Goldenberg
860 Ridge Lane Blvd.
Memphis, TN 38120

The Scott Co.
Attn: Wayne Mixson
P.O. Box 2187
Apopka, FL 32704

Valent USA Corp.
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3700 NW 91st; Bldg. C S
Gainesville, FL 32606

Walt Disney World
Attn: Jerry Hegedorn
P.O. Box 10000
Lake Buena Vista, FL 32830

Wright Pest Control Inc.
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Winter Haven, FL 33880

Yoder Brothers
Attn: Nancy Rechiegl
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United States Postal Service

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Contact Person: Teresa DuChene
Telephone: 813-903-9234

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Lutz, Florida 33549

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