INTERACTION OF PARASITOIDS AND RESISTANT CULTIVARS OF WHEAT ON HESSIAN FLY, *MAYETIOLA DESTRUCTOR* SAY (CECIDOMYIIDAE).

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ABSTRACT

Field-cage and open-field studies evaluated parasitism of Hessian fly infesting five wheat cultivars with no, one, two or three genes for resistance to Hessian fly. Parasitism of Hessian fly by the egg-larval parasitoid *Platygaster hiemalis* Forbes (Platygasteridae) and the pupal parasitoids *Homoporus destructor* (Say) (Pteromalidae) and *Eupelmus allynii* (French) (Eupelmidae) did not vary among wheat cultivars with different levels of resistance. Parasitism rates by pupal parasitoids were independent of host density. The number of adult *P. hiemalis* emerging per host was the same regardless of the level of plant resistance to Hessian fly. The presence of both *P. hiemalis* and pupal parasitoids resulted in multiple parasitism with no net gain in mortality of Hessian fly puparia. Mortality of Hessian fly pupae by parasitoids is compatible with host plant resistance and may extend the usefulness of resistant cultivars by slowing the increase of populations of virulent Hessian fly biotypes.

INTRODUCTION

Since the late 1970s, the acreage of wheat in central Texas and in the southeastern U.S. has substantially expanded (Lidell and Schuster 1990, Buntin and Chapin 1990). Damage to wheat by the Hessian fly, *Mayetiola destructor* Say (Cecidomyidae), in this region has also increased during the time. Widespread and economic infestations of Hessian fly occurred in Georgia in 1977 and 1984 (Johnson et al. 1984), in South Carolina in 1986 (Chapin et al. 1989), in Alabama in 1985 (Estes et al. 1985) and in Texas in 1984 and 1989 (Knutson 1991).

Delayed planting and use of resistant wheat cultivars have been the most effective and economical methods for preventing losses to Hessian fly in the midwestern U.S. (Wellso et al. 1991). Use of area-wide delayed sowing dates (i.e., planting after adult fly activity ceases in the fall) is effective in midwestern and eastern states where the Hessian fly typically completes a single fall and a single spring generation (Wellso 1991). This strategy has less utility in the southern U.S. where warm weather permits Hessian fly to complete two broods in the fall and one or two broods in the spring in Texas and as many as six broods in Georgia (Lidell and Schuster 1990. Buntin and Chapman 1990). Although Lidell and Schuster (1990) in Texas recommended planting wheat after November to escape Hessian fly damage, they also concluded that economic benefits of planting wheat early for livestock forage might outweigh the benefits of delayed planting for Hessian fly control.

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Since the first resistant cultivar was introduced in the 1950s, planting resistant cultivars has been the primary control tactic for Hessian fly (Wellso 1991). Twenty genes, designated *H1* to *H20*, have been identified for Hessian fly resistance in polyploid wheats (Gallun and Rietz 1971, Hatchett 1970). The resistance mechanism is usually antibiosis and results in the death of first-instar Hessian fly larvae shortly after they begin feeding on resistant plants (Gallun and Rietz 1971). An exception is the Marquillo gene which is associated with plant tolerance and is temperature sensitive (Masas et al. 1987). Deployment of resistant wheat cultivars has selected at least 16 Hessian fly biotypes, each based on an ability to infest wheat with one or more resistant genes (Gallun 1977, Ratcliffe et al. 1994). The greater number of broods produced in the southern states favors a rapid increase in populations of virulent biotypes.

Three hymenopterous parasitoids *Homoporus destructor* (Say) (Pteromalidae), *Eupelmus allynii* (French) (Eupelmidae), and *Trichomalopsis subapterus* (Forbes) (Pteromalidae) attack the Hessian fly in Texas (Schuster and Lidell 1990). All are solitary, primary parasitoids that feed externally on the host pupa inside the puparium (Schuster and Lidell 1990). The most common parasitoid attacking the fall brood(s) of Hessian fly in the eastern and midwestern U.S. is *Platygaster hiemalis* Forbes (Platygasteridae) (Hill et al. 1939, Hill 1953). It is a primary, internal, and polyembryonic parasitoid that oviposits in the Hessian fly egg. The parasitoid embryo enters a resting stage until the host larva molts into the non-feeding third instar (puparium). The parasitoid larvae then eclose and feed on host fat bodies. Parasitoid larvae pupate within the Hessian fly puparium (Hill 1926). Efforts to introduce *P. hiemalis* into Texas have not proven successful (Rojas et al. 2000).

Host plant resistance and biological control programs generally are considered compatible methods for insect pest management, although resistant plants can have both positive and negative effects on natural enemies (Boethel and Eikenbary 1986). As an example of a negative effect, the parasitoid *Diaeretiella rapae* (McIntosh) took longer to develop and were fewer in number and smaller in size when parasitizing Russian wheat aphid, *Diuraphis noxia* (Mordvilko), feeding on cultivars of triticale with high levels of antibiosis relative to susceptible cultivars (Reed et al. 1991). The interaction of egg-pupal and pupal parasitoids of the Hessian fly and resistant wheat cultivars is not well understood. Chen et al.(1991) reported that wheat cultivars with high levels of resistance reduced the effectiveness of *P. hiemalis*, whereas, moderate levels of resistance were compatible with the activity of parasitoids.

Integrating biological control by parasitoids with plant resistance could help extend the durability of resistant wheat genes by reducing the survival of virulent Hessian flies. The objective of this study was to measure the interaction of Hessian fly resistant wheat cultivars and parasitism of Hessian fly by egg-pupal and pupal parasitoids. These results will aid in assessing the role of biological control in an integrated pest management program for Hessian fly.

MATERIALS AND METHODS

Hessian flies and *P. hiemalis* used in cage and field experiments were maintained in laboratory colonies as described by Rojas et al. (2000). The laboratory colony of Hessian fly consisted of biotypes GP (34%), A (33%), C (21%), and B, D, E, F and G, each at 5% or less (Ratcliffe et al. 1994).

Cage Studies. Experiments were conducted at the Texas A&M University Research and Extension Center at Dallas, Texas. Field plots, each 1.2 x 5 m, were planted on 5 February, 1992 to one of the following wheat cultivars: 'Collin' (PI 511849, no genes for resistance to

Hessian fly), 'Caldwell' (CI 17897, with one resistant gene, *H6*), 'Chisholm' (PI 486219, with one resistant gene, Marquillo), 'Bradford' (P 470925, with two resistant genes, *H3* and *H5*), and 'Pioneer-2157' (NS 6187405, with three resistant genes, *H3, H5,* and *H6*). These five wheat cultivars provided a gradient of plant resistance effective against the predominant Hessian fly biotypes identified in central Texas and in the laboratory colony (Ratcliffe et al. 1994). Each wheat cultivar was replicated three times in a randomized complete block design.

In April 1992, two cages were placed over wheat plants in each plot when plants were 20 to 30 cm tall. Cages were 0.95 m high and 1.0 m square and constructed of a frame of plastic (PVC) pipe covered with a nylon fabric tent. A zipper (60 cm long) on one side allowed access into the cage for releasing insects and for collecting wheat samples. Each cage was secured to the soil with four U-shaped iron bars (2.0 x 30 cm). Soil was placed on the lower edges of the tent fabric to seal the cage bottom.

During 6-10 April 1992, 50 female and 20 to 25 male Hessian flies were released into each cage. The second cage in each plot was also inoculated with 50 adult *P. hiemalis* (mixed sexes). During 1-10 May, all wheat plants were dug from the soil and removed from each cage in the order in which they were infested with Hessian flies in April. Each cage was relocated in the plot and the experiment was repeated again by releasing Hessian flies and parasitoids in the cages during 3-10 May 1992. Wheat plants were collected from these cages in late May. The cages were relocated to a third site and Hessian flies and parasitoids were again released in the cages from May 25 to 30, 1992. Wheat was sampled from this third repetition on 25 June 1992.

On each sample date, a sub-sample of 28 wheat plants, each having 2-12 tillers, was collected from each cage and dissected to recover Hessian fly puparia. Puparia were placed individually in cells of a tissue culture plate (MicroTest, Falcon) and held for emergence of the adult Hessian fly or parasitoid (Rojas et al. 2000). Puparia that did not produce an adult insect after 60 days were dissected to determine the presence or absence of a parasitoid.

The experiment was repeated in 1994 using the same cultivars and plot size but with the following treatments:1) no parasitoids; 2) *P. hiemalis* only; 3) the pupal parasitoids *H. destructor* and *E. allynii*; 4) all three parasitoid species. Wheat was planted in November 1993 and four cages were placed over wheat plants in each plot in March 1994. From 20-30 April, 50 females and 20-25 male adult Hessian flies were released into each cage. At the same time, 50 *P. hiemalis* adults (mixed gender) were released in each of two cages per plot. During 24-28 May 1994, 14-16 adult *H. destructor* and 4-6 adult *E. allynii* were released into one cage previously infested with Hessian fly only and also into the second cage inoculated earlier with only *P. hiemalis*. Hessian fly densities were determined in each cage on 28 June 1994, at which time all of the Hessian flies were in the pupal stage, and the wheat was dead. A sample of 28 wheat plants was collected from each cage and the plants were dissected to recover Hessian fly puparia. Puparia were held as previously described for parasitoid emergence. Sample data were combined across all three replications (dates) within a year.

Field Studies. The interaction of the five wheat cultivars and Hessian fly parasitoids was also evaluated under open-field conditions in Clay County in 1992-1993 and in Bosque County, Texas, in 1993-1994. The Clay County site was near the Texas-Oklahoma border while the Bosque County site was located in central Texas. Hessian flies were common at both locations because susceptible wheat cultivars planted in previous years in nearby commercial fields were heavily infested with Hessian flies. Study plots, each measuring 1.2 X 5 m, were established in a large, commercial wheat field at each location. Plots were planted to the five wheat cultivars used earlier ('Collin', 'Chisholm', 'Caldwell', 'Bradford', and 'Pioneer 2157') with three replications of each cultivar in a randomized, complete block design. The wheat field surrounding the plots was planted to 'Russian beardless' or

'Weathermaster' cultivar, both susceptible to Hessian fly.

Populations of the extant pupal parasitoids, *H. destructor* and *E. allynii*, were present at both sites as determined from previous sampling. *P. hiemalis*, which was not present at either site, was released into the Clay County plots along with Hessian fly adults to ensure host eggs were present. Each plot was inoculated with 50 adult *P. hiemalis* (mixed gender) and Hessian flies (ca 50 females and 20-25 males) on 8 and 22 March and 12 April. On 29 March 1993, a sample of 28 wheat plants, each with 2-12 tillers, was collected from each plot and dissected to recover Hessian fly larvae and puparia. Sampling was repeated on 21 April, 12 May and 17 June 1993.

At the Bosque County site, wheat was planted in the plots in November 1993. No parasitoids or Hessian fly were released at the study site in Bosque County. Wheat plants were sampled on 28 May 1994 as at the Clay County site and dissected to recover Hessian fly larvae and puparia. Hessian fly puparia were held in tissue culture plates to record the emergence of parasitoids as described previously. Puparia that did not produce an adult after 60 days were dissected to determine the presence or absence of a parasitoid larvae or uneclosed adult. Sample data from all sample dates were combined for each study site.

Data were analyzed using the Statistical Analysis System (SAS) version 6.03 (SAS Institute 1989). Analysis of variance was used to detect significant differences in Hessian fly densities between treatments. Percentage emergence data for adult parasitoids and Hessian fly were transformed by arcsine before analysis. Mean differences were separated using Fisher's Least Significant Difference (LSD) at $P < 0.05$.

RESULTS

Cage Studies. Densities of Hessian fly puparia decreased with increasing levels of plant resistance regardless of the presence of *P. hiemalis* (Table 1). In the absence of *P. hiemalis*, 'Collin', lacking genes for resistance, had significantly more Hessian fly puparia per tiller than did the other four resistant varieties ($F = 70.0$, $P = 0.001$). Densities of Hessian fly puparia in 'Chisholm' were intermediate while no Hessian flies were recovered from 'Pioneer 2157' that carried three genes for resistance. These results demonstrated that the selection of cultivars established the desired range of resistance levels for the Hessian fly biotypes used in the trials.

Percentage parasitism by *P. hiemalis* ranged from 35.5-36.8% and differences between cultivars and density of Hessian fly puparia was not significant (Table 1). This lack of density-dependent response of *P. hiemalis* to densities of host puparia was expected because parasitism by this species occurred in the egg stage, and although densities of host eggs were not measured, they were assumed to be similar because each variety was exposed to the same number of female Hessian flies. Differences in densities of Hessian fly puparia among cultivars were primarily due to mortality of first-instar larvae on resistant plants. Mortality due to parasitism occurred after the puparium was formed.

Parasitism by *P. hiemalis* significantly reduced the percentage of puparia yielding Hessian fly adults in 'Collin' and 'Chisholm' ($F = 59.6$, $P = 0.001$; $F = 24.4$, $P = 0.001$, respectively). Although 35.5% of the Hessian fly puparia were parasitized by *P. hiemalis* in the 'Bradford' cultivar, percentage of emergence by adult flies was not significantly different with or without *P. hiemalis* ($F = 0.16$, $P = 0.69$). The absence of a measurable effect of *P. hiemalis* on Hessian fly in 'Bradford' may have been due to the small number (34 and 36) of host puparia recovered from this cultivar. Plants of 'Caldwell' in cages with *P. hiemalis* were heavily infested with greenbugs, *Schizaphis graminum* (Rondani), and died before Hessian flies completed development to the puparia stage. Sample size was considered too small to

TABLE 1. Density of Hessian Fly Puparia, Percentage Parasitism and Adult Hessian fly Emergence in Different Wheat Cultivars and with and without *P. hiemalis* in Cages, Dallas, TX. 1992.

	Average no. Hessian fly puparia per tiller with *P. hiemalis*:[a]			Percentage adult Hessian fly emergence with *P. hiemalis*:[b]	
Cultivar	Absent	Present	Percentage parasitism[a]	Absent	Present
Collin	3.33 Aa	1.79 Ab	36.8 A	55.1 a	20.7 b
Chisholm	2.04 Ba	1.35 Ab	35.5 A	32.9 a	20.5 b
Caldwell	0.24 Ca	n.d.[c]	n.d.	64.4	n.d.
Bradford	0.06 Ca	0.08 Ba	35.5 A	34.3 a	27.3 a
P-2157	0	0	n.d.	n.d.	n.d.

[a]Means followed by the same lower case letter in a row for the number of Hessian fly puparia are not significantly different at $P = 0.05$; means followed by same upper case letter in a column are not significantly different at $P = 0.05$. (Fisher LSD).
[b]For percentage adult Hessian fly emergence with *P. hiemalis* present or absent, $F = 59.6$, $P = 0.001$ for Collin; $F = 24.4$, $P = 0.001$ for Chisholm; and $F = 0.16$, $P = 0.69$ for Bradford.
[c]n.d. = no data because of an inadequate sample.

TABLE 2. Mean Number of Hessian Fly Puparia per Tiller with and without *P. hiemalis* and Pupal Parasitoids in Cages, Dallas, TX. 1994.

Cultivar	No parasitoids[a]	*P. hiemalis* only[a]	Pupal parasitoids[a]	*P. hiemalis* and pupal parasitoids[a]
Collin	2.55 Aa	2.27 Aa	2.38 Aa	1.63 Aa
Chisholm	1.36 Ba	1.16 Ba	0.17 Bb	0.31 Bb
Caldwell	0.03 Ca	0.08 Ca	0.11 Ba	0.28 Bb
Bradford	0.03 Ca	0.02 Ca	0.16 Bb	0.01 Ba
P-2157	0	0	0	0

[a] Means followed by the same lower case letter in a row for the presence or absence of *P. hiemalis* are not significantly different at $P = 0.05$; means followed by same upper case letter in a column are not significantly different at $P = 0.05$. (Fisher LSD). Statistics for treatment effects by cultivar (within column) are $F = 25.94$, $P = 0.001$ for 'No parasitoid'; $F = 50.6$, $P = 0.001$ for '*P. hiemalis* only'; $F = 25.9$, $P = 0.001$ for 'Pupal parasitoids'; and $F = 50.6$, $P = 0.001$ for '*P. hiemalis* and pupal parasitoids.' Statistics for treatment effects by parasitoid combination (across rows) are $F = 1.53$, $P = 0.21$ for 'Collin'; $F = 15.5$, $P = 0.001$ for 'Chisholm'; $F = 4.12$, $P = 0.007$ for 'Caldwell'; and $F = 3.66$ and $P = 0.012$ for 'Bradford'.

assess treatment effects in 'Caldwell' because only five puparia were recovered from this cultivar.

In the 1994 cage studies, the density of Hessian fly puparia again declined as expected with increasing levels of plant resistance (Table 2). No Hessian fly puparia were recovered from 'Pioneer 2157'. The presence of parasitoids should not have altered the density of Hessian fly puparia because *P. hiemalis*, *H. destructor* and *E. allynii* attack the host only after the puparia is formed. However, the density of Hessian fly puparia with and without parasitoids was significantly different in four of the twelve cultivar and parasitoid combinations (Table 2). A significant reduction in density of puparia in the presence of *P. hiemalis* was also observed in 1992 in 'Collin' and 'Chisholm' ($F = 20.68$, $P = 0.001$, $F = 8.2$, $P = 0.004$, respectively) (Table 1). Hessian fly mortality occurring before the puparia stage could have resulted from *P. hiemalis* adults feeding on host eggs. Also, Hill (1926) observed multiple parasitism of host eggs by *P. hiemalis* but did not determine if this resulted in premature host death. Differences in densities of puparia in cages where the pupal parasitoids were released is unexplained and might have been caused by other factors such as greenbug infestations which competed for plants.

With one exception, parasitism rates by parasitoids alone or in combination with *P. hiemalis* were not significantly different among the four cultivars (Table 3). The exception was the reduction in percentage parasitism of Hessian flies on 'Chisholm' relative to 'Collin' and 'Bradford' in the *P. hiemalis* only treatment. Survival of Hessian fly in 'Caldwell' and 'Pioneer 2157' was too low to evaluate.

Parasitism by the pupal parasitoids and *P. hiemalis* was not additive. Parasitism by *P. hiemalis* ranged from 17.8-50.0% alone but declined to 3.8-5.9% in the presence of the pupal parasitoids *H. destructor* and *E. allynii* (Table 3). Larvae of the pupal parasitoids, which feed externally on their host, apparently consumed *P. hiemalis* larvae, which feed internally on the same host stage. Thus, multiple parasitism resulted in a decline in survival of *P. hiemalis* to the adult stage.

TABLE 3. Percent Parasitism of Hessian Fly by Egg and Pupal Parasitoids Alone and In Combination and Among Different Wheat Cultivars in Cages, Dallas, TX. 1994.

	P. hiemalis		Pupal parasitoids		*P. hiemalis* and pupal parasitoids			
Cultivar	n[a]	only[b]	n[a]	only[b]	n[a]	Pupal parasitoids[b]	*P. hiemalis*[b]	Total
Collin	96	42.7 a	464	39.7 a	384	46.6 a	4.4 a	51.0
Chisholm	90	17.8 b	67	49.3 a	34	50.0 a	5.9 a	55.9
Caldwell	0	n.d.	40	42.5 a	52	42.3 a	3.8 a	46.1
Bradford	16	50.0 a	41	61.0 a	1	(100)	0	100.0
P-2157	0	n.d.[c]	1	(100)	4	(75.0)	0	75.0

[a]n = number of puparia sampled.

[b]Means followed by same letter within a column are not significantly different at $P = 0.05$. Statistics are $F = 7.81$, $P = 0.01$ for *P. hiemalis* only; $F = 1.45$, $P = 0.23$ for pupal parasitoids only; $F = 1.07$, $P = 0.38$ for pupal parasitoids in the presence of *P. hiemalis*; and $F = 0.20$, $P = 0.38$ for *P. hiemalis* in the presence of pupal parasitoids.

[c]n.d. = no data because of as inadequate sample.

Field Studies. As in the cage studies, densities of Hessian fly puparia declined with increasing levels of plant resistance at both field locations (Tables 4 and 5). Parasitism by *P. hiemalis* (Table 4) and pupal parasites (Table 4 and 5) were not significantly different among wheat cultivars. These results confirm those from field cages showing that for both *P. hiemalis* and the pupal parasitoids, plant resistance did not reduce parasitism rates and that parasitism of host puparia was not density-dependent. Hessian fly puparia were recofered from 'Pioneer 2157', indicating a biotype virulent to *H3* , *H5* and *H6* genes was present at both field sites. Extant pupal parasitoids were discovered from puparia on 'Pioneer 2157', demonstrating the ability of pupal parasitionds to find host puparia at very low densities (i.e., 0.01 puparia per tiller) (Table 5).

Parasitism rates by extant pupal parasitoids were low, ranging from 0 to 2.6%, at the Clay County location and therefore are believed to have had little negative impact on parasitism by *P. hiemalis*. In contrast, parasitism by pupal parasitoids was much greater at the Bosque County location (Table 5). At this site, parasitism rates ranged from 56.5 to 100% even though host densities varied more than 100 fold, from 0.01 to 1.52 puparia per tiller, suggesting that pupal parasitoids did not respond to changes in host density. At both locations, parasitism by released *P. hiemalis* or extant pupal parasitoids provided significant and additive mortality to that achieved by resistant wheat cultivars alone.

The average number of adult *P. hiemalis* emerging per host puparia was not significantly different among wheat cultivars collected from either the cage study at Dallas or the field study in Clay County (Table 6). These results provide further evidence that plant resistance expressed by the wheat cultivars in this study was not detrimental to the development of *P. hiemalis* in surviving Hessian flies. Other life history characteristics including adult longevity and sex ratio may have been impacted by plant resistance but were not evaluated in this study.

TABLE 4. Mean Number of Hessian Fly Puparia per Tiller, Adult Hessian Fly Emergence and Percentage Parasitism by Egg and Pupal Parasitoids in Field Studies, Clay County, TX. 1993.

Cultivar	Puparia per tiller[a]	Percentage adult emergence[a]	Percentage parasitism by:		Hessian fly adults per tiller[b]
			P. hiemalis[a]	Pupal parasitoids[a]	
Collin	0.21 a	23.5 a	29.6 a	0.9 a	0.049
Chisholm	0.19 a	11.9 a	34.7 a	0.5 a	0.023
Caldwell	0.13 b	11.9 a	31.9 a	0	0.015
Bradford	0.13 b	19.6 a	31.1 a	2.5 a	0.025
P-2157	0.09 b	27.6 a	18.4 a	2.6 a	0.025

[a]Means followed by the same letter in a column are not significantly different at $P = 0.05$ (Fisher LSD). $F = 2.23$, $P = 0.075$ for percent adult Hessian fly emergence; $F = 1.24$, $P = 0.304$ for percentage parasitism by *P. hiemalis;* $F = 1.26$, $P = 0.294$ for percentage parasitism by pupal parasitoids.

[b]calculated as puparia per tiller X percentage adult emergence

TABLE 5. Mean Number of Hessian Fly Puparia per Tiller and Parasitism by Pupal Parasitoids in Field Studies, Bosque County, TX. 1994.

Cultivar	No. puparia sampled	Puparia per tiller	Percentage parasitism[a]	Hessian fly adults/tiller[b]
Collin	115	1.52 a	61.5 a	0.59
Chisholm	202	0.99 a	56.5 a	0.43
Caldwell	135	0.31 b	71.3 a	0.09
Bradford	122	0.01 b	69.2 a	0.003
Pioneer 2157	76	0.01 b	100	0

[a]Means followed by the same lower case letter in a column are not significantly different at $P = 0.05$ (Fisher LSD). $F = 15.15$, $P = 0.001$ for puparia per tiller and $F = 1.54$, $P = 0.191$ for percent parasitism.
[b]calculated as (1 - percentage parasitism) X (puparia per tiller).

TABLE 6. Mean Number of Adult *P. hiemalis* Emerging per Hessian Fly Puparium Among Different Wheat Cultivars.

Cultivar	Mean no. *P. hiemalis* adults per parasitized puparium[a] (no. puparia sampled)	
	Cage study at Dallas	Field study at Clay County
Collin	3.4 (39)	2.6 (34)
Chisholm	3.2 (58)	3.6 (70)
Bradford	3.4 (25)	3.8 (38)
Caldwell	n.d.[b]	2.7 (43)
Pioneer 2157	n.d.[b]	1.8 (14)

[a]Means are not significantly different within a study. $F = 0.73$, $P = 0.49$ and $F = 1.87$, $P = 0.125$ for cage study at Dallas and for field study at Clay County, respectively.
[b]n.d. = no data because of an inadequate sample.

DISCUSSION

These results and those of Chen et al. (1991) suggest that the use of resistant cultivars is compatible with parasitism by *P. hiemalis*. As in the current study, Chen et al. (1991) found that the number of *P. hiemalis* adults emerging per host larva was not different among cultivars, suggesting resistant genes did not directly influence parasitoid survival. As expected, cultivars with high levels of antibiosis indirectly reduced survival of the egg parasitoid because the host dies on resistant plants before the parasitoid completes development. Our results suggest that parasitism rates by *P. hiemalis* were similar regardless

of the level of plant resistance to Hessian fly. Chen et al. (1991) concluded that the rate of parasitism by *P. hiemalis* was less on resistant wheat cultivars compared to a susceptible cultivar, although no data supporting this conclusion were presented.

Parasitism by *P. hiemalis* provided no additional mortality of Hessian fly when the extant pupal parasitoids were present. Both *H. destructor* and *E. allynii* are primary and secondary parasitoids, and in the case of *E. allynii* there is no evidence it discriminates between parasitized and nonparasitized hosts when ovipositing (Gahan 1933). In Texas, *H. destructor* and *E. allynii* attack Hessian fly only in the spring (May) and are inactive during the fall and winter (Schuster and Lidell 1990), while *P. hiemalis* parasitizes the Hessian fly only in the fall (Hill 1926). Competition for Hessian fly hosts therefore is avoided because of differences in parasitoid phenology.

Parasitism by pupal parasitoids in Texas is highly variable by site and year as shown in the field experiments in this and other studies (Schuster and Lidell 1990), and the impact of parasitoids on Hessian fly populations is difficult to quantify. The extant pupal parasitoids parasitize Hessian fly only in the spring, after Hessian fly larvae have damaged the crop. Thus, parasitoids reduce the number of Hessian fly larvae which enter diapause during the summer and the subsequent number of adults emerging in the fall (Schuster and Lidell 1990). However, because one to two broods can develop in the fall, Hessian fly populations can quickly increase at this time in the absence of parasitoids, particularly given favorable weather conditions and early planting common in Texas. Rather than suppressing Hessian fly populations below economic levels, parasitoids may play a more important role in attacking virulent biotypes that survive on resistant cultivars and thus serve to extend the effectiveness of resistant cultivars. In the present study, pupal parasitoids were very efficient at finding and parasitizing Hessian fly puparia at low densities (i.e., 0.01 puparia per wheat tiller) (Table 5). Gould et al. (1991) concluded that natural enemies could both increase and decrease the rate of herbivore adaptation to resistant plants, but in general, selection for adaptation to a resistant plant was less when pest suppression was achieved by the combined action of plant resistance and natural enemies than by strong resistance alone.

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USE OF THE CENTER PIVOT IRRIGATION SYSTEM FOR REDUCTION OF COTTON APHID[1] SUGARS ON COTTON LINT

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ABSTRACT

The potential for applying water through a center pivot irrigation system to reduce cotton aphid, *Aphis gossypii* Glover, honeydew sugars on cotton lint was investigated for two years in the Texas High Plains. Using a center pivot chemigation simulator, one, two and three applications of water were applied to sticky lint at water volumes of 6,800 and 13,600 gal/A (0.25 and 0.50 inch water depths, respectively) and using overhead and in-canopy (using chemigation splash pads) nozzle positions. After water treatments were applied, numbers of sugar spots on lint were counted using the High Speed Stickiness Detector (H2SD). Irrigation treatments were effective in reducing numbers of sugar spots on lint, removing from 20.0 to 95.8%. In all cases except one, lint that received irrigation treatments had significantly fewer sugar spots than the untreated control. Water volume and nozzle position/configuration had no significant effect on the number of sugar spots on lint, but multiple applications reduced the number of spots with each successive application. Three applications of water significantly reduced the number of spots over one and two applications, and in both years reduced the number of spots to less than ten per H2SD sample. In 1999, lower percentages of sugar spots were removed by washing treatments (when compared to the untreated control) than in 1998.

INTRODUCTION

Insect induced sticky cotton, the result of honeydew deposited on lint by pests such as the cotton aphid, *Aphis gossypii* Glover, and the silverleaf whitefly, *Bemisia argentifolii* Bellows and Perring [= *B. tabaci* (Gennadius) Strain B], has become a significant problem in U.S. cotton. During the spinning process, sugar residues carried by sticky lint accumulate on equipment and interfere with processing (Perkins 1991). In some cases the problem can become so severe that machines must be shut down for cleanup (Ellsworth et al. 1999). In addition, soil particles trapped by the sugars abrade and cause premature wear to spinning machines. During recent years, textile mills have reported instances of insect induced sticky lint in Texas High Plains cotton. This lint contamination has been attributed to late maturing crops accompanied by infestations of the cotton aphid during the fall.

Since 1975, infestations of the cotton aphid have been an annual problem in the Texas High Plains (Rummel et al. 1995). Through the last two decades the problem has escalated. Infestations of a localized nature have become more widespread, affecting entire fields and regions (Leser et al. 1992, Slosser et al. 1998). Widespread use of insecticides to control these infestations has exacerbated the problem, selecting populations of aphids with increased tolerance to previously effective insecticides (Godfrey and Leser 1999).

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In the Texas High Plains, cotton aphid infestations generally reach highest levels in mid-August, then decline (Slosser et al. 1989). However, light aphid infestations can persist throughout the fall until the plants are killed by harvest aid chemical or freezing weather. In some years these late populations can build to moderate levels, often exceeding 50 aphids per leaf. During many years sufficient rainfall occurs during the fall to cleanse the lint of sugars before harvest; however, in recent years dry weather during the fall has allowed aphid sugars on lint to persist until harvest time. Research in California indicated that in the absence of rainfall as few as 10-15 cotton aphids per leaf could result in sticky cotton with enough sugar residue to negatively affect lint quality (Rosenheim et al. 1995).

The use of center pivot irrigation in the Texas High Plains has expanded greatly in the last decade. From 1986 to 1998 the High Plains Underground Water Conservation District No. 1 (HPUWCD) estimated a 237% increase from 3,352 in 1986 to 11,287 in the number of center pivot systems in 1998. In the Llano Estacado Planning Region in 1998, it was estimated that 72% of irrigated acres were irrigated by 16,420 center pivot systems (Llano Estacado Regional Water Planning Group 2001). Managers at the HPUWCD estimate that 60% of these systems are configured for use in the LEPA (low energy precision application) (Lyle and Bordovsky 1981) irrigation management system.

In Israel, rinsing of *B. tabaci* contaminated sticky lint between defoliation and harvest using sprinkler irrigation was reported to be successful in reducing honeydew and was included as part of a stickiness management program (Fishler 1992). With much of the cotton in the Texas High Plains grown under center pivot irrigation, the potential for using these systems to reduce the amount of cotton aphid sugars on cotton lint was of economic interest. The objective of this study was to determine if center pivot irrigation systems could be used to cleanse cotton lint of cotton aphid honeydew sugar residues during years of low rainfall when aphid infestations are present just prior to the harvest period.

MATERIALS AND METHODS

The experiment was conducted at the Texas Agricultural Experiment Station at Halfway, Texas, in 1998-99. Irrigation water was applied to sticky cotton in an effort to reduce the number of cotton aphid honeydew spots on lint. The test was arranged as a completely randomized design in a 3x2x2 factorial arrangement plus a control. The main effects were: 1) number of applications of irrigation water (one, two, and three), 2) volume of irrigation water (6,800 and 13,600 gal/A), and 3) nozzle position and configuration (overhead and in-canopy sprays). The volumes 6,800 and 13,600 gal/A are equal to 0.25 and 0.50 inch depths, respectively. Plots were 10, 40-inch rows wide by 40 feet long. Each treatment was replicated four times.

In each year, two cotton cultivars were planted: 'Paymaster 183', an early maturing cultivar, and 'All-Tex Atlas', an intermediate maturing cultivar. The two separate test areas were established to increase the probability that susceptible cotton with an adequate number of open bolls would be available for the test during the aphid population peak. Each test area contained 60 plots with the best 52 (based on plant stand density) selected for the test. Planting dates were 19 May 1998 and 17 May 1999. Plots were irrigated by the furrow method and normal cultural practices were performed throughout the season.

After test cotton reached the six-leaf stage both test areas were inspected weekly for cotton aphids. Aphid population density was determined by sampling five plants per plot and counting total numbers of aphids on one upper, middle and lower leaf on each plant. In both years, four applications of cyhalothrin at 0.025 lb ai/A were made to stimulate aphid population development (Kidd et al. 1996).

Irrigation treatments were applied with a center pivot chemigation simulator used for research at the Texas Agricultural Experiment Station. The simulator was constructed from a 3910 Model Ford tractor, equipped with 18.4-16.1 rear turf tires and a "creeper" gear to provide slow ground speeds typical of a center pivot. At an engine speed of 750 rpm and the tractor in low gear, a ground speed of 10 feet per minute was achieved. The start/stop movement of a pivot tower was simulated by disengaging and engaging the clutch and brake of the tractor within 60-second cycles. Therefore, pivot advancement of 4-feet per minute was simulated by tractor movement for 40% (or 24 seconds) of each 60-second interval. Super Spray® irrigation nozzles (Senniger Irrigation Inc., Orlando, FL) were used for both in-canopy and overhead application positions. The in-canopy treatment nozzles were located

approximately 8 inches above the soil surface, equipped with cotton chemigation splash pads, and positioned in alternate furrows. This configuration represented an option for LEPA length, low elevation spray drops, and gave upward penetration of the wash water into the canopy. Overhead treatment nozzles were located approximately 36 inches above the soil surface, equipped with flat, medium fine grooved splash pads and positioned above alternate furrows. This represented a general irrigation configuration applicable to center pivot systems equipped with medium elevation drops. Water used for the simulated irrigation treatments was supplied from a 1000-gal tank trailer that was pulled by the tractor as the applications were made. The spray boom was positioned to the side of the tractor, perpendicular to the simulator travel direction, and extended across six, 40-inch rows of cotton. During application, ground speed of the tractor approximated that of a center section of a quarter mile center pivot irrigation system applying water at the respective water application volume (6,800 or 13,600 gal/A).

In both years, the test area planted to Paymaster 183 was chosen for the irrigation treatments because of its advanced maturity and the abundance of honeydew present on the plants. Irrigation treatments were initiated after sticky leaves were abundant and enough open bolls with clearly visible sugar residue were present to complete the test. Water treatments were made on 10, 17 and 24 September 1998, and on 7, 13 and 27 October 1999. Plots assigned treatments with one application were treated on the first date only, those assigned treatments with two applications were treated on the first two dates, and plots assigned treatments with three applications were treated on all three dates. In both years, the full range of overhead and in-canopy treatments at volumes of 6,800 and 13,600 gal/A was completed as planned. Lint samples were taken from the plots within 24 hours following the third group of irrigation treatments. The samples from the 1998 test were submitted to the laboratory at Cotton Incorporated, Cary, NC, for sugar spot counts. Sugar spot counts on the 1999 lint samples were performed at the International Textile Center at Texas Tech University, Lubbock, Texas. In both cases counts were performed using the High Speed Stickiness Detector (H2SD) (Compton 1998). Sugar spot data were analyzed using ANOVA and LSD (SAS Institute 1985).

RESULTS AND DISCUSSION

1998 experiment. Cotton aphid population densities for 1998 are plotted in Fig. 1. The failure of the cotton aphid population to develop naturally was attributed to extremely hot dry weather which extended from early May to mid-August. No aphids were detected in the test plots during June and July. During the first week of August, very low densities of aphids were detected in the test plots. In an effort to stimulate aphid population development, an application of cyhalothrin was made on 6 August. Samples taken on 17 August indicated a very light infestation of 0.2 aphids per leaf (Fig. 1). The aphid population increased sharply following a second application of cyhalothrin on 17 August, and peaked on 4 September at an average of 41.9 aphids per leaf. The aphid population declined following the 4 September peak even though two additional applications of cyhalothrin were made (Fig. 1).

The effects of irrigation treatments on sticky cotton lint are presented in Tables 1-4. Lint collected from untreated control plots had 31.3 spots per H2SD sample, a significantly higher number than lint from all irrigation treatment plots (Table 1). Numbers of sugar spots on lint from plots given irrigation treatments ranged from 14.2 to 1.3, or a 54.6-95.8% reduction over the untreated control (Table 1). There was no significant difference in the mean number of sugar spots in plots treated with 6,800 and those treated with 13,600 gal/A of irrigation water (Table 2). Likewise, no significant difference was found between samples from plots treated using in-canopy and overhead sprays (Table 3). However, the number of irrigation treatments demonstrated a significant effect in reducing aphid honeydew sugars on the lint. The mean number of aphid honeydew spots decreased with each successive irrigation application, and three irrigation applications resulted in significantly fewer spots on lint compared to one and two applications (Table 4). Lint from plots treated with three applications of water had 2.8 spots per H2SD sample, a reduction of 9.8 spots or 77.8% over lint from plots treated with one application (12.6 spots) (Table 4). Irrigation treatment reduced the number of sugar spots from 18.7 to 28.5 over the untreated control, or 59.7-91.1% (Table 4). Lint from plots treated with three applications of water

showed significantly higher reduction in the number of spots (91.1 %) than lint from plots treated with one and two applications (Table 4). There were no significant interactions between the main effects.

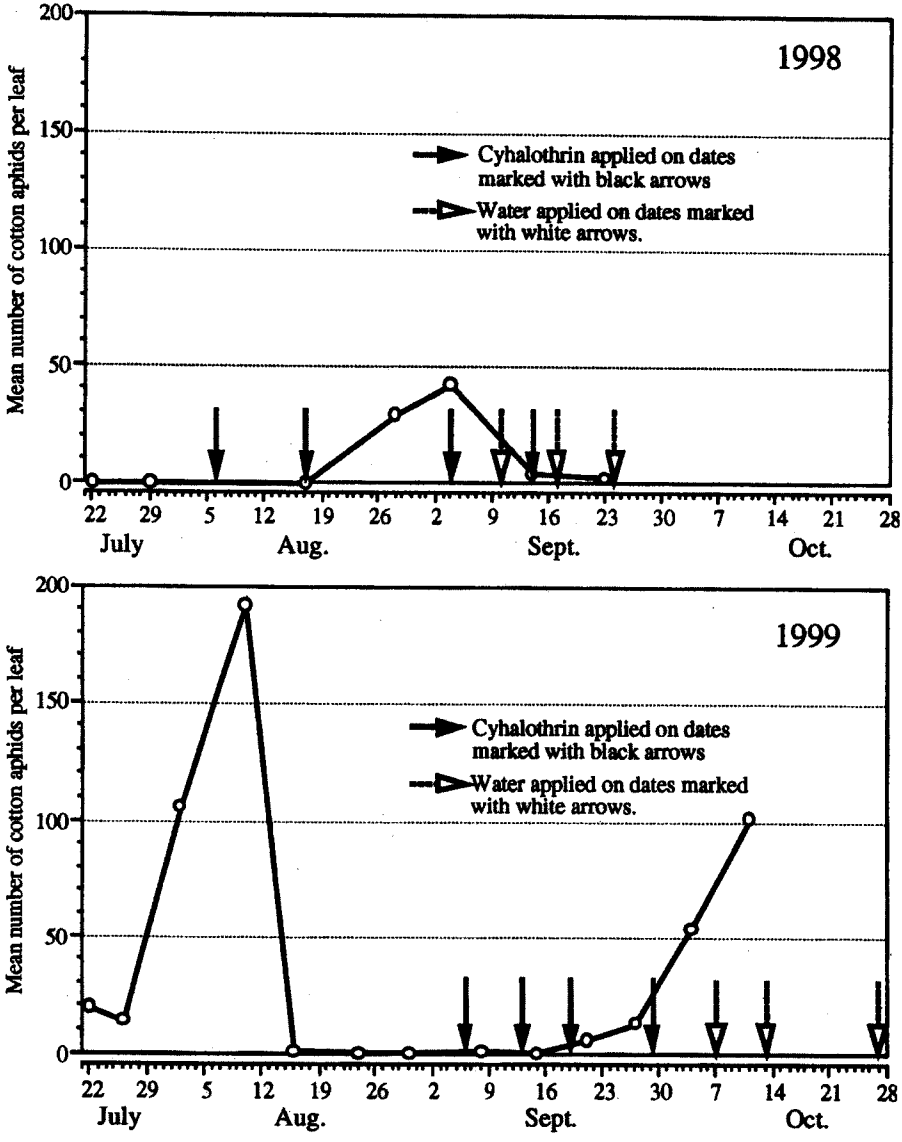

FIG 1. Cotton aphid population trends in plots subjected to applications of irrigation water for removal of aphid sugars from cotton lint, Halfway, Texas.

TABLE 1 Mean Number[a] of Sugar Spots on Cotton Lint, and Spot Reduction Compared to an Untreated Control in Control Plots and Plots Treated with Irrigation Water to Remove Cotton Aphid Honeydew, Halfway, Texas.

Treatment	1998			1999		
No applications[b] Nozzle position Water vol. (gal/A)[c]	Mean no. spots/ sample	Reduction over control # spots	%	Mean no. spots/ sample	Reduction over control # spots	%
control (no applications)	31.3 a			45.1 a		
1 in-canopy 6,800	14.2 b	17.1	54.6	31.4 bc	13.7	30.4
1 overhead 13,600	13.2 bc	18.1	57.8	31.2 bc	13.9	30.8
1 in-canopy 13,600	11.8 bcd	19.5	62.3	36.1 ab	9.0	20.0
1 overhead 6,800	11.3 bcd	20.0	63.9	23.0 cd	22.1	49.0
2 in-canopy 6,800	10.9 bcd	20.4	65.2	23.7 bcd	21.4	47.5
2 overhead 6,800	9.8 bcde	21.5	68.7	20.9 cde	24.2	53.7
2 overhead 13,600	9.2 bcde	22.1	70.6	29.8 bc	15.3	33.9
2 in-canopy 13,600	7.0 bcde	24.3	77.6	22.5 cd	22.6	50.1
3 in-canopy 6,800	4.0 cde	27.3	87.2	15.0 def	30.1	66.7
3 overhead 6,800	3.3 de	28.0	89.5	8.9 ef	36.2	80.3
3 in-canopy 13,600	2.5 de	28.8	92.0	6.6 f	38.5	85.4
3 overhead 13,600	1.3 e	30.0	95.8	7.2 f	37.9	84.0

[a] Means within a column, followed by the same letter are not significantly different (P>0.05, LSD).

[b] Application dates in 1998: 1 application – 10 Sept, 2 applications – 10 and 17 Sept, and 3 applications – 10, 17 and 24 Sept. Application dates in 1999: 1 application – 7 Oct., 2 applications 7 and 13 Oct, and 3 applications – 7, 13 and 27 Oct.

[c] Water volumes 6,800 and 13,600 gal/A are equal to 0.25 and 0.50 inch water depths, respectively.

TABLE 2. Mean Number of Sugar Spots on Cotton Lint Treated with Irrigation Water at Two Volumes to Remove Cotton Aphid Honeydew, Halfway, Texas.

	Mean no. sugar spots per sample[a]	
Water volume (gal./A)[b]	1998	1999
6,800	8.9 a	20.5 a
13,600	7.5 a	22.2 a

[a] Means within a column, followed by the same letter are not significantly different (P>0.05, ANOVA).

[b] Water volumes 6,800 and 13,600 gal/A are equal to 0.25 and 0.50 inch water depths, respectively.

1999 experiment. In 1999, aphid population densities reached higher levels than in 1998 (Fig. 1). Cotton aphid numbers began to increase in mid-July. This aphid infestation was unusually early for the Texas High Plains area, which experiences most cotton aphid population peaks in mid-August (Slosser et al. 1989). The aphid population density averaged 19.3 aphids per leaf on 22 July and increased to a peak of 191.4 aphids per leaf on 10 August (Fig. 1). Unfortunately, no open cotton was present during this early infestation so the irrigation test could not be conducted. The aphid infestation was accompanied by a heavy predator population, mainly *Hippodamia convergens* Guérin-Méneville, and by 16

August, aphid numbers had decreased to less than one aphid per leaf. Cyhalothrin was applied on 6 September in an attempt to increase the cotton aphid infestation. Subsequent applications were made on 13, 19 and 29 September. Inspections conducted on 21 September showed an increasing aphid population averaging 5.9 aphids per leaf, and by 11 October the population had increased to 101.5 aphids per leaf (Fig. 1). Unlike 1998, the cotton aphid population persisted through much of the time period that the water applications were made. The aphid population persisted until 24 oz. of Cyclone® was applied on 20 October, which quickly defoliated the cotton and terminated the infestation.

TABLE 3. Mean Number of Sugar Spots on Cotton Lint Treated with Irrigation Water Applied from Two Nozzle Positions to Remove Cotton Aphid Honeydew, Halfway, Texas.

	Mean no. sugar spots per sample[a]	
Nozzle position[b]	1998	1999
in-canopy	8.4 a	22.5 a
overhead	8.0 a	20.2 a

[a] Means within a column, followed by the same letter are not significantly different (P>0.05, ANOVA).
[b] In-canopy position nozzles were located approximately 8 inches above the soil surface and equipped with cotton chemigation splash pads. Overhead position nozzles were located approximately 36 inches about the soil surface and equipped with medium fine grooved splash pads.

TABLE 4. Mean Number[a] of Sugar Spots and Spot Reductions Compared to an Untreated Control on Cotton Lint Treated with Irrigation Water to Remove Cotton Aphid Honeydew, Halfway, Texas.

	1998			1999		
Number of applications[b]	Mean no. spots/ sample	Reduction over control[c] # spots	%	Mean no. spots/ sample	Reduction over control[c] # spots	%
1 application	12.6 a	18.7	59.7 b	30.4 a	14.7	33.3 b
2 applications	9.2 a	22.1	70.5 b	24.2 a	20.9	46.3 b
3 applications	2.8 b	28.5	91.1 a	9.4 b	35.7	79.1 a

[a] Means within a column, followed by the same letter are not significantly different (P>0.05, LSD).
[b] Application dates in 1998: 1 application – 10 Sept, 2 applications – 10 and 17 Sept, and 3 applications – 10, 17 and 24 Sept. Application dates in 1999: 1 application – 7 Oct, 2 applications 7 and 13 Oct, and 3 applications – 7, 13 and 27 Oct.
[c] Mean number of spots on H2SD samples from control plots: 1998 – 31.3, 1999 – 45.1.

Sugar spot analysis of the 1999 samples revealed a higher level of aphid sugars on lint than in 1998, probably due to the larger and more persistent aphid population infesting the plots in 1999. The mean numbers of sugar spots on lint subjected to the various irrigation treatments are shown in Tables 1-4. With 45.1 sugar spots per H2SD sample, cotton in control plots had significantly more sugar spots than all irrigation treatments except one (one application, in-canopy, 13,600 gal/A, 36.1 spots) (Table 1). Number of sugar spots on lint from plots that received irrigation treatments ranged from 36.1 to 6.6, or a

20.0-85.4% reduction over the untreated control (Table 1). As in 1998, there was no significant difference between the mean numbers of sugar spots on lint from plots treated with 6,800 and 13,600 gal/A of water or on lint treated using overhead and in-canopy nozzle positions/configurations (Tables 2 and 3). Analysis of number of water applications also revealed results similar to those from 1998. Mean number of sugar spots decreased with increasing number of water applications, falling from 30.4 spots in plots receiving one application of irrigation water to 9.4 spots in plots receiving three applications (Table 4), a reduction of 21 spots or 69%. Cotton from plots receiving three applications of irrigation water had significantly fewer sugar spots than those receiving one or two applications (Table 4). Irrigation treatment reduced the number of sugar spots on lint from 14.7 to 35.7 spots compared to the untreated control, or 33.3-79.1% (Table 4). Lint from plots treated with three applications of water showed significantly higher reduction in the number of spots (79.1%) than lint from plots treated with one and two applications (Table 4). As with the 1998 data, there were no significant interactions between the main effects.

In both years, lint from plots treated with the two volumes of water showed no significant differences in the number of sugar spots on lint (Table 2), indicating that the lower volume of 6,800 gal/A (0.25-inch depth) will provide the same reduction of spots at lower cost. Likewise, the number of spots on lint from cotton treated using the two nozzle positions/configurations showed no significant differences in either year (Table 3). This indicates that center pivot systems configured either for overhead irrigation or for use in the LEPA irrigation management system can be used with equal effectiveness to reduce the numbers of aphid honeydew spots.

In both years, irrigation treatments made substantial reductions in the number of sugar spots over the control, ranging from 20.0 to 95.8% (Table 1). All irrigation treatments significantly reduced the number of sugar spots over the untreated control in 1998, and in 1999 all treatments except one produced the same effect (Table 1). Multiple water applications reduced the number of sugar spots with each successive application in both 1998 and 1999 (Table 4). In both years, lint treated with three applications of water had significantly fewer sugar spots and significantly higher percentage sugar spot reduction over the untreated control when compared to one and two applications (Table 4). For the multiple treatment main effect, the lowest mean percentage reduction of 33.3% was observed in 1999 by lint receiving one application of water (Table 4). Though the lowest, this was a substantial reduction, removing one-third of the spots. Three applications of water removed over 75% of sugar spots in both years, and in 1998 removed over 90% (Table 4). This demonstrates the effectiveness of using center pivot irrigation systems for cotton aphid honeydew reduction after contamination by aphids at low to moderate population densities, particularly when multiple applications are made.

In both years, three irrigation treatments reduced the number of sugar spots on lint to less than ten (Table 4). Research at the International Textile Center at Texas Tech University has suggested that contamination of lint by between zero and eleven sugar spots in various mixes of 6.5 to 50% sticky cotton with non-sticky cotton does not seem to influence spinning productivity in the short term, although a long term effect may exist (Hequet et al. 2000). The sugar trehalulose has been shown to be among the stickiest of the insect sugars (Miller et al. 1994). The relative lack of trehalulose in cotton aphid honeydew (Hendrix et al. 1992) probably makes sugar mixtures deposited by cotton aphids less problematic for the spinning process than those deposited by whiteflies (Hequet et al. 2001). Therefore, the sugar spot level at which cotton aphid honeydew contamination will begin to cause problems at the textile mill is in question and is probably higher than that for whiteflies.

In 1999, the percentages of spots removed by multiple applications over the control were consistently lower than in 1998 by 12.0 to 26.4% (Table 4). This suggests that allowing the aphid population to persist through much of the period of water application in 1999 (Fig. 1) reduced the effectiveness of multiple treatments by allowing the deposition of honeydew after the first and second of the three repeated applications had been made. This problem can be avoided by termination of the aphid population prior to the onset of washing treatments. It is also possible that the washing treatments lose effectiveness to some degree (as percentage reduction of spots) as the level of contamination rises. The treatments tested in this study may not give satisfactory results at more severe levels of lint contamination.

Results of this study indicate that center pivot irrigation systems may be used effectively to reduce the amounts of cotton aphid honeydew sugars on cotton lint. As little

as 6,800 gal/A (0 25-inch depth) of irrigation water applied as an overhead spray using medium elevation drops or as an in-canopy spray using low elevation drops and chemigation splash pads can significantly reduce aphid sugars on lint. However, multiple treatments may be required to reduce stickiness to an acceptable level. After contamination by cotton aphids at low to moderate population densities, three applications of water may be adequate to reduce the amount of aphid sugars on lint to an acceptable level. Allowing an aphid population to persist and continue to deposit honeydew during the time period that treatments are applied may reduce the effectiveness of multiple applications. It is recommended that for best results, aphid infestations should be terminated prior to the onset of washing treatments. It is possible that timing of application may have a significant impact on the effectiveness of washing treatments. This effect was not included in this study and should be addressed by future research.

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COMPARISONS ON THE EFFICACY OF DIFFERENT TRAP TYPES IN CAPTURING PECAN NUT CASEBEARER, *ACROBASIS NUXVORELLA*[1]

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ABSTRACT

A two-year study was conducted comparing the effect of trap type (Pherocon® II, IIID, and blacklight) on numbers of adult pecan nut casebearer (PNC), *Acrobasis nuxvorella* Neunzig captured throughout the growing season. No significant differences were observed between sites or years; however, treatments, site by treatment interaction, generations, and generation by treatment interaction were significant. Results obtained from trapping the spring emergent generation revealed that the Pherocon® II mean trap catch was significantly greater than the Pherocon® IIID or blacklight traps. Although the Pherocon® II trap appeared to catch more moths in the second and third flight periods, no significant differences were observed between means of trap types in the F1 and F2 generations.

INTRODUCTION

One of the key pests of pecan, *Carya illinoiensis* (Wangenheim) K. Koch, is the pecan nut casebearer (PNC), *Acrobasis nuxvorella* Neunzig and pecan is the only known host (Neunzig 1972). Pecan nut casebearer larvae damage fruit from early growth until kernel maturity through a series of successive generations. Overwintering larvae emerge from diapause coincident with budbreak and tunnel within rapidly growing shoots (Bilsing 1926, Bilsing 1927). The larvae feed, pupate, and adults emerge to oviposit first generation (F1) eggs. Detection of this adult emergence is critical in anticipation of controlling PNC larvae (F1) that create the greatest amount of economic damage. A sensitive and selective monitoring system for adult moths is of considerable benefit for alerting growers to the need for scouting their orchards for eggs and/or larval damage. Scouting for eggs is a time consuming and difficult process because of their small size. In addition, oviposition sites on developing fruit are often located in grooves and under bracts. Considerable effort can be spent scouting during times when PNC are active. Knowledge of when adult PNC are becoming active may help in reducing the amount of time spent scouting.

Until recently, blacklight traps were the primary and most effective means of estimating the arrival of adult PNC populations; however, assessing the need for an insecticide treatment and determining the optimum time for application rely heavily on scouting for egg and larval stages (Boethel et al. 1979, Calcote 1983, Calcote et al. 1972). Before blacklight traps were used to monitor for adults, treatment decisions were based on scouting for eggs and/or pecan bud and fruit damage and represented the only viable

[1] Lepidoptera: Pyralidae

alternative to prophylactic treatments based on a calendar spray schedule. Blacklight trapping studies have added to knowledge of the basic biology of PNC (Bilsing 1926, Bilsing 1927), established flight activity patterns, determined seasonal population abundance, and facilitated use of economic thresholds for decision-making. Calcote (1983) determined infestation levels when insecticides should be applied, but his determinations were cumbersome and assumed that growers or managers had access to blacklight traps. Because of their high cost and indiscriminate attraction of many different species of insects, use of blacklight traps in pecan production has been abandoned and the focus has shifted to determination and isolation of a potential sex attractant pheromone for PNC.

The PNC sex pheromone has been identified as (9*E*, 11*Z*) - hexadecadienal or (9*E*, 11*Z*) - 16:Ald, from pheromone gland extracts of female moths and verified through pheromone library screening and field-tests (Millar et al. 1996). Different isomers and analogues, (9*E*, 11*Z*) - 15:Ald, (9*E*, 11*Z*, 13*E*) - 16:Ald, and (9*E*, 11*Z*, 13*Z*) - 16:Ald were shown to be only slightly attractive or unattractive to males. In addition, research revealed that rubber septa impregnated with 100 µg synthetic pheromone placed in orange delta flight traps were attractive to male moths. Higher (1,000 µg) and lower doses (0.1, 10 µg) of the pheromone were unattractive to male PNC. Subsequently, Harris et al. (1997) examined the density effect of different numbers of PNC pheromone baited orange delta traps on capture of male PNC moths. They found that capture tended not to decrease significantly as traps per tree increased from one to four traps/tree. Their data indicate the effective trap capture range for PNC pheromone is one tree. Knutson et al. (1998) examined the effects of pheromone dose and lure longevity. They found dosages of 33-100 µg were better suited to capturing adult PNC than dosages above or below this range and septa were still active after 12-14 weeks. Knutson et al. (1998) also tested eight different traps, with pheromone, to determine efficiency of capture but only followed PNC through the spring emergence period, and no comparisons were made with conventional blacklight traps.

Objectives of this experiment were to compare the attractiveness of the new PNC pheromone to blacklight attractiveness, assess which trap (pheromone baited flight or blacklight) monitors flight phenology best over an entire growing season, and to evaluate two different trap designs for use in an adult male PNC flight monitoring program. This experiment tested the hypothesis that PNC pheromone-baited traps are equal to blacklight traps in determining first emergence and that no difference exists between Pherocon® IIID Delta traps and Pherocon® II traps.

METHODS AND MATERIALS

The Pherocon® IIID Delta trap (orange) is constructed of cardboard that must be folded into a triangular shape before deployment in the field. The approximate assembled dimensions are 182 mm long by 118 mm high by 100 mm wide at the base. The ends of the trap are folded inward, creating a small triangular opening 30 mm on each side. The Pherocon® II trap is also constructed of cardboard but is preassembled, making it easier to use. Deployment involves grasping the top and bottom, pulling it apart, and tucking in the bottom-side flaps, creating a diamond-shaped trap. The approximate assembled dimensions are 150 mm long by 142 mm wide by 100 mm high. The end openings are diamond-shaped with dimensions of 140 mm wide by 45 mm high. The interior of both traps is coated with adhesive material to capture insects that enter. Traps were fastened to tree limbs by heavy gauge wire and were reinforced with heavy tape at the point of attachment.

A randomized complete block design consisting of five treatments replicated four times was used at two sites: Knight orchard (1996-1997) in Sapulpa, OK, and Adams orchard (1996) and Alderson orchard (1997) in Stillwater, OK. Treatment 1 consisted of an orange Pherocon® IIID sticky trap (Trécé™, Inc., Salinas, CA) with 100 µg synthetic PNC pheromone (IPM Technologies). Treatment 2 was a Pherocon® II sticky trap (Trécé™, Inc., Salinas, CA) with 100 ug synthetic PNC pheromone. Treatment 3 consisted of a control orange Pherocon® IIID sticky trap without pheromone, similarly treatment 4 was a control Pherocon® II sticky trap without pheromone. Treatment 5 utilized a standard 12W blacklight insect trap model 2851U/T (Bioquip Products, Gardena, CA). At the Adams and Alderson orchards a 12V-DC battery was used, while at the Knight orchard an AC power source was used due to the presence of electricity in the orchard.

Trapping began at least two weeks before the historical first emergence date of moths from the overwintering population (approximately 15 May to 3 June). Pheromone traps baited with rubber septa impregnated with PNC pheromone were positioned at the tips of branches at least 1.5-1.8 m above the ground. Blacklight traps were hung from stronger branches at the same height as pheromone traps. Deep-cycling marine batteries used to power the 12V-DC traps were changed every two days to insure adequate charge. Treatments were randomly assigned within replicates (plot is 1 row of trees) with traps and replicates (rows) located at least 30 m apart to act as a buffer and reduce possible interference of adjacent pheromone treatments. During the studies, orchards were not sprayed to reduce PNC populations. Traps were monitored daily beginning 1 May at the Stillwater sites in order to accurately assess first adult emergence, then at two-day intervals during the remainder of the current flight. All other servicing of traps occurred at two-day intervals during times of PNC emergence. If no new moths were captured over a one-week period during monitoring, it was assumed the generation had ended and traps were checked on seven-day intervals until emergence began for subsequent generations. There are approximately 1,750 degree-days (38°F lower threshold) from first adult emergence for one generation until adult emergence for the next. Data collected from each trap included the number of moths recorded by location and date. Pheromone septa were changed every 30 days and spent septa were removed from the orchard. Traps becoming too contaminated with foreign debris were replaced when changing septa. The pecan budmoth, *Gretchena bolliana* Slingerland, which can be confused with PNC, has been captured in previous trials but care was taken to correctly identify any PNC moths captured. Samples from blacklights were sorted and adult PNC moths counted and removed.

Data on trap type, based on the total number of male PNC moths captured, were analyzed using analysis of variance (ANOVA) procedures (PROC MIXED) with a least squares means option for separation (SAS Institute 1996). A critical value of $\alpha = 0.05$ was used to test for significant differences in the analysis. Count data for each generation were converted using a square root transformation to correct for heterogeneous variances (Steel et al. 1997) and compared for statistical differences.

RESULTS AND DISCUSSION

Use of the new pheromone (9*E*, 11*Z*) - 16:Ald indicates Oklahoma averaged three PNC generations per year during 1996 and 1997 (Figs. 1, 2, 3, and 4). Mulder et al. (1996) also report three generations per year are typical in Oklahoma: adults of spring emergent, overwintering PNC larvae (late May to mid June); F1 (mid July to mid August); and F2 (late September to late October).

Data from 1996 (Figs. 1 and 2) for adult male flights indicate the overwintering generation peaked about 1 June (flight period from 19 May-17 June) and the F1 generation

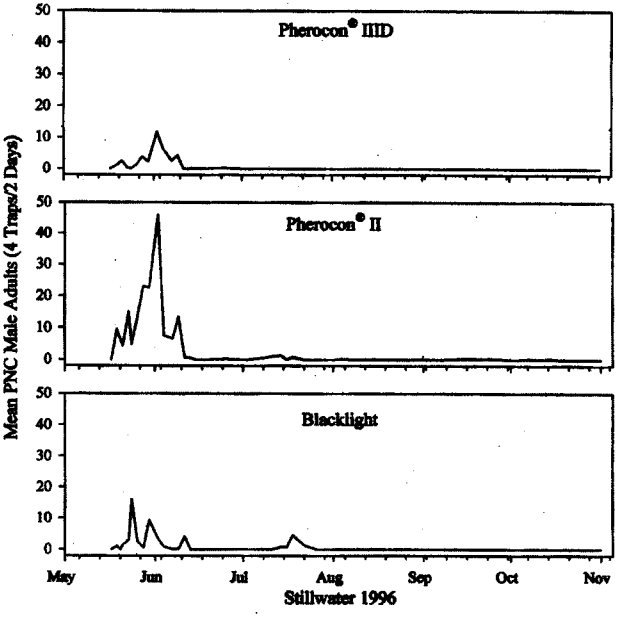

FIG. 1. Pecan nut casebearer flight phenology detected by three different trap types (Pherocon® II, IID, blacklight) in Stillwater, OK, for 1996.

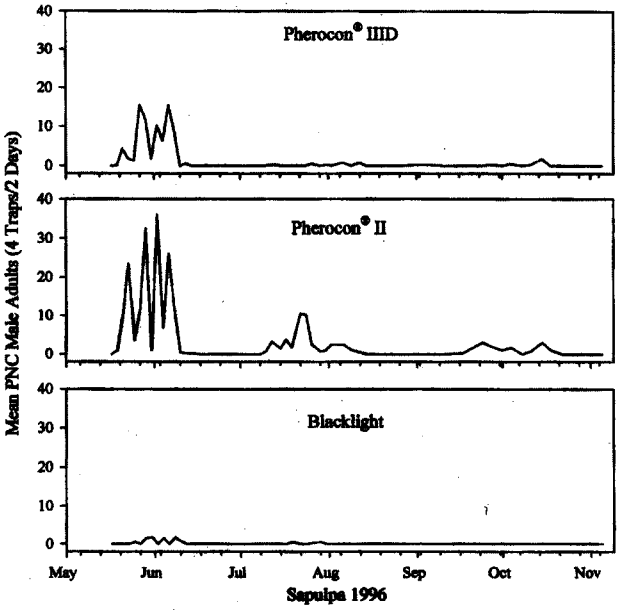

FIG 2. Pecan nut casebearer flight phenology detected by three different trap types (Pherocon® II, IID, blacklight) in Sapulpa, OK, for 1996.

peaked about 20 July (flight period from 8 July-12 August). The F2 generation had no observable peak (flight period from 10 September-14 October). Flight periods for each generation were approximately one month.

In 1997, detection of first emergence was delayed due to a severe freeze of primary bud growth that occurred in mid-April over a majority of the state. Freeze damage in the Stillwater site necessitated moving to another orchard. Peak flight period for the overwintering generation (Figs. 3 and 4) was not evident due to lower nighttime temperatures and rain during early June (flight period from 26 May-20 June). The F2 generation peaked on 28 July (flight period from 17-28 July) and F3 peaked on 12 September (flight period from 25 August-26 September).

Male moths emerging from the overwintering generation produced the largest flight for each season. This can be observed when looking at the combined mean pheromone trap catch for each year: 1996 overwintering generation = 113.5, F1= 11.8, and F2 = 4.4; 1997 overwintering generation = 42.9, F1 = 2.3, and F2 = 0.3. Calcote (1983) noted the proportion of female to male moths that emerged from the overwintering generation varied with years but was usually less than the number of male moths. Females successively outnumbered males in each of the F1, F2, and F3 generations. Because there are fewer males in each of the succeeding generations, the mean trap catch should be less. It is possible also that male sensitivity to the female-produced pheromone varies by generation and proportion of males. Males emerging from the overwintering generation may be more sensitive to the pheromone because of higher male to female sex ratios than those in F1 or F2 generations where the sex ratio is reversed and there are more females producing the pheromone.

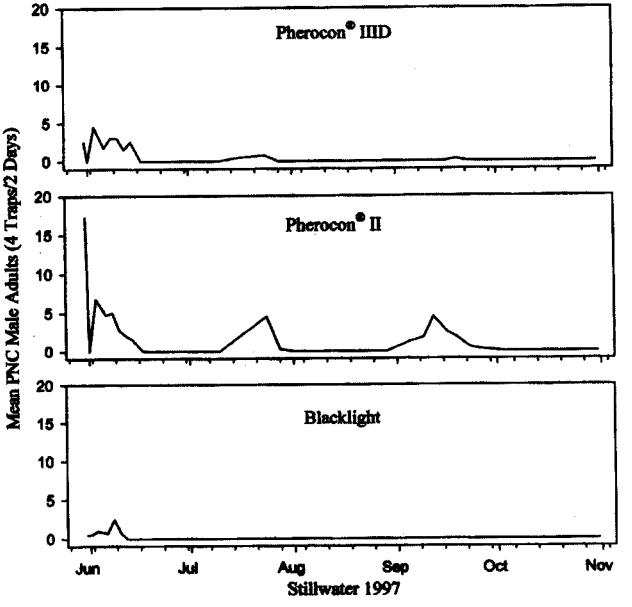

FIG 3. Pecan nut casebearer flight phenology detected by three different trap types (Pherocon® II, IID, blacklight) in Stillwater, OK, for 1997.

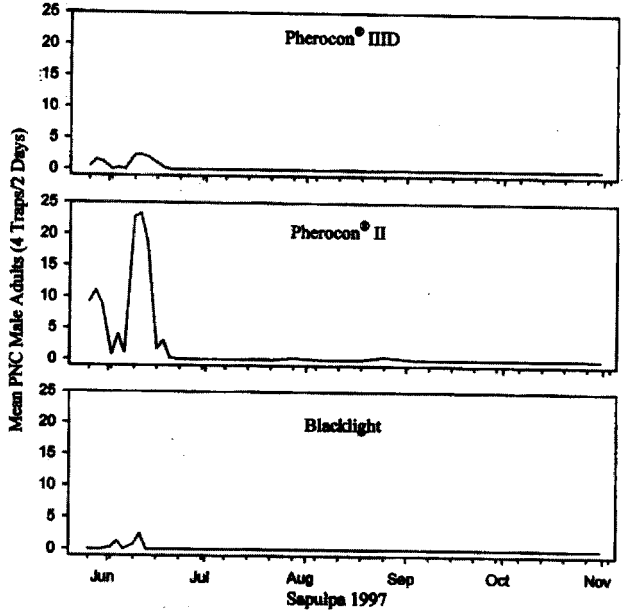

FIG 4. Pecan nut casebearer flight phenology detected by three different trap types (Pherocon® II, IID, blacklight) in Sapulpa, OK, for 1997.

The data for trap type were analyzed utilizing PROC MIXED (SAS Institute 1996). Treatments 3 (Pherocon® IIID without pheromone) and 4 (Pherocon® II without pheromone) were removed from the data set prior to analysis because no moths were captured throughout the experiment in either of these treatments. Analysis revealed no significant differences between the two trapping sites, Sapulpa and Stillwater ($F > 0.4013$). Note that Figs. 5, 6, and 7 show means from raw data while means presented in Table 1 have been transformed (square root).

Analysis of treatment effects across generations indicated significant differences ($P<0.0001$) among Treatment 2 (Pherocon® II) with a mean trap catch of 5.25 ± 4.44 and Treatments 1 (Pherocon® IIID) and 5 (blacklight) (Table 1). Data indicated that the white trap enhanced captures of moths over the orange traps and the blacklight. There were no significant differences in moth response to the orange Pherocon® IIID with pheromone and the blacklight trap with mean trap catches of 2.55 ± 2.66 and 1.77 ± 1.75, respectively. There was a significant site by treatment effect ($P<0.02$), which can be seen in Figs. 5-7. The difference seen between the Pherocon® II and Pherocon® IIID indicates that there may also be some visual cue associated with response to the pheromone. During both years and at both sites, trap captures in baited Pherocon® II traps were significantly greater than those

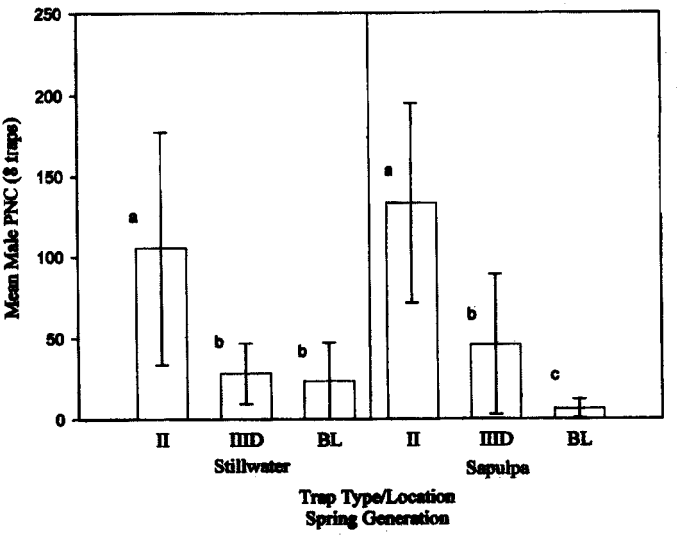

FIG 5. Spring generation mean trap captures comparisons for PNC detected in three different trap types (Pherocon® II, IIID, blacklight), Stillwater and Sapulpa, OK. Means within the same site and with the same letter are not significantly different (P≤0.05), error bars ± SE.

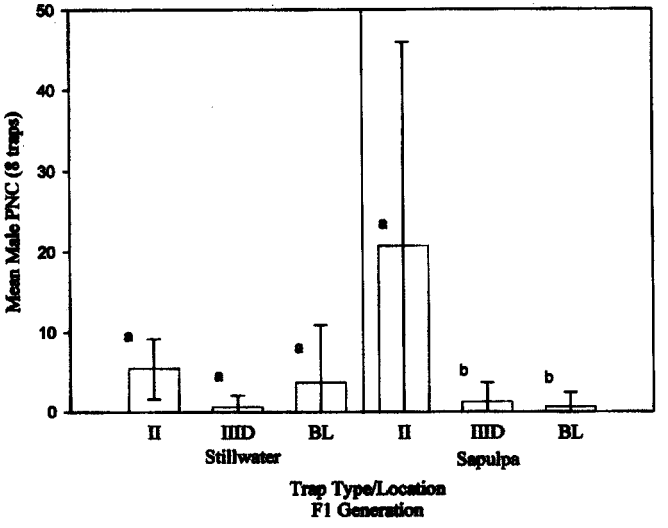

FIG 6. F1 generation mean trap captures comparisons for PNC detected in three different trap types (Pherocon® II, IIID, blacklight), Stillwater and Sapulpa, OK. Means within the same site and with the same letter are not significantly different (P≤0.05), error bars ± SE.

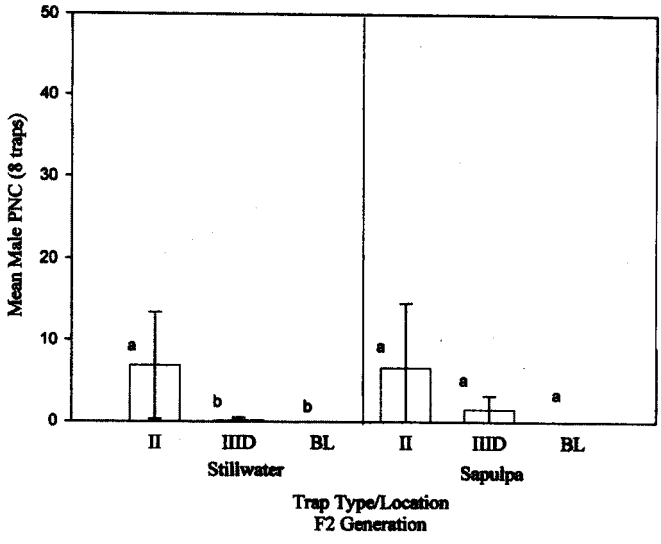

FIG 7. F2 generation mean trap captures comparisons for PNC detected in three different trap types (Pherocon® II, IIID, blacklight), Stillwater and Sapulpa, OK. Means within the same site and with the same letter are not significantly different (P≤0.05), error bars ± SE.

obtained from the orange trap design or the blacklight trap. While blacklight traps are effective at capturing insects in general, several drawbacks discourage their use. First, they are indiscriminate, capturing large numbers of both beneficial and potential pest species. Second, it is difficult to sort and identify the large numbers of insects captured even when the individual has been extensively trained. Finally, blacklight collections are susceptible to degradation from rain, which makes accurate identification very difficult.

Analysis of data showed a significant generation by treatment interaction (P<0.0001). Differences in treatments for the spring generation are presented in Fig. 5 and Table 1. Significant differences were observed in means between Treatment 2 (Pherocon® II, 10.48 ± 0.934) and Treatments 1 (Pherocon® IIID, 5.57 ± 0.934) and 5 (blacklight, 3.37 ± 0.934) (Table 1). Fig. 5 indicates significant differences only among Treatments 2 and 1 or 5 in Stillwater while all three treatments in Sapulpa were significantly different from each other. Fig. 6 depicts differences in treatments for the F1 generation. There were no significant differences observed in means between Treatments 1 (Pherocon® IIID, 1.05 ± 0.934), 2 (Pherocon® II, 2.96 ± 0.934), or 5 (blacklight, 1.23 ± 0.934) (Table 1). In addition, significant generation by treatment effects for the F1 generation at the Sapulpa site were evident (Fig. 6). Fig. 7 shows differences in treatments for the F2 generation. As in the F1 generation, there were no significant differences observed in means between Treatments 1 (Pherocon® IIID 1.03 ± 0.934), 2 (Pherocon® II 2.30 ± 0.934), or 5 (blacklight 0.70 ± 0 .934) (Table 1). Fig. 7 reveals that there was a significant generation by treatment effect for the F2 generation at the Stillwater site.

Based on analysis of data from this study, Treatment 2 that utilized the Pherocon® II with pheromone, was more effective in attracting male moths to traps than Treatment 1, Pherocon® IIID with pheromone. or Treatment 5. backlight trap. Although the Pherocon®

TABLE 1 Least Squares Means of PNC Trap Captures for Treatment and Generation Effects for the Trap Type Study at Sapulpa and Stillwater, OK.

Effect	Estimate of least squares means	±SE	T grouping[a]
Treatment (trap type)			
Pherocon® II	5.25	4.44	a
Pherocon® IIID	2.55	2.66	b
Blacklight	1.77	1.75	b
Generation (trap type)			
Spring Generation			
Pherocon® II	10.48	0.93	a
Pherocon® IIID	5.57	0.93	b
Blacklight	3.37	0.93	b
F1 Generation			
Pherocon® II	2.96	0.93	a
Pherocon® IIID	1.05	0.93	a
Blacklight	1.23	0.93	a
F2 Generation			
Pherocon® II	2.30	0.93	a
Pherocon® IIID	1.03	0.93	a
Blacklight	0.70	0.93	a

[a] Means followed by the same letter are not significantly different ($P \leq 0.05$). Means are for data transformed using a square root transformation.

II trap caught moths in greater numbers, all trap types (with the exception of unbaited traps) were able to detect first emergence of spring generation moths. Knutson et al. (1998) also noted the Pherocon® II trap caught more male PNC moths in their 1996 trial when tested against five other trap types, although they recommend the Intercept-A trap due to ease of servicing and less chance of capturing other insects.

Our data also indicate that the Pherocon® II trap with pheromone is superior to the other two trap types tested in attracting summer (F1, F2) generations of pecan nut casebearer. The trap is also one of the easiest to deploy and maintain. Data provided from these studies suggest that the grower now has information that will be more useful in determining the best monitoring method for utilization in his/her orchard. Research can now focus on determining optimal spray timing utilizing the Pherocon® II trap with the PNC pheromone and a good scouting program.

ACKNOWLEDGMENT

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MONITORING *SOLENOPSIS INVICTA* (HYMENOPTERA: FORMICIDAE) FORAGING WITH PEANUT OIL-BAITED, UV-REFLECTIVE *BEAUVERIA BASSIANA* ALGINATE PELLETS

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ABSTRACT

Several ultraviolet-reflective dyes of different colors were incorporated into alginate pellets of *Beauveria bassiana* that were tested for pathogenicity against the red imported fire ant, *Solenopsis invicta* Buren, under laboratory and field conditions. Mycoses developed after exposure to conidia produced by pellets colored with each ultraviolet-reflective dye, and 100% mortality of ants was measured within four to eight days. *Solenopsis invicta* foragers gathered significantly greater numbers of pellets coated with peanut oil than identical pellets without peanut oil coating. Foragers were not detracted from retrieving brightly colored *B. bassiana* pellets, and the UV-reflective dyes allowed observations of ant foraging in the dark.

INTRODUCTION

Ultraviolet (UV) reflective dyes can be useful tools to study insect behavior. Marking insects with these dyes allows monitoring of foraging, dispersal, and flight behavior using a simple blacklight. When using any marking technique, survival or behavior of the insect must not be compromised. Many techniques for mass-marking insects have been described (Southwood 1978).

Diabrotica virgifera virgifera LeConte (Coleoptera: Chrysomelidae) adults were marked externally with fluorescent powder (Day-Glo™) with no apparent detrimental effects (Lance and Elliot 1990). Incorporating an internal oil-soluble dye (Calico oil red) into the diet of *D. v. virgifera* also was an efficient method of marking the insect without causing harm (Oloumi-Sadeghi and Levine 1990). In comparison, the externally applied fluorescent powder was more simple to apply, and retention time was greater (Naranjo 1990).

Marking aphids with fluorescent powders was used to study patterns of virus transmission by four aphid species (Thomas et al. 1997). After painting aphids with reflective dye and releasing them to source plants, aphids were recollected, and marked individuals were easily identified under UV light. The research concluded that marking aphids could trace dispersal patterns of both alate and apterous aphids.

Marking seeds with UV-reflective paint allowed Bossard (1990) to trace the movement of seeds by ants. By placing marked seeds in ant-populated areas, Bossard discovered where

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ants relocated the seeds.

The successful use of UV-reflective dyes on other insects suggests that they would be useful to mark the red imported fire ant, *Solenopsis invicta* Buren (Hymenoptera: Formicidae). *Solenopsis invicta* foraging behavior differs little throughout a 24-h period (Porter and Tschinkel 1987); therefore, observations of *S. invicta* foraging behavior at night on UV-reflective baits or pellets could be representative of daily patterns. *Solenopsis invicta* are opportunistic omnivores (Lofgren et al. 1975) that feed on living or dead animal material (Hays and Hays 1959, Wilson and Oliver 1969) or plant material (Adams 1986, Lofgren 1986). *Solenopsis invicta* are attracted to oils (Hays and Arant 1960, Lofgren et al. 1961, 1964), and oils have been utilized as attractants formulated in effective chemical controls (Banks et al. 1978). Seasonal temperature changes can account for differences in bait preferences between carbohydrate, preferred at lower temperatures, and proteinaceous, preferred at higher temperatures (Stein et al. 1990). Field studies in Louisiana showed that molasses was more attractive to *S. invicta* over a short exposure period (30 min), but peanut oil was more attractive and resulted in more forager recruitment over a long time period (120 min) (Ali and Reagan 1986).

Because *S. invicta* are voracious competitors, they often dominate their environment by out-foraging other ground-dwelling insects (Porter et al. 1988). Using bait-formulations to target *S. invicta* in areas where they dominate may effectively deliver control agents, including biological and insecticidal.

Beauveria bassiana (Fungi: Deuteromycotina), incorporated in an alginate matrix, can cause mortality in *S. invicta* (White 1995). Effective delivery of *B. bassiana* alginate pellets to *S. invicta* colonies and efficient activation of the pellets are necessary for this technology to be valuable in management of *S. invicta* populations. The objectives of the following studies were to determine the effects of UV-reflective dye incorporation into *B. bassiana* alginate on fungal pathogenicity to *S. invicta*, and to determine the efficiency of peanut oil-baited alginate pellets.

MATERIALS AND METHODS

Beauveria bassiana (ARSEF#2484) was propagated in erlenmeyer flasks (500ml) containing sterile Sabouraud dextrose broth (Difco Laboratories, Detroit, MI) with 1.0% yeast extract (SDBY). Plugged with cotton, flasks were inoculated for five days at 24°C on laboratory shakers (100 RPM). After incubation, the contents of flasks were strained through white muslin cloth to separate mycelia from broth. The separated mycelia were mixed with a 1.0% sodium alginate solution (Bioserv, Frenchtown, NJ) at a rate of 37.0g of wet mycelia per 100ml of sodium alginate solution. The suspension was then mixed in a blender with 2.0g of wheat bran (Hodgson Mill, Inc. Teutopolis, IL). Once mixed, the suspension was added dropwise with a sterile 10-ml pipette to 0.25 M aqueous solution of calcium gluconate. The resulting pellets were removed with a sieve after five minutes and dried on double sheets of waxed paper on a laboratory table. After drying for 24h, pellets were approximately 10% of original volume and were stored in airtight, plastic vials. Success of *B. bassiana* propagation was tested for each batch of pellets before their use in experiments. A sample of pellets was placed in a petri dish with sterilized filter paper (No. 1 Whatman) and incubated at 25°C. Propagation was considered successful if conidiophores and conidia were produced and no other fungal contamination existed.

UV-Reflective Dye Pellet *Trials.* Each of eight UV-reflective dyes was added to a separate fungal matrix slurry and encapsulated in pellets: six poster paint dyes (Palmer Paint Products, Troy, NY), one tracer powder (Ultra-Violet Products, San Gabriel, CA), and an orange dye received from the U.S.D.A. (Gainesville, Fla.). Dyes were added at a rate of 2.8g of poster paint, 1.4g of tracer powder, or 5.0g of USDA orange per 37.0g of wet mycelia. Pellets were allowed to dry and were tested for UV-reflectance. The pellets were tested to

ensure that the dyes had no deleterious effects on the vitality and efficacy of *B. bassiana*. Approximately five pellets from each batch were placed in a petri dish on reverse osmosis (RO) water-moistened filter paper. Five days after conidia and conidiophore production, five *S. invicta* foragers were placed in each petri dish. The ants were infected by contact with conidia, and mortality was recorded. These infectivity tests were run twice, with the first trial having two replications and the second trial having four replications. Data were compared with analysis of variance (ANOVA), and means were separated by least significant differences (LSD) at the critical *P*-value=0.05.

Laboratory Foraging Trials. The goal of this trial was to determine whether coating the fungal pellets with peanut oil affected the rate at which *S. invicta* retrieved pellets. Two treatments tested were *B. bassiana* alginate pellets with and without peanut oil. Six laboratory colonies of similar size and activity were used in these experiments. All colonies were kept in separate 55 x 44 x 13cm trays with a brood box (clear plastic box, 12 x 12 x 3.5cm, with dental plaster filling the bottom one-third) in the center of each tray. Three replications of each treatment were completed.

Twenty pellets were randomly dispersed in each tray, and a dot of yellow, UV-reflective paint was placed next to each pellet as a position marker. Observations were made in the dark using a UV lamp (365nm, 6-volt; Spectronics Corp., Westbury, NY). The position of each pellet was noted every five minutes for 1.5h, and the final location of each pellet was recorded. The number of minutes necessary for the ants to transport pellets into brood boxes was recorded. Additionally, pellets coated with peanut oil were tested for activation by placing 20 pellets in a petri dish with moistened filter paper and comparing activation to pellets with no peanut oil.

Field Foraging Trials. To trace the movement of the *B. bassiana* alginate pellets in the field, 20 pellets (either peanut oil-coated *B. bassiana* alginate pellets or *B. bassiana* alginate pellets with no peanut oil coating) were randomly distributed at the perimeter of three mounds of each treatment. Each pellet was marked with a wooden toothpick painted with yellow, UV-reflective paint. The treatments were applied at dusk, and UV lights were used to observe pellet movement by *S. invicta* at nightfall. The UV lights were held 1.0m above the ground surface. The numbers of pellets that were moved into foraging tunnels were recorded. Two months after treatment, randomly selected mounds were excavated by slowly removing layers of mound soil and searching for *B. bassiana* alginate pellets.

RESULTS AND DISCUSSION

UV-Reflective Dye Pellet Trials. Significant differences in percentage accumulated mortality were detected after four days (Table 1). Mortality in all treatments was significantly higher after six days than the control ($F = 8.13$; $df = 8,27$; $P < 0.001$). Mortality reached 100% in all treatments by day nine except in the control treatment (35.0%). The incorporation of dye into the pellets did not appear to hamper infectivity and pathogenicity of this *B. bassiana* formulation to *S. invicta*.

The bright U.S.D.A. orange dye was chosen from several candidate dyes for subsequent trials because pellets could be easily seen under laboratory lights, in daylight, and at night under UV lamps. Using this tracking technology, pellets were easily followed in laboratory and field trials to study transport of pellets into brood boxes and mounds.

Laboratory Foraging Trials. The fungal pellets that were coated with peanut oil were moved into brood boxes quickly by *S. invicta* (Table 2). Within one hour, *S. invicta* had moved 96.7% of the peanut oil-coated fungal pellets into the brood box; whereas, pellets with no oil were untouched. Though *S. invicta* took the pellets back to the brood chamber where the fungus could potentially activate and infect the colony, the pellets did not hydrate and produce conidia in this trial, perhaps because the pellets were not left long enough for

TABLE 1. Mean Accumulated Percentage Mortality of *Solenopsis invicta* Exposed to *Beauveria bassiana* Alginate Pellets with Different Ultraviolet-reflective dyes.

	Mean Accumulated Percentage Mortality (±SD) [a] Days Post-Treatment						
Pellet Color[b]	3	4	5	6	7	8	9
Blue	5.0 ±10.0a	45.0 ±10.0abcd	80.0 ±23.1a	80.0 ±23.1a	90.0 ±20.0a	100.0 ±0.0a	100.0 ±0.0a
Green	0.0 ±0.0a	45.0 ±34.2abcd	80.0 ±16.3a	90.0 ±11.5a	90.0 ±11.5a	100.0 ±0.0a	100.0 ±0.0a
Pink	5.0 ±10.0a	35.0 ±30.0bcd	70.0 ±34.6a	80.0 ±23.1a	80.0 ±23.1a	85.0 ±19.1a	100.0 ±0.0a
Yellow	5.0 ±10.0a	30.0 ±20.0bcde	65.0 ±19.1a	100.0 ±0.0a	100.0 ±0.0a	100.0 ±0.0a	100.0 ±0.0a
Orange	5.0 ±10.0a	25.0 ±25.2bcde	75.0 ±19.1a	100.0 ±0.0a	100.0 ±0.0a	100.0 ±0.0a	100.0 ±0.0a
Hot pink	5.0 ±10.0a	15.0 ±19.1bcde	70.0 ±38.3ab	90.0 ±11.5a	85.0 ±10.0a	100.0 ±0.0a	100.0 ±0.0a
Green tracer	5.0 ±10.0a	70.0 ±20.0ab	85.0 ±19.1a	100.0 ±0.0a	100.0 ±0.0a	100.0 ±0.0a	100.0 ±0.0a
USDA Orange	0.0 ±0.0a	20.0 ±23.1bcde	50.0 ±25.8ab	75.0 ±25.2a	75.0 ±25.2a	100.0 ±0.0a	100.0 ±0.0a
Control [c]	0.0 ±0.0a	0.0 ±0.0de	5.0 ±10.0b	20.0 ±28.3b	20.0 ±28.3b	30.0 ±34.6b	35.0±44.3b

[a] Means followed by the same letter within a column are not significantly different (ANOVA, LSD; df = 8,27; $P > 0.05$).

[b] Blue, Green, Pink, Yellow, Orange, and Hot pink were poster paint dyes (Palmer Paint Products, Troy, NY), Green tracer powder (Ultra-Violet Products, San Gabriel, CA), and U.S.D.A. orange was an orange dye received from the U.S.D.A. (Gainesville, Fla.)

[c] No exposure to *B. bassiana* pellets

TABLE 2 Movement of *Beauveria bassiana* Alginate Pellets by *Solenopsis invicta* into Brood Boxes of Laboratory Colonies.

	Mean Percentage of Pellets in Brood Box[a]						
	Minutes After Application						
Treatment[b]	0	15	30	45	60	75	90
Peanut oil	0.0a	18.3a	66.7a	86.7a	96.7a	98.3a	98.3a
No oil	0.0a	0.0b	0.0b	0.0b	0.0b	0.0b	0.0b

[a] Same letters within time periods are not significantly different (paired t-test; df=2; $P>0.05$).
[b] Fungal pellets coated with peanut oil or fungal pellets without peanut oil coating.

activation to occur. In activation trials in petri dishes, 100% of the pellets coated with peanut oil activated, and conidiophores and conidia were produced.

Field Foraging Trials. At the time of application, *S. invicta* located the peanut oil-coated pellets almost immediately. After 30 minutes, 63.3% of the peanut oil-coated fungal pellets had been moved into foraging tunnels by *S. invicta* (Table 3). After two hours, 98.3% of the peanut oil-coated pellets were moved by *S. invicta* into foraging tunnels. The lack of movement by *S. invicta* of fungal pellets not coated with peanut oil into mounds contributed to the hypothesis that peanut oil was a phagostimulant. When mounds were excavated, *B. bassiana* alginate pellets were observed in mounds up to two months after treatment in both active (one pellet was found in one of three viable mounds) and non-active mounds (one pellet per mound was found in five of twelve non-active mounds). We were unable to determine conidial production from these pellets, and no dead ants were found. If *S. invicta* had removed the *B. bassiana* alginate pellets from mounds before the fungus was activated, the delivery system and the efficacy of the biological control treatment would be void.

The UV dye incorporated into alginate pellets allowed observations to be made both during the day and at night. Ants were not repelled by the brightly colored pellets and readily retrieved them for colony use. This technology is a useful technique for the study of *S. invicta* foraging in both laboratory and field experiments.

TABLE 3. Movement of *Beauveria bassiana* Alginate Pellets by *Solenopsis invicta* Foragers into Foraging Tunnels in Field Applications on 9 July 1997, Cass. Co., Texas.

	Mean Percentage of Pellets Taken into Foraging Tunnels[a]				
	Minutes After Application				
Treament[b]	0	30	60	90	120
Peanut oil	0.0a	63.3a	70.0a	95.0a	98.3a
No oil	0.0a	0.0b	0.0b	0.0b	0.0b

[a] Same letters within time periods are not significantly different (paired t-test, df=2, $P>0.05$)
[b] Fungal pellets coated with peanut oil or fungal pellets without peanut oil

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AUGMENTATIVE RELEASES OF COMMERCIAL BIOLOGICAL CONTROL AGENTS FOR RUSSIAN WHEAT APHID[1] MANAGEMENT IN WINTER WHEAT

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ABSTRACT

The effectiveness of augmentative biological control in Russian wheat aphid, *Diuraphis noxia* (Mordvilko), management in dryland winter wheat was evaluated. The two species of commercially reared predators that were released for control of Russian wheat aphid were *Hippodamia convergens* Guerin-Meneville and *Chrysoperla rufilabris* Stephans. There were no effects of cage exclusion on Russian wheat aphid per tiller after releases of the natural enemies in 1994 and 1997. Fewer Russian wheat aphid per tiller were found in the plots with no exclusion on two post-release dates for 1995. There were no effects of cage exclusion of natural enemies on percentage infested tillers except for the first post-release sample date in 1994 and the first and second post-release dates in 1995 where fewer percent infested tillers were found in the plots with no exclusion. Differences observed in 1995 could have been due to lower Russian wheat aphid population densities or high precipitation that occurred during the 1995 experimental period. Plant biomass production was not affected by natural enemy exclusion. Grower reported yields in 1994 and 1997 were lower than the county average. The augmentative release of commercial biological control agents did not have a measurable economic benefit in this study.

INTRODUCTION

Since 1986, the Russian wheat aphid, *Diuraphis noxia* (Mordvilko), has been a serious pest throughout the small grain-producing areas in the United States west of the 100th meridian and parts of Canada (Hein et al. 1990). Typical Russian wheat aphid damage on susceptible small grains includes longitudinal white or cream-colored streaks (Peairs 1990), reduced tillering and root development, plant stunting, prostrate growth, leaf rolling, trapped heads, reduced stem and seed production, and reduced seed and head weight (Burd et al. 1998). The total direct and indirect costs due to this pest has been estimated at $893.1 million for the 7-year period between 1987-1993 (Morrison and Peairs 1998).

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Biological control has been implemented as a component of aphid management (DeBach 1974, Huffaker et al. 1976, Carver 1988). Extensive research has been conducted on the classical approach of biological control to aid in the management of Russian wheat aphid populations (Pike and Allison 1991, Gilstrap et al. 1994, Prokrym 1998). Although many native natural enemies have been associated with Russian wheat aphid populations (Bernal et al. 1993, Racette 1993, Mohamed et al. 2000), studies have not been performed to evaluate augmentative aphid biological control. Our study evaluated augmentative biological control of Russian wheat aphid in a commercial dryland winter wheat production system. The goal of our study was to assess the pest management benefits of the use of commercially available natural enemies in a large scale production system.

MATERIALS AND METHODS

The study was performed in 1994, 1995, and 1997 on Kalcevic Farms, a large dryland winter wheat enterprise in east central Colorado with approximately 13,000 hectares in production each year. This area has a history of economic Russian wheat aphid infestations. Between 1986 and 1989, the farm's annual Russian wheat aphid chemical control costs ranged between $350,000 and $400,000. This method of control was not considered profitable, therefore Kalcevic Farms began releasing commercially available lady beetles and lacewings in 1990. Their costs for the purchase and release of beneficial insects was about $75,000 per year, less than one-quarter of the cost of insecticides. However, it was unknown if these releases were cost effective.

Four locations planted to susceptible wheat cultivars that had more than 1% of the tillers infested with Russian wheat aphid at spring regrowth were selected each year (Table 1); locations were separated by at least 2 km. Two species of native natural enemies, convergent lady beetles, *Hippodamia convergens* Guerin-Meneville, and lacewings, *Chrysoperla rufilabris* Stephans, were chosen by the producer for their lower cost and ease of application. Lady beetle adults and lacewing eggs were obtained from commercial sources and released each year to control Russian wheat aphid. In addition, areas that could not be farmed were left undisturbed to provide refugia for these species.

Mechanical exclusion with cages has been used to evaluate the effect of natural enemies on greenbugs and Russian wheat aphids in winter wheat (Rice and Wilde 1988, Hopper et al. 1995, Nechols and Harvey1998, Michels et al. 2001). Two treatments, total exclusion to prevent entry of all natural enemies and no exclusion, were used in this experiment. The exclusion cages used in this study resembled free-standing pup tents. The top of the cage frame was a 1.2-m long, 2.5x2.5 cm section of wood attached to two u-posts which were driven into the ground about 15 cm. Four electric fence posts were modified to support the fine mesh (0.2x0.2 mm), white polyester organza material. The edges of the organza were buried under approximately 5 cm of soil to anchor the cages and to prevent entry of biological control agents. Six cages were placed at each site. In 1995, Sites 1 and 2 were set up prior to any release of natural enemies. Due to excessive rain, Sites 3 and 4 were not set up until 15 May, after which several releases of beneficials already had been made. All 1994 and 1997 sites were established prior to any release of natural enemies. For each enclosure cage there was a corresponding paired 1.2x1.2 m uncaged area to allow a side by side comparison of effects of Russian wheat aphid infestations with and without exposure to natural enemies.

Convergent lady beetle (*H. convergens)* adults and *C. rufilabris* eggs were purchased by the grower from M & R Durango Insectary, Bayfield, CO. Lady beetles were dispersed by the producer with a blower-type mechanism mounted on a pickup truck (Table 1). Lacewing eggs were mixed with finely ground sterile farina in a portable cement mixer. The producer used

a modified grass seeder mounted on an all terrain vehicle to disburse the lacewing egg and farina mixture in 15-m swaths (Table 1). Lady beetles and lacewings eggs were disbursed between 24 April, and 10 May in 1995 and between 20 April and 15 May in 1997. Although the grower was unable to provide exact release dates for 1994, releases for both the lady beetles and the lacewing eggs were made approximately 3 weeks earlier than in 1995 due to the dry conditions.

TABLE 1. Cultivars Planted at Study Sites and Natural Enemy Release Rates and Approximate Costs per Hectare; Last Chance, CO, 1994, 1995, 1997.

		Application of *H. convergens* per hectare		Application of *C. rufilabris* per hectare	
Year	Cultivar (s)	Rate (ml)[a]	Cost	Rate (# eggs)	Cost
1994	'TAM 107'	317	$4.61	2500	$5.00
1995	'TAM 107', 'Baca'	238-950	$3.46-$13.80	5000	$10.00
1997	'TAM 107', 'Duke', 'Longhorn'	317-380	$4.61-$5.52	6250-7500	$12.50 - $15.00

[a] There are approximately 1,900 *H. convergens* per 100 ml.

Aphid samples were collected at 2-week intervals, once before the natural enemies were released and three additional times. Twenty-five tillers were taken at random from each caged and each uncaged area. The number of symptomatic tillers with and without Russian wheat aphid and asymptomatic tillers with and without Russian wheat aphid were recorded for each sample. Samples were then placed in Berlese funnels for 24 hours and collected in 70% ethanol. All Russian wheat aphids in each sample were counted. On each sample date in 1995 and 1997, one hundred 180° arc sweeps were taken with a standard 38-cm sweep net (Ward's Biology, Rochester, NY) at each site in the vicinity of the cages to estimate the abundance of predators and parasites.

All tillers from a 30-cm section of row were taken from each caged and uncaged plot before the wheat had completely dried to provide a measure of biomass production. The plants were cut about 2.5 cm above the ground. Samples were placed in paper bags and dried at 38°C for 24 hours and then weighed.

Data were analyzed using PROC GLM and the SNK procedure was used to compare caged and uncaged treatment means (SAS Institute 1994). A log transformation was performed on Russian wheat aphid counts to insure independence of variances and means.

RESULTS

There were no pre-release differences between exclusion and no exclusion of natural enemies in Russian wheat aphid per tiller in 1995 and 1997 but differences occurred in 1994 (Table 2). Significantly higher numbers of Russian wheat aphids were observed where natural enemies were excluded only in the first and second post-release sample dates for 1995.

Exclusion of natural enemies resulted in a significantly greater percentage infested tillers in the pre-release samples for 1997, the first post-release date for 1994 and the first and second post-release dates for 1995 (Table 3).

TABLE 2 Effects of Presence or Absence of Exclusion Cages on Russian Wheat Aphid per Tiller in Winter Wheat; Last Chance, Colorado, 1994, 1995 and 1997.

Year	Number of Russian Wheat Aphid per Tiller ± SEM[a]		
	Cage Exclusion	No Exclusion	p>F
	Pre-release	Pre-release	
1994	1.0 ± 0.3 B	2.2 ± 0.3 A	0.0037
1995	0.4 ± 0.1 A	0.5 ± 0.2 A	0.4904
1997	2.6 ± 0.6 A	3.2 ± 0.7 A	0.4392
	Post-release 1	Post-release 1	
1994	4.4 ± 0.8 A	6.1 ± 1.0 A	0.2401
1995	3.9 ± 1.5 A	1.3 ± 0.4 B	0.0398
1997	8.3 ± 2.1 A	8.1 ± 1.9 A	0.7473
	Post-release 2	Post-release 2	
1994	51.9 ± 14.5 A	58.5 ± 8.3 A	0.3248
1995	15.8 ± 2.5 A	5.8 ± 1.2 B	0.0012
1997	106.7 ± 19.4 A	69.0 ± 7.3 A	0.1023
	Post-release 3	Post-release 3	
1994	469.9 ± 85.3 A	401.8 ± 48.9 A	0.8802
1995	29.1 ± 5.3 A	17.8 ± 4.4 A	0.2041
1997	134.3 ± 11.0 A	136.3 ± 11.6 A	0.8531

[a]SEM, standard error of the mean. Means in the same row followed by the same letter(s) are not significantly different, SNK (α=0.05).

TABLE 3. Effects of Presence or Absence of Exclusion Cages on Percent Infested Tillers in Winter Wheat; Last Chance, Colorado, 1994, 1995 and 1997.

Year	Percentage Infested Tillers ± SEM[a]		
	Cage Exclusion	No Exclusion	p>F
	Pre-release	Pre-release	
1994	21.6 ± 4.1 A	30.5 ± 3.8 A	0.1270
1995	10.7 ± 2.1 A	11.4 ± 3.1 A	0.8366
1997	25.6 ± 3.8 B	38.1 ± 4.1 A	0.0316
	Post-release 1	Post-release 1	
1994	42.7 ± 7.0 B	64.3 ± 5.7 A	0.0248
1995	31.6 ± 5.3 A	12.8 ± 2.9 B	0.0030
1997	52.8 ± 3.8 A	46.4 ± 5.2 A	0.3279
	Post-release 2	Post-release 2	
1994	83.3 ± 3.9 A	91.3 ± 3.6 A	0.1434
1995	56.3 ± 6.7 A	25.8 ± 5.1 B	0.0007
1997	98.2 ± 1.5 A	93.4 ± 3.5 A	0.2151
	Post-release 3	Post-release 3	
1994	99.0 ± 1.0 A	99.7 ± 0.3 A	0.5336
1995	72.5 ± 8.9 A	50.5 ± 9.0 A	0.0973
1997	100.0 ± 0.0 A	100.0 ± 0.0 A	1.0000

[a]SEM, standard error of the mean. Means in the same row followed by the same letter(s) are not significantly different, SNK (α=0.05).

Sweep samples indicated that there were very few *H. convergens* or *C. rufilabris* recovered until the third post-release sample in 1995 and 1997 (Table 4). Other natural enemies collected in the sweep samples included species of Nabidae, Anthocoridae, Syrphidae, Hymenptera, and spiders. These species were not found in high numbers so they were not included in the study. Sweep samples were not taken in 1994.

TABLE 4. *H. convergens* and *C. rufilabris* per Sweep Collected from Sweep Samples at Each Study Site; Last Chance, CO, 1995, 1997.

Year	*H. convergens*	*C. rufilabris*
	Pre-release	Pre-release
1995	NA	NA
1997	2.3	0.0
	Post-release 1	Post-release 1
1995	0.0	0.0
1997	1.5	0.0
	Post-release 2	Post-release 2
1995	2.5	0.8
1997	1.8	0.0
	Post-release 3	Post-release 3
1995	32.8	0.5
1997	98.8	0.3

Crop biomass production was not affected by natural enemy exclusion in 1995 (F=2.89, P=0.0960) or 1997 (F=0.01, P=0.9326) (Table 5). The grower cooperator reported yields of 1,002, 2,281, and 968-1,452 Kg/ha in 1994, 1995, and 1997, respectively. These averages were lower in two years than the Arapahoe county averages of 1,521, 2,143, and 1,970 Kg/ha in 1994, 1995, and 1997, respectively (Hudson and Fretwell; 1996, 1998). The lower yields in 1994 and 1997 compared to 1995 were likely due to greater Russian wheat aphid abundance, lack of moisture, and higher temperatures.

TABLE 5. Effects of presence or absence of exclusion cages on above ground crop biomass in winter wheat; Last Chance, Colorado, 1995 and 1997.

	Above Ground Biomass (g/m) ± SEM[a]		
Year	Cage Exclusion	No Exclusion	p>F
1995	60.7 ± 4.4 A	71.3 ± 4.5 A	0.0960
1997	26.6 ± 2.1 A	26.9 ± 2.7 A	0.9326

[a]SEM, standard error of the mean. Means in the same row followed by the same letter(s) are not significantly different, SNK (α=0.05).

DISCUSSION

Exclusion of natural enemies by the use of cages did not affect Russian wheat aphid densities in 1994 or 1997, indicating that the releases of commercially available biological control agents were ineffective. In 1995, however, Russian wheat aphid population densities were lower in uncaged areas on two post-release sample dates. This may have been due to the inability of the biological control agents to control Russian wheat aphid at the higher population densities experienced in 1994 and 1997. Michels, et al. (2001) and Elliott and Kieckhefer (2000) found that coccinellids had significantly reduced aphid numbers in high density patches when aphid populations of the entire field were low. However, when the aphid density in a field was high, there was not a significant reduction of aphids by coccinellids. Another explanation may be that caged Russian wheat aphids were protected from the much greater precipitation that occurred during the 1995 experimental period. The precipitation for the 1995 experimental period was 30.96 cm which was nearly three times greater than the 1994 (11.05 cm) and 1997 (11.40 cm) precipitation and nearly twice the average precipitation (17.78 cm). Although it has been reported that rainfall is not a factor between caged and uncaged treatment differences (Hopper et al. 1995), many dead aphids were observed on uncaged wheat plants after heavy rainfall during the 1995 experimental period. Similar observations of decreased numbers of Russian wheat aphids occurring after heavy rainfall were noted by Kriel et al. (1984). Smaller Russian wheat aphid density differences occurred between caged and uncaged treatments in Kansas (Nechols and Harvey 1998). Suspected causes of the cage differences were attributed to natural enemy responses, location and seasonal differences or differences in experimental techniques.

Biomass production was not affected in 1995 or 1997. Also, grower reported yields were generally lower in 1994 and 1997 than the county average. Thus, the release of biological control agents did not have a measurable economic benefit in this study.

The commercial release of biological control agents was not cost effective as neither reductions in Russian wheat aphid population densities nor protection of biomass was attributable to the activity of biological control agents. Alternatives to the augmentative release of biocontrol agents are available. Biological control agents have become established after release in a classical biological control program effort and eventually may prove effective (Prokrym et al. 1998). Five species of exotic parasitoids, *Aphelinus albipodus*, *A. asychis*, *A. varipes*, *Diaeretiella rapae* and *Lysiphlebus testaciepes*, were recovered in Colorado (Burd et al. 2001). Several Russian wheat aphid resistant wheats are now commercially available as a non chemical management alternative (Quick et al. 1996; Quick et al 2001a,b,c,d). Chemical control agents are useful in suppressing sporadic heavy populations of Russian wheat aphid when densities exceed accepted economic thresholds (Archer 1994).

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AERIALLY APPLIED STANDARD RATE MALATHION AGAINST REDUCED RATES OF MALATHION + COTTONSEED OIL FOR BOLL WEEVIL[1/] CONTROL

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ABSTRACT

This study was conducted to determine whether reduced rates of malathion in cottonseed oil were as effective at reducing boll weevil, *Anthonomus grandis* Boheman, populations as the current standard rate of ultra-low volume (ULV) malathion alone. In a field cage residue study, 693 g AI malathion + 553.5 g cottonseed oil per ha and 867 g AI malathion + 415 g cottonseed oil per ha resulted in boll weevil mortality over a five-day sampling period that was not significantly different from the standard rate of 1,040 g AI malathion per ha. In the field cage assay, mortality declined below 36% in all of the malathion treatments on the third day after application. A petri dish assay showed that the malathion treatments had a measurable effect on boll weevil mortality for three days, and that daily percentage mortalities caused by the reduced malathion + cottonseed oil treatments were not statistically different than those induced by the standard rate. However, the numerical differences in mortality between the second and fourth day after treatment suggest that residual activity of the reduced rates in oil might not always be as great as the activity of the standard rate during this period. Populations of nontarget arthropod groups, particularly lacewings (Chrysopidae and Hemerobiidae: Neuroptera) and *Collops* (Coleoptera: Melyridae) were reduced by all of the malathion treatments. In general, differential effects among the malathion treatments on nontarget arthropods were not observed.

INTRODUCTION

Methods of reducing ultra-low volume (ULV) malathion application rates for boll weevil, *Anthonomus grandis* Boheman (Coleoptera: Curculionidae), control have been examined by several groups, with the goal of generating cost savings to cotton producers. The current standard rate for malathion ULV is 1,040 g AI/ha (Hoffmann 2000). Villavaso et al. (1996) estimated that a 25–50% reduction in the amount of insecticide can translate into millions of dollars in savings to cotton growers. A reduced insecticide rate also would help to extend limited stocks of malathion ULV in the event of production shortfalls. Some studies have shown that 693, 1,040, and 1,386 g AI/ha ULV malathion, respectively, resulted in substantial boll weevil mortality (Cleveland et al. 1966, Hopkins and Taft 1967, Villavaso et al. 1996); however, the results of these studies

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were inconclusive regarding the equivalency of the different malathion rates. Cleveland et al. (1966) did not report statistical analyses, only means. Hopkins and Taft (1967) did not present the magnitude of variation, and the treatment plots were not replicated, invalidating the statistical analyses presented. Villavaso et al. (1996) did not report statistical analyses and did not address the possibility that drift from the aerial treatment applications across the 27 m between treatment swaths might have confounded results. For example, weevils placed on leaves taken from the 693 g AI/ha ULV malathion plots suffered 90% compared to only 63% mortality on leaves from plots receiving the highest rate (1,386 g AI/ha) of malathion. Using a petri dish assay, Villavaso et al. (2000) reported that there were no significant differences among 693, 1,040, and 1,386 g AI/ha ULV malathion treatments, respectively, but indicated that there was an overall trend for higher mortality and speed of death with higher rates of malathion.

Mulrooney and colleagues have reported results from a studies on ULV malathion + cottonseed oil. One study suggested that 693 g AI/ha might not be as effective as 1,040 g AI/ha and 1,386 g AI/ha (Mulrooney et al. 1996). Boll weevil mortality caused by ULV malathion applications from an air-assistedsprayer at 950 g AI/ha was not significantly greater than that in the untreated control at 2 and 48 h after treatment (Mulrooney et al. 1997). Results from a petri dish assay suggested that increased amounts of cottonseed oil might be associated with increased mortality (Mulrooney and Smith 2000). Oils have been shown to act as physical poisons that cause hypoxia in insects (Chapman 1967, Butler and Henneberry 1990) and some oils possess compounds that cause chemical poisoning (Hill and Schoonhover 1981). Horticultural, soybean, cottonseed, and essential oils from species of the Labiatae (mint) plant family exhibit repellent activity to several insect and mite species (Butler et al. 1988, Larew 1988). Oils have been used as insecticide additives for many years (Herbert 1933, Barber 1939) and can increase insecticide penetration through the insect cuticle (de Licastro 1983). Matsumura (1975) reported that oils improve the attachment of contact insecticides to the cuticle, dissolve the epicuticular wax, facilitate passage of the insecticide through the wax, and disrupt the internal protein organization of the cuticle. When the toxicity of an insecticide depends on good coverage, as with contact insecticides, the spreading of an oil-based formulation over a surface, such as an insect's integument or a leaf surface, may increase its biological effectiveness (Quraishi and Poonawalla 1969, Boize et al. 1976). Oils also can increase surface residual activity of insecticides (Ware et al. 1980, Cole 1986). The low cost of oils compared to most insecticides, the low human health hazard, and reduced potential for development of arthropod resistance make oils attractive candidates for use in control strategies (Chapman 1967).

Toxicities of insecticides, including organophosphorus compounds, to arthropods were enhanced by the addition of soybean and cottonseed oils (Treacy et al. 1986a, b). However, other studies indicated that oils synergize pyrethroid insecticides while diminishing the effects of organophosphorus and carbamate insecticides (Ochou et al. 1986). ULV malathion combined with cottonseed oil, which is suitable to ULV systems (Hesler and Plapp 1986), was superior to an emulsifiable concentrate (EC) formulation of malathion, and lower rates of malathion in cottonseed oil could replace higher rates of malathion without oil (Wolfenbarger and Guerra 1986). However, 1,040 g AI/ha ULV malathion was superior to 520 g AI ULV malathion + 415 g cottonseed oil per ha in causing boll weevil mortality in preliminary field cage and petri dish assays (Hoffmann 2000).

This study was conducted to compare the efficacies of conventional rates of ULV malathion and reduced rates in cottonseed oil in causing boll weevil mortality, and to examine differential effects of the treatments on nontarget arthropods. The results will help determine if the current standard rate of ULV malathion for boll weevil control can

be replaced with lower rates when cottonseed oil is added without causing additional reductions in beneficial arthropod populations and maintaining desired efficacy.

MATERIALS AND METHODS

The three treatments in this study were 1,040 g ULV 96.5% AI malathion (Cheminova, Lemvig, Denmark, EPA reg. No. 67760-34) per ha, 693 g AI ULV malathion + 553.5 g once-refined cottonseed oil per ha, and 867 g AI ULV malathion + 415 g once-refined cottonseed oil per ha. Each treatment was applied in 22.9-m swath widths by a C-188T fixed wing aircraft flying 200 kph at 0725–0840 h, 1 May 2001, in a 0.5–1.3 mph wind. Treatments were applied through flat fan, Tee Jet® (Spraying Systems, Wheaton, IL) stainless steel nozzle tips oriented straight down. The 1,040 g AI/ha, 867 g AI/ha, and 693 g AI/ha malathion treatments were applied at 261.25 kpa, 288.75 kpa, and 295.63 kpa, respectively (pressures varied because of differences in viscosities and volumes delivered), through 10, 13, and 13 8002 tips, respectively. Applications were made 3 m (boom height) above the plant canopy. Swaths were applied along cotton rows, (102-cm row spacing) 20 m from the field edges in three of four corners of seven selected commercial cotton fields > 25 ha in area in Hidalgo Co., Texas. Four fields were planted to var. Deltapine 388 and three to var. Fibermax 989. The fourth field corner was used as an untreated control, and samples were taken from unsprayed rows 25–30 m from the edge of the field. Each treatment and control area was separated by at least 200 m in every field to avoid the confounding effects of drift. This arrangement of treatments was replicated in each of the seven fields. Samples were taken daily for five days after the day of application. On the fourth day one replicate was discarded because the grower sprayed azinphosmethyl for boll weevil control, and on the fifth day of sampling, another replicate was discarded for the same reason. The cotton was ≈ 45 cm tall, with squares 3-9 mm in diameter, and some of the fields were starting to bloom. One day prior to treatment applications, 25 random squares were examined in each plot.

Boll weevils used in this study were laboratory reared Mississippi strain (Sikorowski et al. 1984) 5–11 day-old adults, fed on the Pharmamedia cottonseed flour diet (Lindig et al.1980) as described by Sikorowski et al. (1984), at the U.S. Department of Agriculture, Animal and Plant Health Inspection Service, Mission Plant Protection Center at Moore Air Field near Mission, TX.

Within 1 h after the aerial treatment applications were made, two white plastic strips of tarp were placed midway along each swath and in each control area over 95 cm of furrow on each side of one row of plants. A 92 x 122 x 92 cm (w x l x h) mesh cage with an open bottom was placed over a single row such that the two tarp strips formed the bottom of the cage. The interface between the bottom sides of the cage and the tarp were sealed with soil to prevent boll weevils from escaping. Twenty live boll weevils were released into each cage. After 24 h, numbers of dead and living boll weevils were counted on the plants, the inner sides of the cages, on and underneath the tarps, and at the bases of the plants. In the cage and the petri dish assays, boll weevils that could move their limbs were judged to be near death and were categorized as dead. After evaluation, the tarps and cages were removed and placed 1–2 m away on the same row. Twenty boll weevils were released and counted after 24 h in the same manner as described above. This process was repeated for a total of five days following treatment applications.

In a laboratory assay, two cotton leaves from the plant canopies were randomly taken from the upper six fully expanded leaves near the centers of each swath area on the day of treatment applications and on five consecutive post-application days. The leaves were collected using disposable gloves and freshly washed scissors to prevent cross

contamination, and placed in zip-locked plastic bags for transport to the laboratory. Each leaf was placed in a separate ventilated 100 mm x 15 mm plastic petri dish with five adult boll weevils. The petri dishes were maintained under 14:10 h L:D fluorescent lighting at 27–28 °C. Two dishes were established for each treatment replicate. Mortality was recorded daily for 5 d after the weevils were released into the dishes.

At 2 and 5 days after treatment, naturally occurring boll weevils and other insects were collected from two 25-m lengths of cotton row near the center of each plot using a tractor-mounted vacuum sampler (Beerwinkle et al. 1997, Raulston et al. 1998) moving at ≈ 2 kph. Different rows were sampled on each date, omitting the row with a cage. The insects collected from the two lengths of row per swath area were pooled for each sampling date. The collections were frozen, segregated from debris, and frozen again for later identification. No pretreatment counts were taken because the control plot was within the same field.

The experiment was designed as a randomized complete block, and statistical differences were detected using ANOVA. Means were separated using Tukey's multiple range test (Analytical Software 1998). The petri dish assay data were expressed as percentage mortality based on the pretreatment populations. Percentages could be used because, unlike the cage assay, all of the released boll weevils were accounted for throughout the study. Data was transformed to ranks for analysis (Conover and Iman 1981).

RESULTS

Pre-treatment square damage was < 1% in each sampling area, so the chance of naturally occurring boll weevils being in the cages was assumed to be negligible. In the field cage assay, there were no significant differences in boll weevil mortality or survival among the three malathion treatments on any of the five post-application sampling days (Table 1). There were significantly more dead boll weevils and fewer survivors in each of the three malathion treatments than in the control plots through the first three days after treatment. However, mortality was not significantly greater than controls on the fourth or fifth day after treatment (Table 1). The numbers of boll weevils that were unaccounted for 24 h after release were not significantly different among treatment and control cages on any sampling day, or within any treatment across sampling days.

Compared to the peak mortality observed after 24 h, boll weevil mortality in the malathion treated cages declined by 26–34%, 66-75%, 77-88%, and 93-96% during the second, third, fourth, and fifth days, respectively. By the third day, boll weevil mortalities in all of the malathion treatments had declined by at least 66% ($P \leq 0.05$).

As in the field cage assay, the petri dish assay indicated toxicity of the malathion treatments against boll weevils for three days after application. By three days after exposure, all malathion treatments produced mortalities that were not statistically different from one another, but statistically greater ($P \leq 0.05$) than in the controls (Table 2). Percentage mortality values from petri dish and field cage assays were similar. At one day residue age, the 1,040 g AI/ha, 867 g AI/ha, and 693 g AI/ha malathion dosages and the control produced 87, 90, 80, and 0% boll weevil mortality, respectively, in petri dish assays and 84, 92, 82, and 4% boll weevil mortality, respectively, in the cage assay. The only major difference between mortality from petri dish (62%) and cage (35%) assays occurred at 3 day-old residues with the standard malathion (1,040 g AI/ha) dose.

The vacuum sampler recovered very few boll weevils in the control plots (Table 3), indicating that natural populations were low during the test period. However, it caught numerous other arthropods that were divided into ten predator groups and seven

TABLE 1. Boll Weevil Mortality and Survival (Mean ± SE) by 24 h after Exposure to Differently Aged Malathion and Malathion + Cottonseed Oil Foliar Residues in Field Cages, 1 – 6 May 2001, Hidalgo County, TX.

Residue Age (d)	Boll Weevils[b]	Treatments[a] 1,040 M	693 M + 553.5 C	867 M + 415 C	Control	*F*	*P*
0-1	dead	15.0 ± 1.2 a	14.6 ± 1.7 a	14.6 ± 1.3 a	0.7 ± 0.4 b	55.85	< 0.0001
	alive	2.9 ± 1.2 b	3.1 ± 1.4 b	1.3 ± 0.5 b	16.3 ± 0.6 a	50.62	< 0.0001
	not found	2.1 ± 0.5	2.3 ± 0.7	4.1 ± 1.1	3.0 ± 0.7	2.14	0.131
1-2	dead	11.1 ± 1.5 a	10.0 ± 1.6 a	9.6 ± 1.4 a	0.4 ± 0.2 b	15.27	< 0.0001
	alive	5.0 ± 1.1 b	5.7 ± 1.7 b	5.9 ± 1.4 b	14.4 ± 0.7 a	14.63	< 0.0001
	not found	3.9 ± 0.7	4.3 ± 0.6	4.6 ± 1.0	5.1 ± 0.7	0.50	0.684
2-3	dead	5.1 ± 1.4 a	4.7 ± 1.0 a	3.7 ± 1.4 ab	0.3 ± 0.2 b	5.82	0.0058
	alive	9.3 ± 1.4 b	9.3 ± 1.9 b	11.0 ± 1.4 ab	14.7 ± 1.3 a	5.28	0.0087
	not found	5.6 ± 1.9	6.0 ± 1.5	5.3 ± 1.0	4.9 ± 1.2	0.16	0.925
3-4	dead	3.5 ± 1.1	1.7 ± 1.1	1.8 ± 0.9	0.8 ± 0.6	1.58	0.237
	alive	10.8 ± 1.7	13.8 ± 2.0	12.8 ± 1.5	13.5 ± 1.2	1.21	0.339
	not found	5.6 ± 1.9	4.5 ± 1.6	5.3 ± 0.8	5.7 ± 1.5	0.30	0.828
4-5	dead	0.6 ± 0.2	1.0 ± 0.8	0.8 ± 0.4	0.6 ± 0.4	0.14	0.931
	alive	16.0 ± 1.1	14.6 ± 0.9	14.4 ± 1.9	14.0 ± 1.2	0.46	0.718
	not found	5.4 ± 1.3	4.4 ± 0.9	4.8 ± 2.1	3.4 ± 1.2	0.32	0.811

[a] Numbers refer to g AI/ha; M, malathion; C, cottonseed oil. Analyzed as a randomized complete block design, df = 3. Means (± SE) followed by different letters in the same row are significantly different ($P \leq 0.05$) using Tukey's multiple range test.
[b] 20 boll weevils were released in each cage at each residue age.

TABLE 2. Mean Percentage (± SE) Mortality of Boll Weevils Exposed to Treated or Untreated Cotton Leaves in Petri Dishes.

Weevil Release Day after Treatment	Treatment[a]	Days after Exposure[b]				
		1	2	3	4	5
0	1,040 M	92 ± 4.8 a	98 ± 1.7 a	100 a	–	–
	867 M + 415 C	97 ± 2.9 a	97 ± 2.9 a	100 a	–	–
	693 M + 553.5 C	100 a	–	–	–	–
	Control	0 b	0 b	3 ± 2.9 b	14 ± 6.1 b	61 ± 6.3b
1	1,040 M	87 ± 9.6 a	93 ± 6.7 a	95 ± 5.0 a	98 ± 1.7 a	98 ± 1.7 a
	867 M + 415 C	90 ± 5.8 a	97 ± 2.9 a	99 ± 1.4 a	99 ± 1.4 a	100 a
	693 M + 553.5 C	80 ± 7.6 a	99 ± 1.4 a	100 a	–	–
	Control	0 b	6 ± 2.0 b	13 ± 1.8 b	34 ± 9.8 b	59 ± 8.0 b
2	1,040 M	82 ± 7.5 a	83 ± 8.0 a	88 ± 7.5 a	92 ± 5.4 a	98 ± 1.7 a
	867 M + 415 C	53 ± 16.0 a	61 ± 16.2 a	69 ± 14.4 a	79 ± 10.8 a	90 ± 8.4 a
	693 M + 553.5 C	61 ± 8.0 a	70 ± 8.2 a	73 ± 8.1 a	83 ± 6.8 a	90 ± 5.4 a
	Control	4 ± 2.0 b	9 ± 4.0 b	9 ± 4.0 b	17 ± 4.7 b	60 ± 5.8 b
3	1,040 M	62 ± 12.2 a	73 ± 12.3 a	78 ± 9.1 a	90 ± 3.6 a	98 ± 1.7 a
	867 M + 415 C	26 ± 10.7 a	31 ± 11.8 b	41 ± 11.8 a	59 ± 10.0 a	89 ± 3.7 a
	693 M + 553.5 C	26 ± 9.0 a	33 ± 9.9 ab	39 ± 12.4 a	60 ± 10.9 a	83 ± 6.8 a
	Control	1 ± 1.4 b	1 ± 1.4 c	4 ± 3.0 b	21 ± 4.0 b	60 ± 6.9 b
4	1,040 M	17 ± 16.7 a	20 ± 16.2 a	23 ± 15.6 a	35 ± 15.6 a	67 ± 11.7 a
	867 M + 415 C	11 ± 5.5 a	19 ± 6.7 a	24 ± 7.5 a	34 ± 8.4 a	79 ± 5.5 a
	693 M + 553.5 C	6 ± 4.3 a	11 ± 6.3 a	17 ± 5.2 a	41 ± 4.0 a	76 ± 4.3 a

	Control	6 ± 3.0 a	6 ± 3.0 a	10 ± 3.1 a	37 ± 5.6 a	79 ± 4.6 a
5	1,040 M	2 ± 1.7 a	2 ± 1.7 b	8 ± 6.5 a	38 ± 6.5 a	80 ± 3.6 b
	867 M + 415 C	9 ± 5.5 a	11 ± 6.0 ab	14 ± 7.2 a	51 ± 4.6 a	89 ± 4.0 ab
	693 M + 553.5 C	10 ± 4.4 a	24 ± 7.2 a	26 ± 7.2 a	63 ± 7.1a	86 ± 3.7 ab
	Control	0 a	1 ± 1.4 b	9 ± 4.0 a	59 ± 7.7 a	94 ± 4.3 a

[a] Grams/ha followed by M, malathion; or C, cottonseed oil.
[b] Means in a column followed by different letters are significantly different ($P \leq 0.05$) at each residual age.

TABLE 3. Mean Numbers (± SE) of Selected Nontarget Arthropods and Boll Weevils Vacuum Sampled per 50 m of Cotton Row Treated with Combinations of Malathion ULV and Cottonseed Oil, Hidalgo Co., TX.

Arthropod Group[a]	Days After Treatment	Treatments[b]			
		1,040 M	867 M + 415 C	693 M + 553.5 C	Control
Predators					
Geocoris[c]	2	1.0 ± 0.4	4.7 ± 3.6	0.9 ± 0.4	1.3 ± 0.6
	5	0.4 ± 0.2 b	2.3 ± 0.6 a	1.2 ± 0.3 ab	2.3 ± 0.4 a
Anthocoridae	2	0.2 ± 0.2	0.6 ± 0.3	0	0.9 ± 0.4
	5	0.4 ± 0.2	0.7 ± 0.3	0.7 ± 0.5	2.2 ± 1.2
Nabidae	2	0 b	0.6 ± 0.2 ab	0 b	1.0 ± 0.4 a
	5	0.2 ± 0.2	1.0 ± 0.4	0.8 ± 0.3 *	1.0 ± 0.4
Reduviidae	2	0	0.1 ± 0.1	0	0.1 ± 0.1
	5	0.2 ± 0.2	0	0	0.2 ± 0.2
Coccinellidae	2	10.0 ± 4.6 ab	11.0 ± 2.7 ab	4.4 ± 0.8 b	21.0 ± 6.7 a
	5	35.4 ± 8.6 *	76.0 ± 29.6	63.0 ± 19.9 *	133.0 ± 49.0 *
Collops[c]	2	0.8 ± 0.5 b	1.6 ± 0.5 b	1.4 ± 0.6 b	6.6 ± 2.4 a
	5	1.2 ± 1.0 b	1.5 ± 0.8 b	2.0 ± 0.4 b	11.8 ± 4.2 a
Wasps[d]	2	3.5 ± 0.7 b	6.7 ± 1.9 ab	4.6 ± 1.9 b	12.0 ± 2.5 a
	5	5.0 ± 2.1 b	10.7 ± 1.3 ab	13.3 ± 4.4 ab *	21.3 ± 2.7 a

Formicidae	2	1.2 ± 0.5	2.0 ± 0.8	1.7 ± 0.9	5.6 ± 2.8
	5	1.2 ± 0.6	3.2 ± 1.5	1.0 ± 0.6	6.7 ± 4.0
Neuroptera[e]	2	0 b	0 b	0 b	0.9 ± 0.1 a
	5	0 b	1.3 ± 0.4 ab *	1.5 ± 0.9 ab	3.7 ± 0.8 a *
Spiders[f]	2	2.8 ± 1.0	2.4 ± 0.9	3.4 ± 0.6	8.3 ± 4.0
	5	2.0 ± 0.4	2.0 ± 0.8	4.3 ± 1.4	6.3 ± 1.6
Herbivores					
Herbivorous Hemiptera[g]	2	3.8 ± 1.1	3.6 ± 1.2	2.1 ± 0.9	22.1 ± 11.3
	5	3.2 ± 1.2 b	3.0 ± 1.0 b	2.7 ± 0.7 b	18.8 ± 5.2 a
Cicadellidae	2	0.3 ± 0.2	0.1 ± 0.1	0.1 ± 0.1	0.1 ± 0.1
	5	0.6 ± 0.4	0.2 ± 0.2	0.2 ± 0.2	0.3 ± 0.3
Diptera[h]	2	25.5 ± 9.4	37.3 ± 10.3	32.9 ± 10.7	22.3 ± 5.5
	5	79.0 ± 33.8	69.8 ± 29.8	39.2 ± 12.5	54.7 ± 11.4 *
Lepidoptera larvae	2	5.8 ± 2.6	11.9 ± 3.5	9.1 ± 3.2	6.9 ± 1.8
	5	20.8 ± 11.3	22.3 ± 3.8	23.3 ± 8.3	26.8 ± 7.0 *
Lepidoptera adults	2	0.2 ± 0.2	0.3 ± 0.2	0.1 ± 0.1	0.3 ± 0.2
	5	0.8 ± 0.5	1.0 ± 0.5	0.3 ± 0.3	0.5 ± 0.2
Herbivorous Coleoptera[i]	2	1.3 ± 0.6	3.7 ± 1.4	2.7 ± 0.9	3.7 ± 0.6
	5	3.2 ± 0.7	9.0 ± 2.7	7.5 ± 3.0	15.2 ± 4.1 *
Boll weevils	2	0	0.4 ± 0.2	0	0.6 ± 0.3
	5	0	0	0	0.2 ± 0.2

[a] Aphids, whiteflies, ana thrips were not counted.
[b] Numbers refer to g AI/ha; M, malathion; C, cottonseed oil. Numbers followed by different letters within a row are significantly different ($P \leq 0.05$). Means in rows without letters are not significantly different; *, day 5 population > day 2 population ($P \leq 0.05$).
[c] *Geocoris* in family Lygaeidae, and *Collops* in family Melyridae.
[d] Mostly Braconidae, Eumenidae, Eupelmidae, Eurytomidae, Ichneumonidae, Pteromalidae, Sphecidae, and Trichogrammatidae.
[e] Chrysopidae and Hemerobiidae.
[f] Mostly Salticidae, Thomisidae, and Linyphiidae.
[g] Mostly Lygaeidae, Miridae, and Pentatomidae.
[h] Mostly Agromyzidae, Calliphoridae, Cecidomyiidae, Ceratopogonidae, Chironomidae, Muscidae, and Tephritidae.
[i] Mostly Anthicidae, Chrysomelidae, and Elateridae.

herbivore groups (Table 3). Numbers of aphids, whiteflies, and thrips were not included because it is likely that the process of freezing and refreezing the samples destroyed them. Each of the malathion treatments suppressed populations of Neuroptera (Chrysopidae and Hemerobiidae, 100%, $P = 0.0001$ on day 2, and ≥ 59%, $P = 0.0093$ on day 5) and *Collops* (≥ 76%, $P = 0.012$ on day 2, and ≥ 83%, $P = 0.0114$ on day 5) most profoundly among the nontarget arthropods. Populations of Nabidae, wasps, naturally occurring boll weevils, and Coccinellidae, to some extent were significantly reduced in the malathion treatments compared to the control plots. Numbers of herbivorous Hemiptera declined ≥ 83% on the second day, but variation in the controls was too high to show statistical significance; the decline was ≥ 85.6% ($P \leq 0.05$) on the fifth day in the malathion treated plots. By the fifth post-application day, populations of Coccinellidae, wasps, Neuroptera, and Lepidoptera larvae had significantly increased in the control plots and in one or more of the malathion treatments. The other nontarget arthropod groups, including Reduviidae, Formicidae, spiders, Cicadellidae, Diptera, Lepidoptera adults and larvae, and herbivorous Coleoptera (excluding boll weevils) appeared to be unaffected by the application of malathion. On day 5 after application, populations of *Geocoris* were significantly lower in the 1,040 g AI/ha plots than in the 867 g AI/ha + cottonseed oil plots (Table 3). Otherwise, we did not observe differential effects among malathion treatments on nontarget arthropods.

DISCUSSION

The field cage assay showed that boll weevil mortality caused by the reduced rates of malathion mixed with cottonseed oil was not statistically different than that caused by the standard rate alone. This agrees with an earlier study that compared higher dosages of malathion + cottonseed oil against an EC formulation of malathion (Wolfenbarger and Guerra 1986). Daily mortalities declined at approximately the same rate in all three treatments, with the greatest decline occurring after 48 h. In a study on boll weevil control, oil enhanced the toxicity of oxamyl and cyfluthrin, but did not increase longevity of efficacy (Treacy et al. 1986a). Villavaso et al. (1996) reported that aerially applied ULV malathion was effective against boll weevils until 48 h after application unless rainfall occurred. Other studies have shown the activity of aerially applied malathion to be at least two (Foster et al. 2001) to four days (Foster et al. 2000). Numbers of boll weevils in this study that could not be accounted for should have little effect on conclusions drawn from mortality and survival data assuming that the ratio of dead:living boll weevils that escaped detection did not significantly differ among the treatments.

The petri dish assay results were similar to those reported for the same doses of ULV malathion in studies designed to evaluate encapsulated malathion formulations (Foster et al. 2000, 2001). Also, Mulrooney and Smith (2000), using petri dish observations, found that malathion + cottonseed oil at 520 g AI/ha + 1.90 liters/ha cottonseed oil and 693 g AI/ha malathion + 0.59 liters/ha cottonseed oil were as effective as malathion at 866 g AI/ha for 48 h after application. They reported 90% mortality of boll weevils placed on excised cotton leaves as long a 4 d after application with 606 and 693 g AI/ha malathion in cottonseed oil. The data are consistent with the findings of the field cage assay in terms of relative efficacy and effect of residues on boll weevil mortality. It is acknowledged that the susceptibility of laboratory reared strains of boll weevils to insecticides might differ from naturally occurring boll weevils (Martin et al. 1996).

The malathion treatments reduced populations of Neuroptera and *Collops* the most. Populations of initially suppressed Nabidae and Coccinellidae appeared to have recovered by day 5. Malathion effects on wasps, Neuroptera, *Collops*, and herbivorous Hemiptera were apparent through five days and possibly beyond. Because numbers of

boll weevils collected in the vacuum sampler were so small, the residual effect of the malathion treatments were not detected on the natural population. Some of the arthropod groups counted, including Anthocoridae, Formicidae, spiders, Diptera, and Lepidoptera larvae, were not reduced by any of the malathion or malathion + cottonseed oil treatments. Mean numbers of Reduviidae, Cicadellidae, and Lepidoptera adults were too small ($\leq$ 1.0) to draw conclusions. In the case of some of the arthropod groups, significantly increased populations on day 5 did not necessarily indicate an initial malathion kill because the populations had also increased in the controls. The malathion + cottonseed oil treatments showed a trend, albeit statistically nonsignificant, toward being less toxic to nontarget arthropods.

In conclusion, cottonseed oil + malathion treatments at the two reduced rates caused boll weevil mortalities that were not statistically different from the standard rate of malathion alone in both the cage and petri dish assays. The petri dish assay suggests that residual activity of the reduced rates in oil may not be as great as the standard. The two reduced malathion rates + cottonseed oil had slightly higher populations of Neuroptera and wasps than those receiving the standard rate alone. Other differential impacts on the nontarget arthropod groups were statistically negligible indicating that the oil did not cause detrimental effects.

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FIELD RESIDUAL ACTIVITY OF SELECTED FORMULATIONS OF MALATHION AGAINST *CATOLACCUS GRANDIS* (HYMENOPTERA: PTEROMALIDAE)

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ABSTRACT

Selected formulations of malathion were field applied and laboratory bioassayed in 1999 and 2000 to test residual activity to adult *Catolaccus grandis* (Burks), a boll weevil parasitoid. Results indicted that rates currently used in boll weevil eradication programs are highly toxic to *C. grandis* adults. Although rate responses were evident, all rates produced high levels of mortality. Microencapsulated formulations were highly toxic although activity was delayed with one of three formulations tested. In 1999 tests, Fyfanon ULV produced 70-91% mortality at 0.37-0.83 kg AI/ha on day 0 and 1-12% mortality on day 7; a microencapsulated formulation produced 3-24% mortality on day 0 but 21-67% mortality on day 7. In 2000, Fyfanon produced 100% mortality on day 0 compared to 22% and 46% with microencapsulated experimental formulations at 0.83 kg AI/ha. One encapsulated formulation produced 34% mortality 14 days after the initial insecticide application.

INTRODUCTION

Eradication programs had eliminated boll weevil as an economic pest from 4.5 million acres of cotton production in eight states by 1998 (Cunningham and Greffenstette 1998). These eradication program protocols have relied heavily on the use of malathion. *Catolaccus grandis* (Burks) has been extensively evaluated for suppression of boll weevil in cotton and has been suggested for use in eradication programs where synthetic insecticide applications are problematic (King et al. 1995, Morales-Ramos et al. 1995, Summy et al. 1995). However, if this parasitoid is used in conjunction with malathion applications, a better understanding of the residual activity of malathion against *C. grandis* is needed. Malathion was the most toxic of ten insecticides tested to an invivo strain of *C. grandis* in laboratory bioassays (Elzen et al. 1999). The objective of this study was to evaluate the residual activity of selected malathion doses and formulations against *C. grandis* in the field.

MATERIALS AND METHODS

The study was conducted in Eddy County, in southeastern New Mexico in 1999 and 2000. The general location and specific fields were selected because of a recent history of boll

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weevil and the fact that no other insecticide treatments were expected. This study was conducted in conjunction with a study to evaluate malathion activity against boll weevil (Foster et al. 2000, 2001).

Nine plots in 1999 and four in 2000 were each selected from separate fields for the study. Plots were 1.3 ha (114m x 114 m), a plot size that accommodated five full aerially applied swaths Plots were of sufficient separation to ensure no contamination from test treatments or from any crop treatments to nearby fields. All test plots were planted in non-Bt upland cotton, Acala 1517. No insecticides other than those in the study were applied to the plots. Each of the plots was sprayed with one of eight treatments in 1999 and 1 of 3 in 2000. One plot was left untreated as a control. Assignment of treatments to plots was randomized. All formulations were applied undiluted.

Fyfanon® ULV was used as the standard for comparison both years. In 1999, Fyfanon ULV was compared to a Cheminova (Cheminova Agro A/S/ Denmark) experimental microencapsulated formulation and Atrapa™ ULV (Griffin Corp, Valdosta, GA). The Cheminova microencapsulated formulation was aerially applied at 0.69, 0.55 and 0.37 kg AI/ha. Fyfanon ULV was applied at the same rates and at 0.83 kg AI/ha. Atrapa was applied at 0.83 kg AI/ha only. In 2000, Fyfanon ULV was compared to two experimental microencapsulated formulations, a Cheminova CS formulation, and a 3-M (3M, Canada, Co.) MEC formulation. All rates were at 0.83kg AI/ha. This rate is equal to the rate commonly used in eradication programs.

All treatments were applied with a Cessna Ag Truck aircraft owned by USDA, Animal and Plant Health Inspection Service (APHIS), and equipped with winglets (DBA-Ag Tips; Clack Oberholtzer, Alberta, Canada). Winglets are added to the spray aircraft to reduce the production of fine droplets and to improve handling characteristics. The aircraft was equipped with a standard commercial spraying system and a differentially corrected guidance and recording system. It was operated by a USDA-APHIS pilot at 1.5-3.0 m (boom height above plant canopy) during application. Ground personnel flagged swath widths and ensured acceptable wind speed, and ground and air temperatures during treatments. All treatments were applied shortly after sunrise. The aircraft and spraying system were calibrated for a 22-m wide swath for all treatments. Prior to application, the aircraft spray system was calibrated to operate under parameters, which resulted in delivery of spray within 1% of the desired rate for each treatment. Each treatment was calibrated by collecting material sprayed through each nozzle for each treatment for a predetermined amount of time, and making appropriate adjustments in pressure until the desired output was achieved. All treatments were applied through Flat Fan Tee Jet stainless steel nozzle tips oriented straight down. In 1999, the encapsulated formulation at 0.69, 0.55 and 0.37 kg AI/ha was applied at 120 mph and 42 psi through 11, 9 and 7 (8003 size) tips, respectively. Fyfanon ULV at 0.83, 0.69, 0.55, and 0.37 kg AI/ha was applied at 125 mph and 39, 41, 45 and 29 psi, respectively, through 10, 8, 6 and 5 (8002 size) tips, respectively. The Atrapa ULV treatment was applied at 125 mph and 39 psi through 10 (8002 size) tips. In 2000, the Cheminova CS formulation was applied at 120 mph and 44 psi through 14 (8003 size) tips. The 3M MEC encapsulated formulation was applied at 120 mph and 50 psi through 24 (8004 size) tips. The equivalent Fyfanon ULV was applied at 125 mph and 40 psi through 10 (8002 size) tips.

C. grandis were reared at the Mission Plant Protection Center, Mission, Texas, and shipped to New Mexico State University for testing. Adult wasps were maintained in cages and fed honey until used for testing. All wasps used for testing were 2-4 day old females. In 1999, leaves were collected from treated plots on day 0, 5 and 7. In each plot, leaves were collected from the three center swaths of each plot, which were used as subplots and as replications in the bioassay. Leaves were collected at canopy level in the center of the

subplot One leaf was collected every five rows in two lines nine meters apart perpendicular to the flight path. Ten leaves were used per replication, with each leaf placed in a 50-ml plastic vial. Five wasps were placed in each vial, for a total of 150 *C. grandis* per treatment. Mortality was recorded after 24h. In 2000, the bioassay protocol was similar to 1999, but 20 leaves were used per replication, for a total of 300 *C. grandis*/treatment. Bioassays were conducted on days 0, 3, 7, and 14. Data analysis compared differences in mortality among formulations, rates and days after application using SAS JMP (SAS 1998). First level interactions were tested for rate, day and formulation. This was followed by analysis of covariance regression or ANOVA as appropriate, depending on interaction results.

RESULTS AND DISCUSSION

In 1999, all formulations were active against *C. grandis* producing high mortality (Table 1). Formulations were compared on individual days due to statistically significant interactions between days and formulations. Fyfanon and Atrapa results were similar with 91 and 80% mortality, respectively, after 24 hours exposure to leaves treated on day 0 at 0.83 kg AI/ha. Encapsulated formulation activity was initially statistically lower than Fyfanon activity. At 0.37-0.69 kg AI/ha, Fyfanon produced 70-87% mortality compared to 3-24% mortality with the encapsulated formulation on day 0 (df 1,18; F=473, P<0.0001); however, after 7 days all but the lowest rate of the encapsulated formulation had significantly higher activity than all doses of Fyfanon and Atrapa. The 0.69 and 0.55 kg AI/ha rates of the microencapsulated formulation produced 40-67% mortality compared to 1-12% in Fyfanon and Atrapa treatments. Check mortality was no higher than 6%.

TABLE 1. Percentage Mortality of *C. grandis* after 24 Hour Exposure to Leaves from Eight Fields Treated in 1999 with Selected Formulations of Malathion.[a]

Formulation	Rate (kg AI/ha)	Days After Treatment (S.E.)		
		0	5	7
Encapsulated	0.69	24 (1.7) b	17 (2.4) a	40 (5.2) ab
	0.55	11 (3.8) b	6 (2.3)a	67 (5.3) ab
	0.37	3 (0.7) b	2 (2.0) a	21 (9.1) bc
Fyfanon ULV	0.83	91 (5.8) a	20 (2.4) a	11 (0.7) cd
	0.69	87 (0.7) a	14 (8.0) a	1 (0.7) d
	0.55	81 (6.9) a	20 (0.3) a	12 (5.0) cd
	0.37	70 (3.6) a	34 (14.0) a	9 (3.3) cd
Atrapa ULV	0.83	80 (3.8) a	10 (4.2) a	8 (4.0) cd

[a] Means within columns followed by the same letters are not significantly different (P<0.05) by Tukeys Multiple Comparison.

Residual activity was also examined with individual formulations due to the interaction. of formulation and day. Activity of the standard ULV formulations decreased over time while activity of the microencapsulated formulation increased over time. The effect of days after application on activity was also examined with individual formulations due to interactions. Mortality rates decreased over 0-7 days with both Fyfanon and Atrapa ULV malathion formulations (Table 2). Linear regression equations indicated percentage mortality decreased 11-12% per day in Fyfanon and Atrapa treated plots. On the other hand, mortality increased over time in the encapsulated malathion treated plots (Table 2), with mortality

increasing 3.5% per day.

TABLE 2. Regression Statistics for the Effect of Days after Malathion Application on Percentage Mortality of *C. grandis* in 1999.

Formulation	r^2	df	F	*P*	B_0	B_1(days)
Microencapsulated	0.24	1, 25	7.8	0.0097	8.0	3.5
Fyfanon ULV	0.89	1, 34	292	0.0001	81.2	-10.9
Atrapa ULV	0.95	1, 7	14.3	0.0001	77.9	-11.9

Data for common rates (0.37 to 0.69 kg AI/ha) were pooled to compare overall activity of Fyfanon and the Cheminova microencapsulated formulation (Fig. 1). Fyfanon produced 82% mortality on day 0 but only 8% mortality on day 7. The microencapsulated formulation produced 13% mortality on day 0 but 44% on day 7.

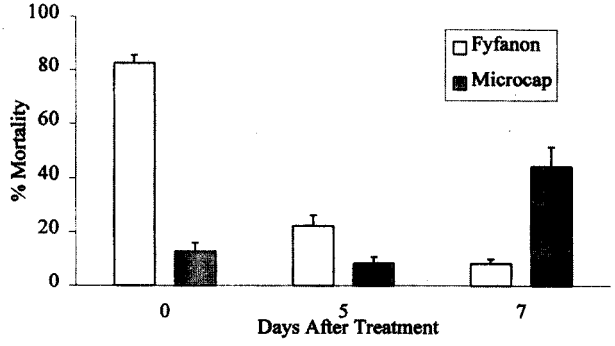

FIG. 1. Activity of Fyfanon and microencapsulated malathion formulations against *C. grandis*, 0-7 days after treatment.

In 1999, rate responses were evident, particularly on day 0 (Fig. 2). Mortality increased with dose for both Fyfanon and the microencapsulated formulation (Fyfanon: df 1,10; $P<0.01$; $r^2=0.50$) (microencapsulated formulation: df 1,7; $P<0.0005$; $r^2=0.83$). All rates of Fyfanon produced high mortality with 70-91% mortality on exposure to leaves treated that morning.

Residual activity of Fyfanon in 2000 was very similar to 1999. Fyfanon produced 100% mortality on day 0 but only 25 and 28% mortality on days 3 and 7, respectively (Table 3). The Cheminova formulation tested in 2000 had faster activity than that tested in 1999 with the highest *C. grandis* mortality (45.8%) on day 0. In 1999, activity peaked on day 8. The 3M formulation was the least active formulation with only 9-38% mortality on days 0-7. The Fyfanon treated field was inadvertently treated on day 14 so data are not available for that day/treatment.

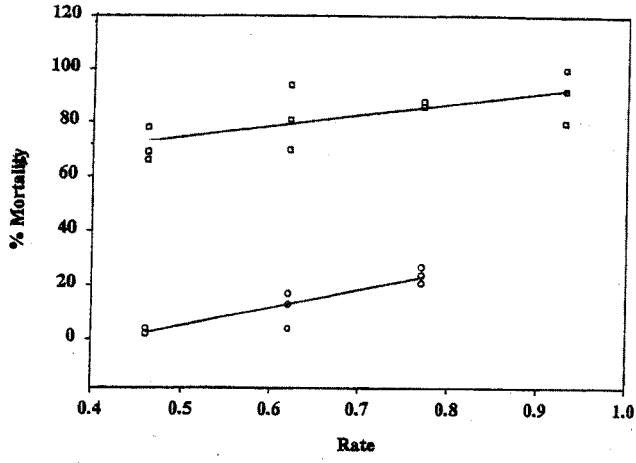

FIG. 2 Percent mortality of *C. grandis* exposed at day 0 for 24 hours to Fyfanon and to microencapsulated malathion: o – microencapsulated malathion; □ – Fyfanon.

TABLE 3. Percentage Mortality of *C. grandis* after 24 Hour Exposure to Leaves from Fields Treated in 2000 with selected Formulations of Malathion at 0.83 lb AI/ha.

	Percent Mortality at Day (S.E.M.)[a]			
Formulation	0	3	7	14
Fyfanon ULV	100.0 (0) a	25.2 (6.5) a	28.0 (5.7) b	----[b]
Cheminova CS	45.8 (8.9) b	25.4 (7.3) a	7.0 (2.6) c	14.0 (4.8) b
3M MEC	22.3 (8.0) c	9.0 (3.4) a	38.5 (6.9) a	33.8 (6.3) a

[a] Means within columns followed by the same letters are not significantly different ($P<0.05$) by Tukeys Comparison.
[b] Not available. See text.

Current field rates of malathion are highly toxic to *C. grandis*. Adjusting rates down will not reduce mortality to acceptable levels. Microencapsulated formulations were also highly toxic although levels of activity were delayed with two of the three formulations tested. It may be possible to use *C. grandis* in conjunction with malathion in eradication programs if *C. grandis* is used primarily in-season with no malathion applications during that period. These results indicate that *C. grandis* could not be released safely within 7-14 days of the last application. More testing will be needed to ensure that there are no adverse effects on *C. grandis* performance within the time frame needed. Releases of *C. grandis* 21 days after malathion application, for example, may still be affected by such applications and would require further testing.

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LIFE CYCLE AND REPRODUCTIVE AND FEEDING BEHAVIOR OF *DIPETALOGASTER MAXIMUS* (Uhler) (REDUVIIDAE:TRIATOMINAE) UNDER LABORATORY CONDITIONS IN BAJA CALIFORNIA SUR , MEXICO

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ABSTRACT

Studies of the life cycle and the feeding and reproductive behavior of a kissing bug, *Dipetalogaster maximus* (Uhler), were performed under laboratory conditions that mimic natural environmental conditions in Baja California Sur. The ambient temperature and relative humidity were recorded and the insects fed on blood from a domestic dove. Courtship and mating behavior were observed and recorded. Courtship was similar to that reported for other species of triatomids. Sixteen matings were observed with a mean time of 62 min for this activity. The period of incubation (mean of 33 days) and nymphal development (mean of 368 days for males and 361 for females) were recorded. Some nymphal instar development was longer when the peaks of mean ambient temperature were lower (i.e., 19.3-25.9°C). Fecundity and fertility periods were measured. Means for the following developmental stage measurements were derived: pre-oviposition period (27 days), eggs per female (21), laying episodes per female (10), and hatching indexes (28%). The mean feeding and defecating time were similar in all stages and adults. The number of specimens that defecated immediately during or after feeding was higher in the second (14%), third (16%), and fifth (15%) nymphal instars. We conclude that the ambient temperature when these insects feed determine the rate of development of the instars. Although the feeding time of *D. maximus* is long, this species does not represent a high risk of infection by *Trypanosoma cruzi* to local human population.

INTRODUCTION

Dipetalogaster maximus (Uhler), is an endemic species of the Cape region in Baja California Sur, Mexico, where it is found among the crevices of large boulders and bare rocks; however, this species recently has been observed in dwellings because of urban growth (Jiménez and Palacios 1999.). *D. maximus* is naturally infected with *Trypanosoma cruzi*, a causal agent of Chagas disease, but generally with low infection rates (Mazzotti 1940, Mazzotti and Dias 1949, Ryckman and Ryckman 1967, Marsden et al. 1979a, Estrada et al. 1995, Jiménez and Palacios 1999).

Although *D. maximus* is not considered epidemically important in Mexico, it is commonly reared under laboratory conditions and used in xenodiagnostic tests (Barreto et. al 1978, 1981; Cuba et al. 1978, 1979; Marsden et al. 1979b) in *T. cruzi* development tests (Marsden et al. 1979a), and to confirm *T. cruzi* infection among kissing bugs by coprophagy (Garcia da Silva et al. 1992). The first study of the longevity, fertility, and fecundity of the species was made by Ryckman and Ryckman (1967). Studies were continued by Barreto et al. (1981), Nakano et al. (1994), and Meireles et al. (1995), who focused on the biological aspects of mass rearing. Costa et al. (1987), reported on the

influence of different diets on the life cycle of this species. Costa et al. (1992) studied the population dynamics of the species, evaluating its growth rate, longevity, and fertility under controlled temperatures. Later, Garcia da Silva (1990) provided data on the influence of temperature on life cycle. Badauy et al. (1999) determined the nutritional interspecies relationship between *T. cruzi* and the life cycle of *D. maximus* under normal conditions and starvation conditions.

All of these previous studies were made under controlled environmental conditions in the laboratory with incubators. Herein, we report our observations of the reproductive, feeding, and biological cycles of this species, under more natural conditions in a closed laboratory environment in Baja California Sur.

METHODS

Specimens of *D. maximus* were collected during 1997 from houses in the city of La Paz, in the state of Baja California Sur, and reared to obtain 30 virgin adults (17 females and 13 males). They were used for the study of the life cycle of this species from June 1998 to November 1999. The insects were maintained in a closed room (1.87 x 2.00 m), with the ambient temperatures and relative humidity recorded once daily.

Observations of mating behavior were made from June to August 1998 between 0900 to 1300h at 5.5°C and 39.4% RH in a glass container (25 x 50 x 15.5 cm) containing broken stones as a substrate. Sixteen virgin insect male/female pairs were used, fed on blood of a domestic dove (*Columba livia*) for 15 minutes according to the technique of Salazar-Schettino (personal communication). Each pair was permitted to mate, after which the females were separated, and each mated female was placed in an individual separated, transparent 500-ml plastic jar, which was numbered sequentially. A piece of circular drying paper had been placed in the bottom of each jar, and another drying paper lined the vertical walls of the interior of each jar; each jar was covered with a fine mesh cloth. Time of mating was recorded. If there was no mating during the first 15 minutes, the couple was separated and one of the pair was replaced by another of that same sex. One week after the mating event, the females were fed to satiation, and subsequently fed every 2 or 3 weeks, depending on the behavior of the specimen, for the remainder of the experimental period.

The length of time covering pre-oviposition, fecundity, and viability were obtained by daily observations, recording the number of egg deposition events, number of eggs per oviposition event, and the viability of each egg hatch for each fertilized female. The eggs were grouped into six lots according to the date of deposition. After eclosion, nymphs were placed individually in jars until the adult emerged. The duration of each nymphal instar was determined by the exuviae collected during daily observations. The first-nymphal instars were fed until the eighth day after their appearance and, together with those of the second to the fifth instars, were fed every two or three weeks according to their need. Adults were fed every 10 or 12 days after their emergence.

Initially, feeding behavior and defecation were observed for 89 specimens (16 females, 13 males, and 60 nymphs) reared under laboratory conditions described above. This number decreased with the death of some of the insects during the study (Table 3). Insects were fed individually on the blood of a domestic dove by placing the bug in a glass tube containing paper filter in its interior and with a cloth mesh covering the mouth of the tube. The mouth of the tube was placed on the skin of the bird to allow the bug to feed through the mesh cover; the total time of feeding and defecation from the first contact of the insect with the skin of the host until it's voluntary retirement was recorded.

RESULTS

Sixteen matings were observed. The female was stimulated by holding her with a

clip forcing her to stridulate and secrete odoriferous substances (pheromones). She was then placed on the stone substratum inside the cage. The male detected her, approached, and attempted to mount her. If the female was not receptive, she resisted by pushing him with her posterior legs, or she moved from one side to another. If she was receptive, she remained motionless while the male mounted her. In the event the female attempted to move, the male mounted her back with his first pair of legs grasping the pronotum and the last two pairs of legs grasping the abdomen. Upon immobilizing her, the male tapped slightly on her head and thorax with his first pair of legs. The female lifted her abdomen and the male assumed a dorsolateral position, placing himself on the right side (rarely on the left). The male parameres were released and used to immobilize the female genitalia to introduce the aedeagus to the ovipore. Copulation was initiated, and the pair remained immobile an average of 6.2 min (maximum=8.3 min; minimum=3.4 min). At the end of the mating episode, the male remained motionless on the female with his parameres exposed for two to four additional minutes. He then departed and the female remained motionless on the substratum. All females, with one exception, accepted mating once. Males were capable of mating with different females several times during the study period.

During the life cycle study, the mean egg incubation period was 33 days (range= 22-45) at a mean temperature of 31.7°C. The average nymphal development of males and females was 368 days (maximum=507; minimum=313) and 361 days (maximum=529; minimum=208, respectively) (Table 1). Analysis of variance did not indicate a significant difference (α=0.05) in the total mean time of development of females compared to males; however, there was a significant difference between sexes in development time from the fourth to fifth-instar stage.

TABLE 1. Mean Time of Nymphal Instar Development Stages and Mean Total Nymphal Period (Days) of *Dipetalogaster maximus* at 21 to 31°C.

Instar	Female	Male
1	32.6 ± 5.8	32.9 ± 6.5
2	23.9 ± 2.0	23.7 ± 2.1
3	73.9 ± 67.8	42.6 ± 46.3
4	65.5 ± 59.4	104.6 ± 90.4
5	95.0 ± 51.7	74.6 ± 31.0
Mean total nymph period	368.5 ± 36.1	361.5 ± 50.0
Maximum	507	529
Minimum	313	208

The development of some-second instar specimens in experimental Groups V and VI; third-instar specimens in Groups III, IV, and V; fourth-instar specimens in Groups I and II; and fifth-instar specimens in all the Groups were considerably prolonged when ambient temperature averages were lower (i.e., 19.3-25.9°C) during January to June. This was not the case when temperatures were higher (33.4-36.1°C) from August to December 1998 and from July to November 1999 (Figs. 1 and 2).

The pre-oviposition period for these experimental populations was 3 to 54 days, with an average of 27 days. The mean number of eggs was 21 per female and the mean number of egg layings per female was ten. The hatching indexes were zero to 80%, with an average of 28% (Table 2).

The mean feeding time was similar among the nymphal instars, but slightly shorter for female (19 min) compared to male adults (24 min). Mean defecation time was similar in all developmental stages except longer for the fifth instar (26 min) and in male adults (26 min). The number of insects that defecated during or immediately after feeding was higher in the second (14 %), third (16 %), and fifth (15%) nymphal instars (Table 3).

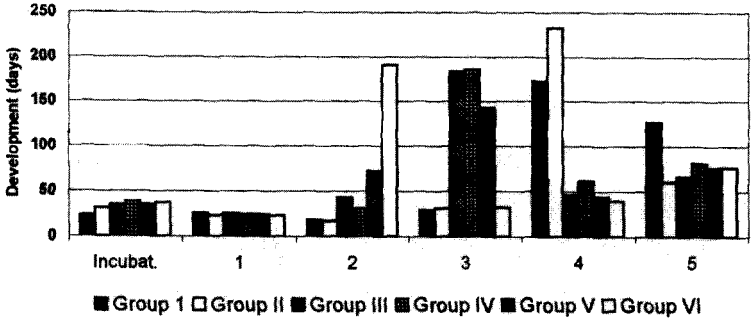

FIG. 1. Mean period of incubation and nymphal instars of *Dipetalogaster maximus* from June 1998 to November 1999. (Incubat. = egg state; I, 2, 3, 4, 5 = instar stage)

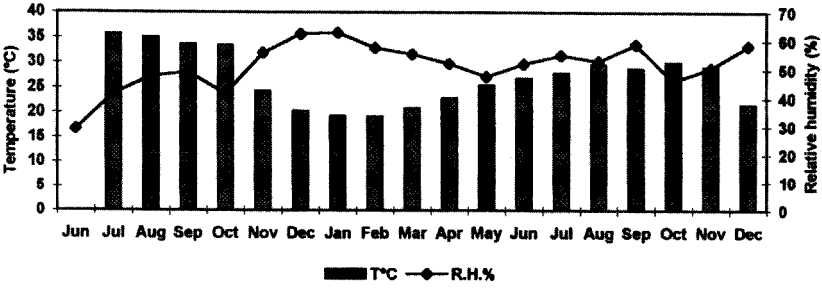

FIG. 2. Mean temperature and relative humidity from June 1998 to December 1999.

TABLE 2. Fecundity and Fertility of *Dipetalogaster maximus* from June to November 1998 at 24.3-36.1°C and Relative Humidity of 29% to 56%.

Female No.	Pre-oviposit period (days)	Total number of layings	Fertility period (days)	Total number of eggs	Hatchings: viable eggs	% of hatching index
1	3	8	89	17	2	11.7
2	27	21	61	41	8	19.5
3	25	11	83	32	10	31.2
4	16	6	24	10	6	60.0
5	21	10	75	12	0	0.0
6	20	12	90	23	2	8.9
7	22	3	9	4	0	0.0
8	22	15	86	23	0	0.0
9	44	16	72	30	0	0.0
10	39	10	59	16	8	50.0
11	42	5	84	5	4	80.0
12	54	4	18	7	0	0.0
13	15	12	56	62	33	53.2
14	25	7	26	9	7	77.7
Total	375	140	832	295	78	28.0
	$\bar{x}$=26.7	$\bar{x}$=10	$\bar{x}$=59.4	$\bar{x}$=20.9	$\bar{x}$=5.5	

TABLE 3 Mean Feeding and Defecating Times in All Instars and adults of *Dipetalogaster maximus* at 19.8 to 31.7 °C.

	Feeding time (min)				Defecation time (min)			Specimens defecating during feeding	
Instar	No.	Mean	Ds	Range	Mean	Ds	Range	No.	%
1	60	21.5	3.86	11.3-52.5	25.1	3.12	13.2-52.5	2	3.3
2	57	21.5	4.37	10.0-50.3	22.8	4.01	10.0-50.3	8	14.0
3	56	21.3	3.17	11.2-71.2	24.1	2.09	13.2-71.2	9	15.7
4	53	21.2	2.10	12.1-45.2	22.8	3.15	13.3-63.3	7	13.2
5	52	21.2	4.09	8.3-42.4	26.3	3.54	8.5-45.2	8	15.3
Female	26	19.4	7.01	10.0-34.3	19.7	7.66	14.4-42.5	0	0
Male	16	23.8	5.81	10.2-31.2	27.5	8.20	14.0-40.3	0	0
Total	320								

DISCUSSION AND CONCLUSIONS

As in other triatomids, courtship and mating behavior of *D. maximus* was not elaborate, consisting only of the female stridulating and releasing pheromones and the male approaching and mounting the female. The average time of mating (5-15 min) is similar to that reported by Lent and Wygodzinski (1979). Examples exist where mating times are briefer (e.g., 3-4 min for *Rodniux prolixus* and *Triatoma vetticeps*) or longer (e.g., 29.3 min for *Panstrogylus megistus*) (Lima et al. 1986). Many species of the family Reduviidae produce sounds or stridulate by rubbing their beaks against a ridged ventral groove on their thorax (Readio, in Schmidt 1994). The sounds are generally produced in response to mechanical disturbances and are interpreted as alarms against predators (Ewing1989). According to our observations, when the females produce those sounds before mating, they also exude perceptible odoriferous substances that activate males. During mating activity, females continue stridulating. Zeledón (1981) stated that adults of *T. dimidiata* stridulate during these acts; therefore, we assume that this excitation, together with the pheromone emanation, served as stimulant to males of *D. maximus* to begin courtship and mating and the alarm function was subdued.

In triatomids, the significance of a right or left mounting position during mating is not known. For this species males preferred the right side of females. According to Lent and Wygodzinski (1979), the torsion of the genital for the introduction of the aedeagus into the female's ovipore is more successful in that position for this species. Our observations of *D. maximus* indicate that the incubation period is slightly longer than recorded by other authors (Ryckman and Ryckman 1967, Barreto et al. 1981). The nymphal period recorded in the current study was shorter than the nymphal period obtained by Ryckman and Ryckman (1967) who recorded 382 days for females and 422 days for males. They did not specify temperature conditions. In contrast, the nymphal period recorded by Barreto et al. (1981) was shorter than our observations. They reported a mean of 164 days at 28-30°C. García da Silva (1990) indicated the average life cycle was 303 days for males and for females at 25°C, and 206 and 205 days for males and females, respectively, at 30°C. Badauy et al. (1999) reported an average life cycle of 205 and 202 days for males and females, respectively, at 28°C.

During the development of nymphal instars, there were no significant differences in developmental time for the first three instar stages destined to be female compared to those destined to be males ($\alpha = 0.05$). However, developmental time for the female third instar appears to be longer, as do the times for the fourth and fifth instars for both sexes, where

there were significant differences. This is probably due to the fluctuation of the ambient temperatures during the course of the experiment (Table 1).

The average time that each nymph needs to molt and pass to the following instar increases gradually from the first instar to the adult (Barreto et al. 1981). This is true, provided the individuals are reared at constant temperatures. According to our results, the low temperatures of the cold months (19.3-25.9°C) considerably prolonged the life cycle of *D. maximus*, as shown in Fig. 1, where some individuals of the second to fifth instars interrupted their development and entered a diapause state. Lent and Wygodzinsky (1979) mentioned that most of the triatomids have long life cycles, but there are some cases, like that of *Triatoma eratyrusiformis*, where the first instar may enter a long diapause. In 1998, we made a study of the incidence of *D. maximus* in houses in La Paz and found the period of inactivity of this species was from autumn to spring, when the ambient temperatures were lower (Jiménez and Palacios 1999). This observations coincides with those obtained in this study.

The low number of offspring obtained in the current study was a result of the single mating per couple, and as a consequence, the total number of eggs, egg laying per female and hatching index were low. In other studies of the life cycle of this species, a larger number of offspring were recorded than was obtained in this work. This is probably the result of males and females being kept together during the whole experiment, thereby causing a higher reproductive index, in these other studies.

Studies of *D. maximus* by Costa (1987), Barretto et al. (1981), García da Silva (1990) and Badauy et al. (1999) have demonstrated that blood meal type (roosters, mice, and doves) and the variation in ambient temperature (28-30°C) are decisive for the length of the life cycle (short=164 days; long=205 days) of this species. Our results showed that insects fed dove blood at 19-31°C had a longer life cycle than specimens studied by these other authors. In our study, some insects (27%) that had not fed sufficiently did not conclude molting and died; the mortality was higher in the fourth and fifth instars (25%) and adults (31%). Some adults developed with deformities in their beaks, antennae, or posterior legs (27%). Barreto et al. (1981) also found deformations in antennae and legs of some adults, without giving an explanation for this phenomenon. The specimens used by those authors and ourselves came from a colony recently established with individuals collected in Baja California. It is probable that deformities are more frequent in insects that are inbred and reared in the laboratory.

In most triatomids, feeding to satiation takes 10 to 20 minutes (Schofield 1994). Large kissing bug species show slightly longer feeding times, and this is directly proportional to the instar size (Zeledón et al. 1977, Zarate et al. 1984). Our results showed that *D. maximus*, because of its large size, takes longer to feed (Table 3). However, average feeding time was similar in all instars, but not in adults. Variations in the feeding time also was influenced by the blood supply of the host's skin where the insect fed. If the location had lower blood flow, the insect took longer to feed.

Experimentally, *D. maximus* has been induced to feed on man, dogs, lambs, cattle, rabbits, rats, and chickens (Ryckman and Ryckman 1967). As in other triatomids species, it can feed on any wild or domestic vertebrate in its natural environment. Results from our simulated natural set of ambient conditions, as well as the work of other researchers, support the conclusion that the length of the life cycle of kissing bugs is a response to environmental conditions, particulary temperature, and the host species.

Defecation patterns during or immediately after feeding by triatomids are important in the transmission of *T. cruzi* (Zeledón 1981). Longer contact between an infected vector and a host/victim increases the possibility that defecation will occur during or immediately after feeding. Therefore, the risk of infection or re-infection may increase. In our study of some 320 observations, only 11% of the nymphs defecated during feeding. Although the feeding time of *D. maximus* is long, we conclude that this species does not represent a high

risk of infection by *T. cruzi*.

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EVALUATION OF *BEAUVERIA BASSIANA* AND *METARHIZIUM ANISOPLIAE* (MITOSPORIC) AGAINST SPECIES OF THE "WHITE GRUB COMPLEX" [1] IN THE SOUTH OF MEXICO.

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ABSTRACT

Laboratory bioassays, at 25±2°C, 80±5% RH and 12:12 L:D, were conducted to evaluate several strains of *Beauveria bassiana* (Balsamo) Vuillemin and *Metarhizium anisopliae* (Metschnikoff) Sorokin against some species of white grubs, *Phyllophaga* spp. All strains of the fungi were pathogenic to third-instar *Phyllophaga* spp. larvae, resulting in mortality from 28.3 to 61.6% by *B. bassiana* and from 21.6 to 90.0% by *M. anisopliae*. The strains that caused higher mortality were Bb26 (61.6%) of *B. bassiana* and MaN7 (90.0%) of *M. anisopliae*. Lethal time (LT_{50}) was 4.9 days (FL_{95} = 3.6 to 7.1) for Bb26 and 6.1 days (FL_{95} = 4.6 to 7.7) for MaN7. Lethal concentration (LC_{50}) was 1.29×10^{8} conidia/g with MaN7 strain and 1.15×10^{9} conidia/g with the Bb26 strain. The application of *B. bassiana* did not reduce the damage done by larvae to plant biomass (total plant weight and stem weight) compared with the controls. Plants in control plots produced greater biomass than did plants infested with white grubs treated with *M. anisopliae*.

INTRODUCTION

Corn, *Zea mays* L., is one of the most important cereal crops in the world, and has been cultivated by Mesoamerican families for thousands of years (Turner and Harrison 1981, Flannery 1985). Insect pests of corn are economically important, but it is difficult to estimate and compare crop losses because of damage diversity, the species involved, and the varied environmental conditions in the regions where this crop is cultivated; however, losses have been estimated at 20-30% (Lagunes et al. 1985). Some of the most damaging pests of corn are white grubs. In Mexico this complex of pest includes 560

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species in the genera *Phyllophaga, Anomala, Dyscinetus, Strategus, Eutheola, Orizabus, Lygurus, Euphoria* and *Cotinis*. Most species (230) belong to the genus *Phyllophaga* (Morón 1984, 1986; Deloya 1993). A few studies have been carried out in Mexico to determine the magnitude of damage caused by white grubs. For example, Rodríguez (1980) estimated crop losses between 400 and 700 kg/ha in Tamaulipas State, and Nájera (1998) reported losses between 350 and 1500 kg/ha in Jalisco State. In Chiapas State, white grubs are a major problem affecting agricultural production because they attack different crops such as corn, vegetables, and fruit trees (Castro et al. 1998). However, damage caused by white grubs has not been quantified, and no information about control methods is available.

Currently, entomopathogenic fungi such as *Beauveria bassiana* (Balsamo) Vuillemin and *Metarhizium anisopliae* (Metschnikoff) Sorokin (Ascomycota:Mitosporic) (Hawksworth 1998) show promise as alternatives for control of white grubs. In Mexico few studies have assessed the effect of these entomopathogenic fungi on white grubs, but Cortés et al. (1994), Nájera (1998), and Berlanga and Hernández (1999) evaluated some strains under laboratory and field conditions in the states of Guerrero, Colima and Guanajuato, respectively. They reported positive results, suggesting that these fungi might be effective biological control agents. The effect of these fungi on white grubs in Chiapas State has not been assessed. This study was undertaken to select strains of *B. bassiana* and *M. anisopliae* potentially effective in controlling *Phyllophaga* spp.

MATERIALS AND METHODS

Strains of *B. bassiana* and *M. anisopliae* were collected from Amatenago del Valle (16° 30′ 35′′ N, 92° 26′ 11′′ W), Bochil (17° 00′ 00′′ N, 92° 54′ 00′′ W), San Cristobal de las Casas (16° 42′ 09′′ N, 92° 38′ 03′′ W) and Villa Comaltitlan (15° 12′ 48′′ N, 92° 34′ 38′′ W) municipalities in the State of Chiapas, Mexico. The strains collected in this study are kept in the collection of entomopathogenic fungi maintained by El Colegio de la Frontera Sur (ECOSUR).

Fungi were propagated on Sabouraud Dextrose Agar (SDA) culture medium (40g dextrose, 10g peptin, 15g agar, and 0.2% yeast extract), to which 1,000ml of distilled water was added. The culture medium was placed in sterilized Petri dishes (90×15mm) and test tubes (22×175mm), and conidia of the fungi were streaked onto the culture medium in a laminar flow cabinet. To induce growth and sporulation, the fungi were maintained at 27±2°C, 80±5% RH and 12:12 L:D. Fifteen days later, conidia were collected with a sterile bacteriological loop and stored at 4°C in 50-cc plastic flasks to maintain the viability of the spores (Smith and Onhions 1994).

Third-instar larvae of *Phyllophaga* spp. were used as recommended by Shannon et al. (1993) in laboratory bioassays. Larvae were collected from a plot sown with corn and grass in the municipality of Villa Comaltitlan. Larvae were taken to the ECOSUR insect pathology laboratory. Each larva was placed with 250g of sterile soil in a 300-ml plastic container (7cm×9.5cm) in which a 25-day-old corn plant was growing. Larvae were placed with new plants every week, or before, if necessary, and kept in the containers for 25 to 30 days, sufficient time to complete the bioassays. Foliage was cut at soil level in the containers to stimulate root growth (Nájera 1998).

Viability of the conidia was determined by means of Jimenez's technique (1992) before carrying out the bioassays. Later, pathogenicity was estimated with the objective of selecting only the most virulent strains of fungi. Three strains of *B. bassiana*, designated Bb4, Bb25 and Bb26, isolated originally from *Hypothenemus hampei* (Ferrari) (Coleoptera: Scolytidae) collected in Ecuador and Mexico were employed (De la Rosa et

al. 1997). Three strains of *M. anisopliae* (Ma3, Ma5 and MaN6) previously isolated from species of Homoptera and Lepidoptera (De la Rosa et al. 1995), and two strains of *M. anisopliae* (MaN7 and MaN8) isolated from *Phyllophaga* spp. larvae (this study) were used. Before the bioassays, larvae were treated with 0.02% sodium hypochlorite to eliminate contamination from the larval body surface (Bruce et al. 1997). Pathogenicity was determined using the technique called the "maximum test" (Shannon et al. 1993). Larvae in Petri dishes were exposed for 20 to 40 seconds to a maximum concentration of spores (1×10^{10} conidia/g of rice, quantified by a Neubauer chamber) of each of the eight strains. Each fungal strain was replicated and evaluated three times, and each replicate consisted of 20 larvae. Thirty days after exposure, the two most aggressive strains, one of each fungus, were chosen for subsequent bioassays.

Lethal time (LT_{50}) bioassays were conducted to determine the time required by the selected strains to kill 50% of the treated larvae (Leucona 1995). The technique proposed by Shannon et al. (1993) was used. Twenty larvae were inoculated with a maximum concentration of conidia of the selected strain (1×10^{10} conidia/g of rice); this procedure was repeated three times (60 larvae in total/strain). Each larva then was placed on a 50-cc (5.5×5cm) plastic tray with sterile soil and a piece of carrot ($1.5cm^3$) as food. The number of dead larvae was recorded daily for 30 days. Dead larvae were placed with wet filter paper in Petri dishes to stimulate the development of fungal mycelia and confirm that the fungus killed the larvae.

Five concentrations of conidia, 1×10^6, 1×10^7, 1×10^8, 1×10^9, and 1×10^{10} conidia per gram of rice, and a control (without conidia) were evaluated. Larvae were inoculated with the strains as described in the pathogenicity bioassays. Larvae inoculated with fungi were fed as discussed above and maintained at 27±2°C, 80±5% RH and 12:12 L:D. White grubs were checked daily and mortality was recorded if the larva did not move when the thorax was touched. To confirm that fungus caused the death, the larva was placed onto wet filter paper to stimulate development of mycelia. Four replicates for each concentration and a control were carried out. Twenty larvae per replication were used (a total of 120 larvae per strain).

The most virulent strains of *B. bassiana and M. anisopliae* selected in the lethal concentration bioassay (LC_{50}) were evaluated under field conditions. For the massive production of the fungi in solid substrate, the method proposed by De la Rosa and Barrera (1997) was used. The experiment was conducted in the municipality of Teopisca, Chiapas, México, 16° 32′ 24″ North Latitude and 92° 28′ 19″ West Longitude, at 1,780 masl (INEGI, 1984). The weather is subhumid-temperate with a rainy summer (total annual precipitation = 1,316mm) and annual mean temperature of 16.6°C; soil types are litosol, regosol and luvisol (INEGI 1988). *Phyllophaga menetriesi* (Blanchard) and *Phyllophaga ravida* (Blanchard) are the most destructive species of white grubs on corn in the study area (Ramírez and Castro 2000). The experiment was conducted using a random block design with six treatments: 1) *P. menetriesi* + *B. bassiana*, 2) *P. ravida* + *B. bassiana*, 3) *P. menetriesi* + *M. anisopliae*, 4) *P. ravida* + *M. anisopliae*, 5) *P. menetriesi*, and 6) *P. ravida*. Ten blocks for each treatment were used. The experimental plot consisted of a cylindrical container (35×45cm) of metal wire (mesh size 4×4mm) to avoid escape by the white grubs. Five kg of sterile soil was deposited in each container. The soil was sterilized by the "solarization method", followed by dry heating (Melera 1998). Fungi were applied first before the corn was sown. The rice, which contained the conidia, was combined with the soil, to form a homogenous mixture within the container. Three seeds of corn of a local variety (criollo) were sown in each container. Forty days after sowing, six third-instar larvae of *P. menetriesi* or of *P. ravida* were introduced into each container. A second application of the fungi was carried out in each plot a day after the

introduction of the larvae (Cortés et al. 1994). A maximum concentration of 1×10^{12} conidia/g of rice (see results of LC_{50}) was used in both applications. Twenty days after the second application, all grubs were removed from each plot. The number of dead larvae was recorded for each treatment. The dead larvae were placed in a controlled humidity chamber to stimulate the development of mycelia. In addition, direct damage to the root (dry weight) and the effect of larval damage upon biomass production, including the weight of foliage and root, total plant weight, plant height and extend of plant cover were evaluated and recorded during one week of observation (Castro et al. 1998).

B. bassiana and *M. anisopliae* strains were selected according to their maximum mortality percentage. For the LT_{50} and LC_{50} tests, original data were corrected by Abbot's formula (1925) and analyzed using the program PcProbit (Camacho 1990). For LC_{50}, the mortality-dose relationship of the strains was established to select the most virulent strains. Original data from the field study were corrected by logx + 1 (Steel and Torrie 1992) to homogenize variances and then subjected to one-way analysis of variance (ANOVA), followed by the Tukey test ($P. < 0.05$) (SAS Institute Inc. 1991-1992).

RESULTS AND DISCUSSION

From the samples taken in the different municipalities, 12 *B. bassiana* and four *M. anisopliae* strains were collected. *B. bassiana* was more frequently collected than *M. anisopliae*. Studies with *Metarhizium* upon *Phyllophaga* spp. have showed that strains present high virulence when they come from the same soil where individuals of the *Phyllophaga* genus are present, but not necessarily from the same species (Shannon 1994). In this study, fungal strains were collected from species such as *P. menetriesi*, *P. ravida*, *P. dasypoda* (Bates) and *P. parvisetis* (Bates). The former two species were found in Teopisca municipality, where they are pests of corn (Ramírez and Castro 2000), while the last two were found in Villa Comaltitlán municipality, Chiapas. In Villa Comaltitlán, two *M. anisopliae* strains (MaN7 and MaN8) were isolated; the rest of the strains were eliminated because of low viability of their conidia. Lack of viability could have been caused by unsuitable conditions during transport from the field to the laboratory. Conditions in the laboratory also may affect original growth features and sporulation (Ouedraogo et al. 1997).

Pathogenicity test results from several *B. bassiana* and *M. anisopliae* strains against third-instar larvae of white grubs 30 days after inoculation are shown in Table 1. In general, all strains tested were pathogenic, although mortality ranged from 21.6 to 90.0%. Five of the eight strains caused 50.0% mortality. Four *M. anisopliae* strains caused mortality from 50.0 to 90.0%; the least pathogenic strain was Ma3 (21.6%) and the most pathogenic was MaN7 with 90.0% mortality. Bb4 and Bb26 strains of *B. bassiana* caused 28.3 to 61.6% mortality, respectively. In general *B. bassiana* strains were less pathogenic to *Phyllophaga* spp. grubs than were strains *M. anisopliae*. Results suggest that future investigations should prioritize *M. anisopliae* strains for the control of *Phyllophaga* spp. Strains derived from other hosts should also be evaluated because they may prove to be even more virulent to this pest (Xu 1988, Prior 1992). Results of the present study agree with those of Shannon et al. (1993) who reported that *M. anisopliae* strains killed 31.3 to 66.7%, while *B. bassiana* strains killed 0.0 to 6.3% of third-instar larvae of white grubs. However, Berlanga and Hernández (1999) found that both fungi caused similar mortality; *B. bassiana* strains killed 46.7 to 93.3% and *M. anisopliae* strains killed 50.0 to 93.3% of third-instar larvae of white grubs.

Lethal time bioassays (LT_{50}) with *B. bassiana* indicated that the time before 50% of white grubs died ranged from 4.9 to 90.9 days, with strain Bb26 having the lowest LT_{50}

value of 4.9 days (FL_{95} = 3.6 to 7.1 days). Three strains differed significantly in the length of time before 50% of the white grubs were killed (Table 2). The mean LT_{50} of all *B. bassiana* strains was 42.8 days (SE = 21.9 days). For the five *M. anisopliae* strains, LT_{50} ranged from 6.1 to 58.0 days, with MaN7 having the lowest LT_{50} value of 6.1 days (FL_{95} = 4,6 to 7,7 days). There was a significant difference among the five strains evaluated (Table 2), with a mean LT_{50} of 25.6 days (SE = 11.2 days) for all *M. anisopliae* strains evaluated.

TABLE 1. Pathogenicity of Several *Beauveria bassiana* and *Metarhizium anisopliae* Strains Against Third-Instar Larvae of White Grubs (*Phyllophaga* spp.).

Strain	Country of origin	Original Host	Treated larva	Larval mortality	% Mycosis
Bb4	Ecuador	*Hypothenemus hampei* (Coleoptera: Scolytidae)	60	17	28.3
Bb25	Mexico	*Hypothenemus hampei* (Coleoptera: Scolytidae)	60	25	41.6
Bb26	Mexico	*Hypothenemus hampei* (Coleoptera: Scolytidae)	60	37	61.6 [a]
Ma3	E.U.A.	Commercial strains	60	13	21.6
Ma5	Mexico	*Spodoptera frugiperda* (Lepidoptera: Noctuidae)	60	30	50.0
MaN6	Mexico	Not identified (Homoptera: Cicadidae)	60	34	56.6
MaN7	Mexico	*Phyllophaga* spp. (Coleoptera: Scarabaeidae)	60	54	90.0 [a]
MaN8	Mexico	*Phyllophaga* spp. (Coleoptera: Scarabaeidae)	60	32	53.3
Control	-	-	60	0.0	0.0

[a] Highest percentages of mycosed larvae

The most homogeneous populations were obtained from Bb26 (0.66) and MaN7 (1.77) because of their steeper slope values, compared with Bb25 (0.33) and MaN6 (0.58), which were the most heterogeneous populations because of their lower slope values (Lagunes 1991). Bb26 and MaN7 strains had the lowest LT_{50} values against third-instar larvae of white grubs and caused the greatest percentages of mortality in the pathogenicity test, with 61.6 and 90.0%, respectively. The LT_{50} values of 4.9 and 6.1 days for *B. bassiana* and *M anisopliae*, respectively, are similar to those from other studies with these fungi. According to Berlanga and Hernández (1999), the LT_{50} values for *B. bassiana* and *M. anisopliae* strains were 4.5 and 5.8 days, respectively. The differences in LT_{50} between the different strains may be caused by several factors such as the type of hosts where strains were isolated, host tolerance, and intrinsic characteristics such as germination time (GT_{50}) and variable ability to infect hosts because different enzymes of each strain (Madelin 1963, Bidochka and Khachatorians 1990). Poprawski and Yule (1991) reported similar results with topical applications of *M. anisopliae* killing 64% of

second-instar and 52% of third-instar larvae of *Phyllophaga* spp., whereas *B. bassiana* caused 39% and 28% mortality, respectively.

TABLE 2. Mean Lethal Time (TL_{50}) of Several *Beauveria bassiana* and *Metarhizium anisopliae* Strains Against Third-Instar Larvae of White Grubs (*Phyllophaga* spp.).

Strain	Treated larvae	% Mycosis	LT_{50}[a] (Days)	FL95% (Days)	Regression line equation	x^2
Bb26	60	61.6	4. 9 a	3.6 - 7.1	Y = 3.95 + 0.66X	1.20
Bb25	60	41.6	32.7 b	11.8 – 56.7	Y = 3.82 + 0.33X	0.57
Bb4	60	28.3	90.9 b	35.1 – 112.2	Y = 2.90 + 0.47X	0.24
MaN7	60	90.0	6.1 a	4.6 - 7.7	Y = 3.61 + 1.77X	1.70
MaN6	60	56.6	8.8 ab	6.0 – 21.3	Y = 3.73 + 0.58X	2.25
MaN8	60	53.3	15.9 ab	10.1 – 44.7	Y = 3.47 + 1.17X	1.14
Ma5	60	50.0	39.1 bc	28.0 – 82.0	Y = 2.30 + 1.69X	4.01
Ma3	60	21.6	58.0 bc	43.2 – 113.7	Y = 2.27 + 0.61X	0.20

[a] Means with the same letter were not significantly different, according to 95% FL.

Virulence tests (LC_{50}) carried out using probit analysis (Finney 1971), showed that MaN7 was the most virulent strain of *M. anisopliae* with an LC_{50} value of 1.29×10^8 conidia/g ($FL_{90} = 4.0\times10^7$ to 4.1×10^9 conidia/g), while Bb26 was the most virulent strain of *B. bassiana*, with a value of 1.15×10^9 conidia/g ($FL_{90} = 2.0\times10^8$ to 6.6×10^{10} conidia/g) (Table 3). Slope values of the regression lines (MaN7 = 0.25 and Bb26 = 0.35) indicated that Bb26 with the higher value gave the most homogeneous response, while MaN7 was more heterogenous. Variation in virulence between the strains might be related to the production and activity of enzymes during penetration of the host cuticle, the species of fungus, the strain (pathotype), and/or host combination assays (Roberts 1989, Bidochka and Khachatorians 1990, Hayden et al. 1992, Ignoffo and Boucias 1992).

TABLE 3. CL_{50} Values for *Beauveria bassiana* and *Metarhizium anisopliae* Against Third-Instar Larvae of White Grubs (*Phyllophaga* spp.).

Strain	CL_{50}[a] (conidia/g)	90% Fiducial limits	CL_{90} (conidia/g)	Regression line equation	x^2
MaN7	1.29×10^8 a	4.0×10^7 - 4.1×10^9	1.77×10^{12}	Y = 4.72 + 0.25X	0.02
Bb26	1.15×10^9 b	2.0×10^8 - 6.6×10^{10}	5.36×10^{12}	Y = 4.27 + 0.35X	0.16

[a] Values with different letters, were significantly different, according to 90% FL.

The application of *B. bassiana* upon the herbivores (Treatments 1 and 2) did not result in significantly greater total weight or stem weight of the corn plants as compared to plots of corn, where *P. menetriesi* and *P. ravida* were present without aplication of *B.*

bassiana (Treatments 5 and 6). However, application of *M. anisopliae* (Treatments 3 and 4) had a negative effect on these two variables with respect to the values found in the controls (Treatments 5 and 6). The other variables evaluated were not affected by application of fungi (Table 4). Also, only a few larvae were affected by application of fungi. The minimal effect of the fungi on *P. menetriesi* and *P. ravida* could be related to environmental factors such as relative humidity, temperature, solar radiation, and moisture content of the soil that can affect germination, survival, and infection by the fungi (Lingg and Donaldson 1981, Bidochka and Khachatorians 1990). Also, the intrinsic characteristics of each strain may affect performance (Goettel and Inglis 1994). On the other hand, the methodology used in the present study may have affected fungal performance. Furthermore, sterilization may have altered soil characteristics, allowing the development of microorganisms antagonistic to the fungi (Villani et al. 1994).

TABLE 4. Development of Corn Plants Attacked by White Grubs (*Phyllophaga* spp.) Treated with Fungi *Beauveria bassiana* and *Metarhizium anisopliae*.

Treatment	Total dry Weight [a] (gr)	Stem Weight [a] (gr)	Root Weight [a] (gr)	Height Increase [a] (cm)	Covering Increase [a] (cm)	Dead Larvae With mycelia	Disease Symptoms On larvae
1. *P. menetriesi* + *B. bassiana*	14.51 a	11.40 a	3.11 a	3.29 a	3.31 a	0	0
2. *P. ravida* + *B. bassiana*	14.03 ab	10.91ab	3.12 a	3.21 a	3.06 a	1	1
3. *P. menetriesi* + *M. anisopliae*	11.77 bc	9.35 bc	2.42 a	2.58 ab	3.25 a	1	3
4. *P. ravida* + *M. anisopliae*	10.30 c	8.34 c	1.96 a	2.37 b	3.76 a	0	1
5. *P. menetriesi*	14.30 ab	11.17 ab	3.13 a	2.81 ab	3.46 a	0	0
6. *P. ravida*	14.00 ab	11.12 ab	2.88 a	2.91 ab	2.53 a	0	0

[a] Means with the same letter were not significantly different according to Tukey's test ($P<0.05$).

The fact that only two larvae showed signs of mycosis could be related to latency of the fungal spores; spores of *B. bassiana* may take up to 8 weeks to germinate under natural conditions (Griffin 1963), longer than the duration of the experiment in the field. Germination in the field is difficult to determine and is not known for *B. bassiana* or *M. anisopliae* in relation to corn. The timing of the experiment was based on the biological cycle of white grubs, with third-instar larvae causing the greatest damage by rapidly devouring roots of the plants (Ramírez and Castro 2000). The presence of fungistatic substances, such as exudates and larval feces in the soil, may interfere with spore germination and mycelial growth and should be considered (Sandhu et al. 1992). At the end of the study, only five grubs showed disease symptoms (brown, darkened lesions on

the dorsal thorax) caused by *B. bassiana* or *M. anisopliae*. These symptoms coincide with those described by Lozano et al. (2000) on *Serica* sp. larvae (Scarabaeidae) attacked by *M. anisopliae*. The duration of the experiment (20 days) probably was not long enough for these fungi to affect white grubs. Other field experiments with a longer exposure time revealed that *M. anisopliae* acts slowly, with the pests continuing to die several months after application of the fungus (Samson et al. 1997). Although *M. anisopliae* is a potential candidate for a biological insecticide against white grubs, it probably will be more successful as a prophylactic rather than remedial control agent (Samson et al. 1997).

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SPATIAL ANALYSIS OF DENGUE CASES IN GUADALUPE, NUEVO LEON, MEXICO 1995-96.

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ABSTRACT

The spatial and temporal distributions of dengue fever cases in Guadalupe, Nuevo Leon, Mexico, were analyzed using a geographic information system (GIS). Data were obtained from the Secretary of Health of Nuevo Leon and mapped for the Municipality of Guadalupe, NL, using CARTALINX and IDRISI programs. Confirmed cases of dengue (689) occurred mostly during October (42.3%) and November (43.9%). More females (59.1%) were infected than males. Individuals 21 to 30 years of age had the highest prevalence (25.3%). Dengue cases were ranked at 5 levels: 1= 1-2 cases; 2= 3-5; 3= 6-7; 4= 8-9, and 5≥ 9, and then mapped. The spatial distribution of 545 cases was concentrated in the southern part of city in 1995 and in the south/central part in October 1996. However, in November the concentration was in the west central region of the city. Factors like human population densities and failure of basic sanitary services contributed to the incidences and distribution of dengue cases in Guadalupe.

INTRODUCTION

The most recent outbreaks of dengue in Bangkok and Puerto Rico, usually vectored by *Aedes aegypti* (L.), indicate that infection rates are higher in young children and women than in adult males (Halstead et al. 1969, Morens et al. 1986). Similarly, children of school age in Puerto Rico showed higher attack rates by mosquitoes than other populations (Rodriguez-Figueroa et al. 1995). In 1996, a record high number of classic dengue cases occurred in Nuevo Leon. In the municipality of Guadalupe, NL, in 1995, 545 cases were officially registered. It is one of the municipalities of the state with a high incidence of dengue (SSA 1997).

The use of geographical information systems (GIS) and remote sensors have been shown to be quick and exact methods in the acquisition of data for the control of mosquitoes in two counties of Michigan (Wagner et al. 1979, Washino and Wood 1994). The satellites LANSAT 1 and 2 were used to detect the larval habitats of mosquitoes (Hayes et al. 1985). Riley (1989) presented a revision of the use of remote sensors in entomology; see also Hugh-Jones (1989) on the application of remote sensors in the study of vector transmitted illnesses.

In Israel, distances between population centers and breeding sites of species of *Anopheles* (Kitron et al. 1994) were calculated to propose systems of surveillance based on GIS. The characterization of the composition of the landscape was evaluated for the exposure risk to Lyme disease in 337 residential properties (Dister et al. 1997). They found significant association with the vegetation and areas with water. Kitron and

Kazmietcsak (1997) compared surveillance measures for cases of Lyme disease in Wisconsin and their association with vegetation; they generated maps of the counties with high risk for the transmission of this disease. Becker et al. (1998) evaluated the geographical epidemiology of 7,330 cases of gonorrhea in Baltimore, Maryland, during 1994 cases were ranked in quartiles and analyzed by means of GIS. Cases of dengue (8,689) were studied in the municipality of Florida, Puerto Rico. Using GIS, Morrison et al. (1998) determined the distance among cases in intervals of time of 0-10, 11-20 and 21-30 days. They found that more than 80% of the pairs of neighbors' cases were within 500 m of one another.

Advances in the GIS technology provide new opportunities for epidemiologists to study associations among exposures in the spatial distributions of illnesses (Vine et al. 1997). Kitron (1998) reviewed the GIS, global positions, remote sensors and statistical space, and tools to analyze and to integrate spatial components in the epidemiology of illnesses transmitted by vectors. Kitron suggested surveillance and control programs based on approaches to the ecological landscape. The purpose of this study was to determine the spatial and temporal distributions of dengue fever cases in Guadalupe, Nuevo Leon, Mexico, during 1995-96 using GIS.

MATERIALS AND METHODS

This survey was conducted in the municipality of Guadalupe, Nuevo Leon, Mexico, to the east of the metropolitan area of Monterrey, population four million, with a surface area of 118.737 km^2 at 25° 37′ 20″ to 25° 44′ 7″ north latitude and 100° 12′ 58″ to 100° 16′ 29″ west longitude (2834190 and 2846303 N and 372051 and 386674 W in UTM). It is the second largest city in size after Monterrey in Nuevo Leon, Mexico, with 618,933 inhabitants. This corresponds to 17.4% of the state's population (INEGI 1995) with 29.34% of this population being over 12 years of age.

Data were provided by the Central Laboratory of the Secretary of Health of the state. The data bases for the cases included years, age and sex. Cases were defined at five levels: 1-2 cases=1, 3-5=2, 5-7=3, 8-9=4, and > 9=5.

Map bases were digitized using aerial photographs with CARTALINX and taken as polygons in the geostatistics basic areas (AGEB's); ID's of polygons were assigned. The data of the dengue cases that corresponded to the months of October and November of every year were added. These maps were exported to IDRISI.

RESULTS

Of the 689 dengue cases recorded in this study, 545 were reported during 1995 and 144 were reported in 1996. Women were more affected (59.1%) than men (40.9%); the ratio of infected women/men was 1.39 and 1.67 in 1995 and 1996, respectively.

Cases of dengue were tabulated according to the year and month in which they were reported (Table 1). The months of highest dengue incidences were October and November (43.9% in both genders), corresponding with the autumn period following highest rainfalls. During the two years included in the study, only ten cases were reported during the first six months of each year; therefore, they were not considered. In July and August, there were five (0.8%) reported cases. In September, after the July-August rains, 53 (7.7%) cases were recorded, and finally the number of cases dropped to 25 (3.7%) in December. The minimum age of an infected individual was one year, while the maximum

ages were 78 and 82 years for women and men, respectively. The corresponding averages were 26.1 years for women and 32.3 years for men.

TABLE 1. Cases of Dengue Reported by Month and Year of Occurrence in 1995 and 1996 in the Municipality of Guadalupe, NL, Mexico.

Month	1995	1996	Total	(%)
Jul.	0	1	1	(0.2)
Aug.	0	4	4	(0.6)
Sep.	23	30	53	(7.7)
Oct.	248	55	303	(43.9)
Nov.	255	48	303	(43.9)
Dec.	19	6	25	(3.7)
Total	545	144	689	(100)

Cases presented by year, sex and patient age interval are given in Table 2. The greatest density of infections (25.3%) was at 21-30 years of age. It is important to note here that at ages up to 10 years, 59 and 55 cases were presented for females and males, respectively, while there were 58 female and 30 male cases for 50 year-old adults and older.

TABLE 2. Numbers of Cases of Dengue Classified by Year, Sex and Age Intervals, for the Municipality of Guadalupe, NL, Mexico.

Year	Sex	Age interval								Total	(%)
		1-10	11-20	21-30	31-40	41-50	51-60	61-70	>70		
1995	W	48	47	79	52	45	31	13	2	317	46.0%
	M	46	42	58	26	30	15	7	4	228	33.1%
1996	W	11	16	18	16	17	5	6	1	90	13.1%
	M	9	9	19	8	5	3	0	1	54	.8%
Total	W	59	63	97	68	62	36	19	3	407	59.1%
	M	55	51	77	34	35	18	7	5	282	40.9%

In Fig. 3, the spatial distribution of the cases of dengue is presented for October (a) and November (b) of 1995 as well as for October (c) and November (d) of 1996. The five levels correspond to 1=1-2 cases, 2=3-5, 3=6-7, 4=8-9 and 5= >9 cases per polygon.

The level one polygon for the most part was randomly distributed over the whole municipality; nevertheless, the greatest density of cases was located in the south of the city. A greater distribution of the polygons was observed with levels 4 and 5 toward the south and southwest during 1995 and toward the center in 1996.

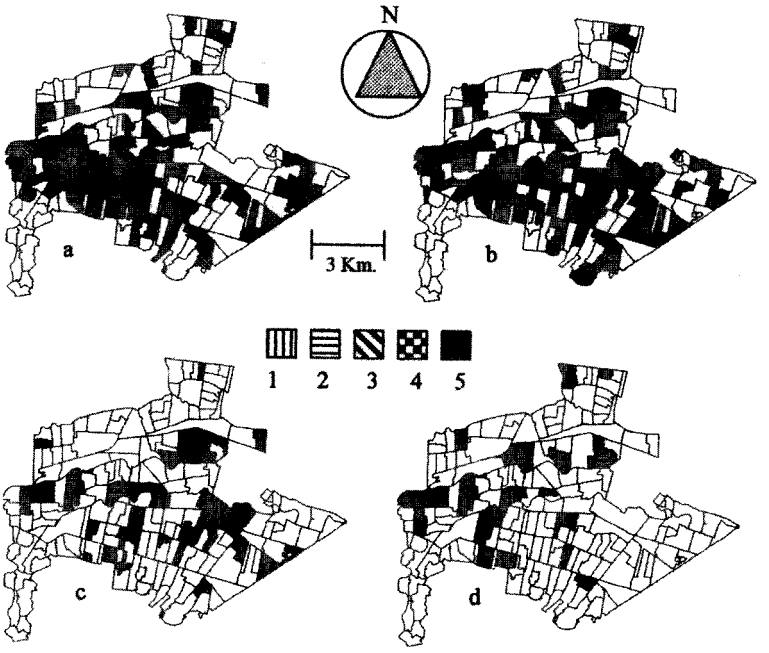

FIG. 3. Spatial distribution of the cases of dengue during a) October, b) November of 1995 and c) October, d) November of 1996 in the municipality of Guadalupe, N. L., Mexico. (Categories: 1=1-2, 2=3-5, 3=6-7, 4=8-9 and 5≥ 9 cases)

DISCUSSION

In northeastern Mexico, the months of higher temperatures are July and August, and those with more rain are August and September. In the current study, October and November were the months of higher dengue incidence in the state of Nuevo Leon, agreeing with data published by Ram-Shobha et al. (1998) for the dengue outbreak in India. Months of the year with higher rainfall and temperatures may trigger conditions for larval habitats, increasing in towns where water is stored (Gubler and Trent 1994). The higher temperatures may produce small females that are forced to take increased numbers of blood meals and therefore are contributing with higher vector bite frequency (Focks et al., 1995).

Age and sex are two factors that influence infection rates in humans. It was found that 59.1% of the total dengue cases were reported in women, and this agrees with studies by Halstead et al. (1969) and Morens et al. (1986). However, 7.9% of cases occurred in

10 years-old girls, which does not agree with data published by Rodriguez-Figueroa et al. (1995) who established that children of school-age presented higher rates of attack by mosquitoes than other groups. Of the total cases, 12.1% were reported for people older than 50 years of age. Most cases by age group were in the 21-30 years-old bracket, with 25.1% of infections in Guadalupe occurring in this age group.

A total of 183 polygons were drawn in the Municipality of Guadalupe, Nuevo Leon, and they agree with the basic geographical units used by the National Institute of Geography and Statistics of Mexico for the handling of demographic data.

The most populated area in the municipality is in the south, where the neighborhoods are relatively new, and some lack appropriate sanitary services. Also people store water in barrels used by *Ae. aegypti* as breeding sites and this agrees with results of Gubler (1994), as far as environmental factors that lend to universal incidences of dengue transmission.

The map bases, generated by the digitalization of the polygons, were exported to the package IDRISI for their spatial analyses. Most of the cases found in 1995 were distributed spatially toward the south and the west of the municipality (Fig. 3). The polygons in the southern part of the city, where there are recently formed neighborhoods, were at the city limits. To the west, there are neighborhoods with businesses and industries near the long-established colonies of Monterrey, Nuevo Leon, that have well-kept neighborhoods.

The spatial distribution of the dengue cases in 1996 (Fig. 3) changed from the south-central part of the city in October to west-central in November, and these two months had the highest dengue incidences in both years of this study. The linear TREND analysis indicates a clear tendency toward the southern portion of the municipality in October and November of 1995, while in 1996, the trend was toward the central part of the city.

In conclusion, GIS is a tool which can be used to efficiently and effectively determine the spatial distribution of dengue cases and to produce risk maps for this disease. Concentrating efforts on those areas demonstrated to be at high risk can effect economies.

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BIOLOGY AND LIFE HISTORY OF *HYLESIA IOLA*[1] DYAR, A CORN-LEAF FEEDER OF TLAXCALA, MEXICO

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ABSTRACT

The life history of *Hylesia iola* Dyar was studied in the field and in confinement at Colegio de Postgraduados, Montecillo, State of México. During the fall, a female *H. iola* lays eggs on twigs of the capulín tree in a mass protected with a ball of foam which she secretes. Eggs hatch in late spring, and the first three instars feed on the leaves of the tree. Larvae of the last three instars feed on corn leaves. After pupation, adults emerge from mid until late fall to start a new cycle.

RESUMEN

El ciclo biológico de *Hylesia iola* Dyar se estudió en el Colegio de Postgraduados, Montecillo, Estado de México. La hembra de *H. iola* deposita sus huevos en masa durante el otoño, cubriéndolos con una bolita de espumalina que ella misma secreta y adhiere a las ramitas terminales del árbol de capulín. Las larvas emergen al final de la primavera y los primeros tres ínstares se alimentan de las hojas del árbol; las larvas de los últimos tres ínstares se alimentan de las hojas del maíz. Después pasan al estado pupal y de mediados a finales del otoño emergen los adultos para iniciar un nuevo ciclo.

INTRODUCTION

The many species of the neotropical genus *Hylesia* Hübner are found from Argentina to México and include some of the smallest species of Saturniidae. Most are inconspicuous brown moths with diffuse markings, although some have red or orange spots on the hind wings. *Hylesia* larvae are leaf-feeders that cause skin irritation known as "papilonitis" or "lepidopterism" [Ferguson 1972, Lamy and Lemaire 1983 (cited by Zamora 1988)]. Adult *Hylesia* spp. are pests in tropical America because of their possession of urticating hairs (Ferguson 1972).

Very little is known about feeding habits of *Hylesia* except that the larvae are leaf-feeders, gregarious on a variety of hosts. *Hylesia alinda* Druce (as cited by Ferguson 1972) feeds on avocado, *Persea americana* Mill., in Costa Rica, and *H. nigricans* Berg. on *Populus* sp. in Uruguay. *Hylesia continua alinda* Druce was reported on *Psidium Friedrichsthalianum* (Berg.) Nied at San Antonio de Coronado, Costa Rica (Calvo 1994). In Argentina, *Hylesia* spp. attack a wide range of plants, including shrubs, forest species, fruit

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trees, ornamental plants, and grasses. *H. nigricans* is an important leaf feeder on forest trees at Chequeno Seco Park in Argentina (Fiorentino et al. 1996). Hosts for *H. frigida* Schaus are *Arbutus glandulosa* Mart. et Gal., at least six species of *Pinus,* and two *Quercus* (Cibrián et al. 1995).

Hylesia species are distributed widely in México and have been found in the states of Nayarit, Colima, Guerrero, Puebla, Veracruz, Tabasco, and Chiapas (Zamora 1988). Beutelspacher and Balcázar (1994) listed 20 *Hylesia* species from different tropical localities and from the high plateau of México. They recorded *H. iola* at San Angel, Distrito Federal and Yautepec, Morelos.

H. iola has been found by corn growers at Huamantla, Tlaxcala, since at least 1990. However, the biology and behavior of the insect and the extent of damage caused to the capulín tree or to corn are unknown; there are no adequate methods for controlling the pest. The purpose of this study was to assess the development and life history of *H. iola* under field conditions and in confinement both in the laboratory and in the field.

MATERIALS AND METHODS

The field site was at Ejido San Luis Huamantla, Municipio of Huamantla, State of Tlaxcala, México, where corn is grown in plots surrounded by capulín trees. The area was visited every other week from April to December 1998 and from January to December 1999, to determine the time at which first hatch occurred and to study *H. iola* development. Activities of the larvae on capulín, corn, and other host plants were observed.

Studies on the life-history of *H. iola* were conducted at the Instituto de Fitosanidad, Colegio de Postgraduados, Montecillo, State of México. About 50 egg masses were collected in the field at Huamantla during April and in May 1998, respectively. Six of the egg masses were randomly selected, dissected, and the numbers of eggs per mass counted. Three of these masses per month were fastened on individual capulín twigs about 25cm long in a manner similar to that of deposition of egg masses by the female in the field. Each twig was placed in a glass jar (25.5cm high × 16cm diameter) with water to keep it turgid. The jars were covered with organdy mesh and placed in a rearing chamber at a temperature ranging from 27 to 29°C and a relative humidity of 20 to 30%. Numbers of eggs hatched, numbers of live and dead larvae, and stages of larval development were recorded daily. Capulín twigs were replaced every other day.

The life history of *H. iola* under natural conditions was studied on a capulín tree on the grounds of the Colegio de Postgraduados. Three egg masses brought from Huamantla were dissected and the eggs counted. In May, each egg mass was tied singly on a twig of the tree and covered with a small organdy bag to confine the larvae following egg hatch and to exclude predators. Data recorded were the same as for the specimens reared in the laboratory.

In October 1998, when *H. iola* reached the pupal stage at Huamantla, about 30 pupae were collected and taken to a laboratory. They were placed in a cage (30×30×30cm) to observe adult emergence. Adults that emerged were killed, pinned, and sent for identification to the Laboratorio de Entomología, Instituto de Biología, Universidad Nacional Autónoma de México.

RESULTS

Female *H. iola* laid eggs in a mass covered with a ball of fibrous foam about 1cm in diameter. The balls, resembling abacus beads, were fastened to terminal twigs of capulín trees where they remained throughout the winter and most of the following spring.

Oviposition began during late November 1998 and late October 1999. In 1998, the first eggs hatched by the end of June; whereas, in 1999 the larvae emerged in early June about 1 week after the rainy season began. Egg hatching therefore may have been stimulated by increasing humidity. Four other egg masses brought from Huamantla were placed in a carton (8.5cm in diameter × 9cm high) with a wet sponge on 3 April 1998; the eggs hatched 2 days later, about 2 months before egg hatch occurred in their natural habitat. In the field, first-instar larvae remained inside the fiber ball for protection for 2 weeks after emergence. The larvae built small tunnel-like galleries through the fiber balls to emerge and fed safely on nearby tree leaves. Upon reaching the third instar, larvae became more active and feeding damage to capulín leaves was more evident. The larvae were not observed feeding during the day; therefore, we assume feeding occured at night.

Larvae reached the fourth instar by the first week of August 1998. At that time, larvae descended the trunk of the tree in lines of 10 to 40 larvae, one behind another. The larvae invaded nearby corn plants as well as "acahual", *Simsia amplexicaulis* Cav. (Compositae), and "chayotillo", *Sicyos angulata* L. (Cucurbitaceae).

In 1999, a different behavior was observed. In late June, more than 20 groups of larvae were observed clustered inside or on the fiber balls. Other groups of larvae were clustered at the ends of dry twigs. In several trees, larvae ranged from second to fourth instar. Some larvae were descending in lines from the tree; most were fourth instars, but a few second and third instars were present among the older larvae. Two colonies of numerous larvae were clustered on different trunks. The larvae were motionless, strongly fixed to the substrate, and did not react to attempts to use a stick to dislodge them from the trunk.

About 2 weeks later, the larvae were sheltered in tubular cells or small bags of silken threads and rolled or folded pieces of corn leaves. Groups of five to 20 larvae were motionless in each cell during the day when observations were made. It was evident that the larvae fed on corn leaves because, 2 weeks after their arrival, most of the leaves except the central vein of infested plants were severely damaged. Damage was more severe on plants closer to the tree but occurred as far as 8-10m away. Two weeks later, the larvae had dispersed throughout the field, infesting plants as far as 35m from the capulín trees. Empty cells or cells containing fewer larvae were observed on plants near the trees. The larvae that had dispersed were isolated on the corn leaves or in small bags formed of rolled leaf fragments which contained one to four larvae. Leaves of the plant were damaged to different degrees, from small portions of the leaf eaten to the entire leaf consumed except the central vein. Larvae did not disperse any farther, and completed six instars. The first pupae were observed during the second week of October 1998 and the first week of September 1999. In 1998, insects were in the pupal stage through November. The first adults were active in the field and there were new egg masses on the capulín trees in early December.

Adult females differed from the males in appearance. They were pink in color and larger in size than the males, with a 65-mm wing span and with filiform antennae. Males were light brown, with a 50-mm wing span and plumose antennae. Females rested on terminal twigs of capulín trees. Other females and males rested on dead stems of weeds or fluttered slowly on the ground. A few adults flew away from the ground in an undefined direction.

In 1999, the first adults were observed in the field by the end of October, about 5 to 6 weeks earlier than in 1998. Adults rested on stalks of mature, dry corn plants. Although no adults were observed on capulín trees, three new egg masses had been deposited on low twigs of each of two capulín trees 300m apart. By the end of November a few adults were actively ovipositing on the twigs, and numerous egg mass balls were observed. Two pair were copulating, and one to ten single adults were counted on each of ten trees examined.

No adults were observed, but egg masses had been deposited on ten other capulín trees. The egg balls were isolated on twigs, or as many as three balls were deposited within 15cm of each other on a single twig.

In laboratory observations, more than 100 eggs (range = 112-198; average = 145 for nine masses) were deposited within each ball. Most of the eggs were deposited in the core, then covered with a fiber foam that was soft at the moment of oviposition. The eggs, about 1mm long, were opaque white, oval, and lightly depressed on the sides, with a hard chorion. Upon hatching, first-instar larvae were about 1mm long and light orange in color, with scoli and bundles of setae along the body; the round head had little sclerotization. Second-instar larvae were similar in appearance to first-instar larvae, but the head, scoli and setae were darker. These structures became more conspicuous in the later instars. Last instar larvae were 50-55mm long and very robust. Molting from each instar to the next was determined by the exuviae.

Some adults copulated in the cage in the laboratory. The male flew or fluttered around the female, climbed on her, and initiated copulation. While copulating, the male remained hanging from the female, keeping the wings open. One to two days later, the female oviposited on previously supplied capulín twig fragments. The eggs were laid and covered with a foam ball.

Larvae from two egg masses developed to the third instar; larvae from the third egg mass reached only the second instar. In one mass (116 eggs), 86% eggs hatched; 78% of the larvae reached the second instar and 55% reached the third instar (Table 1). In a second mass (127 eggs), 81% eggs hatched; 68% of the larvae reached second instar and 32% reached third instar. In a third mass (112 eggs), 74% of the eggs hatched and 67% of the larvae reached second instar. Very likely, rearing chamber conditions were not ideal for development. Even more larvae emerging from the second group of egg masses died before maturing (Table 1).

TABLE 1. Development of *Hylesia iola* Dyar in a rearing chamber. Montecillo, Méx., México.

Egg mass[a]	No. of eggs	% Hatch	Instars		
			1st	2nd	3rd
1	116	86.2	100	90	64
2	127	81.1	103	86	41
3	112	74.1	83	76	0
4	149	50.3	75	0	-
5	162	0	0	-	-
6	119	42.0	50	9	0

[a] Egg masses 1 to 3 set on 15 April 1998, egg masses 4 to 6 set on 11 May 1998.

The life cycle of *H. iola* confined outdoors was completed in one of three egg masses. Of 198 eggs in this mass, 38% hatched; 33% of the larvae developed to second instar and 16% to third, 16% to fourth, 13% to fifth, 9% to sixth, and 8% to adults (Table 2). In the other two egg masses, 150 and 174 eggs were counted. The larvae which developed from these masses reached only to the first or second instar. In general, only half as many eggs hatched but more instars survived than did those in the laboratory.

Table 2. Development of *Hylesia iola* Dyar in a natural environment. Montecillo, Méx., México.

Egg mass[a]	No. of eggs	% Hatch	Instars						No. of adults
			1st	2nd	3rd	4th	5th	6th	
7	198	37.9	75	65	31	31	26	18	16
8	150	58.0	87	0	-	-	-	-	-
9	174	51.7	90	1	0	-	-	-	-

Egg masses 7 to 9 set on 21 May 1998.

The damage caused by larvae feeding was not as important on capulín leaves as it was on corn. The larvae during the first three instars fed on capulín leaves, then moved to corn during the last three instars when they were more voracious. Furthermore, yield of corn might be affected by defoliation caused by the insect when the grain is maturing.

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BITING SITE SELECTION ON A HUMAN VOLUNTEER BY MEMBERS OF THE *LUTZOMYIA LONGIPALPIS*[1] COMPLEX

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ABSTRACT

Biting site distribution by three members of the *Lutzomyia longipalpis* (Lutz and Neiva) complex was documented on a 29-year-old male human volunteer. Observations were carried out in an experimental cage. Populations originated from Curarigua, Lara State, Venezuela; Jacobina, Bahia State, Brazil; and Marajo Island, Pará State, Brazil. Female sand flies from Jacobina displayed a marked preference for biting on the ears; whereas, those from Curarigua and Marajo exhibited less preference for biting on the ears. Biting selection behavior seemed to be odor-mediated as revealed by female sand fly responses to ear extracts. The time required by each population to locate the host was compared and results showed that female sand flies from Jacobina took significantly less time to find the host than the other two members of the complex.

INTRODUCTION

An interesting aspect of haematophagous insect vectors is their preference to bite on specific body sites. The distribution of bites on human volunteers has been analyzed for several species of blood-sucking insects but mainly for mosquitoes. Three main distribution patterns for bite site selection have hitherto been identified: on the head such as *Aëdes simpsoni* Theobald (Diptera: Culicidae) (Gillett 1967), *Aëdes aegypti* (L.) (De Jong and Knols 1996), *Anopheles albimanus* (Wiedemann) (Knols et al. 1994), *Anopheles atroparvus* Van Thiel (De Jong and Knols 1995) and *Sabethes belisarioi* (Neiva) (Gillett 1971); on the upper body such as *Armigeres malayi* (Theobald) (Basio and Magluyan 1975), *Culex quinquefasciatus* Say (Basio and Magluyan 1975); on the legs, ankles and feet such as *Aedes albopictus* (Skuse) (Basio et al. 1978), *Anopheles arabiensis* Patton (Braack & Gerickel 1996), *Eretmapodites* spp. (Gillett 1971), *Culex pipiens fatigans* Wiedemann (Self et al. 1969), and *Simulium damnosum* Theobald (Diptera: Simuliidae) (Duke and Beesley 1958).

Male external morphological differences led Mangabeira (1969) to suggest that the sand fly *Lutzomyia longipalpis* Lutz & Neiva might be represented by different species. Low insemination rates between Brazilian populations of *Lu. longipalpis* were interpreted by Ward et al. (1983, 1988) as being indicative of a species complex. Currently, three

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members of the species complex can be distinguished by comparing their male sex pheromone. One member produces 9-methylgermacrene-B, which has been identified from males captured in Laphina Cave, Brazil (Hamilton et al. 1996a). This chemotype is widespread throughout Central and South America (Hamilton et al. 1996c). The second chemotype is 3-methyl-α-himachelene, which has so far been found in Brazil and Venezuela (Hamilton et al. 1996b). The third chemotype produces cembrene which is a C_{20} diterpene molecule found only in Northeastern Brazil (Hamilton et al., unpublished data).

In spite of the outstanding role of *L. longipalpis* as a vector of *Leishmania infantum* (Nicolle) in South America, no studies on the biting site selection of sand flies have been undertaken. Therefore the primary objective of the present study was to determine whether or not there was a biting site preference. In addition, we investigated whether biting site selection was odor-mediated, and we also compared biting choices between members of *Lu. longipalpis*. Biting rate in humans is considered to be an important component for vector species in Garrett-Jones' (1964) vectorial capacity model. For this reason, we attempted to establish differences in the time needed for different populations of *Lu. longipalpis* to locate and bite the host and to use these data as representative of the biting attack rate.

MATERIAL AND METHODS

Lutzomyia longipalpis colonies originated from females collected in the field in Curarigua (9° 59' S, 69° 55' W) Lara State, Venezuela; Jacobina (11° 11' S, 40° 31' W) Bahia State, Brazil; and Marajo Island (0° 45' S, 48° 30' W) Pará State, Brazil. Larvae were reared following the methodology of Modi and Tesh (1983). Newly emerged adults were released into 16x16x16 cm nylon net Barraud's cages and provided with a saturated sucrose solution ad libitum. Cages were maintained at 27°C, 80% R.H. and a L12: D12 photoperiod. Flies were maintained in the insectary for six to seven days without blood-feeding before being used in the bioassays.

The experimental design was based on that described by Knols et al. (1994). A male volunteer (EART), aged 29, wearing only close-fitting underpants sat in an upright stationary position on a metal stool positioned in the center of a nylon mesh tent. The tent (1.90x1.70x1.50m) was suspended from the walls of a bioassay room with a mean temperature of 28.1 ± 0.7°C, and R.H. 88.3 ± 6.9%. Illumination was provided by two red fluorescent lights. The volunteer did not bathe for at least twelve hours before the experiments; dietary habits were not controlled. Observations were carried out in the bioassay room during from 09:00 h to 14:00 h daily.

Six- to seven-day-old non-blood-fed sand flies were released into a Barraud's cage and placed on a bench in the bioassay room for one hour before observations were made to allow time for acclimatization. These flies were taken from stock cages at different intervals and may or may not have been mated at the time the bioassays were conducted. Because it is unknown whether the nutritional status previous to an assay could influence the sand fly response, access to sugar was denied during the acclimatization period. Four females were taken from the cage with an electrical aspirator and released into a holding chamber, consisting of a small hollow plastic container (7.5 cm diameter at the bottom and 5 cm on the top), with both sides opened. The base was covered temporally with a table tennis ball to prevent flies from escaping. The other side was covered with a fine fabric mesh and a cotton-wool plug which allowed the introduction of flies into the chamber. The chamber containing the flies was then attached at a height of 1m to the front side of the tent, after which the volunteer entered the tent, sat on the stool, and carefully released the flies from the chamber by pulling a piece of string attached to the closing device.

The bioassays were conducted with at least two members of the *Lu. longipalpis* complex on the same day. However, due to an uneven supply of flies from colonies, it was

only possible to follow this scheme in 32% of all the replicates, and the remaining replicates were carried out on different days. To avoid bias toward either side of the body, the position of the volunteer was alternated between facing toward and facing away from the releasing-device. Flies were allowed an arbitrary period of twelve minutes to search for and bite the volunteer freely before being collected and discarded. The locations of the bites and times taken to the first bite were recorded with a stopwatch. Flies biting the volunteer were removed from the tent with a manual aspirator to avoid counting the same fly more than once. To avoid excessive movement by the volunteer during the experiment, the position of all bites were registered in a notebook immediately after each experimental run had finished. This procedure was repeated until one hundred bites from each population had been accumulated.

Because the observed distribution of bites on the volunteer's body showed a preference for the ears, it was decided to carry out bioassays to measure the response of sand flies to ear extracts. This experiment was conducted to determine whether the attraction of sandflies to ears was odor-mediated. The samples were collected using a small piece of cotton-wool soaked with pesticide residue analysis n-hexane (BDH Laboratory Supplies, Poole, UK). The outer side of the ears were then rubbed with the soaked cotton-wool. The ears were cleaned and then the cotton wool was transferred into a clean glass funnel. The extracts were obtained by rinsing the cotton wool thoroughly with 2ml of hexane; the sample was collected in a small glass beaker and then transferred into small vials from of Pasteur pipettes. The vials were sealed off and kept in a freezer at – 20°C for several days until needed for bioassay.

On bioassay day, the vials were opened and the volume of solvent was reduced to 50µl by blowing air over the sample. This amount was then deposited onto a clean 2.5-cm diameter filter paper (Whatman International Ltd, UK) and allowed to evaporate. Similarly, an equal amount of pure solvent was deposited onto another filter paper which acted as control. Both papers were then placed side by side onto a clean glass 10-cm diameter Petri dish, which had previously been inserted into a 16x16x16 cm Barraud cage containing 20 (six- to seven-day-old) female *Lu. longipalpis*. After introduction of the impregnated filter papers, the numbers of flies landing on either side of the Petri dish were recorded. Video-recordings were conducted as previously described by Rebollar-Téllez et al. (1999). The numbers of landings on each side of the Petri dish containing both filter papers were compared. Bioassays were replicated five times for each sand fly population.

The observed numbers of bites on each body site were compared with the numbers of bites which otherwise would be expected if there was no preference for a given site. The expected proportions were established for the five body skin surface areas as follows: head (0.083), ears (0.007), trunk (0.36), arms (0.18), and legs (0.37). The expected proportions for the major body zones (trunk, arms and legs) were taken from Thibodeau and Patton (1993), and the proportions for the ears were based on Fujimoto and Watanabe (1969). The null hypothesis was that female sand flies would bite on the volunteer's body following the expected proportions. Any higher number of bites on a particular site was interpreted as a preference. Data on body site selection between body sites as well as sand fly populations, were compared by 2x5 contingency tables (X^2 test). Because the expected frequency of landings on the ears was <5, it was necessary to calculate the exact *P*-value using the statistical software programme StatXact Turbo. Proportion of bites between back and front of the body was evaluated by the *Z*-statistic test. The time elapsed to the first bite after release was transformed (log n + 1) to normalise the data according to the Anderson-Darling test (MINITAB 11.0, Clecom, UK). Analysis of variance (ANOVA) and Tukey-Kramer's test for comparison of means were conducted on the transformed data. The response of flies towards filter papers containing the ear extracts and solvent was analysed with the X^2 test assuming that landings on filter papers were independent and were

compared with a paired t-test. The response between populations to ear washings was compared with Kruskal-Wallis' non-parametric analysis of variance, where pair comparisons were made using Mann-Whitney's non-parametric two sample test. All statistical tests were considered significant if $P< 0.05$ (Sokal and Rohlf 1995).

RESULTS

From the statistical analysis presented in Table 1, it is obvious that for Jacobina, Curarigua and Marajo populations of *Lu. longipalpis*, choice of biting sites did not occur randomly. Curarigua, Marajo and Jacobina females bit preferentially on the ears. The other body sites were bitten at relatively the same expected frequencies according to relative surface area for each anatomical region. Curarigua and Marajo showed no preference for either the front (Z-test, Z =1.83, $P>0.05$) or the back (Z-test, Z =1.83, $P>0.05$) of the volunteer. However, Jacobina females were more attracted to bite on the front of the body (Z-test, Z =4.37, $P< 0.001$).

TABLE 1. Biting Site Selection of Different Populations of *Lutzomyia longipalpis* (n= 100) on Different Body Zones Compared with Expected Proportions According to Skin Surface.

Population	Head	Ears	Trunk	Arms	Legs	X^{2a}
Curarigua	0.07	0.07	0.39	0.20	0.27	30.08[b]
Jacobina	0.09	0.16	0.28	0.19	0.28	338.5[b]
Marajo	0.12	0.07	0.38	0.17	0.26	31.79[b]
Expected Proportion	0.083	0.007	0.36	0.18	0.37	

[a]2 x 5 contigency table, exact P- values
[b]P< 0.0001

TABLE 2. Response of Female Curarigua, Marajo and Jacobina *Lu. longipalpis* to Ear Skin Extracts Impregnated onto Clean Filter Papers[a].

Population	Extract	Solvent	X^2	*t-test*
Curarigua	27.6 (± 2.54)	9.60 (± 2.06)	*43.54*[b]	*9.38*[b]
Marajo	17.6 (± 2.29)	5.60 (± 0.93)	*31.04*[b]	*6.33*[b]
Jacobina	38.8 (± 1.16)	10.0 (± 1.70)	*85.00*[b]	*23.24*[b]

[a]The biological activity of the extract is represented by average number of landings (±S.E.) of sandflies on the filter papers compared with the solvent as a control by X^2 analysis and by a paired t-test.
[b]Significant at P< 0.001

The results showed that there were significant differences between the three allopatric populations in the time taken to locate the host (F=6.31; d.f.=3, 230; $P<0.001$). Jacobina flies were able to locate the host in a significantly shorter period of time (Tukey-Kramer's test comparisons, P<0.05) than the other two members of the complex. An interesting observation is that fewer Jacobina flies (244 flies) were required to score 100 bites on the volunteer compared with the Curarigua and Marajo (380 flies) populations.

There was a significant difference in the response of the three populations to filter papers containing ear washing extracts (Table 2). Flies from Curarigua were more attracted to filter papers plus the extract than filter paper impregnated with solvent alone. Similarly, Marajo and Jacobina responded significantly more to ear extracts than solvent alone. When comparing the three populations to each other, it was found that they differed significantly in their response to the ear odors (Kruskal-Wallis test H=10.99; d.f.=2; P=0.04). Significant differences were observed between Curarigua (mean=18.0; SEM=1.92) flies and Jacobina

(mean=28.8 SEM=1.24) (Mann-Whitney's test U=15.0; d.f.=5,5; P=0.012), and also between Marajo (mean=12.0; SEM=1.90) and Jacobina (Mann-Whitney's test U=15.0; d.f.=5,5; P=0.012); whereas, Marajo flies were not significantly different from Curarigua (Mann-Whitney's test U=36.5; d.f.=5,5; P=0.074).

DISCUSSION

This is the first report of sand fly biting site distribution which shows that *L. longipalpis* have a preferential biting behavior on humans when given the freedom to do so. Shaw et al. (1972) compared the numbers of bites of *Lutzomyia flaviscutellata* (Mangabeira) received by human subjects when they were either in a standing or sitting position in the field. These authors found that *Lu. flaviscutellata* was a low-height biter; however, this behavior may not be odor-mediated, and perhaps the findings reflect the poor vertical flying ability of this sandfly species. No other report has been published on the biting habits on humans; therefore, the present study contributes significantly to this little investigated topic.

The response of flies to ear extracts supports the idea that preferential behavior is odor-mediated. In the biting experiments, Curarigua, Marajo and Jacobina populations showed a preference for biting on the ears. In a random distribution of bites on the human body, it would have been expected to record no more than one bite on the ears. Considering the small size of ears relative to the rest of the body, the number of bites on ears represented a 10.7-fold (Curarigua and Marajo) and 24.2-fold increase for Jacobina, higher than the expected proportion of bites. The ear extract obtained in this study was not chemically analyzed and its composition remains unknown. To our knowledge, no other species of blood sucking insect has been reported to bite preferentially on ears; although, it has been reported that the addition of rabbit ear wax to membrane feeders increased the response of ticks *Ornithodoros tholozami* (Laboulbene & Megnin) (Acari: Ixodidae) (Ben-Yakir and Galun 1993). At least for *Lu. longipalpis sensu lato*, human ears seem attractive, and perhaps other species of sand flies may respond similarly to ear odors. Distribution of cutaneous lesions (Chiclero's ulcer or Bay sore) due to *Leishmania mexicana* (Biagi) in Belize (Garham and Lewis 1959, Lainson and Strangways-Dixon 1963, Chalmers et al. 1968) and the Peninsula of Yucatán, México (Beltran and Bustamante 1942, Biagi 1953) has clearly shown that more than 50% of these are located in the ears. Traditionally, it has been assumed that protective clothing worn by forest laborers leaves only the head region exposed to the *Leishmania*-infected bites of sand flies and lesions, therefore, are restricted to this area. However, Williams (1970) argued that this assumption did not explain why there were more records of lesions on ears than on the rest of the head including the neck area. Acton (1919) was perhaps the first author to suggest an association between sand fly biting habits and the distribution of cutaneous lesions of leishmaniasis. The observations of this study on the biting distribution of *Lu. longipalpis* have shown that this species of sand fly, especially Jacobina, were attracted mainly to ears during the free choice trials and has also shown that this attraction is odor-mediated. Therefore, it may be possible that other species of sand flies, such as Chiclero's ulcer vectors, are also attracted to ear odors, thus explaining the distribution of lesions in this area.

Studies carried out on mosquitoes have shown that selection of biting sites is also mediated by specific odors (kairomones). For species biting on the head, the explanation is that they are attracted to the highest concentration of carbon dioxide. In contrast, species biting preferentially on the feet and ankles do so because of odors emanating from this body region (De Jong and Knols 1995). Foot odors contain triglycerides in sebum skin that are broken down to fatty acids by the action of bacteria (Nicolaides 1974). Interestingly, Knols and De Jong (1996) found that bacteria of the genus *Brevibacterium* are used in the

ripening of Limburger cheese, and that these bacteria produced carboxylic acids with an aroma similar to that of foot odor. Extracts of Limburger cheese have attracted *An. gambiae* in wind tunnel trials as well as evoked electrophysiological responses (Knols et al. 1997). Other studies (Haddow 1954, Dekker et al. 1998) notwithstanding have shown that biting selection of mosquitoes such as *Eretmapodites* spp. and *An. gambiae* may be guided by the position of the host, and perhaps it is more linked to heat convection currents of the host's body rather than being odor-mediated.

Selection for body site has also been related to body distribution of parasites. Chandra and Hati (1993) found a direct correlation between biting of *Culex quinquefasciatus* and the distribution of *Wuchereria bancrofti* (Cobbold) filariae. These results indicate that parasites may have evolved a mechanism by which they synchronize with the normal biting habit of the vector. This suggests that parasites have evolved a strategy for the maximization of transmission of human filariasis.

The differences in time required to locate the host among the three populations indicates that these sibling members differ in their biting rate attack as defined by Garrett-Jones (1964). Differences observed in the time taken to bite between Jacobina and Marajo flies may reflect natural differences in human biting rate. Other authors, such as Rutledge et al. (1975) interpreted speed of response as a criterion for evaluating and comparing two different strains of *Ae. aegypti* mosquitoes. Schreck et al. (1990) have suggested that difference in response to human odor extracts between *Ae. aegypti* and *An. quadrimaculatus* Say may reflect anthropophilic feeding preferences. Hamilton and Ramsoondar (1994) also reported differences in the response to handled Petri dishes between *Lu. longipalpis* populations from Lapinha and Jacobina. In the present study, no other host was offered for sand flies to choose from; therefore, a feeding preference cannot be inferred directly. However, differences in the times taken to bite correlates with the biting habits reported for Marajo and Jacobina in their natural habitat. Species with a greater biting rate attack (bites/man/hour) are considered more dangerous vectors because it implies that more bites/hour/man would result in a potentially greater number of infected bites. This could explain, at least partially, why there have been many more reports of visceral leishmaniasis in north-eastern Brazil where Jacobina is located than the scanty reports of disease in Marajo Island (Lainson et al. 1983, Ryan et al. 1984).

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SPRAY DRIED MICROENCAPSULATED FORMULATION OF *BEAUVERIA BASSIANA* FOR CONTROL OF *EPILACHNA VARIVESTIS* MULSANT.

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ABSTRACT

Blastospores of *Beauveria bassiana* (Vuill) were produced in basal salts liquid medium supplemented with ammonium sulfate as the nitrogen source and with molasses as the carbon source. Whole cultures of *B. bassiana* containing $2.2x10^9$ blastospores/ml were used to produce a sprayable flour-basal microencapsulated formulation of *B. bassiana* (MF*Bb*) at concentrations of 1, 3 and 5% of *B. bassiana* biomass (w/w) using a spray-drying process. The number of blastospores germinated/mm^2 on SDA agar microplate of these formulations after the drying process was determined. The number of blastospores germinated to each formulation shows statistical differences according to each *B. bassiana* concentration level (LSD, $P < 0.05$). MF*Bb* 1, 3 and 5% had 296 ± 2.59, 376 ± 2.35 and 444 ± 2.86, blastospores/mm^2, respectively. Eight days after the formulations were applied on first-instar larvae of the Mexican bean beetle reared in the laboratory using a dipping leaf bioassay, their toxicities were compared with an unformulated *B. bassiana* (U*Bb*) and with the commercial bioinsecticide Mycotrol™. There were statistical differences of toxicity among treatments (Fc 94.089; $p < 0.05$). Mycotrol was highest with 96% larval mean mortality; U*Bb* resulted in 77.3%, while MF*Bb* 5, 3 and 1% yielded 72, 64 and 44%, respectively. MF*Bb* 5% was the superior microencapsulated formulation; it's toxicity was statistically similar to that of U*Bb*, and also demostrated acceptable toxicity compared to that of the commercial formulation.

INTRODUCTION

The Mexican bean beetle (MBB), *Epilachna varivestis* Mulsant (Coleoptera: Coccinellidae), is a important pest of many kinds of beans cultured in Durango, Mexico. Adults and larvae cause serious economic losses every year. Chemical treatments using carbaryl (1-naphty methyl carbamate) and parathion-720™ (methyl-parathion) are common methods used to control MBB. Biological methods to control *E. varivestis* include sporadic annual inoculative releases of the parasitoid wasp, *Pediobius foveolatus* Crawford (García et al 1993). *Aplomyopsis epilachnae* (Aldrich) and *Perillus bioculatus* (Fabricius) are also important biological control agents of *E. varivestis* larvae; however, their efficacy is poor due to the timing of their appearance, which is in late August when the pest infestation is high (García and Piedra 1994).

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Quattlebaum and Carner (1980) determined that *B. bassiana* can infect and kill *E. varivestis* when applied at concentrations of $4x10^6$-$4x10^8$ spores/ml, while García et al. (1999) found *B. bassiana* infected first-instar larvae of MBB at levels between 1.23 and $1.27x10^7$ spores/ml.

The dominance of *Bacillus thuringiensis* in the microbial insecticide market has resulted in the development of new formulation technologies, such as microencapsulation with spray drying. Starch and flour based *B. thuringiensis* sprayable formulations have been developed for lepidopteran pests to stimulate feeding and provide protection from environmental agents such as UV degradation, a principal reason for the loss of bioinsecticide activity in the field. Moreover, sprays made of dried products must be sufficiently small to be applied by conventional spray equipment (McGuire et al 1996). Tamez et al. (1999a) evaluated a microgranule formulation of *B. thuringiensis* (9.9-40.1 µm of size) based in corn starch and flour mix for MBB control. The strain was active against *E. varivestis* larvae at an LC_{50} of 642 µg/ml. At the field level, *B.thuringiensis* formulations controlled the MBB significantly better than treatments with unformulated *B. thuringiensis*. Tamez et al. (1999b) applied a spray-drying procedure to encapsulate the baculovirus *Anagrapha falcifera* (AfNVP), using pregelatinized corn flour, kraft lignin and sugar formulation; spray-drying conditions were 120°C at inlet and 80°C at outlet.

This study was undertaken to investigate the use of sprayable *B. bassiana* formulations based on starch and nixtamalized corn flour against *E. varivestis*.

MATERIALS AND METHODS

A strain of *B. bassiana* code BbP1 from *Geraeus senilis* (Coleoptera: Coccinellidae), was obtained from the National Center Stock of Biological Control (CNRCB-SAGAR), Mexico. Liquid cultures of *B. bassiana* were grown in shake flasks at 130 rpm and 30°C for 48 hours. The composition of the liquid culture medium used to grow *B. bassiana* was, per liter deionized water: 6g molasses as a carbon source, 6g $(NH_4)_2SO_4$, 3.5g KH_2PO_4, 0.5g $MgSO_4$, 0.1g $CaCl_2$ and 0.1g NaCl. Blastospores of *B. bassiana* were produced using 250-ml erlenmeyer flasks, 100-ml culture volume. Spore inocula were obtained by rearing sporulated cultures of *B. bassiana* grown on SDA agar with 10ml of sterile distilled water. Two ml of spore inocula were used to inoculate a shake flask; blastospore concentrations were measured microscopically using a Neubauer chamber (Cantwell 1970). The *B. bassiana* production was $2.16x10^9$ blastospores/ml, and the incubation time was 6 days.

Whole cultures of *B. bassiana* were formulated with cornstarch (CS) Maizena™ and nixtamalized corn flour (NCF) Maseca™ (CS:NCF) at a ratio of 1:1 (10g). The CS:NCF mixture was combined with 280ml of distilled water, 100g of powdered sugar, 20ml of vegetable oil, 120ml of isopropanol, 0.1g of malachite green oxalate salts, 0.5ml of 37% formaldehyde, and *B. bassiana* at concentrations of 1, 3 and 5% per gram of total solids. Microencapsulated formulations were made using a Niro atomizer spray-drying, following the procedure of Tamez et al. (1999a). The spray drying conditions were: 10 ml/min flow rate; 120°C inlet temperature; 80°C outlet temperature and 4 kilograms pascals (Kps/cm^2) air pressure. The microgranule formulations of *B. bassiana* were stored in a glass jar at room temperature of 26°C. Moisture content of each MF*Bb* was determined using a Mark Moisture Analyzer (Omnimark, Temple, AZ). The moisture content of *B. bassiana* microencapsulation formulation samples 5, 3 and 1% were 2.0, 2.1 and 2.0%, respectively.

Samples of 0.1 gram each microencapsulated formulations were diluted in 9.9ml of distilled water and one drop was used to inoculate a SDA agar microplate; the fungus was grown in a chamber at 38°C and 70% RH for 72 hours. The germinated blastospores on each microplate were counted three times microscopically; the number of blastospores/mm^2

to each formulations were analyzed using analysis of variance, and means were separated with LSD test.

Dilutions of 1% suspensions of the various *B. bassiana* formulations were used to determine the insecticide efficacy of these preparations. The *E. varivestis* colony was reared on "flor de mayo ", beans grown in plastic pots (30cm diameter) in a greenhouse at 25^{0}C and 65% RH First-instar larvae of *E. varivestis* were used in all bioassays. Leaf disks (50mm diameter) were cut from bean leaves and placed in 50x9-mm plastic petri plates with moistened filter paper (Whatman No.1, 42.5 mm diameter). *B. bassiana* blastospores were applied to leaf disk colonized by 5 MBB larvae by dipping the leaf disk in the various spore suspensions for 30 seconds. Five leaf disks were used for each treatment with three replicates. Leaf disk plates were incubated for three days in a laboratory room at 25^{0}C and 65% RH. One untreated bean leaf disk larvae was placed in each petri plate as a control; water was used in this treatment. The experiment was performed in a laboratory room with artificial fine rain which provided the moisture necessary for germination of *B. bassiana* blastospore allowing the development of the fungus on *E. varivestis*. Dead larvae were counted eight days after application. The mean mortality of MBB to each treatment was determined, data were analyzed using analysis of variance, and mean values were separated by LSD test with a Statistic SPSS 8.0 PC program for windows.

RESULTS AND DISCUSSION

The spray-drying process produced a microencapsulated formulation with *B. bassiana* as the active ingredient, able to survive under dried conditions and maintaining toxicity against first-instar larvae of MBB. Table 1 shows the germinated blastospores from microencapsulated formulations samples of *B. bassiana* after the drying process.

TABLE 1. Blastospores Germinated From Dried Microcapsulated Formulations of *B. bassiana*.

MF*Bb* (*Bb* concentration w/w)	Blastospores/mm^2 germinated[a]	Confidence Limits
1%	296 a	± 2.59
3%	376 b	± 2.35
5%	444 c	± 2.86

[a]Average of three replicates. There are significant differences among formulations ($p << 0.05$). Values in a column with same letters are not different LSD (0.05).

The number of germinated blastospores/mm^2 indicated statistical differences among formulations after drying, with MF*Bb* 1% and 3% having 1.5 and 1.18 times lower *B. bassiana* concentration of spores germinated, respectively, than MF*Bb* 5% due to the 5% formulation having a major concentration of *B. bassiana* as the active ingredient.

The mean mortality of first-instar larvae of MBB in each treatment evaluated appears in Table 2. The toxicity results showed that Mycotrol™ provided the best control of *E. varivestis* (96%) compared to the microencapsulated 5% formulation (72%) and the unformulated *B. bassiana* (77.3%); however, these products were significantly more effective in killing larvae of *E. varivestis* compared to the 3% and 1% formulation. The spray-drying process (Tamez et al 1999a) used in this study is an excellent method for encapsulation of *Bacillus thuringiensis*. We determined that *B. bassiana* can survive the spray-drying process and retain its infective effects on first-instar larvae of MBB in the greenhouse. The susceptibility of MBB larvae to unformulated *B. bassiana*, and microencapsulated formulations of *B. bassiana* was confirmed.

TABLE 2. Toxicity of Microencapsulated Formulations, Unformulated *B. bassiana* and Commercial Formulation on First Instar Larvae of MBB.

Treatments	Total mortality[a]	% Mortality[b]
Mycotrol™	72	96.3 a
U*Bb*	58	77.3 b
MF*Bb* 5%	54	72.2 b
MF*Bb* 3%	48	64.1 c
MF*Bb* 1%	33	44.2 d
Control	2	2.6 e

[a]Number of the insects dead per 75 individuals.
[b]Data are percentage of mortality means, after eight days of exposure. There are significant differences among treatments Fc (94.089); $p << 0.05$. Average of sample a week after treatment; values in a column with the same letters are not different LSD (0.05).

The results obtained with unformulated *B. bassiana* blastospores and *B. bassiana* microencapsulated formulations are in agreement with *E. varivestis* susceptibility to *B. bassiana* found by Quattlebaum and Carner (1980) and García et al. (1999) on MBB larvae. The cost of *B. bassiana* microencapsulation formulation is approximately $1.5 US dollar/kg/dosage/ha. This formulation had similar bioinsecticide effects to U*Bb* at the laboratory level. This study demonstrated the potential of spray dried formulations of *B. bassiana* against *E. varivestis.*

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TREATMENTS AFFECTING RED IMPORTED FIRE ANT ABILITY TO CRAWL ON VERTICAL SURFACES

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Hymenoptera, including the Asian weaver ant (*Oecophylla smaragdina* Fabricius), attach to smooth surfaces with a flexible pad, the arolium, between the claws. Adhesion by arolia to smooth surfaces like glass is mediated by a thin liquid film between the arolium and the surface (Federle et al. 2001). The extension of the arolium is coupled with the retraction of the claws. When the claws are placed on a smooth surface and do not achieve traction, they are quickly and automatically retracted until they reach a position perpendicular to tarsi. The arolium is simultaneously unfolded and extended down to the surface, and a gland reservoir pumps liquid into the arolium through hydraulic inflation. This reaction is operative even in severed legs, indicating that it is a passive reaction of the mechanical system to a pull on the leg. When the leg is lifted, releasing the claw-flexor tendon, the pretarsus moves back to the extended position, and the arolium folds up elastically. This system allows the adhesive pad to be deployed only if required, primarily unfolding on smooth surfaces when the claws slip and find no resistance (Federle et al. 2001).

The red imported fire ant, *Solenopsis invicta* Buren, is commonly collected and maintained in laboratory cultures using techniques described by Banks et al. (1981). For field collection, the inner surface of a plastic bucket is liberally dusted with talcum powder. As long as the dusted surface remains dry, the powder prevents the ants from scaling the vertical surface even if it is not very smooth. Once the ants are extracted from the soil, they are placed in plastic buckets with the inner surface painted with a Teflon®-like chemical, ADI Fluon® (ICI Fluro Polymers, Bayonne, NJ). This material is applied as a liquid and dries on the surface. As long as the surface remains dry and scratch-free, and the humidity level in the laboratory remains low, ants are unable to climb up the vertical surface.

Although researchers have speculated about surface properties that allow these types of materials to work, no investigations have been reported to date. Scanning electron microscopy (SEM) photographs were taken of red imported fire ant and tarsi from ants which had walked on treated surfaces and the Fluon® surface on which ants had attempted to crawl.

Red imported fire ant workers which had walked on Fluon® or talcum treated vertical surfaces, and the surfaces on which they had walked were collected and prepared for scanning electron microscopy. Specimens were prepared by fixing in 2.5% (by volume) glutaraldehyde in sodium cacodylate buffer (p.h. 7.2) for 1 hr. at 2°C and washed in a buffer, after which they were dehydrated in a graduated series of ethanol. To produce "clean" ants, specimens were rinsed in acetone and dried, mounted on stubs with two-sided sticky tape, and coated with a 20-nm thick layer of gold-palladium using an ion-sputter coater (Hummer I from Technics, Inc., Alexandria, VA). All Polaroid® photographs of intact specimens were taken using a Japanese Electron Optical LaSamtoris (JEOL) JSM-2552 Scanning Microscope at the Texas A&M Microscopy Center, College Station, Texas.

The scanning electron micrographs of red imported fire ant tarsae are similar to those of Federle et al. (2001) depicting claws and a central arolium (Figs. 1 - 4). Although the

arolium is visible in these figures, it was not retained in a fully expanded form because of the preparation procedure of these ant specimens for SEM. Extension and retraction of tarsal claws coupled with deployment of the arolium can be seen in Figs. 2 and 3. Ants attempting to walk up vertical surfaces can not apparently gain traction on the Fluon® surface with their tarsal claws causing claws to retract and arolium to deploy (see Fluon® on claws in Fig. 4 and "tracks" of attempts to insert claws, Fig. 5). Because adhesion to smooth surfaces is mediated by a thin liquid film between the arolium and the surface, the arolium may be unable to develop liquid surface tension necessary to adhere on vertical Fluon surfaces. However, under humid laboratory conditions, coatings of Fluon® can not prevent ants from climbing on the vertical surfaces (personal observation). It is unlikely, therefore, that the fluid secreted by the arolium partially dissolves the outer layer of the coating to cause "slippage".

Fig. 1. Clean tarsus, 200x

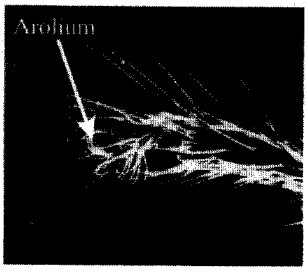

Fig. 2. Claws extended, 200x

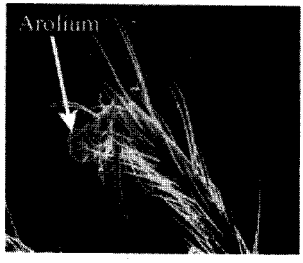

Fig. 3. Claws retracted, 200x

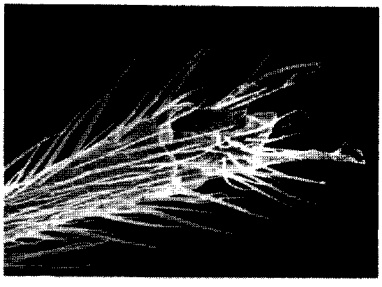

Fig. 4. Fluon®-covered tarsus, 200x

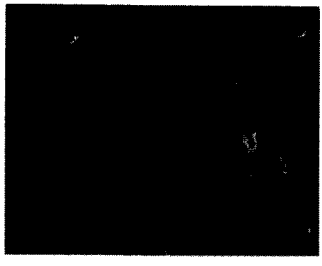

Fig. 5. Fluon® surface, 700x

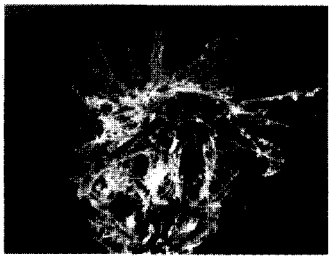

Fig. 6. Talcum covered tarsus, 300x

Fig. 7. Encrusted tarsus, 200x

Talcum may have a different mechanism of preventing ants from climbing up dusted vertical surfaces than does Fluon®. Ants coming into contact with the dust quickly become

coated (Fig. 6), and dust covers legs, antennae, and other body parts. This may affect not only the proper function of these structures, but could cause disorientation or distraction. Furthermore, when moistened the talcum appears to form a coating that can encrust these structures (Fig. 7), reducing the grasping capability of the claws and preventing the arolium from forming a liquid film for establishing surface tension. Conversely, as a drying agent, talcum may absorb the liquid secreted by the arolium.

Both Fluon® and talcum powder are used to maintain imported fire ant colonies in the laboratory. Occasionally, when the Fluon® surface becomes scratched or moistened, ants will escape from the colony box. Dusting these areas with talcum powder can prevent the escape, is easy to apply, and produces an almost immediate effect even under highly humid laboratory or field conditions.

Development of surfaces or barriers onto which red imported fire ants can not or will not crawl could be of use in site-specific Integrated Pest Management (IPM) programs for this pest such as preventing ant foraging onto greenhouse benches, raised animal cages, feeding containers and nesting boxes. Similar effects to Fluon® have been observed with other materials such as Turtle Wax® Polyshell Spray Poly Sealant (Turtle Wax, Inc., Chicago, Illinois), applied as a liquid spray and not buffed, have been observed to produce similar effects (personal observation). Unfortunately, surfaces treated with all of these materials remain effective only when the surface remains dry and not scratched or abraded. Thus, these materials are unsuitable as long-term ant barriers for outdoor situations.

In addition to these materials, other ant barrier products and methods that have been developed include: 1) Teflon®-coated tape (EnviroSafe™ Tape, Envirosafe Solutions Corp., Schuylkill Haver, Pennsylvania); 2) deep pile fabric materials (Ant Boot™, Just Tops, Sarasota, Florida); 3) electrical strips that shock ants attempting to crawl across closely-spaced charged wire or plates (Yaard-Vark™ Electric Fire Ant Killer, Yaard-Vark Corp., Bryan, Texas; Solar Ant Charmer™, Heitman Laboratories, Plano, Texas); 4) use of heated metal strips, plates or wires, 60°C (140°F) or warmer (Drees et al. 1996); and 5) flange elements in the design of insect exclusion devices (Fool-A-Bug® V-M, Alternative Control Systems Corp., Columbia, SC).

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EFFECTS OF COTTON PLANT WATER STRESS ON *BEMISIA TABACI* STRAIN B (HOMOPTERA: ALEYRODIDAE) HONEYDEW PRODUCTION

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ABSTRACT

Honeydew production by sweetpotato whitefly (SPW), *Bemisia tabaci* (Gennadius) Strain B, feeding on water-stressed and non-water-stressed cotton was compared in field and greenhouse studies. In the field in 1999, leaf water potentials, as a measure of water stress, decreased with increasing numbers of days following irrigations. More honeydew sugars were produced by SPW feeding on cotton plants four days after irrigation (non-water stress) compared with 7 or 13 days after irrigation (water stress). In 2000, leaf water potentials of furrow irrigated cotton and furrow irrigated plus supplementary drip irrigated cotton (1 h per day) showed the same decreasing leaf water potential patterns as in 1999, but leaf water potentials decreased less in furrow irrigated plus supplementary drip irrigation compared with furrow irrigation alone. SPW feeding on plants in the field with furrow plus supplementary drip irrigation and in the greenhouse on non-stressed cotton plants produced more micrograms of honeydew sugars per gram of honeydew compared to SPW feeding on plants with furrow irrigation alone in the field and on water-stressed plants in the greenhouse.

INTRODUCTION

The sweetpotato whitefly (SPW), *Bemisia tabaci* (Gennadius) Strain B, also known as *B. argentifolii* Bellows and Perring, has been a serious pest of cotton, vegetables, and ornamentals in Arizona since the early 1980s (Henneberry et al. 1998b). Epidemic infestations as experienced in the early to mid-nineties are rarely encountered at present because of improved SPW management systems (See Henneberry et al. 2000 for review). When economic population levels do occur, one of the damaging impacts is honeydew contamination of the produce in SPW infested crops. For cotton, this can be particularly serious. Honeydew deposits on lint adhere to textile mill machinery parts during lint processing. Sticky cotton can be such a disruptive influence that mill operation efficiency may be reduced and result in as high as 10% lower price differentials imposed on honeydew contaminated lint (Hector and Hodkinson 1989).

Although chemical control approaches provide short-term solutions to reducing SPW populations and the incidence of sticky cotton (Henneberry et al. 1998a), there is an urgent need to develop additional control methods for integration into ecologically sound SPW management systems. Water management in cotton production is an important factor

influencing SPW population dynamics as well as cotton yields. Cotton plant water stress studies showed that seasonal densities of SPW eggs, nymphs, and adults were reduced 45-69% and 22-36% in plots irrigated weekly compared with densities in plots irrigated every other week in each of two years, respectively (Flint et al. 1996). Results of the effects of cotton plant stress on soluble sugars extracted from cotton lint harvested from bolls showed greater amounts from stressed (irrigated every other week) plants in one year and lesser amounts in a second year. Yee et al. (1996) also did not find clear, consistent SPW honeydew production relationships to diurnal differences in leaf water potentials of cotton in the field. Leaf water potential is an index of plant water stress and is expressed as the pressure (-bars) required to cause the exudation of interstitial leaf sap (Meron et al. 1987, Turner 1987).

We conducted field studies in 1999 and 2000 and greenhouse studies in 2001 to further investigate the effect of cotton water stress and cotton cultivar on SPW honeydew production and population development.

METHODS AND MATERIALS

1999 Field: Cultivar and irrigation schedule. Delta and Pineland (DPL) 5415 (Delta and Pineland, Scott, MS) and NuCOTN 33B® (Bt) (Monsanto Co., St. Louis, MO) cotton seeds were planted on 28 April 1999 in eight plots, 16 rows wide by 19m (60 feet) long plots at the Western Cotton Research Laboratory at Phoenix, AZ. Plots were arranged in a randomized complete block design with four replications. Standard production methods were used. Furrow irrigations of about 12.7cm of water were applied every 14 days.

1999 and 2000 Leaf water potentials and ambient temperatures. We measured leaf water potentials at 1200 h to 1300 h on days 4, 7, and 13 following furrow irrigations with a pressure chamber (Soilmoisture Equipment, Santa Barbara, CA) according to standard methods (Meron et al. 1987, Turner 1987). Three leaves were collected from the fifth main-stem node of cotton plants in the center of plots on each sample date. The leaves were placed in plastic bags, sealed, and transported in an insulated storage chest to the laboratory. Readings of leaf water potentials were made in the laboratory within 1 h of leaf excision. All leaves were collected between 1200 h and 1300 h (MST). The 14-day intervals between irrigations resulted in water stress at the end of the irrigation cycles. Maximum-minimum temperatures (°C) were obtained from a nearby University of Arizona weather station.

1999 Honeydew collections and SPW counts. We collected honeydew from each of five SPW-infested DPL 5415 and Bt cotton plants in each plot on days 4, 7, and 13 following furrow irrigations on 29 July and 12 August to compare selected sugars in honeydew produced by SPW feeding on non-stressed and stressed plants. The honeydew was collected in 20.5 x 20.5 cm plastic bags (Plastic Interlocking Seal, Wichita, KS). The open interlocking ends of the bags were slipped over individual leaves and one corner of each of the bags folded back and inside out and held in place with a paper clip. The bottom ends of the bags were cut off to provide air circulation. Honeydew was collected from one leaf per plant on each of five plants in each of the four replicated plots. The leaves were excised from the plant following 24 h of honeydew collection and numbers of whiteflies were counted in the laboratory with the aid of a microscope. Honeydew was washed from the plastic bags with 125ml of warm deionized water and frozen. Frozen honeydew samples were lyophilized and reconstituted in 125µl of deionized water. The amounts of trehalulose, melezitose, glucose, fructose and sucrose in the samples were determined using the high performance liquid chromatography (HPLC) method of Hendrix and Wei (1994). Sampled sugars were quantified by comparison with peaks of known

sugar standards.

2000 Cultivar and irrigation schedule. In 2000, the experiment was repeated with DPL 5415 and NuCOTN 33B® cotton seed planted in eight plots each on 13 April. The 14-day interval furrow irrigation treatment of 12.7cm of water on each date was repeated. In addition, a furrow irrigation plus supplementary T-tape (T-system International, Inc., San Diego, CA) drip irrigation treatment was initiated in plots of each cultivar to determine the effects on honeydew production by reducing water stress between furrow irrigations. Supplementary water was 102 liters per hour per 31m of row. The experimental design was a four replicate split-plot with irrigation treatments (furrow alone and furrow plus drip) as whole plots and cultivars as split plots. The T-tape drip irrigation system was controlled with a timer-operated solenoid valve that was set to apply supplementary water to furrow irrigated plots for 1 h durations on each day.

Honeydew collections and SPW counts. Honeydew collections in bagged leaves were performed as described for 1999. Adult SPW were sampled on days 4, 7, and 13 following irrigation in all plots using the sampling method of Naranjo and Flint (1995). Eggs and immatures were counted on one 3.88cm^2 leaf disk from each of 30 leaves taken on the same dates from the fifth node from the terminal of sampled plants (Naranjo and Flint 1994). We also determined weights of honeydew collected by weighing 2.5cm^2 pieces of aluminum foil on a Mettler-Toledo Mt-UMT balance (Mettler-Toledo GmbH, Switzerland) before they were taken to the field and suspended under SPW infested leaves on stiff wire platforms. Following 24 h exposure periods, aluminum foil pieces were reweighed and numbers of honeydew drops on the foil pieces were counted with the aid of a microscope. Honeydew was washed from the aluminum foil, frozen and processed as described, but only 1ml of deionized water was required for washing. Amounts of trehalulose, melezitose, glucose, fructose, and sucrose were determined using the HPLC methods described. The results were expressed as fractional micrograms/microgram of honeydew to compare concentrations of each sugar in honeydew produced by SPW feeding on stressed and non-stressed cotton plants.

2001 Greenhouse Experiments 1 and 2. Water supply, leaf water potentials, and honeydew collections. SPW used in the studies were reared in the greenhouse on cotton plants. One DPL 5415 seed per 3.8cm diameter x 3.8cm tall Jiffy Pot (Jiffy Products of America, Inc., Batavia, IL), or two to three seeds per soil filled 10cm^2 x 12cm tall plastic flower pot were planted at about one week intervals to assure a continuous supply of plant material. In all greenhouse studies, temperatures were recorded hourly with a hygrothermograph (Weather Tronics, Model 5020-1, Sacramento CA). For Experiment 1, when plants were in the 10 to 12 node stage of plant development, 20 plants were irrigated until free water drained from the bottoms of the pots. After four hours, leaf water potentials (at 1200 h to 1300 h) were measured as discussed. Ten plants were supplied with 100ml of water thereafter on each day for four days and ten plants received 25ml of water on day 1 and 50ml on days 2, 3, and 4 of the experiment. Leaves (fully expanded) picked for leaf water potential measurements were from nodes 8 to 10.

In Experiment 1, honeydew collections were made and leaf water potentials measured on the day the experiment was initiated and on day 4 following the last irrigations. For honeydew collections, five female SPW were placed in each of two, 1.9cm diameter x 0.85cm high leaf-clip cages attached to individual, intact cotton leaves on each stressed (25 or 50ml water/day) or non-water stressed (100ml water /day) plant for 24 h honeydew collections. Each leaf cage had a 2.5cm^2, styrofoam cage bottom. Aluminum foil pieces, 2.5cm^2, overlayed the cage bottoms for honeydew collection. Aluminum foil pieces were handled in all cases with forceps. They were weighed (Mettler-Toledo scale) before being exposed to SPW and after exposure. Numbers of honeydew drops on the foil pieces were also counted. Honeydew, on each collection day, was washed from the foil

with 3ml of warm deionized water and frozen. Sample lyophilization and HPLC sugar determinations were as described.

In Experiment 2, cotton seed, pots and plants sizes and initial watering were as described for Experiment 1. On day one following initiation of the experiment and thereafter at daily intervals 10 plants each were irrigated with 100ml of water per day and ten plants with 25 or 50ml of water per day. Leaf water potentials were measured and honeydew collected on aluminum foil in leaf cages as described on days 2, 6, 8, and 13 after initiation of the experiment.

Statistical Analysis. ANOVA were conducted and means were separated contingent upon a significant F test using the method of least significant differences ($P \leq 0.05$) or using Students "t" test for paired treatment comparisons at the same probability level (MSTAT-C 1989). All percentages were transformed to arcsines before analysis. Regression analyses were performed, when appropriate, to determine relationships between honeydew sugars and leaf water potentials.

RESULTS

1999 Field: Leaf water potentials and ambient temperature. During the experiment in 1999, maximum air temperatures ranged from 37 to 41°C and minimum air temperatures from 19 to 25°C (Table 1). Over both irrigation dates, leaf water potentials for DPL 5415 and Bt cottons averaged -20.7 ± 0.47 and -20.3 ± 0.51 bars, respectively. The differences were not significantly different ($F = 1.01$; $df = 1,75$; $P > 0.05$). Also, over both irrigation dates there were no significant differences between cultivars for the amounts of glucose, fructose, trehalulose, sucrose or melezitose in honeydew produced by SPW feeding on DPL 5415 or Bt cottons (F range = 0.06 to 1.14; $df = 1, 9$; $P > 0.05$). Thus, data for the two cultivars were combined for further analyses and cultivar comparisons are not tabulated.

Leaf water potentials ($\bar{x}$ of both cultivars) ranged from –27.0 bars to –17.5 bars (Fig. 1A). Leaf water potentials decreased significantly on days 7 and 13 following irrigations compared to day 4 (Table 1). Effects of dates of irrigation on leaf water potentials were not significantly different. However, differences between leaf water potentials decreased significantly with increasing days after irrigation.

Honeydew collections and SPW counts. Highest concentrations of all sugars measured in honeydew for leaves enclosed in plastic bags occurred when SPW were feeding on plants with the highest leaf water potentials on day four following irrigation (Table 1). However, only the regression for increasing amounts of trehalulose measured in honeydew was significantly related to increasing leaf water potential (decreased plant stress) (Fig. 2). Dates of irrigation and days following irrigation had no effect on numbers of SPW. More trehalulose, sucrose, and melezitose were produced following the 29 July irrigation compared to the 12 August irrigation (Table 1).

2000 Field: Leaf water potentials and ambient temperatures. Air temperatures were higher in 2000 compared with 1999 (Table 2). Maximum air temperatures ranged from 41 to 43°C and minimum air temperatures from 24 to 26°C. Similar to 1999, there were no significant differences for leaf water potentials or honeydew sugar collections between cultivars (F values ranged from < 0.03 to 2.73; $df = 1, 9$; $P > 0.05$; data are not tabulated). Leaf water potentials on 28 July were –24.6 ± 0.4 and -28.1 ± 0.8 in furrow plus drip and furrow alone irrigations ($t = 4.01$; $df = 14$; $P < 0.01$) (Fig. 1B). Leaf water potentials on each sampling date were higher in plots with furrow plus supplementary drip irrigation compared with furrow irrigation alone (Fig. 1B) and, in general, were lower compared with 1999. Differences between furrow plus drip irrigation compared with furrow irrigation alone were not statistically significant on every sampling date. However,

TABLE 1. Mean (± SE) Cotton Leaf Water Potentials and Honeydew Sugars Produced Per SPW (μg) while Feeding on Plastic Bag Enclosed Cotton Leaves on Days 4, 7, and 13 Following Furrow Irrigations in 1999.

Irrig. Date	Air (°C) Max	Min	Days After Irrig.	Leaf Water Potential (-bars)	SPW /Leaf	Sugar[a] G	F	T	S	M	Total
Jul 29											
	41	22	4	-17.5 ± 0.6 d	2.8 ± 0.7 a	26.5 ± 9.6 a	29.3 ± 10.3 a	15.8 ± 5.2 a	21.6 ± 6.2 a	16.8 ± 5.7 a	110.0 ± 30.8 a
	40	19	7	-20.3 ± 0.5 c	2.6 ± 0.6 a	3.4 ± 0.5 b	5.2 ± 0.8 b	10.9 ± 2.3 a	5.7 ± 0.9 b	2.8 ± 0.5 b	28.0 ± 4.5 b
	37	25	13	-24.4 ± 0.5 b	1.8 ± 0.7 a	1.2± 0.2 b	1.6 ± 0.3 b	3.1 ± 0.6 a	2.8 ± 0.8 b	1.9 ± 0.5 b	10.5 ± 2.1 b
Aug 12											
	39	22	4	-16.7 ± 0.6 d	1.6 ± 0.3 a	5.7 ± 1.4 b	7.6 ± 1.7 b	9.1 ± 1.7 a	6.4 ± 1.0 b	4.0 ± 0.7 b	32.9 ± 4.9 b
	41	25	7	-20.1 ± 0.6 c	1.0 ± 0.0 a	7.6 ± 3.8 b	8.6 ± 4.1 b	6.9 ± 1.9 a	8.0 ± 2.3 b	2.4 ± 0.6 b	33.5 ± 11.7 b
	41	25	13	-27.0 ± 0.3 a	2.3 ± 0.4 a	0.7 ± 0.1 b	1.1 ± 0.2 b	2.0 ± 0.4 a	1.7 ± 0.4 b	0.4 ± 0.2 b	5.8 ± 0.9 b
F[b]	--	--	--	5.04	2.6	4.8	4.4	0.8	6.3	6.6	5.7
P[c]	--	--	--	0.01	>0.05 NS	0.01	0.02	>0.05 NS[d]	0.01	0.02	0.01
Irrigation date effect											
Jul 29	--	--	--	-20.7 ± 0.7 a	2.4 ± 0.4 a	10.3 ± 3.9 a	12.0 ± 4.2 a	9.9 ± 21.1 a	10.0 ± 2.7 a	7.2 ± 2.3 a	49.5 ± 13.4 a
Aug 12	--	--	--	-21.3 ± 0.9 a	1.6 ± 0.2 a	4.7 ± 1.4 a	5.7 ± 1.6 a	5.9 ± 1.1 b	5.3 ± 1.0 b	2.4 ± 0.4 b	24.0 ± 4.9 b
F[e]	--	--	--	1.55	3.5	2.7	2.9	4.4	4.7	6.6	5.4
P	--	--	--	>0.05 NS	>0.05 NS	>0.05 NS	>0.05 NS	0.04	0.04	0.01	0.03
Main effects days after irrigations											
	--	--	4	-17.1 ± 0.4 c	2.2 ± 0.4 a	16.1 ± 5.4 a	18.5 ± 5.8 a	12.5 ± 2.8 a	14.0 ± 3.6 a	10.4 ± 3.2 a	74.4 ± 18.1 a
	--	--	7	-20.2 ± 0.4 b	1.8 ± 0.4 a	5.5 ± 1.9 b	6.9 ± 2.1 b	8.9 ± 1.5 a	6.9 ± 1.2 b	2.6 ± 0.4 b	30.8 ± 6.1 b
	--	--	13	-25.7 ± 0.4 a	2.0 ± 0.4 a	1.0 ± 0.1 b	1.3 ± 0.2 b	2.5 ± 0.4 b	2.2 ± 0.4 b	1.1 ± 0.3 b	8.4 ± 1.3 b
F[f]	--	--	--	122.0	0.37	6.6	7.4	9.8	10.3	9.1	11.5
P	--	--	--	0.01	>0.05 NS	0.04	0.02	0.04	0.03	0.07	0.01

[a] G = glucose, F = fructose, T = trehalulose, S = sucrose, M = melezitose. Means of 8 replications in a column in a group, not followed by the same letter are significantly different. Method of least significant differences, $P \leq 0.05$.

[b] df = 2,35.

[c] P = probability.

[d] NS Not statistically significant.

[e] df = 1,35.

[f] df = 2,35.

Table 2. Mean (± SE) Cotton Leaf Water Potentials, Numbers of SPW and Honeydew Collected on Aluminum Foil from SPW Feeding on Cotton Leaves 4, 7, and 13 Days Following Furrow Plus Drip or Furrow Alone Irrigations.

Irrigation Type[a]	Air Temp (°C) Max	Min	Days After Irrigation	Leaf Water[b] Potential (- bars)	Honeydew[b] Drops/SPW	Honeydew[b] µg/SPW	Number[b] SPW/Leaf
Furrow							
+ Drip	--	--	4	-23.2 ± 0.2 c	19.7 ± 6.0 a	20.3 ± 2.8 a	4.3 ± 0.5 a
Alone	42	24	4	-24.6 ± 0.4 b	19.7 ± 5.8 a	18.7 ± 4.4 a	4.8 ± 1.2 a
Furrow							
+ Drip	--	--	7	-24.4 ± 0.4 bc	19.1 ± 4.4 a	26.7 ± 8.2 a	3.3 ± 0.7 a
Alone	43	25	7	-25.2 ± 0.3 b	14.2 ± 4.4 a	15.0 ± 2.7 a	4.2 ± 0.4 a
Furrow							
+ Drip	--	--	13	-25.1 ± 0.5 b	28.1 ± 16.4 a	56.7 ± 14.8 a	1.2 ± 0.3 a
Alone	41	26	13	-29.2 ± 0.5 a	36.1 ± 18.8 a	27.7 ± 4.2 a	1.6 ± 0.3 a
F[c] (*P*)	--	--	--	9.0 (< 0.01)	0.2 (>0.05 NS)	1.8 (>0.05 NS)	(>0.05 NS)
Main effects days after irrigation							
	--	--	4	-23.9 ± 0.3 c	19.7 ± 4.0 a	19.5 ± 2.6 b	4.5 ± 0.6 a
	--	--	7	-24.8 ± 0.3 b	16.6 ± 3.1 a	20.8 ± 4.4 b	3.8 ± 0.4 a
	--	--	13	-27.1 ± 0.6 a	32.1 ± 12.1 a	42.2 ± 8.3 a	1.4 ± 0.2 b
F[d] (*P*)	--	--	--	32.3 (< 0.01)	1.4 (>0.05 NS)	6.2 (< 0.01)	13.1 (< 0.01)
Furrow							
+ Drip	--	--	--	-24.2 ± 0.3 b	22.3 ± 5.8 a	34.6 ± 6.4 a	2.9 ± 0.4 a
Alone	--	--	--	-26.3 ± 0.5 a	23.4 ± 6.7 a	20.5 ± 2.4 b	3.5 ± 0.5 a
F[d] (*P*)	--	--	--	36.7 (< 0.01)	< 0.1 (>0.05 NS)	5.7 (< 0.05)	1.4 (>0.05 NS)

[a] Furrow irrigation on 28 July, furrow plus drip was furrow irrigation on 28 July plus 1 h of drip irrigation daily thereafter.

[b] Means of 8 replications in a column not followed by the same letter are significantly different. Least significant differences $P \leq 0.05$.

[c] df = 3,63.

[d] df = 1,63

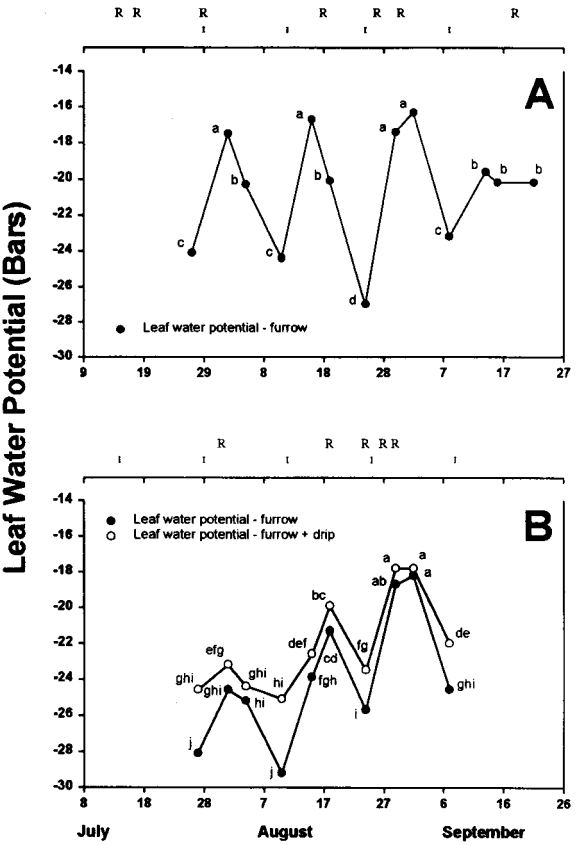

FIG. 1. Cotton leaf water potentials (A) on days 4, 7, and 13 following furrow irrigations in 1999 and (B) on days 4, 7, 13 following furrow irrigations alone and furrow irrigations plus supplementary drip (1 h/day) irrigations in 2000. Data points not followed by the same letter are significantly different. Methods of Least Significant Differences. $P \leq 0.05$. R = dates for rains, I = dates for irrigations.

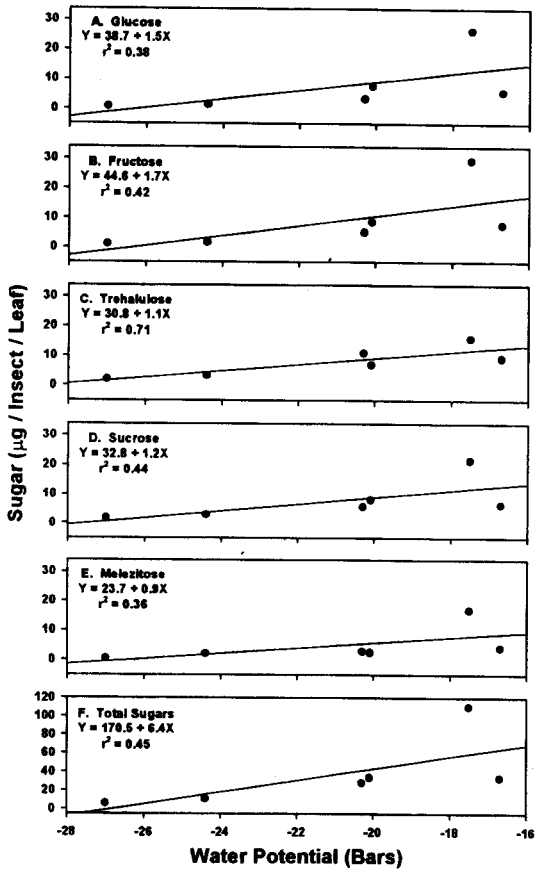

FIG. 2. Mean micrograms (± SE) of sugars in honeydew produced by SPW Strain B feeding on cotton leaves on days 4, 7, and 13 following irrigation, df = 1, 5 for all analyses. F (glucose) = 2.43; P = 0.19; F (fructose) = 2.90; P = 0.16; F (trehalulose) = 9.68; P = 0.04; F (sucrose) = 3.14; P = 0.15; F (melezitose) = 2.20; P = 0.21; F (all sugars) = 3.33; P = 0.14.

average differences for all sampling dates showed higher leaf water potentials for plants in plots with furrow plus drip irrigations compared with furrow alone (Table 2). Also, leaf water potentials were significantly higher on day 4 compared with 7 and 13, and on day 7 compared with day 13 after irrigation.

Honeydew collections and SPW counts. Numbers of SPW were not significantly different in furrow plus drip compared with furrow alone irrigated cotton (Table 2). However, significantly fewer SPW (1.4 ± 0.2) occurred on day 13 after irrigation compared with day 7 (3.8 ± 0.4) and day 4 (4.5 ± 0.6) after irrigation. Numbers of honeydew drops per SPW collected on aluminum foil were not statistically different on days 4, 7 or 13 after irrigation, but the weight (µg) of honeydew collected on day 13 following irrigation was higher compared with day 4 or 7 (Table 2). Weight of honeydew was also higher for SPW feeding on furrow plus drip compared with furrow alone irrigated cotton. For individual sugars, micrograms of sucrose collected was greater on day 13 compared with day 4 or 7 (Table 3). Also, micrograms of sucrose collected were greater in furrow alone compared with furrow plus drip irrigated cotton. This was true for all individual sugars measured and their totals, but the results were not statistically significant. However, micrograms of fructose, trehalulose, melezitose, and total sugars per microgram of honeydew were significantly greater on day 13 following irrigation compared with day 4 (Table 4).

For honeydew collected in plastic bag enclosed cotton leaves with SPW, highest concentrations of all individual sugars measured and their total occur in honeydew collected on day 13 following irrigations compared with days 4 and 7 (Table 5). More of the individual sugars occurred in honeydew produced by SPW feeding on furrow plus drip irrigated cotton compared with furrow alone irrigated cotton.

2001 Greenhouse: Experiments 1 and 2. Water supply leaf water potentials and honeydew collections. At the initiation of greenhouse Experiment 1, all plants were supplied 100ml of water per pot resulting in leaf water potentials of -6.7 and -7.9 bars for wet or dry designated plants (Table 6, Experiment 1). After four days, plants receiving 100ml of water per day had leaf water potentials averaging -8.6 bars compared to -24.0 bars for plants receiving 25ml of water per day. Maximum (36° to 41°C) and minimum (21° to 26°C) air temperatures occurred during 1200 h to 1600 h and 0100 h to 0400 h, respectively.

Honeydew collections. In Experiment 1, SPW produced 125.1 honeydew drops in 24 h feeding periods on 100ml per day watered plants and 80.1 honeydew drops feeding on plants receiving 25ml of water per day. The average total micrograms of all sugars (glucose, fructose, trehalulose, sucrose and melezitose) and for each sugar, except sucrose, produced per SPW were significantly higher when feeding on plants that received 100ml of water daily compared to SPW feeding on plants receiving 25 to 50ml of water per day (Table 6, Experiment 1). Amounts of individual sugars (micrograms), except for glucose and sucrose, per µg of honeydew were greater when SPW were feeding on 100ml per day watered plants compared with 25ml of water-per-day plants.

For Experiment 2, leaf water potentials at the initiation of the experiment (all plants 100ml of water) averaged –5.8 bars for the wet treatment plants and –5.5 bars for the designated dry treatment plants (Table 6, Experiment 2). Honeydew drops and micrograms of honeydew and SPW, total and individual sugars per SPW were not significantly different. Thereafter, leaf water potential averaged -13.8 bars for the wet treatment and -19.5 bars for the dry treatment. Numbers of honeydew drops per SPW were significantly higher for SPW feeding on leaves of wet compared with dry plants. Weights (µg) of honeydew per SPW feeding on leaves of wet plants were also greater compared with dry plants. Total sugars in honeydew per SPW were also significantly greater for SPW feeding on leaves of wet plants compared with dry plants (Table 6, Experiments 1

Table 3. Mean (± SE) Micrograms (x 100) of Honeydew Sugars Per SPW Collected on Aluminum Foil from SPW Feeding on Cotton Leaves 4, 7, and 13 Days Following Furrow Plus Drip or Furrow Alone Irrigations.

Irrigation Type[a]	Days After Irrigation	Sugar[b] G	F	T	S	M	Total
Furrow + Drip	4	0.1 ± < 0.1 a	0.9 ± 0.2 a	2.6 ± 0.5 a	1.5 ± 0.4 a	0.8 ± 0.2 a	6.9 ± 1.3 b
Alone	4	0.2 ± < 0.1 a	0.7 ± 0.2 a	2.8 ± 1.0 a	2.0 ± 0.7 a	0.4 ± 0.2 a	6.1 ± 1.6 b
Furrow + Drip	7	0.6 ± 0.4 a	1.6 ± 0.6 a	2.9 ± 0.6 a	2.0 ± 0.2 a	0.8 ± 0.3 a	7.9 ± 1.5 b
Alone	7	0.5 ± 0.4 a	1.4 ± 0.7 a	2.4 ± 0.7	2.4 ± 0.5 a	0.7 ± 0.4 a	7.5 ± 1.9 b
Furrow + Drip	13	0.3 ± 0.1 a	0.9 ± 0.2 a	2.4 ± 0.5 a	2.2 ± 0.3 a	0.4 ± 0.2 a	6.2 ± 0.9 b
Alone	13	0.8 ± 0.3 a	2.3 ± 0.1 a	4.2 ± 1.7 a	3.7 ± 0.3 a	0.4 ± 0.1 a	11.5 ± 2.1 a
F (*P*); df = 2,35	- -	0.8 (>0.05 NS)	1.9(>0.05 NS)	1.2(>0.05 NS)	0.9 (>0.05 NS)	0.7 (>0.05 NS)	2.6 (≤0.05)
Days after irrigation							
	4	0.2 ± < 0.1 a	0.8 ± 0.2 a	2.7 ± 0.5 a	1.8 ± 0.4 b	0.6 ± 0.1 a	6.0 ± 1.0 a
	7	0.6 ± 0.3 a	1.5 ± 0.4 a	2.7 ± 0.4 a	0.2 ± 0.3 b	0.8 ± 0.2 a	7.7 ± 1.2 a
	13	0.6 ± 0.2 a	1.6 ± 0.3 a	3.3 ± 0.9 a	3.3 ± 0.3 a	0.4 ± 0.1 a	8.9 ± 1.3 a
F (*P*); df = 2,35	- -	1.7 (>0.05 NS)	1.8 (>0.05 NS)	0.5 (>0.05 NS)	3.6 (< 0.05)	1.4 (>0.05 NS)	2.2 (>0.05 NS)
Furrow + Drip	- -	0.3 ± 0.1 a	1.1 ± 0.2 a	2.6 ± 0.3 a	1.9 ± 0.2 b	0.7 ± 0.1 a	6.7 ± 0.7 a
Alone	- -	0.5 ± 0.1 a	1.5 ± 0.3 a	3.1 ± 0.7 a	2.7 ± 0.3 a	0.5 ± 0.1 a	8.4 ± 1.1 a
F (*P*); df = 1,35	- -	0.5 (>0.05 NS)	0.9 (>0.05 NS)	0.6 (>0.05 NS)	4.8 (≤0.05)	0.1(>0.05 NS)	2.3 (>0.05 NS)

[a] Furrow irrigation 28 July, furrow plus drip irrigation on 28 July plus 1 h supplementary drip irrigation daily thereafter.

[b] G = glucose, F = fructose, T = trehalulose, S = sucrose, M = melezitose. Means of 8 replication in a column not followed by the same letter are significantly different. Method of least significant differences $P \leq 0.05$.

TABLE 4. Mean Micrograms (± SE) of Sugar Per Microgram of Honeydew Collected on Aluminum Foil and Produced Per SPW Feeding on Cotton Furrow Plus Drip or Furrow Alone Irrigated Cotton.

Irrigation type	Days after irrigation	Sugar[a]					
		G	F	T	S	M	Total
Furrow							
+Drip	4	0.02 ± 0.01 a	0.17 ± 0.05 a	0.50 ± 0.12 a	0.26 ± 0.06 a	0.15 ± 0.05 a	1.12 ± 0.27 a
Alone	4	0.04 ± 0.02 a	0.12 ± 0.04 a	0.41 ± 0.12 a	0.54 ± 0.36 a	0.07 ± 0.04 a	1.18 ± 0.48 a
Furrow							
+ Drip	7	0.18 ± 0.12 a	0.38 ± 0.21 a	0.60 ± 0.10 a	0.53 ± 0.16 a	0.13 ± 0.04 a	1.82 ± 0.47 a
Alone	7	0.14 ± 0.12 a	0.33 ± 0.21 a	0.38 ± 0.11 a	0.39 ± 0.13 a	0.14 ± 0.08 a	1.38 ± 0.55 a
Furrow							
+ Drip	13	0.15 ± 0.05 a	0.50 ± 0.17 a	1.62 ± 0.83 a	1.24 ± 0.40 a	0.14 ± 0.07 a	3.64 ± 1.43 a
Alone	13	0.22 ± 0.06 a	0.70 ± 0.23 a	1.44 ± 0.77 a	1.01 ± 0.13 a	0.13 ± 0.04 a	3.49 ± 1.07 a
F[b]		.23	0.38	0.01	0.66	0.56	0.06
P		>0.05	>0.05	>0.05	>0.05	>0.05	>0.05
Days after irrigation							
	4	0.03 ± 0.01 a	0.15 ± 0.03 b	0.46 ± 0.08 b	0.40 ± 0.18 b	0.11 ± 0.03 a	1.15 ± 0.27 b
	7	0.16 ± 0.08 a	0.35 ± 0.14 ab	0.49 ± 0.08 b	0.16 ± 0.10 b	0.14 ± 0.04 a	1.60 ± 0.35 b
	13	0.19 ± 0.04 a	0.60 ± 0.14 a	1.53 ± 0.55 a	1.12 ± 0.21 a	0.13 ± 0.04 a	3.57 ± 0.86 a
F[b]		2.13	3.74	4.25	5.90	0.19	5.84
P		>0.05	0.03	0.02	0.02	>0.05	0.02
Furrow							
+ Drip	- -	0.12 ± 0.04 a	0.35 ± 0.09 a	0.91 ± 0.29 a	0.68 ± 0.16 a	0.14 ± 0.03 a	2.19 ± 0.54 a
Alone	- -	0.13 ± 0.05 a	0.38 ± 0.11 a	0.74 ± 0.27 a	0.65 ± 0.14 a	0.11 ± 0.03 a	2.02 ± 0.47 a
F[c]		0.04	0.06	0.23	0.02	0.52	0.08
P		>0.05	>0.05	>0.05	>0.05	>0.05	>0.05

[a] G = glucose, F = fructose, T = trehalulose, S = sucrose, M = melezitose. Means of 8 replications in a column in a group not followed by the same letter are significantly different. Method of least significant differences $P \leq 0.05$.

[b] df = 3,63.

[c] df = 1,63.

Table 5. Mean (± SE) Micrograms (x 100) of Honeydew Sugars Per SPW Feeding on Cotton Leaves Enclosed in Plastic Bags on Days 4 7, and 13 Following Furrow Plus Drip or Furrow Alone Irrigations.

Irrigation Type	Days After Irrigation	Sugar[a] G	F	T	S	M	Total
Furrow + Drip	4	5.0 ± 1.4 c	11.0 ± 2.8 bc	6.6 ± 1.8 bc	5.4 ± 1.1 cd	6.7 ± 2.3 b	34.8 ± 8.5 bcd
Alone	4	3.7 ± 1.3 c	6.8 ± 2.0 c	3.5 ± 1.1 c	2.9 ± 0.6 d	1.8 ± 0.6 b	18.7 ± 5.0 d
Furrow + Drip	7	9.8 ± 2.1 bc	19.4 ± 3.8 bc	17.7 ± 5.0 b	12.2 ± 2.1 b	9.9 ± 2.6 b	69.0 ± 13.8 b
Alone	7	6.8 ± 1.1 bc	14.2 ± 2.5 bc	14.7 ± 5.0 bc	8.8 ± 1.8 bc	8.2 ± 2.8 b	52.7 ± 12.5 bcd
Furrow + Drip	13	33.0 ± 5.8 a	61.0 ± 9.3 a	32.5 ± 7.8 a	24.0 ± 3.3 a	22.7 ± 6.9 a	173.1 ± 25.0 a
Alone	13	13.1 ± 3.4 b	22.8 ± 6.1 b	10.0 ± 2.5 bc	8.4 ± 2.1 bc	3.3 ± 1.2 b	57.4 ± 14.1 bc
F[b] (*P*)	- -	5.7 (< 0.05)	6.9 (< 0.01)	3.23 (< 0.03)	6.6(< 0.01)	4.2 (< 0.01)	7.5 (< 0.01)
Days after irrigation							
	4	4.4 ± 0.9 b	8.9 ± 1.8 b	5.1 ± 1.1 b	4.2 ± 0.7 c	4.2 ± 1.3 b	26.7 ± 5.2 c
	7	8.3 ± 1.2 b	16.8 ± 2.3 b	16.2 ± 3.4 a	10.5 ± 1.4 b	9.1 ± 1.9 ab	60.8 ± 9.2 b
	13	23.0 ± 4.2 a	41.9 ± 7.3 a	21.2 ± 4.9 a	16.2 ± 2.8 a	13.0 ± 4.2 a	115.3 ± 20.4 a
F[b] (*P*)	- -	21.7(< 0.01)	22.8 (< 0.01)	7.6 (< 0.01)	18.4 (< 0.01)	4.6 (< 0.01)	19.6 (< 0.01)
Furrow + Drip	- -	12.9 ± 2.6 a	24.8 ± 4.6 a	16.0 ± 3.0 a	11.6 ± 1.7 a	10.8 ± 2.3 a	76.0 ± 12.8 a
Alone	- -	6.8 ± 1.2 b	12.7 ± 2.1 b	8.6 ± 1.6 b	6.0 ± 0.8 b	4.0 ± 0.9 b	38.1 ± 5.6 b
F[c] (*P*)	- -	9.9 (< 0.01)	13.2 (< 0.01)	6.9 (< 0.01)	17.5 (< 0.01)	10.3 (< 0.01)	15.9 (< 0.01)

[a] G = glucose, F = fructose, T = trehalulose, S = sucrose, M = melezitose, To = total. Means of 8 replications in a column not followed by the same letter are significantly different. Method of least significant difference $P \leq 0.05$.

[b] df = 3,63.

[c] df = 3,63.

[d] df = 1,63

TABLE 6. Mean (± SE) Cotton Leaf Water Potentials, Numbers of Honeydew Drops, Total Micrograms of Sugars Per SPW and Per Microgram of Honeydew from SPW Feeding for 24 h on Greenhouse Grown, Well Watered or Dry Cotton.

Days of drying	Soil condition	Water/ day (ml)	Leaf water potential (-bars)	Honeydew/SPW		Total Sugar (µg) Per	
				Drops (No.)	µg	SPW[a]	µg of Honeydew
				Greenhouse Experiment 1[b]			
0	Wet	100	-6.7 ± 0.1 a	64.7 ± 17.1 a	10.5 ± 2.2 a	2.65 ± 0.90 a	0.21 ± 0.05 a
	Dry	100	-7.9 ± 0.3 a	83.4 ± 15.7 a	14.7 ± 3.4 a	3.70 ± 1.26 a	0.23 ± 0.05 a
t[c]	- -	- -	1.8	0.8	0.8	0.9	0.3
4	Wet	100	-8.6 ± 0.8 a	125.1 ± 13.6 a	33.0 ± 11.7 a	7.41 ± 2.05 a	0.26 ± 0.04 a
	Dry	25	-24.0 ± 3.4 b	80.1 ± 16.6 b	19.3 ± 10.1 a	0.56 ± 0.32 b	0.05 ± 0.02 b
t[c]	- -	- -	2.8	2.1	1.9	3.5	4.5
				Greenhouse Experiment 2			
0	Wet	100	-5.8 ± 0.7 a	80.5 ± 12.6 a	13.0 ± 2.9 a	1.29 ± 0.37 a	0.10 ± 0.03 a
	Dry	100	-5.5 ± 0.9 a	67.1 ± 67.1 a	14.7 ± 4.2 a	1.55 ± 0.43 a	0.12 ± 0.01 a
t	- -	- -	0.3	1.8	0.3	0.5	0.6
7	Wet	100	-13.8 ± 2.0 a	96.8 ± 6.3 a	29.1 ± 2.2 a	5.42 ± 0.99 a	0.19 ± 0.03 a
	Dry	25	-19.5 ± 2.1 b	64.9 ± 4.9 b	19.8 ± 2.9 b	2.25 ± 0.41 b	0.12 ± 0.02 a
t	- -	- -	4.1	4.0	2.6	3.1	1.6

[a] Glucose, fructose, trehalulose, sucrose and melezitose.

[b] Means of 8 pairs (2 leaf cages per plant, 5 SPW adults females per cage) not followed by the same letter are significantly different.

[c] Students t test. $P \leq 0.5$, df = 10.

and 2). For individual sugars, significant differences per SPW feeding on leaves of wet verses dry plants occurred in most cases (Table 7, Experiments 1 and 2). However, for micrograms per microgram of collected honeydew, the only significant difference was for more trehalulose produced by SPW feeding on leaves of wet verses dry plants.

DISCUSSION

In the greenhouse, Isaacs et al. (1998) reported that SPW feeding rates, as measured by weights of honeydew produced when feeding on water-stressed melons, were about half those compared with SPW feeding on unstressed melons. Carbohydrate concentrations of phloem sap from water-stressed plants were higher compared to unstressed plants. SPW feeding on water-stressed plants with the higher carbohydrate concentrations produced honeydew that had higher concentrations of carbohydrates per gram of honeydew collected compared with those feeding on unstressed plants. A greater proportion of the honeydew produced by SPW feeding on stressed plants was glucose and trehalulose. The authors suggested that isomerization of the more complex (trehalulose) sugar while feeding in phloem indicated a mechanism of osmoregulation used by SPW to maintain its internal water status. Under field conditions in 1999 with cotton furrow irrigated every 14 days, we found that leaf water potentials increased following irrigation but decreased to levels indicating water stress (Radin et al. 1992) by day 13 after irrigation. Amounts of all sugars measured (glucose, fructose, trehalulose, sucrose and melezitose) in excreted honeydew were higher when whiteflies were feeding on non-water-stressed cotton compared with water-stressed cotton. We did not weigh the honeydew but increased amounts of individual sugars in honeydew per SPW feeding on cotton appears to agree with those of Isaacs et al. (1998) for increased amounts of honeydew produced by SPW feeding on non-stressed compared with water stressed melons. Our results also appear to agree with the laboratory results of Salvucci et al. (1997) that *B. argentifolii* Bellows and Perring (= SPW Strain B) feeding through membranes on solutions containing increasing amounts of sucrose excreted honeydew containing increased amounts of trehalulose. Honeydew sugar composition may also be affected by other factors. For example most of the sugar in cotton phloem tissue is sucrose (Tarczynski et al. 1992); whereas galactosides such as raffinose and stachyose as well as sucrose are found in phloem in melons (Byrne and Miller 1990). Other plants may contain sugar alcohols such as inositol, mannitol and sorbitol (Davis et al. 1993). These polyol, non-sucrose sugars, and their metabolic products can also appear in honeydew of insects feeding on the various host plants (Byrne and Miller 1990, Hendrix et al. 1992, Davis et al. 1993). Polyol accumulations are known mechanisms for thermoprotection and a case in point is the recently demonstrated ten-fold increase in sorbitol content in *B. argentifolii* in response to elevated temperatures (Wolf et al. 1998).

Our results in the field in 2000 with honeydew collected on aluminum foil showed that weights of honeydew produced by SPW feeding on furrow plus drip irrigated cotton were higher compared with furrow irrigated alone cotton which appears to support our results in the field in 1999. Also, higher micrograms of individual sugars (fructose, trehalulose, and sucrose) per microgram of honeydew produced by SPW feeding on cotton 13 days following irrigation provides additional evidence that the changes in the composition of sugars that occur in honeydew by SPW feeding on the water stressed cotton indicate a mechanism for maintaining water balance as suggested by other authors (Issac et al. 1998, Salvucci et al. 1997).

Flint et al. (1996) in the field demonstrated higher SPW populations in non-stressed cotton irrigated weekly as opposed to cotton irrigated every-other-week (stressed). The results remain unexplained, but higher concentration of carbohydrates and/or some other

TABLE 7. Mean (± SE) Micrograms of Honeydew Sugars Per SPW and Per Microgram of Honeydew from Greenhouse Grown, Well Watered or Dry Cotton (Experiment 1 and 2).

Days of drying	Soil conditions	ml Water/ day	Sugar[a] G	F	T	S	M
Experiment 1				Sweetpotato Whitefly			
0	Wet	100	0.37 ± 0.01 b	0.25 ± 0.08 a	1.90 ± 0.77 a	0.02 ± 0.02 a	0.11 ± 0.04 a
	Dry	100	0.58 ± 0.01 b	0.44 ± 0.13 a	2.77 ± 1.02 a	0.06 ± 0.04 a	1.17 ± 0.03 a
t	--	--	1.3	1.3	0.7	0.7	0.2
4							
	Wet	100	0.37 ± 0.08 b	0.36 ± 0.09 a	6.34 ± 1.75 a	0.05 ± 0.04 a	2.74 ± 0.07 a
	Dry	25	1.30 ± 0.03 a	0.04 ± 0.03 b	0.45 ± 0.03 b	0.03 ± 0.04 a	0.01 ± 0.01 b
t	--	--	2.9	3.4	3.4	0.5	3.7
				Micrograms of Honeydew			
0	Wet	100	0.04 ± 0.04 a	0.02 ± <0.01 a	2.14 ± 0.05 a	<0.01 ± <0.01 a	0.01 ± <0.01 a
	Dry	100	0.04 ± 0.04 a	0.03 ± 0.01 a	0.15 ± 0.04 a	0.01 ± 0.01 a	0.01 ± 0.01 a
t	--	--	0.6	0.5	0.2	0.3	0.3
4							
	Wet	100	0.02 ± 0.01 a	0.01 ± <0.01 a	222.10 ± 0.04 a	0.01 ± <0.01 a	0.01 ± <0.01 a
	Dry	25	0.01 ± 0.01 a	<0.01 ± <0.01 a	0.03 ± 0.02 b	<0.01 ± <0.01 a	<0.01 ± <0.01 a
t	--	--	0.8	2.8	4.5	0.8	3.8
Experiment 2				Sweetpotato Whitefly			
0	Wet	100	0.19 ± 0.04 a	0.18 ± 0.04 a	0.86 ± 0.31 a	0.01 ± 0.01 a	0.04 ± 0.02 a
	Dry	100	0.14 ± 0.03 a	0.13 ± 0.05 a	0.88 ± 0.34 a	0.35 ± 0.35 a	0.05 ± 0.04 a
t	--	--	1.2	0.9	0.1	1.0	0.2
7	Wet	100	0.31 ± 0.09 a	0.27 ± 0.04 a	4.35 ± 0.83 a	0.38 ± 0.12 a	0.12 ± 0.01 a
	Dry	25-50	0.15 ± 0.02 b	0.14 ± 0.02 b	1.74 ± 0.36 b	0.10 ± 0.02 b	0.11 ± 0.04 a
t	--	--	8.11	2.6	2.9	2.3	0.2
				Micrograms of Honeydew			
0	Wet	100	0.02 ± 0.01 a	0.02 ± 0.01 a	0.06 ± 0.02 a	<0.01 ± <0.01 a	<0.01 ± <0.01 a
	Dry	100	0.02 ± 0.02 a	0.01 ± 0.01 a	0.08 ± 0.02 a	0.01 ± 0.01 a	<0.01 ± <0.01 a
t	--	--	0.7	0.9	0.7	0.9	0.8
7	Wet	100	0.02 ± 0.01 a	0.01 ± 0.01 a	0.15 ± 0.01 a	0.01 ± 0.01 a	0.01 ± 0.01 a
	Dry	25-50	0.02 ± 0.01 a	0.01 ± 0.01 a	0.09 ± 0.01 b	0.00 ± 0.01 a	0.01 ± 0.01 a
t	--	--	0.1	1.3	1.9	<0.1	0.3

[a] G = glucose, F = fructose, T = trehalulose, S = sucrose, M = melezitose. Means of 8 replications (2 leaf cages per replication, 5 SPW adult females per cage) not followed by the same letter are significantly different. Students t test. $P \leq 0.05$, df = 10.

nutrients in the phloem of water-stressed cotton may provide some physical or nutritional advantages for SPW that was reflected in higher reproductive rates. We did not find higher SPW populations on water-stressed vs non-water-stressed cotton in our studies. This was probably a result of our small plot size (0.03 ha) compared with 0.1 ha plots for Flint et al. (1996).

The results of our current studies and those of Isaacs et al. (1998) and Flint et al. (1996) show the interactions of melon and cotton host response to water management and subsequent effects on SPW feeding, honeydew production and population dynamics. Further studies should be conducted to identify other crop production inputs as well as water management that may affect SPW biology.

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EFFECT OF IRRIGATION REGIMES AND PLANT POPULATIONS ON GREENBUG (HOMOPTERA: APHIDIDAE) ABUNDANCE IN GRAIN SORGHUM

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ABSTRACT

In a three-year study (1998-2000), we determined the effect of evapotranspiration-based irrigation regimes and plant population density on peak greenbug abundance in grain sorghum. Although yearly temperatures, total precipitation and seasonal distribution of precipitation varied greatly, when peak greenbug abundances were compared within an irrigation regime across plant population levels, peak densities generally decreased significantly (p=0.05) as plant populations increased. When peak greenbug abundances were compared within a plant population across irrigation regimes, peak greenbug abundances either increased significantly (p=0.05) as irrigation levels increased or were not significantly different. Our results compared favorably to previous research which examined sorghum plant density and canopy coverage.

INTRODUCTION

Sorghum, *Sorghum bicolor* (L.) Moench, producers continuously seek ways to maximize irrigated grain sorghum profits using minimum inputs. Variations in plant population densities and scheduled irrigations are two interrelated practices being examined. As these new agronomic practices are considered or implemented, the potential impact on insect pests should be examined as well.

The greenbug, *Schizaphis graminum* (Rondani), is a major pest of sorghum and small grains in the United States (Porter et al. 1997). Ground cover, plant density, and surface residue influence greenbug abundances in sorghum. Harvey et al. (1982) noted that greenbug were collected in significantly fewer numbers from traps in sorghum fields with closed canopies than from traps in fields with open canopies. When comparing greenbug-resistant and greenbug-susceptible sorghum hybrids, Harvey and Thompson (1988) found greenbug abundance per plant on the same hybrid to be significantly less on dense plant populations than on low plant populations. They also found no significant difference in greenbug abundance on resistant hybrids planted at a reduced plant population density and susceptible hybrids planted at an increased plant population density, concluding that planting resistant hybrids in thick stands may keep greenbug infestations below the economic injury level. Burton and Krenzer (1985) and Burton et al. (1987) reported fewer greenbugs in wheat and sorghum, respectively, grown in reduced tillage plots with greater surface residue that in clean-tilled plots with little surface residue.

Water stress in sorghum also affects greenbug abundance in sorghum. Kindler and Staples (1981) concluded that the greenbug economic injury level is less in water-stressed sorghum than in well-watered sorghum. Michels and Undersander (1986) demonstrated that water stress negatively influenced greenbug reproduction in sorghum when water potential

on the stressed plants fell below –0.3 MPa. In addition, greenbugs were distributed toward the top of water-stressed plants, and toward the bottom of well-watered plants.

The objective in this research was to determine the effect of sorghum plant population densities and scheduled center-pivot irrigation practices on greenbug abundance in grain sorghum.

MATERIALS AND METHODS

The field study was conducted at the USDA-ARS, Conservation and Production Laboratory, Bushland, TX.

Irrigation was supplied by a center pivot irrigation system equipped with LEPA nozzles attached to 1.5-m long drop tubes. The center pivot system was 293 m long, measured from the pivot to the end of six, 49-m spans, and covered 13.46 ha. The field covered one half of a full circle and was divided into five, 2.69 ha wedges (Fig. 1). From left to right in Fig. 1, wedge one was planted to continuous wheat. Wedges two through five were rotated on a 4-year schedule between corn, sorghum, sugar beets and wheat. Therefore sorghum was planted in a different wedge each year. Each wedge was subdivided into six replicates. Sorghum variety Cargill 647 was planted on May 15 in 1998, 1999 and 2000, at five population densities (74,132, 172,974, 259,461, 333,592, and 420,079 plants per ha, respectively) in twelve-row plots (76 cm row spacing) in each replicate, beginning at span two from the pivot point and extending to span six. Plant population was randomly assigned to a replicate. The portion of the field located under span one was not used in order to preclude extremely small plot size. For convenience, we designate the plant populations as 74k/ha, 173k/ha, 260k/ha, 333k/ha, and 420k/ha, respectively.

The land under the center pivot was cultivated in a circle to reduce runoff and improve irrigation distribution uniformity. Irrigation regimes consisted of three application rates based on the potential evapotranspiration rate (PET) within the field. PET was determined through climatic data collected from an on-site weather station (Campbell Scientific, Logan UT). The irrigation system was manually adjusted to apply a specific amount of water across a replicate within a wedge. As a base for computing PET, 6.35 cm of water was applied preplant to bring the soil water profile to capacity at the beginning of each season. Afterwards, water was applied as necessary (usually each week) to bring the soil water profile to the desired level. For example, if the PET calculations determined that 2.54 cm of water evaporated from the field in a given week, PET100 treatments received 2.54 cm the following week. The PET075 and PET050 treatments would receive 1.91 and 1.77 cm, respectively. Rainfall modified irrigation events and rates, and weekly irrigations were not always needed. The irrigation system was limited to a maximum application of 6.35 cm at any one irrigation event.

The field was monitored for greenbugs beginning in late May or early June of each year. Once greenbugs were found, weekly sampling began. At each sampling date, five plants from each replicate each were examined from each treatment (a total of 30 plants/plant population/irrigation regime). Plants were randomly selected from areas in the field near neutron probes which had been placed in the field in the spring of the year. Using the neutron probes as location markers ensured that the samples came from the same general area of the field throughout the season. All samples were taken within 15 m of the neutron probe within a treatment. All greenbugs were counted on each plant, with no distinction made between adults and nymphs. Data collection ended when greenbugs were no longer evident in the field.

Initial examination of the greenbug data for the three years suggested that an analysis of greenbug abundance by plant population by irrigation regime for each sampling date would prove to be unwieldy. Therefore we limited the analyses to the average of three

sampling dates (one week before the peak greenbug abundance, at peak greenbug abundance, and one week following the peak greenbug abundance) each year.

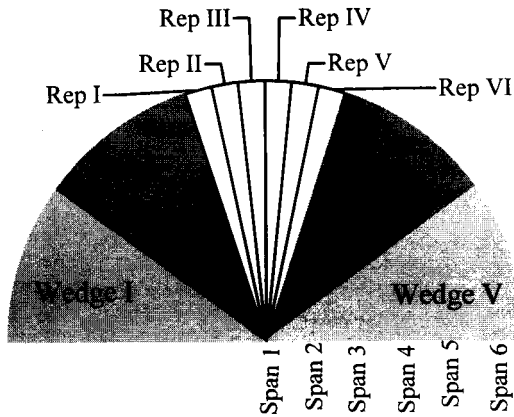

FIG. 1. Experimental center-pivot plot layout at Bushland, TX. Sorghum plots followed a wheat-sorghum-sugarbeet-fallow rotation, therefore the sorghum plot in a given year could be located in Wedges II through V in a given year, depending on the rotation. Wedge I was restricted to continuous wheat production. Note that Wedge III, for clarity, is not labeled. Top of the figure is north.

Peak greenbug abundance occurred on 17 August, 21 July, and 11 August in 1998, 1999, and 2000, respectively. Therefore, sampling dates used to generate the average peak greenbug numbers were: 18 August, 17 August and 24 August in 1998; 14 July, 21 July, and 28 July in 1999; and 4 August, 11 August, and 18 August in 2000.

Each year's data were analyzed as a 2x2 factorial design following the general linear model: greenbug abundance = plant population density + irrigation regime + (plant population density*irrigation regime). A *ln+1* transformation was used on all data prior to analyses to normalize the relationship between the means and variances (Steel and Torrie 1960). Because there was a significant plant population density by irrigation regime interaction each year, mean greenbug abundance were subjected to least square means analyses and a corresponding *t*-test, p=0.05, (SAS 1999) to determine significant differences within each year among means by irrigation regimes within plant populations and plant populations within irrigation regimes.

To develop a general visual description of the relationship between peak greenbug abundance, plant populations and irrigation regimes, the combined data were plotted in three dimensions using S-Plus 6 (2001).

RESULTS

The research was conducted through three cropping seasons, 1998-2000. Varied rainfall and temperatures were observed each year. Fig. 2 and 3 illustrate the seasonal average daily temperatures and rainfall/irrigation distribution throughout the three-year study, respectively. Rainfall and irrigation data for the three years are contained in Table 1.

For reference, the 60-year average for rainfall at Bushland, TX, from 1 May through 2 October is 33 cm. Average temperatures can be better gleaned from Fig. 2.

TABLE 1. Summary of Rainfall and Irrigation Water Applied, Bushland, TX, 1998-2000.

Year	Rainfall	Irrigation water applied(cm/ha) [a]			Total water (cm)		
	(cm)	PET 050	PET 075	PET 100	PET 050	PET 075	PET 100
1998	8.43	34.70	45.21	61.72	43.13	53.64	70.15
1999	30.28	6.48	6.48	12.70	36.75	36.75	42.98
2000	7.37	30.56	49.25	61.37	37.92	56.62	68.73

[a] Irrigation water applied to compensate for 50, 75 or 100% of potential evapotranspiration (PET).

Briefly summarizing the three years: 1998 was a dry summer with about one fourth the average rainfall, and was much warmer than the 60-year average; 1999 was an average summer in terms of rainfall, and was generally cooler than the 60-year average; 2000 was another dry summer, similar to 1998, but temperatures were almost equal to the 60-year average throughout the growing season. In addition to being very dry, the distribution of rainfall during the summers of 1998 and 2000 varied considerably. In 1998 total rainfall was low, but distributed throughout the season. On the other hand, rainfall during the 2000 cropping season came early, prior to 24 July, and there was no precipitation from July 24th through October 2nd. Because of these rainfall differences, irrigation applications were made at regular intervals throughout the season in 1998, but were grouped toward the latter half of the season in 2000.

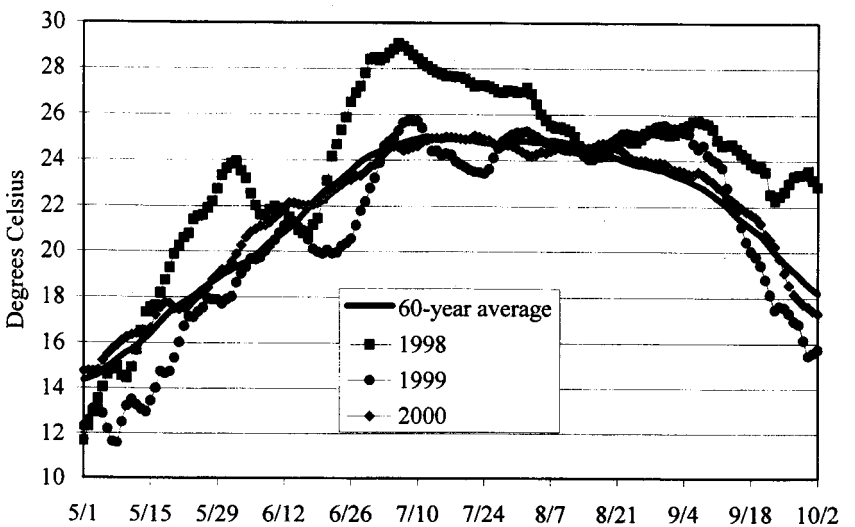

FIG. 2. Comparison of average daily temperatures in 1998-2000 to the 60-year average at Bushland, TX.

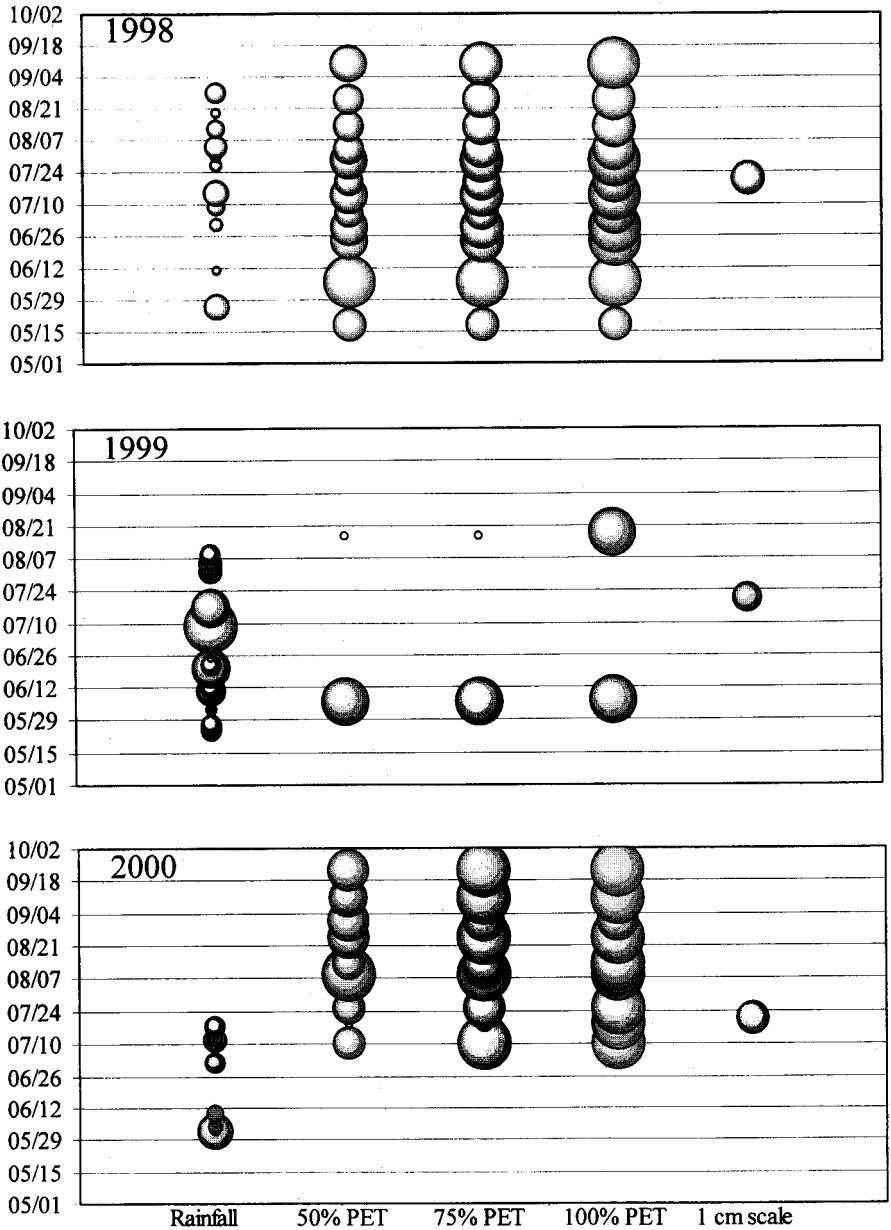

FIG. 3. Comparison of rainfall and irrigation water applied to compensate for 50, 75, and 100% of potential evapotranspiration (PET) in grain sorghum plots at Bushland, TX, 1998-2000.

Peak greenbug abundance by irrigation regime and plant population are found in Tables 2a and 2b. A graphic representation of these results is found in Fig. 4. During the

TABLE 2a. Comparison of Yearly and Aggregate Peak Greenbug Densities in Grain Sorghum, Three Irrigation Regimes within Five Plant Populations. Bushland, TX, 1998-2000.

Irrigation regime [a]	Plants per hectare	Peak greenbug abundance	Std. error	i	t-test comparing mean $_i$ to mean $_j$ [b]														
					j														
					1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
					1998														
PET050	74k	305	35.9	1															
PET075	74k	1,671	183.8	2	↓														
PET100	74k	1,356	140.5	3	↓	ns													
PET050	173k	165	36.8	4															
PET075	173k	525	56.9	5				↓											
PET100	173k	557	99.7	6				↓	ns										
PET050	260k	11	2.1	7															
PET075	260k	459	64.3	8							↓								
PET100	260k	543	108.0	9							↓	ns							
PET050	333k	28	5.2	10															
PET075	333k	91	14.1	11										↓					
PET100	333k	115	17.9	12										↓	ns				
PET050	420k	10	1.6	13															
PET075	420k	44	4.8	14													↓		
PET100	420k	243	54.5	15													↓	↓	
					1999														
PET050	74k	8	1.4	1															
PET075	74k	8	2.1	2	ns														
PET100	74k	7	1.1	3	ns	ns													
PET050	173k	8	1.5	4															
PET075	173k	5	1.1	5				ns											
PET100	173k	2	0.5	6				↑	ns										
PET050	260k	3	0.5	7															
PET075	260k	6	1.1	8							ns								
PET100	260k	10	1.4	9							↓	↓							
PET050	333k	4	0.8	10															
PET075	333k	6	1.1	11										ns					
PET100	333k	3	0.7	12										ns	↑				
PET050	420k	2	0.5	13															
PET075	420k	2	0.5	14													ns		
PET100	420k	3	0.6	15													ns	ns	

TABLE 2a cont. Comparison of Yearly and Aggregate Peak Greenbug Densities in Grain Sorghum, Three Irrigation Regimes within Five Plant Populations. Bushland, TX, 1998-2000.

Irrigation regime [a]	Plants per hectare	Peak greenbug abundance	Std. error	*i*	*t*-test comparing mean $_i$ to mean $_j$ [b] *j* 1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
					2000														
PET050	74k	14	4.2	1															
PET075	74k	26	4.2	2	↓														
PET100	74k	27	4.5	3	↓	ns													
PET050	173k	8	1.9	4															
PET075	173k	23	4.2	5				↓											
PET100	173k	19	3.2	6				↓	ns										
PET050	260k	9	2.3	7															
PET075	260k	17	3.5	8							ns								
PET100	260k	22	4.4	9							↓	ns							
PET050	333k	3	1.0	10															
PET075	333k	7	1.6	11										ns					
PET100	333k	22	3.6	12										↓	↓				
PET050	420k	3	1.0	13															
PET075	420k	10	2.0	14													↓		
PET100	420k	12	3.1	15													↓	ns	
			Aggregate (1998-2000)																
PET050	74k	114	13.8	1															
PET075	74k	508	73.5	2	↓														
PET100	74k	417	57.9	3	↓	ns													
PET050	173k	72	11.6	4															
PET075	173k	178	22.8	5				↓											
PET100	173k	185	33.1	6				↓	ns										
PET050	260k	26	0.9	7															
PET075	260k	158	23.1	8							↓								
PET100	260k	186	34.8	9							↓	ns							
PET050	333k	30	1.7	10															
PET075	333k	51	4.8	11										↓					
PET100	333k	60	6.2	12										↓	ns				
PET050	420k	24	0.6	13															
PET075	420k	36	1.9	14													↓		
PET100	420k	94	17.2	15	↓	↓					↓		↓	↓			↓	ns	

[a] irrigation regime to compensate for 50, 75 or 100% of potential evapotranspiration (PET)

[b] H_o: mean $_{(i)}$ = mean $_{(j)}$, p=0.05. Comparisons are made within yearly or aggregate subheadings only. Ns-no significant difference between mean i and j;↑-mean j significantly greater than mean i; ↓-mean j significantly less than mean i.

TABLE 2b. Comparison of Yearly and Aggregate Peak Greenbug Densities in Grain Sorghum, Five Plant Populations within Three Irrigation Regimes. Bushland, TX, 1998-2000.

Irrigation regime [a]	Plants per hectare	Peak greenbug abundance	Std. error	*i*	*t*-test comparing mean $_i$ to mean $_j$ [b]														
					j														
					1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
					1998														
PET050	74k	305	35.9	1															
PET050	173k	165	36.8	2	↑														
PET050	260k	11	2.1	3	↑	↑													
PET050	333k	28	5.2	4	↑	↑	↓												
PET050	420k	10	1.6	5	↑	↑	ns	↑											
PET075	74k	1,671	183.8	6															
PET075	173k	525	56.9	7						↑									
PET075	260k	459	64.3	8						↑	↑								
PET075	333k	91	14.1	9						↑	↑	↑							
PET075	420k	44	4.8	10						↑	↑	↑	ns						
PET100	74k	1,356	140.5	11															
PET100	173k	557	99.7	12											↑				
PET100	260k	543	108.0	13											↑	ns			
PET100	333k	115	17.9	14											↑	↑	↑		
PET100	420k	243	54.5	15											↑	↑	↑	ns	
					1999														
PET050	74k	8	1.4	1															
PET050	173k	8	1.5	2	ns														
PET050	260k	3	0.5	3	↑	ns													
PET050	333k	4	0.8	4	↑	ns	ns												
PET050	420k	2	0.5	5	↑	↑	ns	ns											
PET075	74k	8	2.1	6															
PET075	173k	5	1.1	7						↑									
PET075	260k	6	1.1	8						ns	ns								
PET075	333k	6	1.1	9						ns	ns	ns							
PET075	420k	2	0.5	10						↑	ns	↑	↑						
PET100	74k	7	1.1	11															
PET100	173k	2	0.5	12											↑				
PET100	260k	10	1.4	13											ns	↓			
PET100	333k	3	0.7	14											↑	ns	↑		
PET100	420k	3	0.6	15											↑	ns	↑	ns	

TABLE 2b (cont. Comparison of Yearly and Aggregate Peak Greenbug Densities in Grain Sorghum, Five Plant Populations within Three Irrigation Regimes. Bushland, TX, 1998-2000.

Irrigation regime [a]	Plants per hectare	Peak greenbug abundance	Std. error	*i*	*t*-test comparing mean $_i$ to mean $_j$ [b] *j* 1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
					2000														
PET050	74k	14	4.2	1															
PET050	173k	8	1.9	2	ns														
PET050	260k	9	2.3	3	ns	ns													
PET050	333k	3	1.0	4	↑	ns	ns												
PET050	420k	3	1.0	5	↑	ns	ns	ns											
PET075	74k	26	4.2	6															
PET075	173k	23	4.2	7						ns									
PET075	260k	17	3.5	8						ns	ns								
PET075	333k	7	1.6	9						↑	↑	↑							
PET075	420k	10	2.0	10						↑	ns	ns	ns						
PET100	74k	27	4.5	11															
PET100	173k	19	3.2	12											ns				
PET100	260k	22	4.4	13											ns	ns			
PET100	333k	22	3.6	14											ns	ns	ns		
PET100	420k	12	3.1	15											↑	ns	↑	↑	
					Aggregate (1998-2000)														
PET050	74k	114	13.8	1															
PET050	173k	72	11.6	2	ns														
PET050	260k	26	0.9	3	↑	↑													
PET050	333k	30	1.7	4	↑	↑	ns												
PET050	420k	24	0.6	5	↑	↑	ns	ns											
PET075	74k	508	73.5	6															
PET075	173k	178	22.8	7						↑									
PET075	260k	158	23.1	8						↑	ns								
PET075	333k	51	4.8	9						↑	↑	↑							
PET075	420k	36	1.9	10						↑	↑	↑	ns						
PET100	74k	417	57.9	11															
PET100	173k	185	33.1	12											↑				
PET100	260k	186	34.8	13											↑	ns			
PET100	333k	60	6.2	14											↑	↑	↑		
PET100	420k	94	17.2	15				↓	↓	↓					↑	↑	↑	ns	

[a] irrigation regime to compensate for 50, 75 or 100% of potential evapotranspiration (PET.)

[b] H_o: mean $_{(i)}$ = mean $_{(j)}$, p=0.05. Comparisons are made within yearly or aggregate subheadings only. Ns-no significant difference between mean i and j;↑-mean j significantly greater than mean i; ↓-mean j significantly less than mean i.

three years in which the study was carried out, overall greenbug abundance was greater in 1998, and much less in 1999 and 2000.

When peak greenbug abundance was compared within a plant population level across irrigation regimes (Table 2a), with two exceptions (1999, PET100, 173k/ha and 1999, PET100, 333k/ha), peak greenbug abundance was either significantly greater as the irrigation level increased, or not significantly different.

The strongest effects were observed in 1998 when greenbug infestations were much greater than in 1999 and 2000, and at or near economically damaging levels. In 1998, peak greenbug densities at PET075 and PET100 were always significantly greater than at PET050, regardless of plant population. Peak greenbug abundance between PET075 and PET100 was not significantly different except at 420k/ha when peak greenbug abundance was significantly greater in PET100 than in PET075 treatments.

Peak greenbug abundance in all treatments was much less in 1999 than in 1998. Greater peak densities were observed with increasing amounts of water, but it was rarely significant. In 2000, when peak greenbug abundance was still relatively low, but greater than in 1999, peak greenbug abundance was always significantly greater in PET100 treatments than in PET050 treatments. Peak greenbug abundance was numerically greater in PET075 treatments than PET050 treatments across plant populations and significantly greater in three out of five plant population levels; the two lowest plant populations, and, as in 1998, at the greatest plant population.

In the analysis of the combined data (Table 2b), peak greenbug abundance was always significantly greater in treatments with 74k/ha or 173k/ha than in treatments with 333k/ha or 420k/ha. Treatments with 260k/ha seemed to be the pivot point, with aphid densities becoming similar to those found at the lesser plant density treatments as PET levels increased.

DISCUSSION

The results of this research supports the findings of Harvey and Thompson (1988) and Harvey et. al. (1982) where greater aphid densities were found in treatments with fewer plants. Harvey and Thompson (1988) had plant populations of 74,100 and 222,300 plants per ha which they designated "High" and "Low" density, which correspond to our lowest (74k/ha) and middle (260k/ha) plant densities. Harvey and Thompson (1988) commented on possible reasons for differences in greenbug densities between high and low plant populations, but did not come to a specific conclusion. They reasoned that spreading the same greenbug population over different plant populations could result in fewer greenbugs per plant in dense planting and thus reduce damage. However, they also pointed out that sorghum plants in denser planting are typically smaller, and cited Teetes and Johnson's (1973) findings that small plants will not tolerate as many greenbugs per plant as large plants. Burton and Krenzer (1985) and Burton et al. (1987) demonstrated that in wheat and sorghum, respectively, greenbugs were repelled by the increased surface residues found in no-tillage practices and complete crop canopies, but were attracted to plantings with decreased surface residue and a greater amount of visible soil surface. They argued that plantings with increased surface residues or dense canopies were more reflective than those with low or no surface residues, and could repel greenbugs. All of our plots were conventionally tilled, therefore the soil surface would conform to Burton et al.'s (1987) low-residue, disturbed soil surface. If greenbug abundance was affected by surface reflectance, our plots should have been equally effective in attracting greenbugs early in the season when plants were small, but plots with greater plant densities may have become less attractive later in the season as the canopy filled more rapidly in the high-plant-density treatments. Burton et al. (1987) also indicated that a complete grassy weed canopy made sorghum fields highly unattractive ($p<0.01$) to greenbugs, and was dominant to the residue effect. Therefore, in our

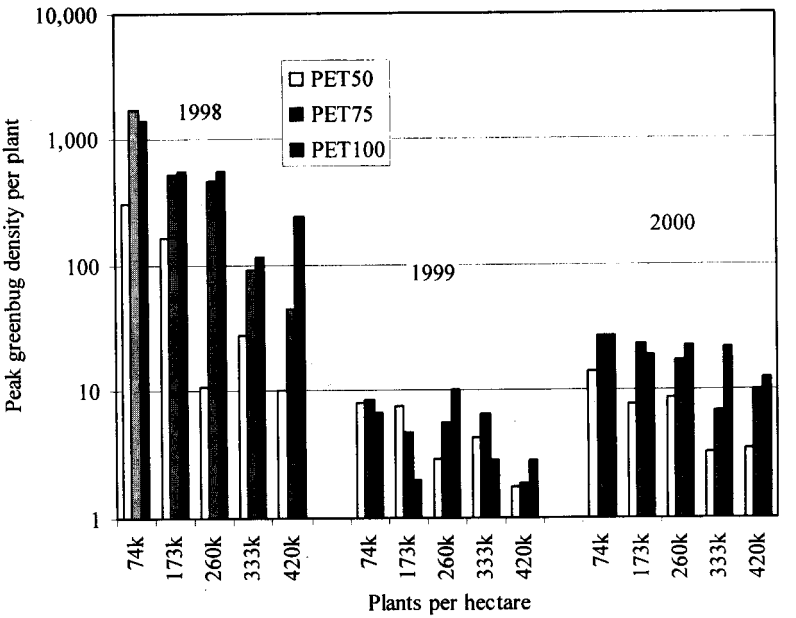

FIG. 4. Peak greenbug abundance per plant at three irrigation regimes and five plant populations in grain sorghum. Bushland, TX 1998-2000.

research, canopy effect may have had a pronounced effect on greenbug infestations at greater plant populations.

Water also played a significant role in our results. Greenbug abundance was typically greater within a plant population treatment when more water was applied. However, each year must be examined individually since rainfall incidence and amount, and therefore the total amount of irrigation water applied based on evapotranspiration, varied from year to year. Michels and Undersander (1986) reported that greenbug reproduction decreased as drought stress increased in grain sorghum; therefore; lesser greenbug densities observed in this research would be expected in grain sorghum plots that received less water than fully-watered plots. It is doubtful that irrigation had a direct effect on greenbug abundance, such as mechanically removing or drowning aphids, because LEPA nozzles were used. These nozzles directed water to bottom of the plants, eliminating effects that could be found with standard drop or overhead nozzles.

A graphic representation of greenbug abundance per plant by total water applied to the field (irrigation plus rainfall) is presented in Fig. 5. The graph was constructed in S-Plus 6 (2001) using a bivariate, three nearest neighbors interpolation.

It is obvious that the greatest greenbug abundance was found at the lesser plant population levels and greater water levels. It is especially notable that at dense plant populations greenbugs were almost absent, and the data reflect primarily uninfested or lightly-infested plants with a heavily infested plant scattered at infrequent intervals. At these greater plant populations, when an occasional plant was found to be heavily infested, it was also found at the greater total water levels. If canopy plays an important role in reducing

greater plant populations, when an occasional plant was found to be heavily infested, it was also found at the greater total water levels. If canopy plays an important role in reducing greenbug colonization of sorghum plants, as suggested by Burton et al. (1987), the isolated peaks found at the greater plant populations may be typical of the type of infestation that can be expected with a full canopy and sufficient water. On the other hand, in fields with plant populations below 240,000 plant/ha and moderate to heavy amounts of water, the likelihood of a significant greenbug infestation is much greater.

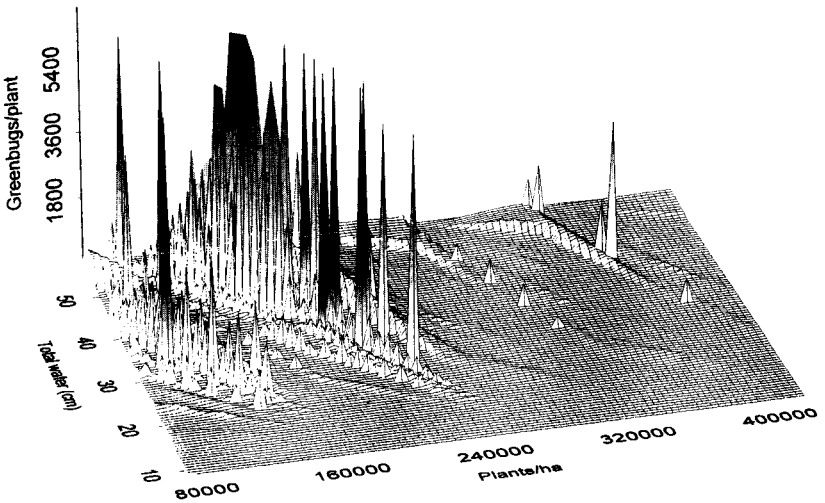

FIG. 5. Graphic representation of greenbug densities associated with varying plant population and total water applied through the season in grain sorghum at Bushland, TX, 1998-2000.

The authors realize that plant population and irrigation regimes are not the only factors impacting greenbug densities in grain sorghum on an annual basis. Obviously, resistant grain sorghum varieties, which were not evaluated in the research, play a role in regulating greenbug infestations. Predator and parasitoid abundance and the occurrence of other aphid species also play a significant role in greenbug infestations each year. Data collected in furrow-irrigated grain sorghum fields at Bushland over a number of years (Michels, unpublished data) indicate that corn leaf aphids (*Rhopalosiphum maidis* Fritch) colonize grain sorghum early in the season and act as a food source for predators. Low corn leaf aphid densities result in low predator densities and increase the chance for a greenbug infestation that reaches economic levels. However, the results from the current study indicate that plant populations and irrigation regimes in grain sorghum play a significant role in the development of greenbug infestations and that these relationships are relatively stable at heavy or light levels of greenbug infestations.

From an applied standpoint, producers raising grain sorghum under irrigation should consider planting grain sorghum at the greatest plant population per ha that can be maintained with available water to maximize yields and reduce chances for economically-damaging greenbug infestations.

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RESPONSES OF *PLUTELLA XYLOSTELLA*[1] and *COLEOMEGILLA MACULATA*[2] TO SELECTED INSECTICIDES[3] IN A RESIDUAL INSECTICIDE BIOASSAY

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ABSTRACT

Immatures of *Plutella xylostella* (L.) and *Coleomegilla maculata* (DeGeer), from laboratory culture, were exposed to selected insecticides and an entomopathogen using a foliar insecticide residue bioassay. Insecticides tested were CGA-293743, neem oil, azadirachtin, emamectin benzoate, abamectin, spinosad, endosulfan, and *Bacillus thuringiensis* Berliner. The entomopathogen tested was *Beauveria bassiana* (Balsamo) Vuillemin. *Bacillus thuringiensis* was significantly more toxic than other treatments to *P. xylostella*. *Bacillus thuringiensis* was also low in toxicity to *C. maculata*. Spinosad, endosulfan, and abamectin were also highly toxic to *P. xylostella*; however, spinosad was non-toxic to *C. maculata*. The remaining treatments were moderately toxic to *P. xylostella*, with the exception of CGA-293743, which was lower in toxicity than any other treatment. CGA-293743, neem oil, and azadirachtin were also low in toxicity to *C. maculata*. *Beauveria bassiana* (both rates) combined with azadirachtin was significantly more toxic to *P. xylostella* than either treatment alone. Similar synergy was found when this combination was applied to *C. maculata*.

INTRODUCTION

Diamondback moth, *Plutella xylostella* (L.), a worldwide pest of cabbage and other crucifers, has developed resistance to synthetic organic insecticides (Liu et al. 1981, Sun et al 1986) and *Bacillus thuringiensis* Berliner (Shelton et al. 1993, Tabashnik et al. 1990). Natural enemies are an important component of management programs for *P. xylostella*; however, control of *P. xylostella* through the use of foliar-applied insecticide is often necessary when an economic threshold is exceeded (Shelton et al. 1983). Because *P. xylostella* has developed resistance to many available insecticides (Shelton et al. 1993), it is important to search for other potentially useful insecticides. In addition, this search should include examination of the toxic effects of these newer insecticides on important natural enemies of *P. xylostella* in order to predict their impact in integrated pest management programs.

[1]Lepidoptera: Plutellidae

[2]Coleoptera: Coccinellidae

[3]Mention of a commercial or proprietary product does not constitute endorsement by USDA

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Coleomegilla maculata (DeGeer) is a polyphagous predator that can complete its development on pollen (Smith 1960), aphids (Smith 1965), and lepidopteran eggs (Conrad 1959, Warren and Tadic 1967). Given its good mobility and reproductive capacity (Wright and Laing 1980), *C. maculata* offers potential as part of an integrated pest management program.

The objective of this research was to determine the toxicity of conventional and newer insecticides with novel modes of action to *P. xylostella* and *C. maculata*. A further objective was to determine the effects of the entomopathogenic fungus *Beauveria bassiana* (Balsamo) Vuillemin alone and when mixed with azadirachtin on *P. xylostella* and *C. maculata*.

MATERIALS AND METHODS

The *P. xylostella* culture was originally collected from cabbage, *Brassica oleracea capitata* L., near Weslaco, Hidalgo County, TX, in 1999, and reared in the laboratory on cabbage. *Coleomegilla maculata* were collected concurrently with *P. xylostella* and were reared in the laboratory on cabbage aphids, *Brevicoryne brassicae* (L.).

Formulated insecticides tested were endosulfan [Phaser 3 suspension concentrate (SC); AgrEvo USA Co., Wilmington, DE], spinosad [Spintor 2 (SC); Dow Agrosciences, Indianopolis, IN], emamectin benzoate [Proclaim 5 soluble granule (SG); Novartis Crop Protection, Greensboro, NC], abamectin [Agri-mek 0.15 emulsifiable concentrate (EC); Novartis Crop Protection, Greensboro, NC], neonicotinyl [CGA 293343 25 wettable granule (WG); Novartis Crop Protection, Greensboro, NC], *Bacillus thuringiensis* Berliner [Dipel dry flowable (DF); Abbott Laboratories, North Chicago, IL], azadirachtin [Neemix 4.5 (EC); Thermo Trilogy Corp., Columbia, MD], and neem oil [Trilogy 5.46 (EC); Thermo Trilogy Corp., Columbia, MD].

Beauveria bassiana (GHA strain) was cultured on Sabouraud dextrose agar supplemented with 1% yeast extract (SDAY) for 18 days at 25°C. This strain of *B. bassiana* is the same one that is the active ingredient of Mycotrol products (Mycotech Corp., Butte, MT). Spores were harvested by scraping them off the culture surface with a sterile spatula; they were then mixed in 0.1% Silwet until an even suspension was obtained. The mixture was then filtered through sterile miracloth (Calbiochem, LaJolla, CA) and diluted to $4.4x10^7$ and $8.8x10^7$ spores/ml and 0.01% Silwet. Silwet was used as a surfactant because the spore powder is hydrophobic. Final spore concentration was $2.11x10^{10}$ spores per 8 oz ($8.8x10^7$ spores/ml). Treatment rates alone and with azadirachtin are shown in Table 2.

Spore viability was tested before each experimental run by plating spores on potato dextrose agar and then incubating them at 25°C. After 24 h, the spores were observed under a phase contrast microscope at 400x, and the number of spores germinated in the first 500 spores observed was determined. Spore viability was 94%.

Rates of formulated insecticides applied to cabbage plants were selected by referring to an appropriate control guide (Sparks 1997) or from manufacture's recommendations (in the case of newer or non-registered materials). These rates are shown in Table 1. Cabbage plants, grown in 4x4 cm pots in a greenhouse, were treated with insecticides using a laboratory spray chamber (DeVries, Mfg., Hollandalle, MN) (Elzen et al. 1998). Air was exhausted from the chamber through a built-in exhaust fan, and the chamber and spray system were washed with water between treatments. The sprayer was calibrated to deliver 282 l/ ha using three TXVS-6 nozzles (two on drops) at 2.2kg/cm2 and 4.8km/h. All treatments contained 0.1% Silwet.

Each treatment consisted of three replicates of 15 cabbage plants. Controls were treated with water plus 0.1% Silwet. One *P. xylostella* or one *C. maculata* (both as second instars) was placed on each plant 30 min after spraying and confined to each plant within a 590-ml ventilated paper cup. Immatures exposed to each compound were held for 96-h at 26 ± 2° C, 55-60% RH, and a 14L:10D photoperiod. Immatures exposed in tests with azidirachtin and *B. bassiana*

alone or mixed were held for 10 days under the same conditions. Mortality was assessed by failure of movement when prodded with a probe. Control mortality was never greater than 10.0 %; data were corrected for control mortality using Abbott's (1925) formula. Percentage mortalities were arcsine transformed and analyzed by analysis of variance; means were separated by least significant difference [$P \leq 0.05$ (SAS Institute 1988)].

RESULTS AND DISCUSSION

Bacillus thuringiensis was significantly more toxic than other treatments to *P. xylostella* (Table 1). As expected, *B. thuringiensis* was low in toxicity to *C. maculata*. Spinosad, endosulfan, and abamectin were also highly toxic to *P. xylostella*; however, spinosad was non-toxic to *C. maculata*. Elzen and Elzen (1999) found that spinosad was non-toxic to the big-eyed bug, *Geocoris punctipes* (Say), in the same foliar insecticide residue bioassay.

TABLE 1. Toxicity of Selected Insecticides to *P. xylostella* and *C. maculata* Immatures in a Foliar Insecticide Residue Bioassay, 96 h Post-treatment.

		% Mortality ± SE[a]	
Treatment[b]	Kg (AI)/ha	*P. xylostella*	*C. maculata*
CGA-293743	0.051	15.7 ± 0.9a	11.9 ± 5.1ab
Neem oil	2.3 l/ha	26.2 ± 4.8a	14.8 ± 6.1ab
Azadirachtin	1.2 l/ha	35.0 ± 6.2ab	7.8 ± 4.0a
Emamectin benzoate	0.017	54.8 ± 5.1bc	31.9 ± 8.0b
Abamectin	0.011	73.5 ± 2.8cd	42.7 ± 9.3b
Spinosad	0.052	79.4 ± 7.4cd	0.0 ± 0.0a
Endosulfan	1.10	87.3 ± 5.2d	28.1 ± 11.7b
Bacillus thuringiensis	1.10	100 ± 0.0e	11.1 ± 9.1a
		$F = 24.28$	$F = 2.15$
		df = 7, 16	df = 7, 16

[a]Values within a column followed by the same letter are not significantly different ($P \geq 0.05$; least significant difference [SAS Institute 1998]).
[b]All treatments contained 0.01% Silwet.

These data are consistent with the study of Murray and Lloyd (1997) who reported that spinosad was not disruptive to predator populations in Australian cotton and suggested that the product has an important role in integrated management programs. Elzen et al. (1998) also found that spinosad was non-toxic to the convergent lady beetle, *Hippodamia convergens* Guerin-Meneville. Further, Hendrix et al. (1997) reported that spinosad was less harmful to beneficials than many insecticides, and Pietrantonio and Benedict (1999) rated spinosad as harmless (causing <25% mortality) to the parasitoid *Cotesia plutellae* (Kurdjumov) in laboratory studies. The remaining treatments were moderately toxic to *P. xylostella*, with the exception of CGA-293743, which was lower in toxicity than any other treatment. CGA-293743, neem oil, and azadirachtin were also low in toxicity to *C. maculata*, while endosulfan toxicity was slightly higher. In contrast, Elzen (2001) found that endosulfan was one of the more toxic insecticides tested on *G. punctipes* and the minute pirate bug, *Orius insidiosus* (Say), using the foliar insecticide residue bioassay. However, a higher rate of endosulfan (1.70 kg [AI]/ ha) was tested in that study, making comparison difficult. To further complicate matters, Elzen et al. (1998) found that endosulfan was one of the least toxic insecticides tested (1.70 kg [AI]/ha) on *H. convergens*. Also, England et al. (1997) found 76% mortality for *O. insidiosus* exposed to endosulfan (0.57 kg [AI]/ha) treated cotton leaves 24-h after exposure. Therefore, *B. thuringiensis*, spinosad, and possibly endosulfan may be useful in control programs for *P.*

xylostella where conservation of beneficial arthropods such as *C. maculata* is important (and resistance to *B. thuringiensis* is not present).

Beauveria bassiana (both rates) and azadirachtin were low in toxicity to *P. xylostella*. However, when mixed, there was a synergistic effect (Table 2). *Beauveria bassiana* (both rates) combined with azadirachtin was significantly more toxic than either treatment alone. A similar trend was found with *C. maculata* except that azadirachtin was slightly less toxic.

TABLE 2. Toxicity of Azadirachtin and *Beauveria bassiana* to *P. xylostella* and *C. maculata* Immatures in a Foliar Insecticide Residue Bioassay, 10 d Post-treatment.

		% Mortality ± SE[a]	
Treatment[c]	Rate	*P. xylostella*	*C. maculata*
B. bassiana	1.25[b]	8.9 ± 1.8a	11.1 ± 6.4a
B. bassiana	2.50[b]	13.3 ± 3.1a	17.8 ± 4.8a
Azadirachtin	1.2 l/ha	28.9 ± 1.8a	15.5 ± 4.8a
B. bassiana +	1.25[b]		
Azadirachtin	1.2 l/ha	57.8 ± 6.3b	40.0 ± 3.2b
B. bassiana +	2.50[b]		
Azadirachtin	1.2 l/ha	73.3 ± 3.1c	64.4 ± 4.8c
		$F = 23.12$	$F = 13.41$
		df = 4, 10	df = 4, 10

[a]Values within a column followed by the same letter are not significantly different ($P \geq 0.05$; least significant difference [SAS Institute 1998]).
[b]x 10^{13} spores/ha.
[c]All treatments contained 0.01% Silwet.

Vandenberg et al. (1998) showed the potential of *B. bassiana* in significantly reducing larval counts in an overall program for controlling *P. xylostella*. *Beauveria bassiana* could be used successfully in a management program for control of *P. xylostella* when tank-mixed with azadirachtin, but these mixtures may not be the best choice for preserving *C. maculata* or similar species.

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LANDSCAPE MATERIALS AS REPELLENTS OF RED IMPORTED FIRE ANTS[1]

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ABSTRACT

Anecdotal evidence suggests that certain natural materials used in landscaping may repel red imported fire ants, *Solenopsis invicta* Buren. The repellency properties of various landscaping materials to *S. invicta* colonies are addressed in this study. The numbers of *S. invicta* on or near water-soluble extracts of materials were compared to numbers on control treatments. Sage (*Saliva* sp.), pine needle, and cedar shaving water suspensions were repellent to *S. invicta* colonies in the laboratory. Juniper extracts, DEET, neem, and naphthalene may offer short-term repellency and may contribute to integrated pest management of *S. invicta* in private and public outdoor areas.

INTRODUCTION

Few materials have been reported as being repellents to ants. Argentine ant, *Linepithema humile* Mayr, foraging in lemon trees was suppressed by a band of cotton twine permeated with farnesol and Stickem® placed around tree trunks (Storey et al. 1996). Farnesol was likely repellent through the ant gustatory sense, mostly through contact with the feet. Mixtures of chemicals having different modes of sensory input may achieve greater ant repellency (Storey et al. 1996). The pyrethroids, bifenthrin and tefluthrin, mixed in potting soil repelled red imported fire ants, *Solenopsis invicta* Buren, and effects were greater with higher chemical concentrations (Oi and Williams 1996). Water and ethanol extracts of sesbania (*Sesbania exaltata* (Rafinesque-Schmaltz) Cory; Leguminosae: Fabaceae) repelled and caused mortality of *S. invicta*. Sesbania, a summer cover crop, showed almost 100% repellency to *S. invicta* for 8 d, whether the plants were aphid-infested or not (Kaakeh and Dutcher 1992). Octanoic acid prevented newly mated *S. invicta* queens from founding colonies in treated potting soil, and several other repellent chemicals were identified by Vander Meer et al. (1994).

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Anecdotal evidence suggests that certain natural products used in landscaping may repel *S. invicta*. Thorvilson et al. (unpublished report) noted that areas along highway rights-of-way and rest areas with dense oak and pine leaf litter had fewer *S. invicta* mounds than did areas without these materials. At highway rest areas, *S. invicta* mounds were not observed in landscaped areas where sage (*Saliva* sp.) was present. Small quantities of finely ground mulches, especially red cedar mulch, showed some repellency to *S. invicta*, (Thorvilson and Rudd 2001). In our studies, the objective was to identify in a laboratory setting the most promising natural landscaping and other materials for *S. invicta* repellence. If such materials can be identified, they may be used to ameliorate problems of *S. invicta* invasion of highway rest area facilities, electrical equipment, and habitations.

MATERIALS AND METHODS

Colony Collection and Maintenance. Twenty-one *S. invicta* colonies were collected with shovels on 24 April 1997, 14 colonies were collected on 23 April 1998, and 19 colonies were collected on 15 October 1998 near Lake Kirby, Taylor Co., near Abilene, Texas. Only colonies that had brood and, therefore, were ranked ≥ 6 (Harlan et al. 1981) were collected. Colonies were transported to Texas Tech University in 19-liter plastic buckets. The top of each bucket was coated with talcum powder to prevent escape of ants. Ants and brood were removed from soil using a water-drip method (Banks et al. 1981).

After ants and brood were removed from soil, each colony was maintained in a 38× 52×7.6-cm plastic tray, the top of which was coated with Fluon® (Northern Products, Woonsocket, RI) to prevent ant escape. Colonies were provided with water ad libitum and chunks of apple and vienna sausages (Hormel®, Austin, MN) every two to three days. In the center of each tray was an 11×11×3.5-cm plastic brood box with 1.5 cm of hardened dental plaster (Castone®; JB Dental Supply Co., Irving, TX) on the bottom. Each box had two 5.0-mm access holes in opposite corners of the top. Dental plaster was soaked with reverse osmosis (RO) water to provide a favorable, high humidity, micro-climate and to prevent desiccation of queens, workers, immatures, and eggs. Colonies of various sizes were collected and used in experiments. All colonies were maintained in the Fire Ant Research Laboratory at Texas Tech University at temperatures of 21-27°C and relative humidity between 70 and 85%.

Leachate Study I. Water-soluble compounds were extracted from five mulch materials and tested for repellency to *S. invicta*. Pine needles (dead), oak leaves (dead), and green sage (*Salvia* sp.; live) were collected from local plants. Green sage branches were placed in a drying oven at 80°C for seven hours before water extraction. Pine bark mulch (Elliott's Agri-Service, Inc., Pineland, TX) and cypress bark mulch (Natchez Forest Products, Inc., Natchez, MS) were purchased at a local garden center. Cedar shavings, often used as pet bedding, (Cimarron Cedar Co., Oklahoma City, OK) were purchased.

One thousand grams of each material were mixed in separate containers with 1.0-liter RO water and allowed to soak for 48 h so water-soluble substances would leach out as they might under field conditions of precipitation. These mixtures were randomly shaken every two hours. Each suspension was filtered through a 2-mm mesh screen, and the liquid was stored in foil-wrapped, sterilized bottles in a refrigerator before testing. A control solution of 1.0 liter of RO water was similarly stored.

Colony trays were cleaned of debris (dead ants, uneaten food, soil, etc.) and numbered for identification purposes before treatments were initiated. Each colony in a separate tray received two treatments, a control (RO water) treatment and one of the six leachates which were presented in the following manner. Two, 9.0-cm diameter, dry tagboard circles were placed into each tray, equal distances from a water source and the brood box. On each tagboard circle was placed a 6.0-cm diameter, inverted, plastic petri dish lid containing one,

5.5-cm diameter filter paper (Whatman, catalog #1001055). One ml of a leachate solution was placed on the filter paper of one dish, and 1.0 ml of RO water (control) was placed on the filter paper in the other dish. The control was labeled using a pencil mark to distinguish it from the treated dish. Ants were counted in zones around the dishes at regular time intervals. The zones consisted of: Zone A, the tagboard circle 1.5-cm radius around the dish; Zone B, the edge of the petri dish; and Zone C, inside the dish, in contact with the filter paper (treated with a leachate or RO water).

Tests were conducted three times using three replications (colonies) for each trial (n=9). Statistical analyses consisted of paired t-tests (critical *P*-value = 0.05) that compared mean numbers of *S. invicta* in a treated dish with a control dish in the same colony tray.

Leachate Study II. The second repellency study was conducted on 30 *S. invicta* colonies using natural landscape materials, insect repellents, and controls. Trays housing colonies were cleared of debris (dead ants, uneaten food, and soil) about 24 h before trials were initiated. Water was removed 12 h before trials began. Each colony was exposed to 10 treatments: (1) control (dry, no leachate); (2) red cedar mulch (Cimarron Cedar Company, Oklahoma City, OK; (3) cypress mulch (Natchez Forest Products, Inc., Natchez, MS); (4) hardwood bark mulch (Elliott's Agri-Service, Inc., Pineland, TX); (5) juniper (*Juniperus ashei* Bucch.); (6) neem (0.09% azadirachtin; VPG A Co-op Gardening Group, Bonham, TX); (7) pecan (*Carya illinoinensis* [Wang.] K. Koch); (8) pine bark mulch (Elliott's Agri-Service, Inc., Pineland, TX); (9) pyrethrin (0.02%, Green Light Company, San Antonio, TX); and (10) reverse osmosis water.

Juniper and pecan branches were each collected from three trees on 24 October 1998. Twigs were broken into 10-15-cm segments. Leaves, twigs, nuts (pecans), or berries (juniper) were included in the mixture. Each material was dried for 48 h at 37-38°C.

One part mulch (red cedar, cypress, hardwood bark, juniper, pecan, pine bark) per three parts RO water (500 g mulch:1,500 ml water) was placed into separate 11.4-liter plastic containers (Sterilite® Corporation, Townsend, MA). Suspensions were gently shaken for 30 s at 0, 6, 12, 20, 30, and 40 h after placement of water on mulch. Water was maintained on mulches for 48 h at 26-27°C. After 48 h, each liquid was filtered using a 2- mm-mesh screen and placed into separate, sterile glass bottles for storage (3-4°C) until used in repellency trials. Neem insecticide was diluted in water (3 fl. oz:1 gal.), and pyrethrin was used at the commercial 0.02% concentration.

All 10 treatments were presented to each colony (n=30) at the same time. For each treatment, a 9.0-cm tagboard circle that had been soaked in a leachate for 15 min was placed in the colony tray. On each tagboard circle, a lid from a 6.0-cm diameter, plastic petri dish containing 5.5-cm diameter filter paper (Whatman catalog #1001055) was placed. Two ml of a leachate were placed on the filter paper, and increments of 1.0 ml of a leachate was added at 4, 6, 12, 24, and 30 h.

Leachate study II differed from the first study in that all treatments were presented at the same time to a colony, control treatments were dry filter papers, and especially, that a piece of sausage (4.0 g of vienna sausage; Hormel®, Austin, MN) was placed in the center of each filter paper as an attractant food source. Ants had to crawl across the soaked tagboard, over the petri dish lid, and across leachate-soaked filter paper to get to the food sausage.

Ants were counted at 0.5, 1, 2, 4, 6, 12, 24, and 48 h after placing petri dishes in trays. All ants occurring on a 9-cm diameter tagboard circle (Zone A), the edge of the petri dish (Zone B), and inside the dish, in contact with the filter paper or sausage (Zone C) were counted. Only ants in contact with the petri dish (Zones B and C) were included in the analyses as determined a priori. Previous data had shown that Zones B and C were most useful because of greater direct contact with the leachate compounds (personal observation). Numbers of ants in zones were analyzed separately and as pooled data. Mean numbers were calculated from the sums

of *S. invicta* per petri dish from eight count periods.

The experimental design was a randomized complete block design with each colony serving as a block, and each petri dish served as an experimental unit. Each colony (n=30) was subjected to all 10 treatments (complete block). One-way, multivariate analysis of variance (MANOVA) was used to test the hypothesis that the number of ants within each zone (side of dish, bottom of dish, and total ants on dish) (dependent variables) varied among the 10 treatments (independent variables). We used Wilks' λ as the test criterion (Johnson and Wichern 1992). Following a significant ($\alpha = 0.05$) MANOVA, one-way analysis of variance was used for each dependent variable. The numbers of ants per zone were log-transformed ($\log_{10}[x + 1]$) because the mean was positively correlated with the variance (Sokal and Rohlf 1981). Means were transposed back to original units for presentation purposes. We used Fisher's LSD ($\alpha = 0.05$) as the mean separation test (Milliken and Johnson 1992).

Repellency Study. In this study, five known or suspected repellents, one attractant (1.0 g apple) and RO water (control) were used. These tests were conducted on the same colonies as in Leachate Study I but were conducted after three weeks of no testing or other disturbances.

Neem (0.09% azadirachtin; VPG A Co-op Gardening Group, Bonham, TX), cedar wood oil (Fisher Scientific Co., Fair Lawn, NJ), DEET (95% N, N Diethyl meta toluamine; OFF® liquid, non-aerosol pump; S. C. Johnson & Son, Inc., Racine, WI), pyrethrin (0.02% with 0.20% PBO, Green Light Co., San Antonio, TX), and naphthalene crystals (laboratory grade, Fisher Scientific Co., Fair Lawn, NJ) were used as suspected repellents. Neem insecticide was diluted in water (3 fl. oz:2 gal.). Pyrethrin and DEET were both used at the commercial level of 0.02% and 95%, respectively. The same methodology as Leachate Study I was used, and tests used 1.0 ml of a liquid and 1.0 g of solids (apple and naphthalene). Counts in the three zones were done as before. These tests were conducted three times with three replications each time. Statistical analysis was conducted as paired t-tests (critical *P*-value = 0.05).

RESULTS

Leachate Study I. After 1 h of exposure, significant differences were found in four of the tests (Table 1). In the zone of contact with the leachate (Zone C), dried sage, pine needle, and cedar shavings solutions had significantly fewer *S. invicta* than in the paired control dishes (paired t-tests, df = 8, $P<0.05$). After 4 h, the cedar shavings solution continued to have significantly fewer *S. invicta* in contact with the leachate ($t = -2.7$; df = 8; $P = 0.029$), whereas no differences were detected among other leachates and controls. After 24 h, significantly fewer *S. invicta* were counted in Zone C of the sage treatment. At the end of 48 h, sage leachates in Zones A and C significantly repelled *S. invicta* ($t = -5.7$, df = 8, $P<0.001$; $t = -3.3$, df = 8, $P = 0.012$, respectively).

Leachate Study II. The numbers of *S. invicta* among treatments differed in Zones B and C and in both zones combined (Wilks' $\lambda = 0.788$; $P < 0.001$). The numbers of *S. invicta* in treatments varied for the total count ($F = 21.01$; df = 9, 261; $P < 0.001$), side of petri dish ($F = 6.95$; df = 9, 261; $P < 0.001$), and bottom of petri dish ($F = 24.8$; df = 9, 261; $P < 0.001$) (Table 2). Substantial overlap in numbers of *S. invicta* in treatments existed, but generally the control (dry) was most attractive. Numerically, hardwood bark mulch, pine bark mulch, and juniper were most repellent to *S. invicta*, but few statistical separations were found. Results are difficult to interpret because of lack of mean separations. Another problematic result was the significant differences between the control (dry) and RO water treatments. It appears that the physical placement of water was a deterrent. Future trials may be run with dry homogenized materials.

TABLE 1. Number of *Solenopsis invicta* Responding to Leachates at Four Time Periods.

		Mean Number of *S. invicta*[a] ±SD							
		1h		4h		24h		48h	
Leachate	Zone[b]	Treated	Control	Treated	Control	Treated	Control	Treated	Control
Dried Sage	A	22.7 ±14.9a	27.2 ±20.3a	17.8 ±16.1a	23.9 ±19.3a	14.7 ±12.9a	19.4 ±13.5a	13.2 ±8.0a	20.3 ±10.7b
	B	13.7 ±12.9a	13.4 ±10.0a	12.2 ±19.0a	11.7 ±13.0a	12.1 ±9.1a	10.3 ±7.6a	9.3 ±5.7a	9.7 ±7.3a
	C	1.6 ±2.1a	13.2 ±14.4b	16.2 ±19.1a	27.1 ±26.3a	7.2 ±8.0a	16.0 ±15.2b	3.4 ±3.3a	9.6 ±8.0b
Pine Bark Mulch	A	43.2 ±30.8a	41.0 ±32.9a	41.4 ±34.7a	39.7 ±27.7a	34.6 ±32.5a	32.6 ±25.6a	33.2 ±36.1a	32.8 ±17.9a
	B	28.2 ±38.6a	20.8 ±25.6a	28.2 ±31.8a	22.8 ±21.3a	22.6 ±28.5a	25.8 ±24.4a	24.6 ±25.4a	24.2 ±19.8a
	C	30.7 ±49.0a	20.9 ±28.2a	57.6 ±46.8a	47.7 ±38.8a	30.9 ±46.6a	35.1 ±43.9a	16.7 ±17.5a	19.8 ±20.2a
Pine Needles	A	45.0 ±25.9a	53.9 ±40.1a	30.0 ±25.5a	36.8 ±27.8a	32.8 ±21.5a	30.1 ±15.6a	27.8 ±17.3a	30.9 ±23.9a
	B	24.6 ±15.6a	32.6 ±27.7a	14.8 ±13.8a	25.8 ±19.0a	17.2 ±11.8a	23.2 ±18.4a	15.0 ±8.3a	21.1 ±18.0a
	C	21.3 ±26.5a	45.1 ±34.8b	42.9 ±64.4a	49.5 ±46.9a	15.8 ±17.5a	25.4 ±23.4a	13.9 ±10.5a	12.2 ±9.0a
Oak Leaves	A	29.8 ±26.4a	42.2 ±47.2a	36.8 ±50.7a	48.2 ±62.4a	23.0 ±25.3a	27.6 ±29.6a	21.3 ±23.5a	29.7 ±30.2a
	B	11.9 ±13.7a	22.8±30.0a	16.1 ±19.9a	22.4 ±30.2a	14.3 ±15.6a	21.3 ±30.1a	9.2 ±9.6a	13.3 ±17.8a
	C	8.6 ±15.1a	23.0 ±32.1a	26.4 ±32.1a	34.2 ±46.6a	13.2 ±15.5a	17.6 ±23.6a	8.1 ±12.8a	14.1 ±23.9a
Cedar Shavings	A	41.9 ±42.7a	31.3 ±32.3a	37.1 ±48.5a	33.0 ±31.6a	23.9 ±22.9a	24.8 ±24.8a	26.8 ±25.4a	27.2 ±27.2a
	B	24.4 ±32.4a	26.9 ±33.7a	16.6 ±20.4a	24.1 ±31.1a	17.0 ±19.2a	19.3 ±24.3a	18.3 ±22.5a	17.1 ±15.7a
	C	10.8 ±14.7a	17.3 ±19.8b	17.1 ±20.4a	45.6 ±48.3b	14.1 ±15.1a	23.6 ±31.7a	17.3 ±21.1a	14.1 ±19.9a
Cypress Mulch	A	40.2 ±29.9a	38.1 ±25.0a	33.2 ±32.4a	36.4 ±32.2a	22.9 ±17.8a	21.9 ±21.4a	26.7 ±15.6a	28.6 ±25.4a
	B	32.1 ±23.6a	26.2 ±25.6a	17.9 ±18.6a	16.0 ±16.4a	19.6 ±18.1a	19.2 ±22.2a	16.1 ±10.8a	15.7 ±18.3a
	C	18.7 ±22.9a	24.7 ±30.6a	35.1 ±23.1a	36.2 ±42.6a	17.7 ±21.7a	20.1 ±29.5a	11.6 ±8.1a	14.7 ±13.9a

[a] Means followed by the same letter within time periods and zones are not significantly different (Paired t-tests; df = 8; $P > 0.05$). Mean of nine replications (n = 9).
[b] Zone A = 1.5-cm radius around dish; Zone B = the edge of the petri dish; Zone C = inside the dish in contact with the filter paper.

TABLE 2. *Solenopsis invicta* Responses to Leachates Used in Repellency Trials ($n = 30$).

	Mean[a] Number of *S. invicta* (±SE)					
	Total		Side of dish		Bottom of dish	
Treatment	$\bar{x}$	SE	$\bar{x}$	SE	$\bar{x}$	SE
Control (Dry)	73.73 a	15.18	15.47 a	3.13	58.26 a	12.29
RO water	26.97 b	6.33	8.13 b	2.07	18.83 b	4.47
Pyrethrin	24.87 bc	6.81	7.64 bc	1.95	17.23 bc	4.96
Red cedar mulch	24.70 bcd	7.49	7.43 bc	2.15	17.27 bc	5.00
Cypress mulch	24.06 bcd	7.01	8.33 bc	2.06	15.73 c	5.08
Pecan	20.37 cd	5.62	7.20 bc	1.68	13.17 c	4.14
Neem	22.43 cd	6.54	8.23 b	2.03	14.20 c	4.72
Hardwood bark mulch	18.60 cd	4.78	5.77 bc	1.24	12.83 c	3.61
Pine bark mulch	19.00 cd	5.07	6.73 bc	1.77	12.27 c	3.45
Juniper	19.80 d	5.77	5.90 c	1.69	13.90 c	4.32

[a] Means are the average total number of ants counted from eight time periods (0.5, 1, 2, 4, 6, 12, 24, and 48 h) on the side and bottom of petri dishes. Means followed by the same lower case letter within columns are not different (ANOVA, Fisher's LSD; $P > 0.05$).

Low densities of *S. invicta* were also observed in Leachate Study II treatments. The large number of treatments ($n = 10$) per colony trial probably lowered overall response to each treatment. Future trials may use an incomplete block design to reduce the number of treatments per colony. Additionally, the good food target may have overwhelmed possible negative effects of leachates, causing a lack of differences among means. Nonetheless, the use of sausage as an attractant is an improvement over previous trials designed to test repellency.

Repellency Study. After 1 h of exposure, three materials produced significantly different results than did the corresponding control. DEET and naphthalene each had significantly fewer *S. invicta* around dishes and on the edges of dishes than did the controls (paired t-tests, $P<0.05$; Table 3). Apple, as expected, attracted significantly more *S. invicta* to all three zones than did the control. At 24 h, Neem and DEET had significantly fewer ants in Zone B than did control treatments. Interestingly, DEET and naphthalene attracted more *S. invicta* after 48 h than did the controls in Zone C, directly upon the materials. Neem retained significant repellency after 48 h. After 48 h, apple remained very attractive to *S. invicta*, perhaps because chemicals of decay such as ethylene were being released. In this study, cedar oil did not show significant repellency to *S. invicta*; however, granular and liquid formations of *Juniperus virginiana* (CedarCide®, CedarCide Industries, The Woodlands, TX) have been marketed as repellents to many insects, including fire ants.

DISCUSSION

Water solutes of various mulches and materials were tested in the trials reported herein.

TABLE 3. Number of *Solenopsis invicta* Responding to Various Materials at Four Time Periods.

		Mean Number of *S. invicta*[a] ±SD							
		1h		5h		24h		48h	
Chemical	Zone[b]	Treated	Control	Treated	Control	Treated	Control	Treated	Control
Neem	A	11.9 ±15.2.a	16.6 ±18.4a	13.3 ±15.3a	23.3 ±17.6a	10.1 ±10.7a	9.9 ±10.3a	6.8 ±6.1a	11.6 ±9.5b
	B	5.8 ±8.6a	9.9 ±12.7a	14.0 ±18.4a	22.7 ±28.2a	2.2 ±3.4a	6.0 ±7.1b	2.3 ±5.2a	7.9 ±7.8b
	C	7.2 ±13.9a	14.2 ±15.8a	11.3 ±16.3a	42.3 ±51.2a	6.6 ±16.4a	5.7 ±8.6a	10.7 ±24.2a	5.6 ±7.8a
Cedar Oil	A	8.6 ±10.4a	20.2 ±31.2a	7.3 ±3.1a	16.0 ±10.1a	5.3 ±4.6a	5.8 ±7.0a	6.1 ±7.3a	6.4 ±7.9a
	B	2.9 ±4.1a	14.3 ±25.2a	0.7 ±1.2a	6.7 ±5.0a	1.1 ±1.6a	2.8 ±3.6a	1.0 ±1.4a	4.4 ±6.7a
	C	0.7 ±1.3a	22.2 ±48.2a	2.7 ±2.3a	12.0 ±12.1a	1.9 ±2.3a	3.1 ±3.4a	4.4 ±3.4a	2.3 ±3.4a
Deet	A	7.7 ±5.0a	15.4 ±11.7b	5.7 ±3.8a	10.0 ±9.5a	5.8 ±4.9a	10.0 ±8.8a	11.2 ±4.8a	6.6 ±6.5a
	B	1.1 ±1.8a	11.4 ±11.3b	2.0 ±2.6a	3.0 ±1.7a	0.9 ±1.1a	7.4 ±6.9b	1.4 ±2.8a	2.7 ±3.6a
	C	2.1 ±2.9a	13.6 ±23.9a	3.3 ±2.5a	10.7 ±8.3a	6.3 ±5.5a	6.3 ±8.2a	20.9 ±15.4a	2.8 ±4.9b
Pyrethrin	A	25.0 ±31.7a	29.1 ±39.9a	24.0 ±31.4a	8.7 ±14.2a	8.2 ±9.3a	10.1 ±10.6a	9.0 ±9.4a	9.2 ±9.7a
	B	8.8 ±8.8a	13.6±16.8a	12.3 ±15.7a	6.7 ±11.5a	6.9 ±7.7a	5.1 ±6.5a	5.4 ±5.5a	4.6 ±4.7a
	C	7.3 ±7.5a	11.1 ±14.6a	13.7 ±12.7a	10.0 ±13.0a	12.9 ±18.0a	3.9 ±4.4a	14.9 ±20.5a	4.8 ±7.5a
Naphthalene	A	9.4 ±10.4a	16.1 ±13.8b	3.3 ±5.8a	5.7 ±8.1a	5.9 ±4.9a	9.0 ±9.2a	4.2 ±2.9a	8.0 ±8.1a
	B	1.8 ±3.1a	13.9 ±18.3b	0.3 ±0.6a	1.7 ±1.5a	1.7 ±2.0a	5.6 ±9.7a	0.6 ±1.0a	4.6 ±5.4b
	C	20.4 ±18.1a	13.8 ±16.8a	22.3 ±16.6a	4.3 ±4.0a	53.0 ±32.1a	3.9 ±5.3b	62.0 ±29.0a	4.1 ±6.4b
Apple	A	33.4 ±25.2a	14.3 ±11.6b	37.0 ±31.6a	11.7 ±12.6a	17.0 ±12.8a	10.0 ±10.8b	18.8 ±14.8a	9.7 ±8.3b
	B	36.1 ±23.7a	12.7 ±10.7b	50.0 ±43.6a	5.0 ±5.0a	11.9 ±10.9a	7.6 ±9.1a	18.9 ±19.6a	7.8 ±8.1b
	C	105.2 ±83.2a	13.2 ±17.0b	210.0 ±115.3a	14.7 ±9.2a	21.7 ±21.8a	10.7 ±12.2a	25.6 ±20.7a	4.1 ±5.6b

[a] Means followed by the same letter within time periods and zones are not significantly different (Paired t-tests; df = 8; $P > 0.05$). Mean of nine replications (n = 9).

[b] Zone A = 1.5-cm radius around dish; Zone B = the edge of the petri dish; Zone C = inside the dish in contact with the filter paper.

Sage is considered an insect repellent (Simon et al. 1984) and showed significant contact repellency in our first study. Pine needle and cedar shaving water suspensions were likewise repellent to laboratory *S. invicta* colonies. Juniper extracts, DEET, neem, and naphthalene may be promising repellents for short-term repellency. In contrast, Thorvilson and Rudd (2001) tested small quantities of dry and moistened, finely ground landscaping mulches and observed some non-preference by *S. invicta* to red cedar.

Precipitation and irrigation remove water-soluble components from landscaping mulches, and extracts seep into soil. Living plants may produce root exudates, and dropped leaves and stems decompose, releasing organic compounds into soil. Data from the present studies suggest that coniferous materials may have repellent properties to *S. invicta*. However, a replicated field study using large, thick beds of mulch in *S. invicta*-infested habitats must be completed to determine if *S. invicta* colonies will establish in these areas. In addition, the study must progress through several years to find if natural weathering of mulches affect colony success. If repellence is identified, mulches or mulch components may be recommended to ameliorate *S. invicta* problems in public and private areas as part of an integrated pest management program.

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TOXICITY OF INGESTED PHOTOACTIVE DYES TO ADULTS OF THE BOLL WEEVIL (COLEOPTERA: CURCULIONIDAE)

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ABSTRACT

Photoactive dyes are increasingly considered potential alternatives to conventional insecticides. Expansion of eradication programs for the boll weevil, *Anthonomus grandis* Boheman, has accentuated the need for new toxicants to reduce dependency on organophosphates and minimize environmental impacts. However, toxicity of photoactive dyes to adult weevils has not been thoroughly investigated. We developed methods to assay dye activity against the adult boll weevil, compared the efficacies of selected dyes, and examined the influence of dye elimination on efficacy. Doses of dyes from 0 – 1.0 µg/weevil were orally delivered in aqueous sucrose and mortality was induced by illumination with a high-pressure sodium lamp. The methods provided consistent mortality responses to rose bengal and phloxine B, and results indicated that mortality occurred earlier as dose increased. Methylene blue, eosin Y, rhodamine B, and erythrosin B failed to consistently produce substantial mortality. Examination of the influences on efficacy of dye elimination by excretion or detoxification indicated that phloxine B was significantly more effective when weevils were exposed to the light source immediately after dye ingestion than when exposure was delayed for 24 or 48 h. Feeding on cotton (*Gossypium hirsutum* L.) squares after dye ingestion did not reduce mortality. Our results indicate the high-pressure sodium lamp provides light of sufficient intensity to permit laboratory examination of photoactive dye toxicity against relatively opaque insects such as the boll weevil, and that both the time-course of mortality and the potential for dye elimination should be considered in interpreting results of dye assays.

INTRODUCTION

Broad-spectrum insecticides which target adult populations of the boll weevil, *Anthonomus grandis* Boheman, represent the primary control tactic used to manage or eradicate this pest. Despite the long standing success of insecticide-based management efforts, this control tactic is characterized by several undesirable qualities. The short residual activity of conventional insecticides against the weevil usually creates the need for multiple applications to achieve or maintain adequate levels of control (Bottrell 1976, Johnson et al. 1996), and use of these materials is often associated with outbreaks of secondary pests (Bottrell 1976, Brazzel et al. 1996, Summy et al. 1996). In addition, considerable concern exists for the deleterious impacts of conventional insecticides on environmentally sensitive

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areas. Finally, the potential for the boll weevil to develop resistance to currently registered materials is of considerable concern to eradication programs because no economical alternative toxicants are currently available. Although Klassen (1976) specifically identified the need to develop new insecticides that are effective against the boll weevil as replacements for the organophosphates, little progress has been made in this regard.

In recent years, photoactive dyes have received increasing attention as insecticides. The insecticidal properties of such dyes have been investigated against a number of insects including the house fly, *Musca domestica* L. (Fondren et al. 1978), Mexican fruit fly, *Anastrepha ludens* (Loew) (Moreno and Mangan 1995), southern and western corn rootworms (*Diabrotica undecimpunctata howardi* Barber and *D. virgifera virgifera* LeConte, respectively) (Schroder et al. 1998), and grasshoppers (*Schistocerca americana* (Drury) and *Melanoplus sanguinipes* (F.)) (Capinera and Squitier 2000). Callaham et al. (1975) evaluated the toxicity of four xanthene dyes against the adult boll weevil by providing the dyes in artificial diet, but no study has examined the toxicity of controlled doses of the photoactive dyes against the boll weevil. Our objectives were: 1) develop appropriate methodology for the laboratory assay of photoactive dyes against the adult boll weevil, 2) evaluate the respective toxicities of selected Food, Drug and Cosmetic (FDC) or Drug and Cosmetic (DC) approved dyes relative to the toxicity of rose bengal, and 3) examine the potential influences of both feeding after dye ingestion and excretion of the dyes on mortality from dye activity.

MATERIALS AND METHODS

Adult boll weevils used in all experiments were reared from field collected squares of cotton, *Gossypium hirsutum* L., using procedures described by Spurgeon and Raulston (1998). After eclosion, mixed-sex groups of adults were held in the laboratory at room temperature in screened cages, and fresh squares with bracteoles removed were provided three to five times weekly until the weevils were used in the respective studies.

Methods for delivering known doses of ingested dyes in 5% sucrose solutions (w/v) were devised in preliminary studies. Dye solutions were presented to individual weevils in a fixed-needle 10 µl syringe marked in 0.1 µl increments (Hamilton, Reno, NV). The syringe was modified by cutting the glass barrel beyond the base of the needle and grinding the tip of the barrel to a conical shape. The modified syringe was charged with dye solution by capillary action. When weevils were dosed they were either held between the thumb and index finger, or were grasped by the lateral aspects of the prothorax using forceps, which usually caused the weevil to extend the legs. The tip of the rostrum was then made to contact the dye solution in the bore of the syringe, and the weevil was allowed to grip the conical tip of the syringe barrel until the desired dose of dye solution was ingested. All dyes used were certified grade except rhodamine B, and all were obtained from Sigma Chemical (St. Louis, MO).

Effects of Dye Concentration on Ingestion. Before toxicity studies were initiated, the volume of dye solution that would be consistently ingested by the weevils was determined. Weevils collected from commercial cotton in the Lower Rio Grande Valley of Texas were used when they were 7- to 9-d-old. Weevils were removed from food the day before the dye solutions were presented. Ingestion of phloxine B and rose bengal was examined, each at concentrations of 0, 0.5, 1.0, and 2.0 mg/ml. In each repetition of the experiment, five weevils were assigned to each treatment combination of dye and concentration. Weevils were individually introduced to the syringe containing the dye solution and allowed to feed for 3 min, at which time the volume of solution ingested was recorded to the nearest 0.1 µl. The entire experiment was repeated five times.

The effect of dye concentration on the volume of solution ingested was examined by analysis of variance using the GLM procedure of SAS (SAS Institute 1998). Dye type, concentration, and repetition of the experiment were main effects in the model. All interactions were also included in the model. Means of treatments with more than two levels were examined for differences using the REGWQ option of PROC GLM (SAS Institute 1998).

Efficacy of Phloxine B and Rose Bengal under a High-Pressure Sodium Lamp. Preliminary efforts to conduct tests on the efficacy of photoactive dyes indicated that assays using natural sunlight were difficult to interpret because of the inability to control light intensity and temperature, while assays using fluorescent lights, as previously reported in studies of fruit flies by Moreno and Mangan (1995), failed to produce measurable mortality (unpublished data). Thus, it was apparent that a light source that was controllable and of sufficient intensity for dye activation through the boll weevil cuticle was needed to obtain reproducible results. For this purpose we used a greenhouse lamp fixture (240 V, 1000 W, Model G4599-4, Ruud Lighting, Racine, WI) equipped with a Lucalox LU1000 high-pressure sodium lamp (General Electric, Fairfield, CT). The lamp was mounted on a metal storage rack with its surface about 56 cm from a shelf on which the weevils were placed. Groups of weevils were exposed to the lamp in 100 x 15-mm plastic petri plates with modified lids. The center of each lid was removed to within 3-4 mm of the outer surface of the rim, and a nylon tulle (T-310, Mandel Fabrics, New York, NY) was glued in its place to contain the weevils. Air was circulated over the assay platform by an oscillating fan to prevent exposure of the weevils to excessive temperatures.

Weevils in these initial assays were 9- to 12-d-old and were collected in the Lower Rio Grande Valley. Toxicities of rose bengal and phloxine B were examined at doses of 0, 0.05, 0.25, 0.5, and 1.0 μg/weevil. In each replication, each combination of dye type and concentration was represented by one petri plate of five weevils. Weevils were removed from food the day before doses were administered. Weevils received doses of dyes in the afternoon and then were held in darkness until the following morning when assays were initiated. On the day of the assay, the sodium lamp was turned on and the plates of weevils were placed on the assay shelf. Mortality was recorded each 30 min until 450 min from the start of the test. A weevil was considered dead if handling failed to elicit any response. Six replicates were conducted involving a total of 30 weevils for each dose of each dye.

Mortality responses to doses of the dyes were examined by probit analysis using the $\log_{10}$ of dose as the independent variable (LOG10 option of PROC PROBIT, SAS Institute 1998). Because the dose-response relationship suggested that a critical dose was necessary to produce mortality, and that the most prominent differences among effective doses were reflected by the time-courses over which mortality occurred, the relationships between mortality and duration of exposure to the lamp for each combination of dye and dose were compared by repeated measures analysis of variance using the REPEATED statement of the SAS procedure GLM (SAS Institute 1998). The ANOVA model contained terms for dye type, dosage, time of observation (the repeated factor), and their interactions. Because the sphericity test rejected the Huynh-Feldt condition at $P < 0.0001$, Wilk's Lambda was used to assess the statistical significance of the repeated factor (time of observation) and of interactions containing that factor. Mortality levels corresponding to combinations of dye and dose (the dye type*dose interaction) were compared at each observation time using the PDIFF and ADJUST=TUKEY options of the LSMEANS statement of PROC GLM (SAS Institute 1998).

Comparative Toxicities of Selected Dyes. Preliminary assays indicated that rose bengal was much more efficacious against adult weevils than was phloxine B. However,

rose bengal is not an FDC or DC approved dye, and thus is unlikely to be approved for use in insect baits. Therefore, we assayed additional approved dyes to determine their effectiveness relative to rose bengal and phloxine B. Dyes included in this comparison were rose bengal, phloxine B, methylene blue, eosin Y, rhodamine B, and erythrosin B.

Experimental procedures used in the preliminary assays were further refined for the comparative studies of selected dyes. A new lamp support was constructed to suspend the sodium lamp 56 cm above a 60 x 75-cm plywood platform painted flat white. A grid of 10 x 10-cm squares was marked on the surface of the platform, and with the lamp warmed up to peak output (~15 min), the light intensity at each intersection of grid lines was measured with a LI-190SA quantum sensor equipped with a 2290 millivolt adapter (Li-Cor, Lincoln, NE). The area of the platform within which light intensity varied <10% from the highest reading was delineated, and all subsequent toxicity testing was performed within this area. During the assays the platform was cooled by an oscillating fan.

Dyes were assayed at doses of 0, 0.05; 0.25, 0.5, and 1.0 µg/weevil, and were delivered as in the previous experiment. Weevils were 6- to 14-d-old mixed-sex adults reared from squares collected in the Brazos Valley of Texas and held as previously described. Weevils were removed from food on the day before dyes were administered. Following administration of dyes on the afternoon before the assay, groups of treated weevils were held in petri plates in an unlighted environmental chamber at 23.9°C until they were moved to the assay platform on the following morning. Because space in the delineated portion of the platform was limited, only two dyes were assayed on a given day. Thus, pairs of dyes for assay were randomly selected without replacement until all dyes had been tested. A single 100 x 15-mm petri plate with a lid ventilated with nylon tulle and containing ten weevils was assigned to each dose of each dye. Individual plates of weevils were randomly assigned to positions within the delineated area of the platform each day. This experiment was repeated three times for a total of 30 weevils of each combination of dye and dose.

The sodium lamp was allowed to warm up for ~15 min before plates of weevils were placed on the assay platform. Mortality of weevils was recorded hourly for 9 h, after which the light was turned off. A final check of mortality was recorded 24 h after the assay began. Only weevils that failed to respond to handling in any way were recorded as dead. During the assays platform temperature was monitored by a HOBO temperature logger (Model H08-001-02, Onset Computer, Bourne, MA). Also, light intensity was measured at the center of the delineated area using the quantum sensor, and readings were recorded at 2-h intervals starting at the beginning of each assay. When a weevil died in the untreated control, mortality for other dosages of that assay were corrected before analysis (Abbott 1925). Mortality responses to doses of the dyes, except for responses to phloxine B after 24 h, were examined by probit analysis of the $\log_{10}$-transformed data (LOG10 option of PROC PROBIT, SAS Institute 1998) as previously described. Mortality responses to phloxine B after 24 h of exposure were examined by probit analysis without the $\log_{10}$-transformation because the probit model did not adequately fit the transformed data. Time-courses of mortality for different doses of the dyes that consistently produced measurable mortality (rose bengal and phloxine B) were examined by repeated measures analysis as in the previous study.

Influence of Subsequent Feeding and Elimination on Dye Activity. Although phloxine B was the most effective of the FDC or DC approved dyes evaluated in the preceding experiment, efficacy was lower than would be desired for its eventual incorporation into a control tactic for adult boll weevils. However, traces of the dye were frequently observed in petri plates that contained the weevils between the times of dose administration and the beginning of the assays. Therefore, we suspected some of the ingested dye was excreted or detoxified in this interval, and that efficacy might be improved

if elimination of the dye could be prevented. Further, weevils ingesting the dye in the field would likely have the opportunity to feed on the fruit of cotton shortly after ingesting the dye. Because feeding weevils are typically enclosed by the bracteoles of the squares, they would be shielded from the light necessary for dye activation until feeding ceased and the weevil moved to another location. Therefore we wished to know if such feeding, and the associated delay in exposure to sunlight, would decrease efficacy by facilitating elimination of dye in the frass.

A single dosage level of phloxine B (1.0 µg/weevil delivered in 0.5 µl of 5% sucrose solution) was used to examine the influences of delayed exposure to light and square feeding on dye-induced mortality. Weevils were 6- to 14-d-old mixed-sex adults reared from squares collected in the Brazos Valley and were removed from food on the day before dyes were administered. Weevils ingesting dyes were assayed by exposure to the high-pressure sodium lamp for 8 h, using the equipment and procedures described for the evaluation of dye types. Three time intervals (0, 24, and 48 h) between dye ingestion and exposure to the light source were examined. In addition, two feeding regimes (fed, unfed) were examined for weevils exposed to the lamp at 24 and 48 h after dye ingestion. Unfed weevils to which the dye was administered were held in darkness at room temperature (~24°C) until they were exposed to the light source. Fed weevils were held likewise except that cotton squares (6-9 mm diameter) were supplied daily at a rate of one square per five weevils. Thus, five different combinations of time before exposure to the lamp and feeding were used (0-d, unfed; 1-d, unfed; 1-d, fed; 2-d, unfed; and 2-d, fed). A sixth treatment (0.5 µl of 5% sucrose, unfed) was included in each assay to estimate background mortality. Ten weevils in a ventilated petri plate were assigned to each treatment combination, and assays of times before exposure to the lamp (0, 24, and 48 h, respectively) were conducted on consecutive days. Fed and unfed treatments within a given time before exposure were examined on the same day. This procedure was repeated three times on different weevil cohorts yielding three replications of each treatment.

Mortality responses to the dye within the various treatment combinations were examined by repeated measures analysis (the REPEATED statement of PROC GLM, SAS Institute 1998). The ANOVA model contained terms for times before exposure to the lamp, feeding status, time of observation (the repeated factor), and their interactions. When a weevil died in the untreated control, mortality observed in other treatments of that assay were corrected before analysis (Abbott 1925). Because the Huynh-Feldt condition was rejected at $P < 0.0001$, Wilk's Lambda was used to assess the statistical significance of the repeated factor (time of observation) and interactions containing that factor. Means of mortality levels corresponding to times before exposure to the light source were separated using the TUKEY option of the MEANS statement of PROC GLM (SAS Institute 1998).

RESULTS

Effects of Dye Concentration on Ingestion. Dye concentration significantly influenced ingestion of the dye solutions ($F = 18.97$; df = 3, 160; $P < 0.01$). The mean volume of solution ingested in 3 min was greater for the control (0.0 mg/ml dye; 1.20 µl) than for a concentration of 0.5 mg/ml (0.90 µl). Ingestion of the solutions with 1.0 (0.71 µl) and 2.0 mg/ml (0.67 µl) were not different from each other, but were significantly less than for the other concentrations. Mean volume of dye solution ingested in 3 min was not different for the two dyes ($F = 0.15$; df = 1, 160; $P = 0.701$; rose bengal, 0.88 µl; phloxine B, 0.86 µl). Based on these results, we adopted a standard dosage volume of 0.5 µl for use in subsequent dose mortality studies.

Efficacy of Phloxine B and Rose Bengal under a High-Pressure Sodium Lamp. In contrast to our previous efforts to obtain consistent mortality responses to the photoactive dyes under natural sunlight or fluorescent lighting, exposure of treated weevils to the sodium lamp provided results that were reasonably consistent. Although the probit model adequately fit the data for phloxine B ($\chi^2 = 1.52$, df = 2, $P = 0.47$), mortality for the highest dose at the final observation time was only 23% and a reliable LD_{50} could not be estimated. The probit model also fit the data for rose bengal ($\chi^2 = 3.26$, df = 2, $P = 0.20$) and provided an estimated LD_{50} of 0.17 µg ($n = 150$, slope = 4.10, SE = 0.74, 95% fiducial limits = 0.12 – 0.21 µg).

Repeated measures analysis indicated that rose bengal provided higher levels of mortality than did phloxine B ($F = 327.08$; df = 1, 40; $P < 0.01$) (Fig. 1). The analysis also indicated that mortality generally increased with increasing time of exposure to the light source (Wilk's Lambda = 0.074; $F = 24.09$; df = 14, 27; $P < 0.01$), that the rate at which this mortality increased was faster for rose bengal than for phloxine B (Wilk's Lambda = 0.112; $F = 15.26$; df = 14, 27; $P < 0.01$), and that mortality occurred earlier at higher dye doses than at lower doses (Wilk's Lambda = 0.042; $F = 3.66$; df = 42, 80.86; $P < 0.01$) (Fig. 1). Examination of levels of the dye type*dose interaction ($F = 46.16$; df = 3, 40; $P < 0.01$) indicated that mortality produced by rose bengal was ≥50% after 60, 150, and 300 min of exposure for doses of 1.0, 0.5, and 0.25 µg, respectively (Fig. 1). Thus, within this dosage range, the time required to attain ≥50% mortality was approximately halved each time dose was doubled. By the final observation period (450 min) mortality by the three highest doses of rose bengal were not statistically different, while the lowest dose (0.05 µg) produced no mortality (Fig. 1). The mortality produced by phloxine B occurred more slowly, and differences among doses could not be detected until the last observation period. By the end of the assay, the mortality at the highest dose of phloxine B (1.0 µg) was greater than that at the lowest two doses (0.05 and 0.25 µg) of phloxine B and the lowest dose of rose bengal (0.05 µg), but remained at a level less than those observed for the other doses of rose bengal (0.25 – 1.0 µg) (Fig. 1).

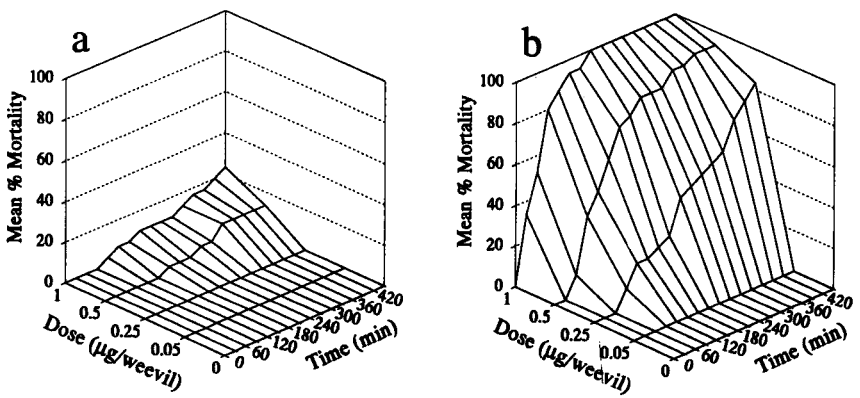

FIG. 1. Temporal patterns of mortality of adult boll weevils fed controlled doses of photoactive dyes and exposed for 7.5 h to a high-pressure sodium lamp. Dyes were administered on the afternoon before exposure to the lamp the following morning; a, phloxine B; b, rose bengal.

Comparative Toxicities of Selected Dyes. Light intensities on the assay platform varied from 909 to 1098 μmol s^{-1} m^{-2} (usually 1000 to 1070 μmol s^{-1} m^{-2}). These intensities represent approximately half that of full sunlight during midday. Temperatures varied from 30.7 to 36.1°C, but were mostly between 32 and 35°C. A total of four weevils in the untreated controls died during the assays, and no more than a single control weevil died in a given assay.

Only methylene blue, phloxine B, and rose bengal produced uncorrected weevil mortality that was >10%. Because of the low levels of mortality observed, probits could not be fit to the dose responses of eosin Y, erythrosin B, or rhodamine B (Table 1). The mean mortality for the highest dosage of methylene blue at 24 h after exposure to the light was only 33% so a valid LD_{50} could not be estimated. Further, results among the individual assays of this dye were not consistent. This variation was likely caused by inconsistencies in the actual doses delivered to weevils. Methylene blue produced an extremely intensely colored solution. This property prevented us from determining with certainty that the dye was completely dissolved, and the highest concentration used was in excess of the solubility of this dye under standard conditions.

TABLE 1. Mortality responses of boll weevil adults to ingested doses (0, 0.05, 0.25, 0.5, and 1.0 μg/weevil) of photoactive dyes after a 9-h exposure to a high-pressure sodium lamp.

Assessment time (h)[a]	Dye	*n*	Slope (SE)[b]	LD_{50}(μg)	95% FL	χ^2	df	*P*
9	Phloxine B	150	2.92 (0.76)	1.07	0.81-2.00	0.002	2	>0.99
	Rose bengal	150	3.63 (0.54)	0.12	0.09-0.15	0.49	2	0.78
	Methylene Blue	150	0.71 (0.34)	--	--	3.57	2	0.17
	Rhodamine B	150	-0.09 (0.79)[c]	--	--	3.00	2	0.22
	Erythrosin B	150	2.39 (1.41)[c]	--	--	0.22	2	0.90
	Eosin Y	150	-0.51 (0.48)[c]	--	--	2.82	2	0.24
24	Phloxine B[d]	150	2.52 (0.41)	0.89	0.76-1.08	1.36	3	0.72
	Rose bengal	150	3.58 (0.68)	0.07	0.06-0.10	0.06	2	0.97
	Methylene Blue	150	1.92 (0.67)	--	--	1.71	2	0.43
	Rhodamine B	150	-0.09 (0.79)[c]	--	--	3.00	2	0.22
	Erythrosin B	150	0.96 (0.65)[c]	--	--	0.42	2	0.81
	Eosin Y	150	-0.07 (0.39)[c]	--	--	0.68	2	0.71

[a] Mortality was assessed at the conclusion of the 9-h exposure period and 24 h after initiation of the exposure.
[b] Slopes were calculated using $\log_{10}$(dose).
[c] Slopes were not significantly different from zero (α=0.05).
[d] The slope was calculated without $\log_{10}$ transformation of dose.

Phloxine B produced mortality by the end of the 9-h exposure period that was slightly higher than that observed by 7.5 h in the previous experiment (Fig. 2), and this mortality was high enough to permit estimation of the LD_{50}. Although rose bengal caused slightly higher levels of mortality after 9 h of exposure to the light (Fig. 2) compared with those observed in

the previous experiment, fiducial limits of the LD_{50} (Table 1) overlapped those previously estimated. Assessment of mortality at 24 h after initiation of the light exposure period indicated even higher mortality levels because weevils that were moribund at the end of the assay died during the additional 15 h. Thus, the LD_{50} for rose bengal calculated from mortality at 24 h after initial exposure to the lamp (Table 1) was lower than that observed in the previous experiment, although neither it nor the LD_{50} estimated for phloxine B were different from respective estimates for the 9-h mortality observations.

The repeated measures analysis indicated that rose bengal again induced higher levels of mortality than did phloxine B (F = 246.05; df = 1,16; $P < 0.01$) (Fig. 2). As in the analysis of the previous experiment, mortality tended to increase with increasing time of exposure to the light (Wilk's Lambda = 0.032; F = 27.01; df = 9, 8; $P < 0.01$), this increase in mortality was greater for rose bengal than for phloxine B (Wilk's Lambda = 0.118; F = 6.65; df = 9, 8; $P < 0.01$), and mortality tended to increase more rapidly at higher doses of the dyes (Wilk's Lambda = 0.022; F = 2.37; df = 27, 24.006; P = 0.02).

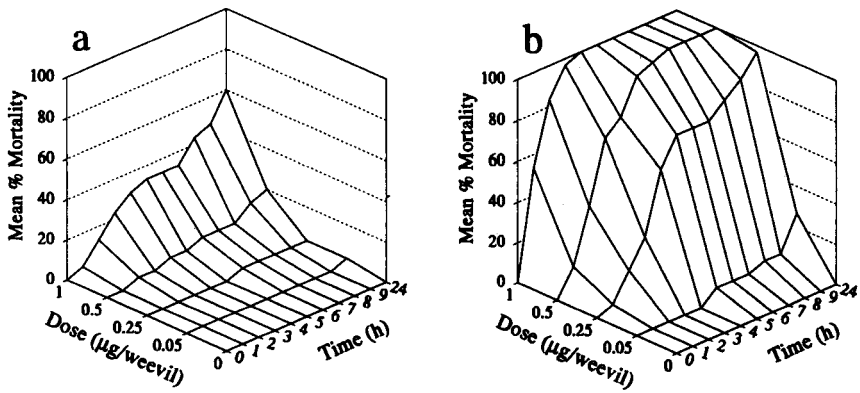

FIG. 2. Temporal patterns of mortality of adult boll weevils fed controlled doses of photoactive dyes and exposed for 9 h to a high-pressure sodium lamp. Dyes were administered on the afternoon before exposure to the lamp the following morning, and final observations of mortality were taken 24 h after initial exposure; a, phloxine B; b, rose bengal.

The slightly higher overall mortality levels observed using the modified assay procedures probably resulted from the extended times of exposure and mortality assessment, and from greater control of light intensities on the assay platform. Regardless, examination of the levels of the dye type*dose interaction (F = 17.50; df = 3, 16; $P < 0.01$) indicated that temporal mortality responses to doses of phloxine B and rose bengal were generally similar to those observed in the previous experiment (Fig. 2). From the first through the fourth hours of observation mortality generally increased with increasing dose of rose bengal. By the fifth hour and thereafter, mortality at the highest two doses of rose bengal (1.0 and 0.5µg) were not different. Between the fifth and eighth hours of exposure to the light, mortality at the 0.25 µg dose of rose bengal and the 1.0 µg dose of phloxine B were equivalent and higher than at the 0.05 µg dose of rose bengal and other doses of phloxine B. The only change in this trend by 9 h after exposure to the light was that mortality at the 0.5 µg dose of phloxine

B was higher than that at the lower two doses. By the last observation time (24 h after initial exposure to the light), mortality at the highest three doses of rose bengal were equivalent and higher than for other doses of either dye. Mortality at the 1.0 μg dose of phloxine B was also higher than for other doses of this dye, and also higher than at the 0.05 μg dose of rose bengal.

We also noted that some petri plates containing the weevils between the time of dye administration and initial exposure to the light source contained small droplets of dye. The boll weevil adult empties the midgut in a short time after feeding (Suh and Spurgeon 2001), and the presence of the dye droplets most likely indicated that some of the ingested dye was eliminated before the assays began. These observations prompted investigation of the effects of delayed exposure of weevils to the light source after dye ingestion.

Influence of Subsequent Feeding and Excretion on Dye Activity. Repeated measures analysis indicated mortality was considerably enhanced by exposure of treated weevils to the sodium lamp immediately after ingestion of phloxine B compared with exposure delayed 1 or 2 d after dye ingestion (F = 18.25; df = 2, 10; $P < 0.01$) (Fig. 3). However, we could not detect an effect of feeding status on mortality (F = 2.35; df = 1, 10; P = 0.16). Thus, feeding after dye ingestion did not substantially hasten elimination of the dye. Although mortality generally increased with increasing time of exposure to the lamp for all treatments (Wilk's Lambda = 0.007; F = 80.66; df = 7, 4; $P > 0.01$), this increase occurred at different rates that depended on whether or not weevils were exposed immediately after dye administration (Wilk's Lambda = 0.021; F = 3.37; df = 14, 8; P = 0.04). Mortality corresponding to the respective treatments were similar through the third hour of exposure to the light, but was significantly higher for weevils exposed immediately after dye administration by the fourth observation period and thereafter.

DISCUSSION

Most investigations of the toxic action of photoactive dyes do not employ the application of known doses. Rather, dyes are generally provided to subject insects in baits (Fondren and Heitz 1978, Moreno and Mangan 1995, Schroder et al. 1998) or artificial diet (Callaham et al. 1975), and dye-mortality relationships are considered on the basis of dye concentrations in the substrate (Moreno and Mangan 1995, Schroder et al. 1998, Capinera and Squitier 2000) or in the whole insect (Callaham et al. 1977, Fondren and Heitz 1978). Callaham et al. (1975) reported that rose bengal supplied in artificial diet neither stimulated nor inhibited feeding by adult boll weevils. In contrast, we found the concentration of both rose bengal and phloxine B significantly influenced ingestion of dye solutions. Nevertheless, our results demonstrate a technique for delivering known doses of photoactive dyes to the boll weevil that with modification may be applicable to other species.

The relative effectiveness of various photoactive dyes appears to vary among insect species, but rose bengal, phloxine B, and erythrosin B are reported among the most effective (Heitz 1995). Moreno and Mangan (1995) indicated that rose bengal, erythrosin B, eosin Y, and phloxine B were sufficiently active to kill Mexican fruit flies when they were provided in baits. Callaham et al. (1975) reported that rose bengal was more effective against the boll weevil adult than phloxine B, erythrosin B, and eosin Y when dyes were supplied in artificial diet. Their results indicated the LD_{50} for phloxine B was about 3-fold that of rose bengal, and that eosin Y was not an effective mortality agent. We observed consistent mortality responses only to rose bengal and phloxine B, with the LD_{50} of rose bengal estimated at nearly one-tenth that of phloxine B under the conditions of our second experiment.

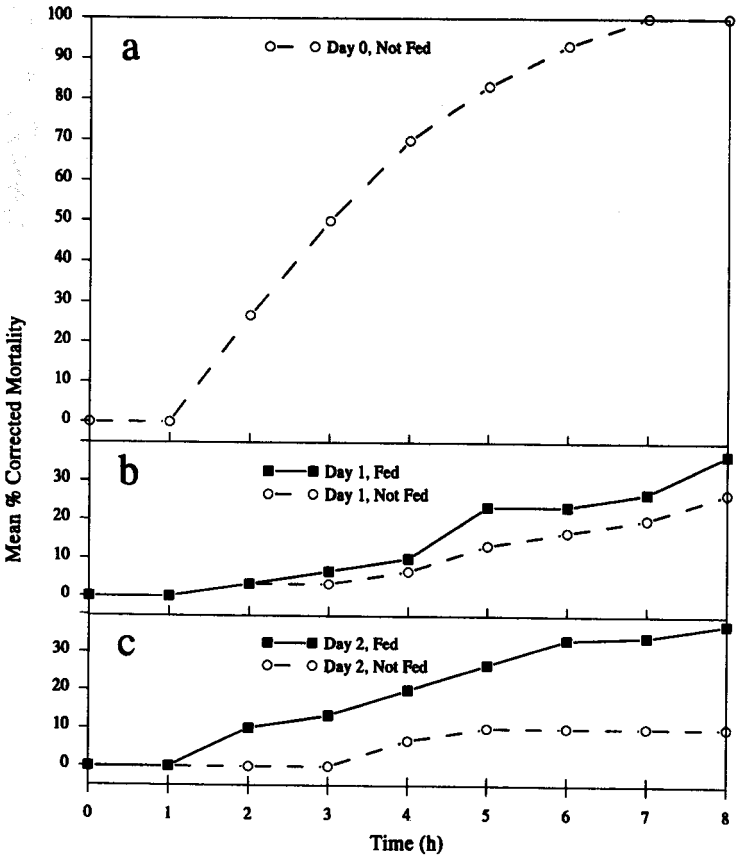

FIG. 3. Temporal patterns of mortality of adult boll weevils fed or not fed squares after ingestion of 1.0 μg phloxine B and exposed for 8 h to a high-pressure sodium lamp beginning at different times after dye ingestion. Dyes were administered a, immediately before (day 0); b, 24 h before (day 1); or c, 48 h before (day 2) exposure to the lamp.

Our results also illustrate the need to consider the time-course of mortality in studies of dye activity. This is especially informative in the case of highly efficacious dyes like rose bengal, because examination of the time-course of mortality provides information regarding the effects of dosage that will not be obtained from a single, final mortality observation. Such observations may be particularly important in selecting dye concentrations for use in field-applied baits. In those cases rapid mortality may be desirable to prevent the possibility of subsequent recovery by the intoxicated insect or to facilitate accurate assessment of mortality.

The target tissue(s) in the boll weevil that are affected by the dyes are unknown. However, examinations of dead weevils that were not too desiccated to permit dissection indicated that traces of the dyes were often visible in the alimentary canal, and the

Malpighian tubules were usually conspicuously colored by the dyes (unpublished data). Our observations regarding the influence on mortality of the time elapsed between dose administration and exposure to the light source clearly indicate that the boll weevil is capable of eliminating at least some of the ingested dye, and that feeding after dye ingestion does not hasten this elimination. Presence of these materials in the Malpighian tubules further demonstrates that the dyes enter the hemolymph, and suggests the dyes may subsequently be excreted. These results also indicate the importance of initiating assays of dye evaluations before substantial elimination has occurred, and that the specific methodology employed should be considered when interpreting results of assays.

In general, our results demonstrate the utility of a high-intensity artificial light source in photoactive dye studies that involve a relatively opaque insect. These methods offer the investigator a practical means of examining light-induced mortality while avoiding the variations in light intensity and temperature conditions associated with assays conducted under natural sunlight.

ACKNOWLEDGMENT

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A NEW TECHNIQUE FOR LABORATORY ASSESSMENT OF RED IMPORTED FIRE ANT MOUND DRENCH TREATMENTS

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ABSTRACT

A new laboratory technique is described for assessing candidate liquid mound drench treatments against the red imported fire ant using inexpensive, readily-available equipment. The method utilizes cotton swab sticks to provide a walking surface for ants enclosed in soda straws pinched at both ends using clips to provide an encapsulation which can be submerged in candidate liquids. The method was used to assess contact insecticide properties of treatments on worker ant samples. Treatments evaluated included various dilutions of liquid dishwashing detergent and plant oil (citrus oil containing d-limonene) and plant oil-containing products as follows: Garden-Ville Soil Conditioner (30% orange oil plus 70% liquid compost), Citrex™ (78.2% d-limonene), Concern® (5.8% d-limonene), Exxant (14.2% terpentine plus 0.2% ammonia) and TFA Super-Kill™ (89% pine oil). Data generated using this technique can help in the rapid development of ant mound drench treatments prior to labor-intensive and costly field trials. This method is also suitable for assessing effects of variables such as exposure time, concentration, and temperature of solutions on efficacy.

INTRODUCTION

Conducting field trials to determine the efficacy of mound treatments to control the red imported fire ant, *Solenopsis invicta* Buren (Hymenoptera: Formicidae), is labor intensive and seasonally limited to periods with mild temperatures and available soil moisture when ants actively nest near the soil surface. Movement of ant colonies to new locations or formation of multiple nests (colony "shattering" or splitting to form "satellite" nests) in field plots is difficult to assess because "new" ant mounds appearing in a plot could either contain surviving members of the treated colony or ants migrating into the plot from surrounding untreated areas (Francke 1983).

The technique described herein was developed to provide a relatively quick, inexpensive, and easy method to assess the toxicity of candidate mound treatments to worker fire ants. Using this method, numerous variables that may affect the performance of a treatment (i.e., exposure time, temperature of solutions, and concentration of active ingredients) can be explored. This is the first method described for assaying candidate liquid ant mound drenches in the laboratory.

MATERIALS AND METHODS

Worker ants were obtained from *S. invicta* colonies collected from the field and brought into the laboratory or obtained directly from ant mounds in the field. Colonies were collected from the field (Brazos County, Texas) by shoveling dirt from the ant mound into a 19 l plastic buckets on 18 November 2000. The inner surface of each bucket was kept liberally dusted with baby powder (Albertson's® brand) to prevent ants from escaping. The bucket was brought into the laboratory and placed into a second bucket containing soapy water (60 ml Dawn®/gal, Proctor and Gamble, Cincinnati, Ohio) for secondary protection against ant escape. Colonies were maintained indoors at 15.5 to 21.1° C and were used to extract worker ants for trials described herein. For some trials, worker ants were directly obtained from disturbed ant mounds in the field by placing a container with the inner vertical surface dusted with baby

powder on top. Worker ants crawled up the outside of the container and fell inside.

For each treatment, approximately 10 worker ants (range: 7-31) were allowed to crawl onto a cotton swab stick (Equate® Gentle & Safe Cotton Swabs) from which the cotton swab ends had been removed. Using forceps, the ant-covered swab stick was slipped into a soda straw (Glad® Flexible Straws - See Thru...For Fun, 19.37 length x 0.60 cm dia.), of which one end was clamped shut using a small binder clip (Office Depot® Binder Clips - 19 mm). The second clamp was placed at the other end of the swab stick on the open side of the straw (Fig. 1).

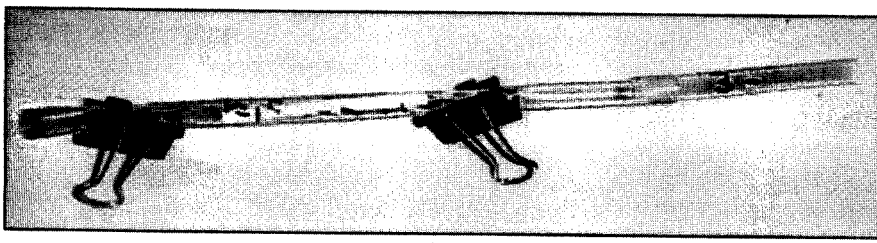

FIG. 1. Soda straw containing cotton swab stick and imported fire ant workers.

Solutions were prepared by mixing appropriate concentrations of candidate materials and placing approximately 353 ml of solution into 473 ml plastic cups (Solo® Cup Company, Jack Frost Plastic Cups). A pipet bulb was used to provide suction and air pressure to help move the solution through the straws more quickly and to assure that no solution remained in the straw following exposure (Fig. 2). Ant-containing straws were dipped into the solution for desired periods of time, but usually just long enough for the solution to completely fill the straw (5 to 10 seconds, usually less than 30 seconds).

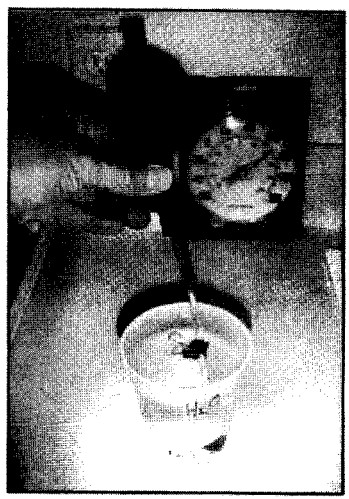

FIG. 2. Submerging confined worker ants in treatment solution.

Following exposure, straws were placed horizontally (Fig. 1) on a counter surface, and ants were observed periodically over time (i.e., 3, 10 min and 1 , 4, 12 and 24 h or more) to document mortality or survival. Upon termination of each trial, all ants in a straw were expelled into a dish containing water and counted. Temperature was recorded throughout these trials, but most were conducted at room temperature (20.5-21.7° C).

Data from these trials were analyzed to determine mortality over time, with number of active ants in the pre-treatment versus 24-hr post-treatment data being subjected to statistical analysis. Arcsine transformations were performed to normalize the data. One-way ANOVA and Tukey-Kramer HSD were performed to observe the effect of treatments on ant survival and to detect the differences, if any, between the treatments within each experiment. Otherwise, the number of active ants per straw was converted to a standardized number of active ants per 10 ant samples and listed with standard deviation for comparison purposes.

Trial 1: Liquid dishwashing detergent concentrations. Worker ant survival was monitored 10 min and 1, 6, 12, 24, and 48 h following exposure to treatments, which included: 1) water, or 2) Dawn® Original Scent, containing biodegradable anionic and nonionic surfactants and no phosphate (Proctor and Gamble, Cincinnati, Ohio) at various concentrations: (0.9 mls/3.8 l, 3.75 ml/3.9 l,15 ml/3.8 l, and 60 ml/3.8 l).

Trial 2: Liquid dishwashing detergent timed exposures. Worker ant survival was monitored 10 min and 1, 6, 12, and 24 h following treatments, which included: 1) water, 10 min; 2) Dawn 3.75 ml/3.8 l at various exposure (submersion) times: (10 min., 5 min., 1 min., and 5 sec.).

Trial 3: Plant oil-containing products . Worker ant mortality was monitored 10 min and 1, 6, 12, 24 and 48 h following 5-min treatments, which included: 1) water; 2) Exxant Fire Ant Killer (Lang Laboratories, College Station, TX); 3) TFA Super-Kill™ Fire Ant Eliminator (TFA Products, Inc., Houston, TX); 4) Garden-Ville Liquid Compost Fire Ant Control (Garden-Ville Fertilizer Company, San Antonio, TX); 5) Citrex™ Fire Ant Killer (EnviroSafe Labs, LLC, Conroe, TX); and, 6) Concern® Citrus Home Pest Control Kills and Repels Ants (Necessary Organics, Inc., New Castle, VA). Solutions of products evaluated were prepared following manufacturers instructions.

Trial 4: Plant oil-containing product dilutions and exposure time. Worker ant mortality was monitored 10 min and 1, 6, 12, and 24 h following exposure to various treatments and exposure times, which included: 1) water (5 min); 2) Garden-Ville 180 ml/3.8 l at two exposure times (5 min and 5-10 sec);and 3) Citrex (5-10 sec) at different concentrations (240 ml/3.8 l, 60 ml/3.8 l, 15 ml/3.8 l and 3.75 ml/3.8 l).

Trial 5: Liquid dishwashing detergent plus orange oil. Worker ant mortality was monitored 15 min and 4.5, 10.5 and 24 h following exposure to 5- to 10-sec dip treatments, which included: 1) water; 2) Dawn (5 ml/3.78 l); and, 3) Dawn + McCormick orange oil (5 ml + 5 ml/3.8 l). Costs of these ingredients were: 1) Dawn - $4.79/42 fl oz or $0.018/5 ml; and 2) McCormick Orange Oil - $2.69/29 ml = $0.45 /5 ml.

RESULTS

Trial 1: Liquid dishwashing detergent concentrations. Worker ants dipped for 5 to 10 sec in dilute liquid dishwashing detergent almost immediately stop moving and appeared dead. Higher concentrations (15 ml and 60 ml Dawn/3.8 l) provided significant increases in mortality 24 h after exposure relative to lower concentrations or water (Table 1). However, observations over time revealed that several ants initially immobilized recovered over the next 5 to 21 h.

Trial 2: Liquid dishwashing detergent timed exposures. Exposure time to detergent treatments did not significantly change ant survival, although all exposures provided significant mortality compared to 10 min submersion into water (Table 2). Again, a few ants initially rendered inactive recovered and become active over the 24-hr period of observations.

Trial 3: Plant oil-containing products . All treatments killed ants after 24 h of exposure (Table 3). For ants submerged in the Exxant solution, mortality was significantly greater than water and less than other product treatments. Garden-Ville Liquid Compost Fire Ant Control did not cause rapid mortality, with ant activity persisting for 6 h after submersion, while products containing d-limonene (Citrex™ Fire Ant Killer and Concern® Citrus Home Pest Control Kills and Repels Ants) killed ants almost immediately.

Trial 4: Plant oil-containing product dilutions and exposure time. Citrex™ Fire Ant

Killer is a relatively expensive treatment that often causes phytotoxic reactions to turf grass. The product can be diluted to 15 ml/3.8 l and continue to provide 100% mortality of worker ants 24 h after being soaked in the solution for 10 sec. Even the lowest rate evaluated (3.75 ml/3.8 l) caused significant mortality (Table 4). Additional time that ants were submerged in

TABLE 1. Mean Number of Active Worker Ants Following Exposure to Various Dilutions of Dawn Liquid Dishwashing Detergent.

	No. active fire ant workers/10 ± S. D.					
	Time					
Treatment	10 min	1 h	6 h	12 h	24 h[a]	48 h
Water	10.00 ± 0.00	9.70 ± 0.60	9.30 ± 1.14	9.25 ± 0.70	9.10a ± 0.54	8.53 ± 1.36
Dawn®						
0.9 ml/3.8 l (1/32 fl. oz./gal.)	9.73 ± 0.55	9.50 ± 0.66	9.33 ± 0.99	9.38 ± 0.58	8.90a ± 0.77	8.33 ± 0.96
3.75 ml/3.8 l (1/8 fl.oz./gal.)	3.00 ± 2.27	4.55 ± 1.67	7.48 ± 0.86	8.05 ± 0.44	7.85a ± 0.34	6.28 ± 0.38
15 ml/3.8 l (½ fl. oz./gal)	0.60 ± 0.80	2.15 ± 1.12	4.63 + 1.63	4.50 ± 1.64	4.38b ± 1.57	3.70 ± 1.09
60 ml/3.8 l (2 fl. oz./gal)	0.00 ± 0.00	1.33 ± 1.44	2.20 ± 2.42	2.20 ± 1.85	1.85b ± 2.10	1.68 ± 1.78

[a] Means followed by the same letters in a column are not significantly different (One-way ANOVA; $F_{4,15} = 28.20$, $P < 0.0001$; Mean separation by Tukey-Kramer HSD, $n = 4$; $P < 0.05$).

TABLE 2. Mean Number of Active Worker Ant Following Exposure to 3.75 ml Dawn® Liquid Dishwashing Detergent per 3.81 l Water (12:00 p.m., Nov. 25, 2000).

	No. active fire ant workers/10 ± S. D.				
	Time				
Treatment	10 min	1 h	6 h	12 h	24 h[a]
Water					
10 min	7.70 ± 3.96	9.18 ± 0.71	10.0 ± 0.00	9.65 ± 0.41	9.85b ± 0.30
Dawn®					
3.75 ml/3.8 l	0.45	1.03	2.35	2.45	3.33a
10 min	± 0.53	± 0.95	± 1.53	± 1.53	± 1.36
5 min	0.23 ± 0.45	1.10 ± 0.84	3.30 ± 2.93	4.20 ± 3.49	3.53a ± 2.81
1 min	0.00 ± 0.00	0.45 ± 0.52	1.13 ± 1.13	2.73 ± 2.89	3.65a ± 3.34
5 sec	1.13 ± 1.32	0.90 ± 1.05	2.78 ± 3.15	2.50 ± 2.73	3.35a ± 2.57

[a] Means followed by the same letters in a column are not significantly different (One-way ANOVA; $F_{4,15} = 13.17$, $P < 0.0001$; Mean separation by Tukey-Kramer HSD, $n = 4$; $P < 0.05$).

TABLE 3. Mean Number of Active Worker Ants Following Exposure to Various Individual Mound Treatment Product Solutions for 5 Min.

	No. active fire ant workers/10 ± S. D. Time					
Treatment	10 min	1 h	6 h	12 h	24 h[a]	48 h
Water	10.00 ± 0.00	9.52 ± 0.55	10.0 ± 0.00	10.0 ± 0.00	9.50c ± 1.00	9.50 ± 1.00
Exxant 30 ml/3.8 l	3.35 ± 0.79	3.25 ± 1.73	6.38 ± 2.69	7.18 ± 2.09	5.43b ± 3.34	5.43 ± 3.34
TFA Superkill™ 150 ml/3.8 l	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00a ± 0.00	0.00 ± 0.00
Garden-Ville 180 ml/3.8 l	6.13 ± 2.83	1.30 ± 1.19	10.0 ± 0.00	10.0 ± 0.00	0.23a ± 0.45	0.0 ± 0.00
Citrex™ 240 ml/3.8 l	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00a ± 0.00	0.00 ± 0.00
Concern® (ready-to-use)	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00a ± 0.00	0.00 ± 0.00

[a] Means followed by the same letters in a column are not significantly different (One-way ANOVA; $F_{5,18} = 50.12$, $P < 0.0001$; Mean separation by Tukey-Kramer HSD, $n = 4$; $P < 0.05$).

TABLE 4. Mean Number Active Worker Ants Following Exposure to Various Individual Mound Treatment Product Solutions and Exposure Times.

	No. active fire ant workers/10 Time				
Treatment	10 min	1 h	6 h	12 h[a]	24 h
Water (5 min)	10.0 ± 0.00	9.33 ± 1.35	9.33 ± 1.35	9.33 ± 1.35	9.33 ± 1.35
Garden-Ville (180 ml/3.8 l) 5 min	0.58 ± 0.29	0.38 ± 0.45	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
5-10 sec	4.48 ± 2.61	1.23 ± 1.70	1.55 ± 2.02	1.73 ± 1.86	1.18 ± 1.70
Citrex™ (5-10 sec) 240 ml/3.8 l	0.00 + 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
60 ml/3.8 l	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
15 ml/3.8 l	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
3.75 ml/3.8 l	2.88 ± 0.26	0.58 ± 0.73	0.83 ± 1.18	1.88 ± 1.81	1.83 ± 2.38

[a] Means followed by the same letters in a column are not significantly different (One-way ANOVA; $F_{6,21} = 45.45$, $P < 0.0001$; Mean separation by Tukey-Kramer HSD, $n = 4$; $P < 0.05$).

the Garden-Ville Liquid Compost Fire Ant Control solution did not significantly increase mortality after 24 h. However, initial knockdown of worker ants was greater with longer (5 min) exposure to the solution.

Trial 5: Liquid dishwashing detergent plus orange oil. Both dishwashing detergent and detergent with citrus oil resulted in significant mortality of worker ants after 24 h (Table 5). In this trial, the addition of citrus oil did not significantly kill more ants than dishwashing liquid, alone. Evaluation of additional rates of these ingredients both in the laboratory and in field trials should allow for the development of an effective ant mound drench treatment for use as a home remedy.

TABLE 5. Mean Number of Active Worker Ants Following Exposure to Dawn®, Dawn Plus Orange Oil or Water in a 5 to 10 Sec Treatment Exposure.

	No. active fire ant workers/10			
	Time			
Treatment	15 min	4.5 h	10.5 h	24 h[a]
Water	10.0 ± 0.00	10.0 ± 0.00	10.0 ± 0.00	10.0b ± 0.00
Dawn® (5 ml/3.7 l)	0.00 ± 0.00	1.30 ± 1.28	1.73 ± 1.37	3.43a ± 2.76
Dawn + orange oil (5 ml + 5 ml/3.7 l)	0.00 ± 0.00	0.38 ± 0.57	0.65 ± 0.85	1.03a ± 1.35

[a] Means followed by the same letters in a column are not significantly different (One-way ANOVA; $F_{2,9} = 70.77$, $P < 0.0001$; Mean separation by Tukey-Kramer HSD, $n = 4$; $P < 0.05$).

DISCUSSION

The trials described herein were selected to illustrate the value of this new technique for the laboratory assessment of red imported fire ant mound drench treatments: concentration of active ingredient(s) (Trial 1), exposure time (Trial 2), product comparisons (Trial 3), methods of improving product performance (Trial 4), and development of new treatments or home remedies (Trial 5). Low mortality observed in water treatments reveal how this method is ideal for handling *S. invicta* and possibly other delicate insects without injury. Trials can be conducted with relative ease by the researcher and without a high probability of being stung while handling imported fire ants. In contrast to current field trial techniques, this method is inexpensive, can be used throughout the year, eliminates the problem of ants leaving treated mounds following treatment (mound abandonment), and data generated from replicated trials can be statistically analyzed. However, this technique should always be followed up with field trials to verify findings from laboratory studies. Soil type and characteristics such as soil moisture affect not only ant mound structure, but also influence the speed and thoroughness solutions percolate through the colony. These factors would affect the duration that ants in these colonies are exposed to drench solutions and success of treatment.

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BARRIER TREATMENTS FOR RED IMPORTED FIRE ANTS *SOLENOPSIS INVICTA*[1] IN COMMERCIAL HONEY BEE OPERATIONS

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ABSTRACT

The efficacy of using a support pallet, either with or without an insecticide treatment, and a soil area application of insecticide was evaluated for preventing red imported fire ant, *Solenopsis invicta* Buren, foraging on apiary equipment. Four replicated treatments were evaluated: 1) a single control pallet and two beehives (hive bodies), 2) support pallet treated with Lorsban-4E® (active ingredient chlorpyrifos) under a single pallet with beehives, 3) untreated support pallet with no insecticide application under a single pallet with beehives, and 4) soil area treatment consisted of a Lorsban-4E® sprayed $3m^2$ area with bee hives on a single pallet. Ant activity was determined by placing several olive oil-soaked index cards ($2.54cm^2$) on or next to bee equipment for 45 min once each week for six weeks. Control and untreated support pallets all tested positive for ant activity on the equipment. Lorsban 4E® treated support pallets and soil treatments eliminated ant activity on all apiary equipment for up to six weeks after insecticide application. Results showed that some vegetation could grow into natural "bridges" over treated pallets providing ants access to apiary equipment. These results have implications for a variety of quarantined commodities that can be stored on support pallets in *S. invicta*-infested areas, for example nursery stock, hay bales, and sod.

INTRODUCTION

Red imported fire ants, *Solenopsis invicta* Buren (Hymenoptera:Formicidae), among other pest ant species (e.g., Argentine ants, *Linepithema humile* Mayr) can invade honey bee, *Apis mellifera* L. (Hymenoptera:Apidae), colonies and feed on pollen and developing bees (Buys 1990, De Jong 1990, Taber 1994). Weak bee colonies are more susceptible to ant invasions than healthy, vigorous colonies. Often, fire ants build their nests directly against, under, or on apiary equipment (Anonymous 1999). Several ant species, as well as *S. invicta*, may use apiary equipment for colony thermoregulation, as refuges to escape localized flooding events, and to take advantage of better nesting conditions during dry conditions (Burrill 1926).

Commercial pollination with honey bees is a highly mobile business, and bee colonies are frequently moved, on pallets, among holding yards and over-wintering and pollination sites. In the United States, most beekeepers living in northern latitudes over-winter their bees in the southern latitudes of the country where *S. invicta* are abundant. These activities increase the probability that *S. invicta* will be inadvertently transported from fire ant-infested areas to non-infested areas with beehives or in soil adhering to apiary

[1]Hymenoptera:Formicidae

equipment. In fact, *S. invicta* infestations of California's Central Valley have been linked to interstate transport of beehives for almond pollination (Freeman and Coviello 1998). Consequently, beehives have become a regulated item by the United States Department of Agriculture (USDA, APHIS, Code of Federal Regulations, Title 7, Volume 5, Chapter 3, Pgs. 79-94, Revised January 2001). According to the state of Texas and federal regulations, any item may be considered infested with *S. invicta* if any living state of development of the ant, including worker ants, is detected (Texas Administrative Code Title 4, Part 1, Chapter 19, Subchapter J, Rule ξ 19.102 and USDA, APHIS, Program Aid No. 1670). Therefore, an item does not have to contain or harbor an entire ant colony to be considered infested.

No quarantine treatments have been currently approved for assuring that transported hives are *S. invicta*-free. Also, no insecticides have been registered specifically for treating apiary equipment to eliminate *S. invicta*. However, several products on the market are labeled for *S. invicta* under a variety of conditions. For example, Lorsban 4-E® (active ingredient: chlorpyrifos) (Dow AgroSciences, Indianapolis, Indiana) is an approved contact insecticide for control of various insects, including *S. invicta*, in certain agricultural sites [e.g. alfalfa, orchard floors, field corn, popcorn, sweet corn, cotton, cranberries, sorghum, and soybeans, (see http://www.dowagroscience.com; MSDS/Label Product ID: 627)]. Historically, chlorpyrifos has been used as seed treatments and band applications to protect crops from *S. invicta* as barrier treatments (Drees et al. 1992). Chlorpyrifos is toxic to bees contacting treated surfaces. However, chlorpyrifos may be useful in preventing *S. invicta* from foraging on bee equipment if it is applied where bees do not normally crawl, such as on support pallets or soil areas. The objective of this study was to evaluate the efficacy of chlorpyrifos applied to a support pallet or the soil area, as a barrier treatment, around apiculture equipment in preventing *S. invicta* activity, such as foraging or nesting, on apiary equipment.

MATERIALS AND METHODS

Research was conducted September-October 2000 in Royalty Pecan Orchard, Burleson County, Texas. Four treatments with four replicates of each treatment were used for a total of 16 sample units in a randomized block design to compare *S. invicta* infestation levels among treatments. Treatments were arranged randomly under pecan trees in four blocks (i.e., tree rows) in the orchard. Grass was mowed in each replicate unit before empty beehives and pallets were set up in the orchard. We used a typical commercial beekeeping arrangement, which comprised a migratory hive pallet with clips and two hive bodies with lids without bees, in all treatments. Used, wooden, hive bodies were used, cleaned, newly painted and free of any honey or wax residue. Support pallets were wooden all-purpose pallets donated by a local supermarket. Treatments included: 1) a single control pallet with two beehives (hive bodies), 2) Lorsban-4E® treated support pallets under a single pallet with beehives, 3) support pallet untreated under a single pallet with beehives, and 4) Lorsban-4E® $3m^2$ area soil treatment with a single pallet and beehives. Insecticide applications followed printed label directions (4.73 ml Lorsban 4E® per 3.78 liter water).

Three locations on each beehive set-up were sampled for foraging ants: on the soil, pallet, and hive body. We used $2.54cm^2$ olive oil (Bertolli Classico Olive Oil®) soaked index cards to attract ants and monitor foraging (Drees 1994). In the orchard, two oil-soaked bait cards were placed next (ca. 20 - 30 cm) to each pallet or adjacent to a treated soil area. Foraging activity on the apiary equipment was assessed using four oil-soaked bait cards on the hive pallet and two on top of the hive boxes. All baits were placed on or next to the bee equipment under good *S. invicta* foraging temperatures (25-30° C). Ant sampling was conducted for 45 min for one day each week for six consecutive weeks. A ranking system was used to record the approximate number of ants per bait card after 45 min (i.e., 0 = no ants, 1 = 1 - 24 ants, 2 = 25 - 49 ants, 3 = 50 - 74 ants, 4 = 75 + ants). To assess treatment

efficacy, we compared *S. invicta* infestation levels on bee pallets by comparing mean ranks of *S. invicta* infestation for each week for each treatment with a Kruskal-Wallis nonparametric rank sums test (JMP SAS, Cary N.C.). Significant differences among the means were determined using Tukey-Kramer honest significant difference (HSD) method (JMP statistics). We did not statistically compare *S. invicta* infestation levels on the tops of the hives because ants were invariably detected on the tops, only to a lesser degree due to a lag time involved in finding and recruiting to baits of hives, if they also were detected on bee pallets.

RESULTS

S. invicta foraged on oil-soaked bait cards in high numbers throughout the six-week study. Ant foraging intensity was greatest on the bait cards that were on the soil surface (Table 1). Efficacy of treatments used to eliminate ant foraging on the bee equipment were significantly different ($\chi^2 = 19.19$; d.f. = 3; $P = 0.0002$). No significant difference was found in the mean ranks of *S. invicta* infestation levels between single (mean = 1.39) and double support (mean = 1.33) pallet set-ups without insecticide application (Tukey's HSD = 0.2984). The double pallet set-up with the bottom support pallet sprayed with Lorsban 4E® and soil area insecticide application significantly reduced *S. invicta* infestation levels compared to the untreated bee equipment over the six-week monitoring period [Tukey's HSD = 0.2984, (Table 1)]

TABLE 1. Means ± 1SD of Six-Weekly Mean Ranks of *Solenopsis invicta* Infestation Levels on Lorsban 4E®, (Active Ingredient: Chlorpyrifos), Protected and Unprotected Apiary Equipment in Royalty Pecan Orchard, Burleson County, Texas.

	Means ± 1SD of mean ranks[b] of *S. invicta*		
Treatment[a]	Soil	Pallet	Hive
Lorsban 4E®			
Soil	0.042 ± 0.102	0.000 ± 0.000a	0.000 ± 0.000
Double pallet	3.125 ± 0.447	0.021 ± 0.051a	0.000 ± 0.000
No Lorsban 4E®			
Single pallet	2.813 ± 0.259	1.396 ± 0.393b	0.458 ± 0.246
Double pallet	3.104 ± 0.374	1.333 ± 0.298b	0.354 ± 0.146

[a] Indicates treatments: 1) Lorsban 4E® soil area treatment consisted of spraying a $3m^2$ area with Lorsban-4E® and placing a single pallet and two bee hives on it, 2) Lorsban 4E® double pallet-support pallets were treated with Lorsban-4E® and placed under single pallets with beehives, 3) No Lorsban 4E® treatment on single pallets and two beehives (hive bodies), and 4) No Lorsban 4E® double pallet– untreated support pallets placed under single pallets with beehives.

[b] Mean ranks followed by the same upper case letter within a column are not significantly different (Tukey's HSD = 0.2984).

No *S. invicta* foraging was detected on the bee equipment in the support pallet insecticide treatment except in one sampling period. Four weeks after insecticide application, a few *S. invicta* (rank 1) were found on two of the hive set-ups that were sitting on treated support pallets. Closer inspection revealed *S. invicta* climbing on blades of grass that had grown tall enough to be used as natural "bridges" to cross over treated pallets and onto untreated (top) bee pallets. After removing these grass "bridges" no other ants were found on the bee equipment after subsequent sampling. Results from the soil insecticide application showed that *S. invicta* were prevented from foraging on the ground area next to the apiary equipment as well as on the apiary equipment up to the sixth week of sampling.

During the last sampling period, which followed a week of intense rainy weather (ca. 3-4cm), a few ants (rank 1) were found on a bait card on the ground in the treated area next to one of the apiary units. This suggests a "protection" interval of approximately six-weeks until the chlorpyrifos degrades enough to allow ant foraging on the apiary equipment, thus rendering these articles "infested" according to *S. invicta* quarantine guidelines.

As expected, bait cards on top of hive boxes collected fewer ants than bait cards on pallets or the ground (Table 1). This may be attributed to the amount of time required for ants to discover and recruit other ants up to these bait cards. It is assumed that if sampling were conducted for a longer duration, (i.e., > 45 min), ant activity levels on upper level bait cards in the untreated units would have reached similar activity levels as lower level bait cards.

DISCUSSION

We detected significant ant foraging and recruitment to our baits in the pecan orchard and on our unprotected apiary equipment. Our results differ significantly from those of Desillppe and Melvin (2001), who reported finding no evidence of *S. invicta* foraging on apiary equipment in a *S. invicta*-infested apiary with or without bees. Our data document ant foraging and significant recruitment on our unprotected apiary equipment. This indicates that beekeepers are faced with a risk of *S. invicta* infestation when part of their bee operations are in *S. invicta*-infested areas of the United States.

In this study, we have shown that soil or supporting pallet application of Lorsban 4E®, a long-residual contact insecticide, is a reasonable and effective technique to prevent *S. invicta* infestation or foraging on apiary equipment for up to six weeks. This technique fits into most commercial bee operations where bees are being moved among established pollination sites and holding yards. Results indicate that simply stacking apiary equipment on supporting pallets or similar structures does not eliminate *S. invicta* foraging on apiary equipment. However, double pallet arrangements have several advantages over single pallet arrangements. Supporting pallets can be treated with insecticides to prevent ant foraging on apiary equipment, can reduce or eliminate time spent on hive pallet sanitation (i.e., soil removal), and may even extend the life of the supported hive pallet. Monitoring and removing any bridges, such as over-grown vegetation, fallen tree leaves and limbs, or accumulations of bee trash, that may form across treated areas between the soil surface or treated support (bottom) pallets and the apiary equipment are necessary.

Treated support pallets and soil applications of contact insecticides may be used in loading yards for short-term hive storage before moving them to new locations. By applying Lorsban 4E® to selected treated surfaces or support (bottom) pallets a day or so prior to moving apiary equipment containing bees onto treated surfaces, foraging honey bees can be protected from direct contact with the insecticide. These practices can make the targeted use of contact insecticides a viable option for beekeepers and provide two potential *S. invicta* quarantine treatment options.

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TOLERANCE TO ACEPHATE IN TARNISHED PLANT BUG (HETEROPTERA: MIRIDAE) POPULATIONS IN THE MISSISSIPPI RIVER DELTA

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ABSTRACT

Tarnished plant bugs, *Lygus lineolaris* (Palisot de Beauvois), were collected from weeds in the fall of 1998, 1999, and 2000 at twenty locations in the Mississippi River Delta of Arkansas, Louisiana, and Mississippi and tested with a glass-vial bioassay for tolerance to acephate. The same twenty collection locations were used each of the three years. Plant bug populations with high levels of resistance to acephate were not found. The highest resistance found was 4.6-fold, and, at most locations in all three years, resistance was 2-fold or less. Based on mean resistance ratios among populations within each year, there appeared to be a slight increase in tolerance to acephate over the three year period. Tarnished plant bug populations at 18 of 20 test locations in 2000 had higher tolerance than was found in populations tested from the locations in 1998. However, the increase was significant in plant bugs from only 7 of the 18 test locations. Acephate should still be an effective insecticide for control of tarnished plant bugs in cotton in the Mississippi River Delta.

INTRODUCTION

Adults and nymphs of the tarnished plant bug, *Lygus lineolaris* (Palisot de Beauvois), damage cotton by feeding on terminals, squares, blooms and bolls. Early season tarnished plant bug infestations in cotton can delay fruiting, cause loss of squares, reduce yield, and delay crop maturity (Scales and Furr 1968, Tugwell et al. 1976, Hanny et al. 1977, Scott et al. 1985). Young bolls are sensitive to feeding damage by tarnished plant bugs, and feeding damage can cause boll abscission (Pack and Tugwell 1976, Russell et al. 1999). Williams (2000) reported that tarnished plant bugs infested an estimated 1.2 million acres of cotton in Mississippi in 1999. About one-half of this acreage was treated with insecticides for plant bug control, yet the yield loss was still estimated to be over 6,000 bales (1.31×10^6 kg).

Tarnished plant bugs are controlled in cotton exclusively with insecticides. Some pyrethroid insecticides were very effective for plant bug control in cotton during the 1980's. However, in 1993 plant bugs were found in cotton in the Delta of Mississippi that were highly resistant to pyrethroid insecticides with multiple resistance to some organophosphate and cyclodiene insecticides (Snodgrass 1996a). Pyrethroid resistance was found to be widespread in tarnished plant bug populations in the Delta of Arkansas, Louisiana, and Mississippi (Pankey et al. 1996, Hollingsworth et al. 1997, Snodgrass and Scott 2000). This resistance changed each year with the most resistant populations found in the fall (after exposure of plant bugs to insecticides in cotton during the growing season) compared to the spring (Snodgrass and Scott 2000).

Among the insecticides recommended for tarnished plant bug control in cotton by the Cooperative Extension Service in Arkansas, Louisiana, and Mississippi, only acephate (Orthene), dicrotophos (Bidrin), and dimethoate (Cygon) are commonly recommended in all three states. The most widely used of the three insecticides is probably acephate. Pyrethroid resistant tarnished plant bug populations have been tested several times in laboratory bioassays and in field tests for resistance to acephate (Snodgrass and Elzen 1995, Snodgrass 1996a, Pankey et al. 1996, Snodgrass and Scott 2000). Little or no resistance to acephate was found in these studies. Efficacy of acephate for plant bug control in cotton can vary widely and tends to be lower at rates less than the highest rate recommended of 0.56 kg AI/ha. In recent efficacy studies on control of plant bugs in cotton in the Delta with insecticides that included acephate at 0.56 kg AI/ha as a treatment, percentage control in the acephate treatment, compared to an untreated check at this rate averaged 66% in the eight studies (Kharboutli and Allen 1998, 2000; Lorenz et al. 2000; Robbins et al. 1999; Scott et al. 2000; Seay et al. 1999; Teague et al. 1998, 2000). Howell et al. (1998) found that over the period from 1980 to 1996, percentage control for plant bugs for all organophosphate insecticides tested in cotton in Mississippi averaged about 50%.

Because of insecticide resistance in plant bugs to several insecticides, the trend for reduced efficacy of many insecticides in Mississippi (Howell et al. 1998), and the limited number of insecticides recommended for tarnished plant bug control, acephate is a very important insecticide for tarnished plant bug control in cotton production in the Delta. We conducted a three-year survey to determine if resistance to acephate was present in tarnished plant bug populations found in the Delta of Arkansas, Louisiana, and Mississippi.

MATERIALS AND METHODS

Tarnished plant bugs were collected for resistance testing from wild host plants using a sweep net at twenty locations in the Delta (Table 1). These locations were easily accessed areas along highways near areas where cotton was grown, and were areas where wild hosts could be found in the fall each year. They were typically near undisturbed drainage ditches. The same twenty locations were used each year and collections were made from mid-September through mid-October. Plant bugs were primarily collected from pigweed, *Amaranthus* spp., giant ragweed, *Ambrosia trifida* L., goldenrod, *Solidago altissima* L., and horseweed, *Erigeron canadensis* L. Bugs were aspirated from sweep nets after collection and placed into paper ice cream cartons (0.95 liter) with green beans, *Phaseolus vulgaris* L. The green beans were washed in detergent and soaked in a 3% sodium hypochlorite solution as described in Snodgrass (1996b) to remove or oxidize any insecticide residue on them. The bugs were held in these containers under laboratory conditions of 24-26°C. Humidity was not controlled for a 24-h period before testing to allow any bugs injured during collection to die.

Adults from each collection location were tested for resistance to acephate using a glass-vial bioassay developed by Snodgrass (1996b). In this bioassay, adults were placed into 20-ml glass liquid scintillation vials (two adults per vial) that were previously prepared by coating their inner surface with acephate. Acephate was applied to each vial by pipetting 0.5 ml of acephate diluted in acetone (pesticide grade, Fisher, Fair Lawn, NJ) into the vial. The vial was then rolled on a hotdog cooker (Star MFG, Smithville, TN). This process evaporated the acetone and left the insecticide as a residue on the inner surface of the vial. In all tests, the acephate was mixed with acetone and applied to the vials on the same day the test was performed. Technical grade acephate was purchased from Chem Service, West Chester, PA and was 98% pure. Prior to adding the adults to a vial for testing, a piece of green bean which had been washed as described previously was added to the vial for food. Green beans were cut transversely into pieces about 3-mm thick and one piece was used in each vial. The bean

TABLE 1. Locations along Highways in the Mississippi River Delta where Tarnished Plant Bugs Were Collected in the Fall from Weed Hosts in 1998-2000 and Tested for Resistance to Acephate.

County/Parish	Nearest city	Highway[a]
	Arkansas	
Ashley	Parkdale	165
Chicot	Lake Village	82
Desha	Gould	65
Lincoln	Grady	65
	Louisiana	
East Carroll	Lake Providence	65
East Carroll	Transylvania	65
	Mississippi	
Coahoma	Clarksdale	61
Holmes	Thornton	49
Leflore	Minter City	49
Leflore	Greenwood	49
Quitman	Marks	6
Sharkey	Rolling Fork	61
Sunflower	Indianola	82
Sunflower	Indianola	49
Sunflower	Ruleville	49
Tunica	Tunica	61
Warren	Vicksburg	61
Washington	Avon	1
Washington	Greenville	82
Washington	Winterville	1

[a] Highway 6 is a state highway, 1, 49, 61, 65, 82, and 165 are U. S. Highways.

pieces were dried on tissue paper before placement into the vials. After plant bugs were placed in a vial, a cotton ball was placed in the vial opening to confine the bugs. During a test the vials were held in an upright position at laboratory conditions of 24-26°C, and humidity was not controlled. At least six doses (as many as nine) were tested, and each test was replicated three times. Each replication had five vials, each containing two adults. Control vials were treated only with acetone, and control mortality was rare and never >3.3%. Data were corrected for control mortality using Abbott's (1925) formula before analysis. Data from the glass-vial bioassays were analyzed assuming the probit model (Proc Probit: SAS Institute 1989). LC_{50} values for each location were compared to the LC_{50} for acephate for susceptible bugs. The susceptible bugs were collected near Crossett, AR, and tested with acephate in September 1998. Forestry is the main industry around Crossett and no cotton is grown near this city. The LC_{50} value determined at a collection location in 1998 was also compared to the LC_{50} determined at the same location in 1999 and in 2000. Differences in LC_{50} values were considered significant if the 95% CL of the resistance ratio at LC_{50} did not include 1.0 (Robertson and Preisler 1992).

RESULTS

Tarnished plant bugs from three of the four locations in Arkansas and seven of fourteen locations in Mississippi had significantly higher tolerance to acephate compared to the

susceptible plant bugs from Crossett, AR, in 1998 (Table 2). Only the resistance ratios found at Tunica and Indianola, MS, were greater than two, which indicated little resistance or only a low level of tolerance to acephate in the plant bug populations tested in 1998.

TABLE 2. Mortality of Adult Tarnished Plant Bugs from Twenty Locations in the Mississippi River Delta Exposed to Acephate in a Glass Vial Bioassay in 1998.

Location	*n*	Slope ± SE[a]	LC_{50}	95% CL	χ^2	$P > \chi^2$	RR_{50}[b]	95% CL
				Arkansas				
Parkdale	270	1.00 ± 0.13	5.6	4.3-6.9	5.8	0.5	1.8*	1.4-2.4
Gould	270	1.46 ± 0.19	5.8	4.9-6.8	7.1	0.1	1.9*	1.5-2.4
Grady	210	1.76 ± 0.23	5.5	4.7-6.3	5.4	0.3	1.8*	1.4-2.2
Lake Village	180	1.88 ± 0.31	3.2	2.5-3.8	1.5	0.7	1.0	0.8-1.3
Crossett	270	1.76 ± 0.2	3.1	2.6-3.6	5.8	0.2		
				Louisiana				
Lake Providence	210	1.24 ± 0.16	2.6	2.0-3.1	1.2	0.9	0.8	0.6-1.1
Transylvania	300	1.10 ± 0.13	3.8	3.1-4.6	6.2	0.4	1.2	0.9-1.6
				Mississippi				
Clarksdale	180	1.63 ± 0.26	3.6	2.9-4.3	1.2	0.6	1.2	0.9-1.5
Rolling Fork	240	1.15 ± 0.16	5.0	3.9-6.0	5.1	0.4	1.6*	1.2-2.1
Vicksburg	210	1.40 ± 0.21	3.8	2.9-4.6	3.1	0.4	1.2	0.9-1.6
Marks	210	1.47 ± 0.20	4.6	3.8-5.4	4.2	0.4	1.5*	1.2-1.9
Tunica	240	1.39 ± 0.17	7.4	6.2-8.5	4.9	0.4	2.4*	1.9-3.0
Thornton	180	1.51 ± 0.25	3.5	2.7-4.2	1.8	0.6	1.1	0.9-1.5
Greenwood	180	1.31 ± 0.22	4.0	3.1-4.9	7.3	0.1	1.3	1.0-1.7
Winterville	210	1.27 ± 0.22	4.1	3.1-4.9	4.7	0.2	1.3	1.0-1.7
Avon	180	2.26 ± 0.35	3.5	3.0-4.0	0.7	0.9	1.1	0.9-1.4
Greenville	270	1.06 ± 0.13	5.5	4.3-6.7	4.3	0.6	1.8*	1.4-2.3
Indianola[c]	180	1.49 ± 0.23	4.5	3.6-5.3	6.4	0.1	1.4*	1.1-1.8
Indianola	270	1.24 ± 0.19	9.6	7.2-12.5	11.9	0.1	3.1*	2.4-4.1
Minter City	180	1.45 ± 0.22	5.1	4.2-5.9	5.8	0.1	1.6*	1.3-2.1
Ruleville	210	1.85 ± 0.25	2.2	1.8-2.6	1.9	0.6	0.7	0.5-0.9
Mean (SE)							1.5 (± 0.12)	

[a] Acephate concentrations are micrograms per vial; survival was scored at 24 h.

[b] RR_{50} (resistance ratio) and 95% CL calculated by the formula of Robertson and Preisler (1992). Differences in LC_{50} values are significant if the 95% CL does not include 1.0 and are indicated by an *. The RR_{50} value in the column compares the LC_{50} value for plant bugs from the listed location to the LC_{50} value for susceptible plant bugs from Crossett, AR.

[c] Two locations near Indianola, MS, were used. The first location was south of Indianola along U.S. Highway 49, the second location was west of Indianola along U. S. Highway 82.

In 1999, plant bugs from Parkdale, Gould, and Grady, AR, again had significantly higher tolerance to acephate compared to susceptible bugs (Table 3). The resistance ratio of 4.6 found in 1999 at Grady was the highest found in the study for a comparison with susceptible bugs. Plant bugs from both locations in Louisianna also had significantly higher tolerance to acephate as compared to susceptible bugs. Comparison of the LC_{50}'s for acephate obtained in 1998 for plant bugs at the three locations in AR and both locations in LA to those found in 1999 at these locations showed that the increases in tolerance were significant from 1998 to 1999 (Table 4). Tolerance to acephate declined at most locations in MS in 1999 and only one location (Indianola) had tolerance significantly higher than susceptible plant bugs (Table 3).

TABLE 3. Mortality of Adult Tarnished Plant Bugs from Twenty Locations in the Mississippi River Delta Exposed to Acephate in a Glass Vial Bioassay in 1999.

Location	*n*	Slope ± SE [a]	LC_{50}	95% CL	χ^2	$P > \chi^2$	RR_{50} [b]	95% CL
				Arkansas				
Parkdale	240	1.31 ± 0.16	9.8	8.4-11.4	7.8	0.2	3.2*	2.5-4.0
Gould	210	1.65 ± 0.20	12.7	10.8-14.5	3.3	0.7	4.1*	3.3-5.1
Grady	240	1.59 ± 0.19	14.3	12.6-16.3	8.4	0.1	4.6*	3.7-5.7
Lake Village	180	1.11 ± 0.16	3.4	2.7-4.2	4.5	0.2	1.1	0.8-1.4
Crossett	270	1.76 ± 0.22	3.1	2.6-3.6	5.8	0.2		
				Louisiana				
Lake Providence	210	1.47 ± 0.21	10.9	9.4-12.6	4.2	0.4	3.5*	2.8-4.4
Translvania	240	1.70 ± 0.20	10.2	8.9-11.6	8.7	0.1	3.3*	2.7-4.1
				Mississippi				
Clarksdale	180	1.55 ± 0.20	2.9	2.4-3.5	2.4	0.5	0.9	0.7-1.2
Rolling Fork	210	1.43 ± 0.18	4.0	3.3-4.7	4.6	0.3	1.3	1.0-1.6
Vicksburg	180	1.72 ± 0.23	3.6	3.0-4.3	6.4	0.1	1.2	0.9-1.5
Marks	180	1.31 ± 0.18	2.4	1.9-2.9	2.9	0.4	0.8	0.6-1.0
Tunica	180	1.52 ± 0.20	3.2	2.6-3.8	0.9	0.8	1.0	0.8-1.3
Thornton	180	1.41 ± 0.19	2.5	2.0-3.0	3.5	0.3	0.8	0.6-1.0
Greenwood	180	0.98 ± 0.18	1.1	0.6-1.5	1.7	0.7	0.4	0.2-0.5
Winterville	180	0.86 ± 0.15	1.4	0.9-2.0	0.5	0.9	0.5	0.3-0.7
Avon	240	1.13 ± 0.14	4.2	3.3-5.0	4.0	0.6	1.3	1.0-1.7
Greenville	180	1.16 ± 0.17	1.9	1.4-2.3	2.2	0.5	0.6	0.4-0.8
Indianola[c]	180	1.88 ± 0.26	4.9	4.2-5.6	5.8	0.1	1.6*	1.3-2.0
Indianola	210	0.90 ± 0.13	2.0	1.3-2.6	4.7	0.3	0.6	0.4-0.9
Minter City	210	0.88 ± 0.12	2.7	1.9-3.4	7.1	0.1	0.9	0.6-1.2
Ruleville	180	0.92 ± 0.25	1.8	0.1-3.6	9.3	0.1	0.6	0.3-1.0
Mean (SE)							1.6 (± 0.29)	

[a] Acephate concentrations are micrograms per vial; survival was scored at 24 h.

[b] RR_{50} (resistance ratio) and 95% CL calculated by the formula of Robertson and Preisler (1992). Differences in LC_{50} values are significant if the 95% CL does not include 1.0 and are indicated by an *. The RR_{50} value in the column compares the LC_{50} value for plant bugs from the listed location to the LC_{50} value for susceptible plant bugs from Crossett, AR.

[c] Two locations near Indianola, MS were used. The first location was south of Indianola along U.S. Highway 49, the second location was west of Indianola along U. S. Highway 82.

All populations in Arkansas and Louisiana, and most in Mississippi had significantly higher tolerances to acephate relative to the susceptible Crossett population in 2000 (Table 5). Tolerance to acephate found in plant bugs tested from both locations in Louisiana and all locations in Arkansas (except Lake Village) was significantly higher in 1999 compared to 2000 (Table 4). Comparison of the tolerance to acephate found in plant bugs tested at the 14 locations in Mississippi in 1999 to results for these locations found in 2000, showed that significant increases in tolerance occurred at 12 of the locations (all but Rolling Fork and Vicksburg) (Table 4).

Comparison of tolerance to acephate found in 1998 to that found in 2000 at the same locations gives a better insight into the stability of the tolerance. Only two locations, Gould, AR, and Tunica, MS, had significantly higher tolerance levels to acephate in 1998 compared to 2000 (Table 4). Although the remaining 18 locations all had higher tolerance to acephate in 2000 compared to 1998, the increase in tolerance was significantly higher at only seven

TABLE 4. Yearly Comparisons of Mortality in Adult Tarnished Plant Bugs from Twenty Locations in the Mississippi River Delta Exposed to Acephate in a Glass-Vial Bioassay.

Location	1998 vs 1999		1999 vs 2000		1998 vs 2000	
	RR_{50} [a]	95% CL	RR_{50}	95% CL	RR_{50}	95% CL
			Arkansas			
Parkdale	1.8**	1.3-2.3	1.8*	1.5-2.2	1.0	0.8-1.4
Gould	2.2**	1.8-2.7	2.9*	2.3-3.7	1.4*	1.1-1.7
Grady	2.6**	2.2-3.2	2.0*	1.6-2.5	1.3	1.0-1.6
Lake Village	1.1	0.8-1.4	1.3	1.0-1.7	1.4**	1.1-1.8
			Louisiana			
Lake Providence	4.3**	1.7-6.9	1.8*	0.7-4.5	2.4**	1.9-3.2
Transylvania	2.7**	2.1-3.4	1.7*	1.3-2.3	1.5**	1.1-2.1
			Mississippi			
Clarksdale	1.2	0.9-1.6	1.4**	1.1-1.9	1.2	0.9-1.5
Rolling Fork	1.2	0.9-1.6	1.1	0.8-1.4	1.1	0.9-1.5
Vicksburg	1.0	0.7-1.3	1.2	0.9-1.5	1.1	0.8-1.5
Marks	2.0*	1.5-2.6	2.2**	1.7-3.0	1.2	0.9-1.5
Tunica	2.3*	1.8-2.9	1.6**	1.2-2.0	1.5*	1.2-1.8
Thornton	1.4*	1.1-1.9	2.0**	1.5-2.6	1.4**	1.1-1.9
Greenwood	3.8*	2.4-6.0	3.9**	2.4-6.2	1.0	0.8-1.4
Winterville	2.9*	1.8-4.5	3.9**	2.6-5.8	1.3	1.0-1.8
Avon	1.2	0.9-1.5	1.8**	1.4-2.2	2.1**	1.7-2.5
Greenville	3.0*	2.1-4.1	3.0**	2.2-4.1	1.0	0.8-1.3
Indianola[b]	1.1	0.9-1.4	1.8**	1.4-2.2	1.9**	1.5-2.5
Indianola	5.0*	3.4-7.2	6.3**	4.5-8.9	1.3	1.0-1.6
Minter City	1.9*	1.4-2.7	2.2**	1.6-3.0	1.1	0.9-1.5
Ruleville	1.2	0.7-2.1	2.9**	1.7-4.9	2.3**	1.8-3.1

* Indicates that the plant bug population at the location increased in susceptibility over the comparison dates, while ** indicates that the population decreased in susceptibility over the comparison dates.

[a] RR_{50} (resistance ratio) and 95% CL calculated by the formula of Robertson and Preisler (1992). Differences in LC_{50} values are significant if the 95% CL does not include 1.0.

[b] Two locations near Indianola, MS were used. The first location was south of Indianola along U.S. Highway 49, the second location was west of Indianola along U. S. Highway 82.

TABLE 5. Mortality of Adult Tarnished Plant Bugs from Twenty Locations in the Mississippi River Delta Exposed To Acephate in a Glass Vial Bioassay in 2000.

Location	*n*	Slope ± SE[a]	LC_{50}	95% CL	χ^2	$P > \chi^2$	RR_{50}[b]	95% CL
				Arkansas				
Parkdale	180	1.62 ± 0.23	5.5	4.6-6.3	0.6	0.9	1.8*	1.4-2.2
Gould	180	1.52 ± 0.23	4.3	3.5-5.1	4.6	0.2	1.4*	1.1-1.8
Grady	210	1.24 ± 0.18	7.0	5.8-8.3	6.2	0.2	2.3*	1.8-2.9
Lake Village	180	1.74 ± 0.25	4.5	3.7-5.2	2.5	0.5	1.5*	1.2-1.8
Crossett	270	1.76 ± 0.22	3.1	2.6-3.6	5.8	0.2		
				Louisiana				
Lake Providence	210	1.44 ± 0.10	6.3	5.3-7.3	6.5	0.2	2.0*	1.6-2.5
Transylvania	180	0.99 ± 0.16	5.9	4.4-7.4	1.4	0.7	1.9*	1.4-2.6
				Mississippi				
Clarksdale	180	1.36 ± 0.22	4.2	3.3-5.0	1.1	0.8	1.3	1.0-1.8
Rolling Fork	180	1.41 ± 0.22	4.3	3.4-5.2	6.2	0.1	1.4*	1.1-1.8
Vicksburg	180	1.64 ± 0.24	4.2	3.4-4.9	1.8	0.6	1.4*	1.1-1.7
Marks	210	1.11 ± 0.17	5.3	4.1-6.4	6.1	0.2	1.7*	1.3-2.2
Tunica	180	1.91 ± 0.26	5.0	4.3-5.7	2.8	0.4	1.6*	1.3-2.0
Thornton	180	1.30 ± 0.21	5.0	4.0-5.9	0.4	0.9	1.6*	1.2-2.1
Greenwood	210	1.35 ± 0.20	4.1	3.2-5.0	1.7	0.8	1.3	1.0-1.7
Winterville	210	1.86 ± 0.25	5.5	4.7-6.3	1.9	0.8	1.8*	1.4-2.2
Avon	210	1.94 ± 0.25	7.3	6.4-8.3	3.2	0.5	2.4*	1.9-2.9
Greenville	180	1.74 ± 0.24	5.6	4.8-6.4	5.3	0.2	1.8*	1.4-2.3
Indianola[c]	240	1.15 ± 0.14	8.6	7.1-10.2	2.0	0.9	2.8*	2.2-3.5
Indianola	270	1.47 ± 0.16	12.3	10.7-14.1	2.1	0.9	4.0*	3.2-4.9
Minter City	180	1.47 ± 0.22	5.8	4.8-6.8	4.2	0.2	1.9*	1.5-2.4
Ruleville	180	1.24 ± 0.20	5.1	4.1-6.2	4.8	0.2	1.7*	1.3-2.1
Mean (SE)							1.9 (± 0.14)	

[a] Acephate concentrations are micrograms per vial; survival was scored at 24 h.

[b] RR_{50} (resistance ratio) and 95% CL calculated by the formula of Robertson and Preisler (1992). Differences in LC_{50} values are significant if the 95% CL does not include 1.0 and are indicated by an *. The RR_{50} value in the column compares the LC_{50} value for plant bugs from the listed location to the LC_{50} value for susceptible plant bugs from Crossett, AR.

[c] Two locations near Indianola, MS, were used. The first location was south of Indianola along U.S. Highway 49, the second location was west of Indianola along U. S. Highway 82 locations.

The overall mean for the resistance ratios found at all locations was 1.5, 1.6, and 1.9 for 1998, 1999, and 2000, respectively (Tables 2, 3 and 5). This showed that tolerance to acephate was slowly increasing in the plant bug population.

DISCUSSION

Despite the use of acephate for over two decades in cotton for control of tarnished plant bugs, only low levels of tolerance to acephate have developed. Adults tested in the glass-vial bioassay were collected and tested in the fall of each year following the cotton growing season, and at this time, plant bug populations should have had their highest levels of insecticide resistance. Tolerance to acephate changed from year to year at most of the test locations, and the overall change was for slowly increasing tolerance to acephate. Acephate is very useful for tarnished plant bug control in the Mississippi River Delta because of widespread resistance to pyrethroid insecticides in this area, and the fact that pyrethroid resistant plant bugs are still susceptible to acephate. However, researchers, growers, and

consultants should realize that acephate may not control a large plant bug population in cotton with a single application. The average percentage control of 66% reported in recent studies that included the efficacy of acephate at 0.56 kg AI/ ha showed that fields must be scouted after each application to determine if additional treatment is necessary.

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EFFICACY OF FIPRONIL AERIALLY APPLIED IN OIL ADJUVANTS AND DRIFT RETARDANTS AGAINST BOLL WEEVILS, *ANTHONOMUS GRANDIS* BOHEMAN (COLEOPTERA:CURCULIONIDAE)

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ABSTRACT

Results of aerial application tests in the field and insecticide transfer tests in the laboratory showed that cottonseed oil was the most effective oil adjuvant to use with fipronil for controlling boll weevils under field conditions and for transferring fipronil from cotton leaf surfaces to boll weevils. The mineral oil and mineral oil + drift retardant more effectively transferred fipronil from cotton leaves to boll weevils than fipronil in water. Fipronil mixed with mineral oil was not as effective as fipronil mixed with cottonseed oil. Results of low volume aerial application of fipronil in aqueous mixtures with drift retardants showed that drift retardants slightly increased deposition of fipronil on cotton leaf surfaces compared to the standard application with water. Boll weevil mortalities from drift retardant-fipronil mixtures were slightly greater than an aqueous mixture of fipronil immediately after application. At 2 or 3 days after application, only drift retardant HM9733-A produced greater boll weevil mortality than that of fipronil in water .

INTRODUCTION

Fipronil is classified as a pyrazole insecticide that has excellent activity against insects infesting cotton. It was discovered in 1987 by Rhone Poulenc scientists and has been shown to be effective when applied to the soil, as a seed treatment, as a bait, or as a foliar spray. Fipronil interferes with the passage of chloride ions through the gamma-aminobutyric acid regulated chloride channel, disrupting activity of the central nervous system and causing death at high doses (Colliot et al. 1992).

Fipronil's effectiveness at low rates against boll weevils, *Anthonomus grandis* Boheman, (Scott et al. 1996) has drawn attention from the Animal and Plant Health Inspection Service (APHIS) Methods Development personnel involved in eradication of the boll weevil. Fipronil's low use rate (Shaw and Yang 1996) combined with its effectiveness against plant bugs (Shaw et al. 1997) make it well suited for use in Boll Weevil Eradication Programs. Although the ULV formulation of malathion is the primary insecticide presently used in Boll Weevil Eradication Programs throughout the country, fipronil has been identified as a possible alternative insecticide for use in eradication. Fipronil was shown to be effective against boll weevils at a rate of 28.0 g(AI)/ha applied in a volume of 0.58 l/ha with cottonseed oil as the adjuvant (Mulrooney et al. 1998).

The use of oils as adjuvants for insecticides increased with the increase in popularity of ultra-low-volume application of insecticides to cotton in the early 1980's. Oil diluents have been

proposed to have several advantages over water as a carrier. These include a more uniform droplet size, better coverage and canopy penetration, and greater persistence on the plant surface (McDowell et al. 1991). In a study conducted by Ochou et al. (1986), both plant (soybean and cottonseed) and petroleum oils synergized various pyrethroids against larvae of tobacco budworm, *Heliothis virescens* (Fab.), and adult house flies, *Musca domestica* L. Conversely, Ochou et al. (1986) also found that these same oils were less synergistic or even antagonistic with more water-soluble organophosphate and carbamate insecticides. These authors contend that the mechanism of insecticide synergism by oils is unclear, but probably relates to polarity of the test insecticides. The more polar organophosphates and carbamate insecticides were less synergized than the less polar pyrethroids. Similarly, Treacy et al. (1986) demonstrated that soybean oil enhanced toxicity of the pyrethroid, cyfluthrin, against the boll weevil, *Anthonomus grandis* Boheman, more that it did for selected carbamate and organophosphate insecticides. While Wolfenbarger and Guerra (1986) found permethrin/petroleum oil mixtures to be more toxic to boll weevils than a permethrin/cottonseed oil mixture, Smith and Luttrell (1987), in field efficacy tests of a pyrethroid mixed in different oils, showed that the poorest efficacy against tobacco budworms in soybeans occurred when the test insecticide was applied with petroleum oil as an adjuvant.

Optimization of the aerial application of fipronil is needed if fipronil is to be used in eradication programs. This research was conducted to evaluate several oil adjuvants for use in ultra low volume (ULV) application and to evaluate several drift retardants for use in low volume (LV) application of fipronil by aircraft.

MATERIALS AND METHODS

Insecticide Transfer Test. A series of tests were conducted to determine the ability of the oils used in these field tests to transfer fipronil from cotton leaves to boll weevils walking across the surface of a treated leaf. Leaves were collected from plants grown in the field. Fipronil (0.056 kg/ha) mixed in the different oils was applied to excised cotton leaves using a spray chamber equipped with an air-assisted spraying system (Mulrooney et al. 1997). Application was made at a 1.17 l/ha volume. The treated leaves were transported to the laboratory where boll weevils, marked with white acrylic paint, were placed on the leaves one weevil at a time. The distance traveled by the weevil over the leaf surface was measured using a VideoMex-V motion analysis system (Columbia Instruments, Columbus, Ohio). There were five leaves per treatment with five weevils per leaf. This test was repeated three times. After a weevil had walked across the leaf, it was transferred to a 35-ml plastic diet cup containing a diet plug. Mortality was recorded 48 h after exposure to the treated leaf.

Cumulative mortality was regressed on distance traveled using a Weibull function to model cumulative mortality (y):

$$F(y) = \max[1-1/\exp(\text{distance}/mu)^{rate}]$$

The estimates of parameters from the Weibull function describe the following:

max - maximum cumulative mortality (%).

mu - distance (cm) at which half of the maximum mortality occurs.

rate - slope of the curve.

F-tests were used to compare estimates of these parameters for each treatment.

Oil Adjuvant Test. The efficacy of fipronil in different oil adjuvants was determined. The three oil adjuvants tested were once refined cottonseed oil from Yazoo Valley Oil Mill (Greenwood, MS), Orchex 796, and WS2908. Orchex 796 and WS2908 are horticultural mineral oils developed by Exxon Chem. Co., Baytown, TX. WS2908 is a blend of Orchex 796 and EX-100, a drift retardant.

Sprays were applied by aircraft on 7 and 20 July 1998 at Stoneville, MS. Fipronil was

applied at 0.056 kg (AI)/ha in a 1.17 l/ha volume of each of the oils using an Air Tractor 402 aircraft equipped with 18, 8002 flat-fan nozzles (Spraying Systems, Wheaton, IL). Pressure and air speed were 262 kPa and 225 km/h, respectively. Application was made parallel to the rows (east - west) in plots 27 m wide by 246 m long during mid-morning.

Leaves were collected for bioassay from the fourth node down from the terminal at 0, 1, 2, and 3 days after treatment. Thirty leaves per treatment were bioassayed in plastic petri dishes (100 mm diameter) using five boll weevils per leaf. Mortality readings were taken 48 h after placing the weevils on treated leaves.

Drift Retardant Test. Three experimental drift retardants, developed by Helena Chemical Co., were used in these tests. The drift retardants and their use rates were HM 9733-A (178 ml/378.2 l), HM9810 (1.0 % v/v), and HM9850 (454 g/378.5 l). Fipronil was mixed with each drift retardant in an aqueous mixture at a 0.019 kg/ha rate and applied in a 9.6 l/ha total volume. Applications were made with an Air-Tractor 402 equipped with Cp nozzles (Cp Products, Mesa, AZ), on 27 July, 4 August, and 25 August 1998. The aircraft was flown at 217 km/h with pressure set at 207 kPa. Application was made parallel to the rows (east - west) in plots 27 by 246 m during mid-morning.

Leaves were collected at 0, 1, 2, and 3 days after treatment for bioassay. Thirty leaves per treatment were bioassayed in petri dishes using five boll weevils per leaf. Mortality readings were taken at 48 h after placing the weevils on treated leaves.

Droplet size analyses of each fipronil/drift retardant mixture were conducted in our laboratory using a Malvern Spraytec RTS 5000 (Malvern Instruments, Inc., Southborough, MA). Mixtures were sprayed through a single TX-6 nozzle (Spraying Systems, Wheaton, IL) at a pressure of 206.8 kPa and at 18.7 l/ha volume. The nozzle was positioned 35.6 cm above the laser beam. There were six replicates of each mixture.

Each plot in the aerial application tests consisted of two swaths of the aircraft and six measurements were made within each plot. The data were analyzed as a randomized complete block with six treatments replicated in time. Measurements within each plot were subsamples. All data were subjected to an ANOVA using SAS's PROC MIXED (Littell et al. 1996). Least square means were separated using the PDIFF option.

RESULTS

Insecticide Transfer Test. Curves of the predicted percentage cumulative mortality are shown in Fig. 1. Notice that with water, Orchex 796, and WS2908, increases in distances traveled did not result in increases in mortality after maximum mortality was reached. This may indicate that within a short time frame, weevils traveling over insecticide treated cotton leaves reach a saturation point and additional contact with insecticide residues does not result in increased mortality. Salt and Ford (1984) observed that the competition for permethrin between cabbage leaf surfaces and *Spodoptera littoralis* Boisd. larvae crawling over them resulted in a steady initial accumulation of insecticide by the insect, which led to a steady state when the rate of transfer to the insect equaled the rate of detachment from the leaf.

Maximum mortality was greatest ($F=84.64$; $df=3, 159$; $P=0.05$) for cottonseed oil (38%), while mortalities for Orchex 796, WS2908, and water were 20, 16, and 8% respectively (Table 1).

Mu is defined as the distance at which half of the maximum mortality occurs. The value of Mu for the cottonseed oil treatment was 7.04 cm. This means that a level of 19 % mortality, or one half the maximum mortality of 38%, would occur if weevils traveled 7.04 cm over a cotton leaf treated with fipronil mixed in cottonseed oil. The shortest ($F=5.69$; $df=3, 159$; $P=0.05$) Mu occurred when weevils walked over cotton leaves treated with fipronil mixed in water; however, this Mu only resulted in 4% mortality.

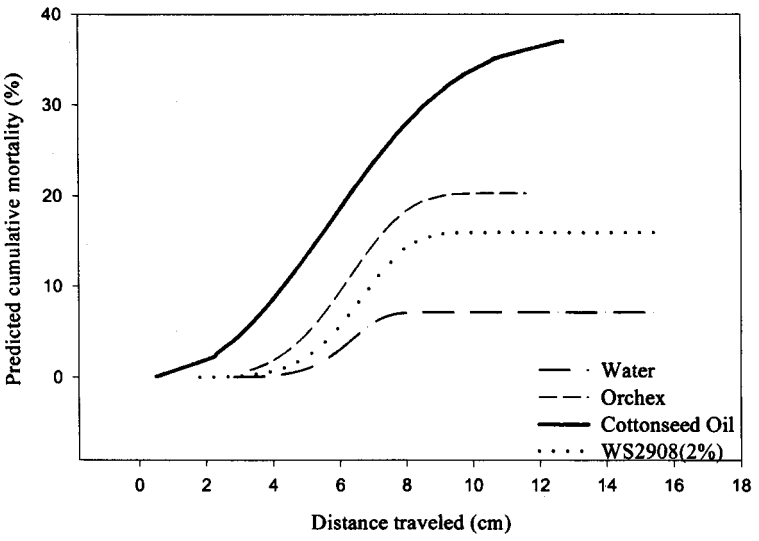

FIG. 1. Cumulative mortality of boll weevils crawling over leaves treated with fipronil in different oil adjuvants.

TABLE 1. Comparison of Parameters of Weibull Function of Cumulative Boll Weevil Mortality over Distance Traveled across Fipronil Treated Cotton Leaves.

Treatment	Max	Mu	Rate
Cottonseed Oil	38 a	7.04 a	2.39 c
Orchex 796	20 b	6.64 a	4.64 c
WS2908 (2%)	16 c	6.94 a	5.74 b
Water	8 d	6.48 b	7.58 a

Rate is the slope of the curve. The lowest (F=83.22; df=3, 159; P=0.05) rates among the treatments were those of cottonseed oil (2.39) and Orchex 796 (4.64), meaning that it took a greater distance for 50% of the maximum cumulative mortality (Mu) to be reached when these oils were used as adjuvants. However, cottonseed oil and Orchex 796 have higher Max's than the other treatments. If the distance where 95% of the maximum mortality occurred for each treatment is calculated and if each of these distances is divided into 95% of the Max mortality for each treatment, then the result is the percentage mortality for each centimeter traveled. When this was done, using cottonseed oil as an adjuvant resulted in 3.24% mortality for every

centimeter traveled. This was the highest among all the treatments. The next highest percentage mortality/centimeter traveled (2.3%) occurred when Orchex 796 was used as an adjuvant. When WS2908 and water were used as adjuvant and carrier, 1.8 and 1.0% mortalities were observed, respectively.

Oil Adjuvant Test. Immediately after treatment, there were no differences in percentage mortalities of boll weevils, which ranged from 98 to 100 (Table 2). At 1 day after treatment, mortality was lowest (92%) ($F=7.67$; $df = 2, 123$; $P>F = 0.0007$) when Orchex 796 was used as an adjuvant compared to that of cottonseed oil (98%) and WS2908 (100%). Mortality from the cottonseed oil treatment was greater than that of WS2908 for the remainder of the test and greater than that of Orchex 796 at days 2 and 4. At 0 ($F=2.67$; $df=2, 28$; $P>F=0.0239$) and 1 ($F=7.67$; $df=2, 28$; $P>F=0.0007$) day after treatment, a greater amount of fipronil was recovered from leaves treated with the fipronil and CSO mixture than when Orchex 796 and WS2908 were used as adjuvants (Table 3). At 2 and 3 days after treatment, there were no differences in the amount of fipronil recovered from leaves treated with different fipronil + adjuvant mixtures.

TABLE 2. Percentage Mortality (48 h) ± SEM of Boll Weevils in Leaf Bioassay of Cotton Treated with Fipronil [56 g(AI)/ha] Mixed in Different Oils and Aerially Applied at 1.17 l/ha.

	Days after treatment				
Adjuvant	0	1	2	3	4
Orchex 796	98 ± 0.7 a	92 ± 2.2 b	51 ± 5.9 c	51 ± 5.3 a	38 ± 5.7 b
Cottonseed Oil	100 ± 0.0 a	98 ± 0.9 a	85 ± 3.0 a	42 ± 4.5 a	60 ± 6.8 a
WS2908@ 3%	99 ± 0.7 a	100 ± 0.0 a	70 ± 4.8 b	20 ± 4.3 b	18 ± 4.0 c

TABLE 3. Fipronil Residues (ng/cm^2) ± SEM on Cotton Leaves Treated with Fipronil [56 g(AI)/ha] Mixed in Different Oils and Aerially Applied at 1.17 l/ha.

	Days after treatment			
Adjuvant	0	1	2	3
Orchex 796	47.3 ± 5.6 b	20.6 ± 3.9 b	9.3 ± 4.0 a	1.7 ± 0.6 a
Cottonseed Oil	104.5 ± 15.6 a	40.6 ± 5.32 a	9.4 ± 3.7 a	9.0 ± 5.1 a
WS2908@ 3%	60.6 ± 18.8 b	15.9 ± 3.7 b	9.6 ± 3.3 a	8.7 ± 2.6 a

Drift Retardant Test. Except for HM9810, mortalities from fipronil mixed with drift retardants were higher ($F= 3.15$; $df=3, 351$; $P>F = 0.0252$) than that of the standard application in water on day 0 (Table 4). If boll weevil mortality is considered an indicator of the amount of fipronil on the surface of leaves, then adding HM9733-A and HM9850 to the fipronil + water mixture resulted in an increased deposition of fipronil on the cotton plant. However at 1 day after treatment, the standard treatment with water resulted in higher ($F=12.87$; $df=3, 236$; $P>F = 0.0001$) mortality than all of the drift retardants. This may be indicating that these drift retardants are binding fipronil to the plant surface. At 2 days after application there was no difference in mortalities between that of the standard water treatment and HM9733-A. Both of these treatments caused higher ($F=28.85$; $df=3, 230$; $P>F = 0.0001$) mortalities than HM9850. At 3 days after treatment, boll weevils exposed to leaves treated with fipronil mixed with HM9733-A had mortalities that were higher ($F=4.75$; $df=3, 111$; $P>F = 0.0037$) than all the other treatments, including the standard. This result seems to indicate that HM9733-A increased

the longevity of fipronil on the leaf surface. Greater amounts of fipronil were found on the leaf surface at 0 (F=6.60; df=3, 62; P>F=0.0006) and 1 (F=5.37; df=3, 63; P>F=0.0023) day after treatment when mixed with HM9850 and HM9733-A (Table 5). The fipronil/HM9733-A mixture had the greatest (F=4.36; df=3, 63; P>F=0.0075) residue among treatments at 2 days after treatment; while, fipronil mixed with HM9850 was found in the greatest (F=7.18; df=3, 14; P>F=0.0037) quantity at 3 days after treatment.

TABLE 4. Percentage Mortality (48 h) ± SEM of Boll Weevils in Leaf Bioassay of Cotton Treated with Fipronil [19 g(AI)/ha] Mixed in Different Drift Retardants and Aerially Applied at 9.36 l/ha.

	Days after treatment			
Adjuvant	0	1	2	3
Water	91 ± 1.6 b	94 ± 1.7 a	71 ± 3.8 a	30 ± 5.5 b
HM9810	92 ± 1.3 ab	65 ± 4.9 c	54 ± 4.3 b	29 ± 4.3 b
HM9733-A	95 ± 1.2 a	84 ± 2.8 b	63 ± 3.8 ab	50 ± 5.4 a
HM9850	96 ± 1.0 a	74 ± 3.8 c	21 ± 4.3 c	26 ± 5.5 b

TABLE 5. Fipronil Residues (ng/cm^2) ± SEM on Cotton Leaves Treated with Fipronil (19 g[AI]/ha) Mixed in Different Drift Retardants and Aerially Applied at 9.36 l/ha.

	Days after treatment			
Adjuvant	0	1	2	3
Water	52.9 ± 8.9 b	23.8 ± 7.2 b	9.8 ± 4.6 b	0.9 ± 0.9 b
HM9810	61.9 ± 10.6 b	9.19 ± 2.7 b	4.7 ± 2.6 b	3.6 ± 2.5 b
HM9733-A	90.2 ± 10.2 a	56.5 ± 11.0 a	34.5 ± 11.5 a	4.7 ± 1.3 b
HM9850	111.2 ± 11.6 a	37.3 ± 11.0 a	8.5 ± 3.0 b	15.6 ± 5.1 a

Droplet size analyses using a Malvern Spraytec RTS 5000 showed that droplet median diameters (Dv_{50}) of HM9850 and HM9733-A were 523 and 154 μm, respectively, while droplet median diameters of HM9810 and water were 146 and 144 μm, respectively. While droplet size may explain the greater deposition of HM9850 because larger droplets weigh more and are more subject to gravity than smaller droplets, it does not explain the increased deposition of HM9733-A.

Based on maximum mortality, CSO was shown to be the most effective oil adjuvant for transferring fipronil from the surface of cotton leaves to boll weevils in laboratory tests. In field tests, aerial application of fipronil in CSO proved to be the most effective treatment against boll weevils.

In aerial application tests, two of the drift retardants, HM9733-A and HM9850, seemed to enhance the deposition of low volume applications of fipronil. However, only HM9733-A seemed to significantly enhance fipronil's effectiveness against boll weevils.

One interesting result from these tests is the effectiveness of fipronil at 19 g(AI)/ha which is one-third the recommended rate of 56 g[AI]/ha.

Application of fipronil for boll weevil eradication would seem to be more expeditiously done with ultra-low-volume application using oil as a carrier as compared to low-volume application

of aqueous solutions. Oils have several advantages over water as a carrier and vast acreage can be treated more effectively with ultra-low-volumes of insecticide because aircraft are able to spend more time spraying and less time filling and ferrying to and from the airstrip.

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EXTERNAL MORPHOLOGY OF THE WAX GLANDS OF *EPIPTERA WOODWORTHI* (HEMIPTERA: FULGOROMORPHA: ACHILIDAE)

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ABSTRACT

External morphology of the wax gland plates and wax gland pores of the fourth-instar nymph of the achilid planthopper, *Epiptera woodworthi* (Van Duzee) (Hemiptera: Fulgoromorpha: Achilidae), is described for the first time, based on scanning electron microscope observations. Six large, oblong wax gland plates are located on lateral regions of the 6th-8th abdominal tergites. Each wax gland plate has numerous, very small, disc-shaped wax gland pores, each of which is composed of a middle disc (3.94-4.25 μm in diameter) surrounded by 5-7 microtubules (six being the most common number encountered) that are separated by 4-6 ridges (five being the most common number encountered). Wax gland pores are separated by numerous rounded papillae located in shallow cavities which have smooth rims with the side adjacent to the wax pore being distinctly ridged. Wax gland pores observed in *Epiptera woodworthi* are externally very similar to those found in nymphs of species of Cixiidae and Dictyopharidae.

INTRODUCTION

The achilid planthopper *Epiptera woodworthi* (Van Duzee), was originally described under *Elidiptera* Spinola by Van Duzee in 1916 from California. It was later listed in Van Duzee's (1917) Catalogue of Hemiptera North of Mexico. Muir (1922) questioned the generic placement of this species. Ball (1933) transferred it to *Epiptera* Metcalf. Since then, the species has not been reported in the literature, except in Metcalf's (1948) catalog of the world Achilidae. The species appears endemic to California.

Epiptera is a northern genus with nymphs and adults usually associated with conifers (O'Brien et al. 1991). Emeljanov (1991) indicated that the New World *Epiptera* Metcalf are actually Old World *Cixidia* Fieber. However, this has not been formally accepted by American hemipterists and *Epiptera* is still widely used in North America. Nymphs of *Epiptera* are the most frequently seen achilid nymphs in the U.S. and Canada. They are usually found under bark of fallen trees (O'Brien and Wilson 1985). Based on observations of *E. fusca* (Walker), Hepburn (1967) believed that nymphs of *Epiptera* species probably feed on fungi, either under the loose bark of dead trees or in leaf litter. Van Duzee (1916) noted

that adults of *Epiptera woodworthi* were collected on Jeffrey pine, but that nymphs as well as adults were beaten from cypress bushes growing on the same slope.

Nymphs and female adults of most fulgoromorphan species secrete wax through distinct sclerotized structures that act as molds to produce structurally different forms at different positions on the body. These wax-producing structures have been termed pores, and ducts and the types and distributions of these structures are useful characters for taxonomic identifications and phylogenetic inferences (O'Brien et al. 1991; Liang, unpublished data). The wax can function as protection from predators and parasites in nymphs, adults, and eggs (Eisner et al. 1978) or from flooding for underground species (Cumber 1953).

Nymphs of *Epiptera* are unique in having large, distinctive wax gland plates, covered with many small wax gland pores which secrete wax, present on the 6th-8th abdominal tergites Fennah (1950) illustrated a fifth-instar nymph of *E. fusca* (Walker) with white, filamentous wax threads on the 6th-8th abdominal tergites but provided no description. Abdominal wax glands are usually absent from adult female Achilidae.

Very few descriptions or illustrations of wax glands of Achilidae nymphs have been published. Previous investigations of wax plates and wax glands of *Epiptera* have only utilized light microscopy (Osborn 1922, Fennah 1950, Yang and Yeh 1994); there have been no scanning electron microscopy studies. During morphological studies of fulgoromorphan immatures, we made scanning electron microscopic observations of the wax gland plates and wax gland pores of nymphs of *Epiptera woodworthi*. Herein, we present a description of the external morphology and distribution of the wax plates and wax pores found on the 6th-8th abdominal tergites of this species.

MATERIALS AND METHODS

Dry, pinned museum specimens were examined from the California Academy of Sciences, San Francisco. The nymphs were collected at Cazadero, California, USA. For scanning electron microscopy (SEM) study of wax gland ultrastructure, the whole nymph or abdomen was first cleaned and dewaxed with 10% KOH, then washed with distilled water, mounted on aluminum stubs with double-sided sticky tape, air-dried at room temperature, and coated with gold-palladium using a sputter coater. Observations were made with a JEOL JSM-6301F (Japanese Electronic and Optical Ltd., Tokyo, Japan) scanning electron microscope, operated at accelerating voltage of 5 kV. Morphological terminology largely follows that of Foldi (1997).

OBSERVATIONS AND DISCUSSION

Gross morphology of nymph. General morphology (Figs. 1, 2) as in *Epiptera opaca* (Say), as described by Wilson (1983), but differs from the latter in abdominal segments with distinct median ridges and in having distinct lateral sensory pits in columns beyond abdominal median ridges.

Wax gland plates. As in other *Epiptera* species, six large, distinct wax gland plates are present on lateral regions of abdominal tergites 6-8 of the fourth-instar *Epiptera woodworthi* nymph (Figs. 1-3). The wax plates are large and transversely oblong, occupying approximately one-half the area to almost all of the tergite, and are covered with numerous small cuticular structures for wax molding, the wax pores (Figs. 4-11).

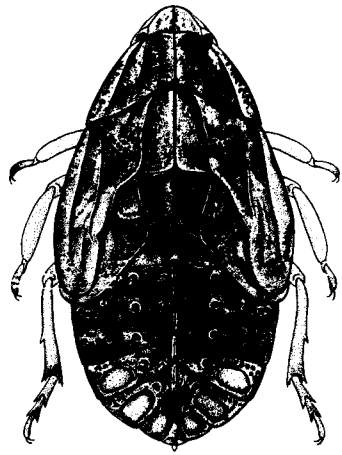

FIG. 1. *Epiptera woodworthi* (Van Duzee), fourth-instar nymph, California: Cazadero, dorsal view.

Some differences in size, shape, and position exist among the wax gland plates on the 6-8th abdominal tergites (Figs. 1-3). Wax gland plates on the 6th abdominal tergite are shortest, narrowest and smallest, being less than one-half the area of the 6th tergite, and being nearer to its posterior margin; those on the 7th abdominal tergite are largest and longest, being greater than one-half the area of the entire tergite, with the inner part relatively narrow and the outer part relatively broad. Wax gland plates on the 8th abdominal tergite are intermediate in size, occupying almost all of the tergite, being close to the anterior and posterior margins of the tergite, with the inner part relatively narrow and the outer part relatively broad.

Wax gland pores. Wax gland pores in nymphs of *Epiptera woodworthi* are disc-shaped (Figs. 4-11). Each glandular pore unit consists of a main circular disc surrounded by 5-7 microtubules which are separated by 4-6 cuticular ridges (Figs. 5-11). The discs are rounded and elevated and measure approximately 3.94-4.25 μm in diameter and about 1.22 μm in height. Each disc has a raised circle in the center which is probably the site of wax secretion emission. Each disc is surrounded by 5-7 microtubules (six being the most common number encountered), which are separated by 4-6 cuticular ridges (1.52-2.12 μm long) (five being the most common number encountered) (Figs. 5-11).

The microtubules are very slender and short, about 0.86 μm in length and about 0.50-0.60 μm in diameter. They are hollow centrally with the apex slightly expanded (Figs. 5-11). Their function is unknown, but they may secrete the fluid material to strengthen or solidify the wax threads arising from the central raised circle of the disc. The number of microtubules varies slightly. Six and seven microtubules (seven being the more common number encountered) are observed in the wax plates on tergite 6 (Figs. 5-7), while 5-7 microtubules (six being the most and five being the least common number) are observed in tergites 7 and 8, respectively (Figs. 8-11).

FIG. 2-7. *Epiptera woodworthi* (Van Duzee): 2. Fourth-instar nymph, California: Cazadero; 3. Abdominal tergites 6-8, showing wax gland plates; 4. Wax gland plates on right abdominal tergites 6-8; 5-7. Wax gland pores in wax gland plates of abdominal tergite 6.

The disc-shaped wax gland pores are surrounded by many small, rounded papillae (1.21-1.82 μm in diameter) located in shallow cavities (2.16-2.61 μm in diameter) (Figs. 5-11). The rim of each cavity is smooth but the side of the rim adjacent to the wax gland pore is distinctly ridged. The length of these elevated ridges is about 2.12-3.03 μm. The function of these papillae is unknown.

Wax gland plates and wax glands are morphologically very diverse within Fulgoromorpha but only a few have been studied with scanning electron microscopy [e.g., Cixiidae (Pope 1985, Sforza et al. 1999), Lophopidae (Liang 1997, 2000), Meenoplidae

(Bourgoin 1997) and Kinnaridae (Liang 2001)]. The present study shows that the wax gland plates and wax gland pores of *Epiptera woodworthi* provide many potentially informative characters for identification and phylogenetic study. Nymphs of the *Epiptera* species have the largest wax gland plates within the Achilidae. The number (six), shape (oblong or ovoid), and position (lateral regions of the 6th-8th abdominal tergites) of the wax gland plates in *Epiptera* differ from those in the nymphs of the Cixiidae, Dictyopharidae, and other fulgoromorphan families.

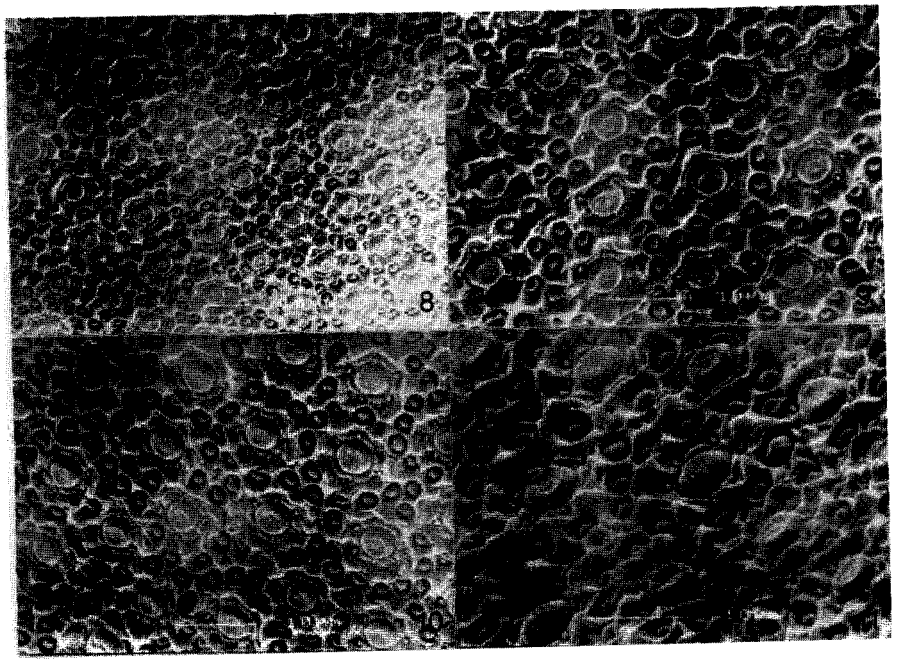

FIG. 8-11. *Epiptera woodworthi* (Van Duzee): 8-9. Wax gland pores in wax gland plates of right abdominal tergite 7; 10-11. wax gland pores in wax gland plates of right abdominal tergite 8.

Wax gland pores in *Epiptera woodworthi* are externally very similar to those in nymphs of species of Cixiidae (Pope 1985, Sforza et al. 1999) and Dictyopharidae, with all having a central rounded disc surrounded by several microtubules and cuticular ridges, and separated by a series of small rounded papillae situated in shallow cavities, but distinctly different from those seen in nymphs of other families (e.g., Fulgoridae, Issidae, Tropiduchidae, Flatidae, Lophopidae, and Eurybrachidae) (Liang, unpublished data).

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DISTRIBUTION OF *STETHORUS NIGRIPES* KAPUR (COLEOPTERA: COCCINELLIDAE), A PREDATOR OF BANKS GRASS MITE [*OLIGONYCHUS PRATENSIS* (BANKS)] IN THE SOUTHERN UNITED STATES

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Members of the genus *Stethorus* Weise, with approximately 70 species worldwide, are obligate predators of spider mites (Acari: Tetranychidae) (Gordon and Chapin 1983). Many of these mites are important pests of various crops, trees, and ornamentals. Almost all North American research on *Stethorus* and its potential role as an agent of biological control of spider mites was done with *S. punctum punctum* (LeConte) and its control of the European red mite (*Panonychus ulmi* (Koch)) and two-spotted mite (*Tetranychus urticae* Koch) in eastern U.S. apple orchards (*e.g.*, Colburn and Asquith 1971, Hull et al. 1977, Houck 1986). Unfortunately, little work has been done on other Nearctic species of *Stethorus*, in different areas of North America. *Stethorus p. punctum* is one of six species or subspecies of *Stethorus* in America, north of Mexico (Gordon and Chapin 1983). As with many other groups of lady beetles, various species of *Stethorus* have been introduced into non-native areas for control of pest insects or mites. According to Gordon (1985), approximately 12 species of *Stethorus*, most not formally named to species, have been introduced into California. The countries of origin of these species are diverse and include Australia, China, Guatemala, India, Morocco, Pakistan, South Africa, and Turkey.

One of the Australian species that was introduced into California is *S. nigripes* Kapur. This species has been acknowledged as an important predator of *P. ulmi* and *T. urticae* in Australian apple and peach orchards (Edwards and Hodgson 1973, Field 1979). Because of its effectiveness as a natural enemy in its native range, *S. nigripes* was introduced into California from 1978-80 for control of mites in almond orchards (Hoy and Smith 1982). Attempts to establish this species were discontinued after a few years, because *S. nigripes* showed significant sensitivity to pesticides, did not consume the desired prey, and needed to have ample numbers of prey during the winter.

The first documented establishment of *S. nigripes* in North America did not occur in California, where it had been released in large numbers, but rather in the Texas Panhandle (Gordon 1993). Large numbers of *S. nigripes* were found in July, 1991, in a corn field in Deaf Smith County, TX, and it was assumed that the species had become established there. Since then, no further work has been done to document the presence of *S. nigripes* in Texas or surrounding states.

In the summer of 2000, a new project was begun at the Texas Agricultural Experiment Station, Bushland, TX. The main objectives were to document the species diversity of *Stethorus* in the Texas High Plains (and adjacent areas as practical), and to describe the immature stages for these species. In this geographical area, the Banks grass mite (*Oligonychus pratensis* (Banks)) is an important pest of corn, sorghum, winter wheat

and other grasses (Walter and Wene 1956, Gilstrap et al. 1980). Therefore, as a prelude to any concerted effort at using *Stethorus* species in biological control of *O. pratensis*, it was decided that a general survey of the species present in the area was an important prerequisite.

Sampling for *Stethorus* species began in early June, 2000, using Bushland, TX as the focal point. Initially, efforts were concentrated on native grasses occurring in ditches, and/or shady or moist places, as crops in the area were not at a suitable stage of development for mite attractiveness. *Stethorus nigripes* adults were collected from several species of native grasses from early June to about mid-July, at which time numbers on the grasses dropped significantly. At this point, the sampling focus was shifted to corn fields, most of which were mature enough to be attractive to large numbers of mites (especially *O. pratensis*), and presumably, therefore, also to *Stethorus*. Through extensive sampling of all local corn-growing areas, specimens of *S. nigripes* were collected from about 30 different corn fields in the Texas Panhandle, the Oklahoma Panhandle and southwest Kansas (Fig. 1). Indeed, with the exception of corn fields that had been treated for western corn rootworm (*Diabrotica virgifera* LeConte), *S. nigripes* was present in almost every field that had obvious infestations of *O. pratensis*. Large numbers of adults and larvae could be found within and beneath the webbing along the central rib of the corn leaf, the characteristic feeding site for this species of spider mite. It is unclear whether the presence of *S. nigripes* had a significant effect on *O. pratensis* densities in the field, but it seems probable that some control was effected by these beetles, considering their extensive range, and locally high population densities.

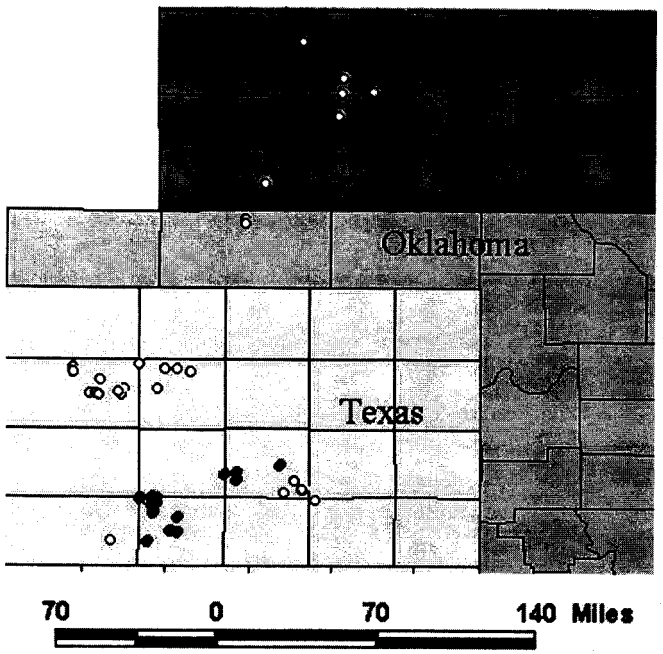

Figure 1. Known distribution of *Stethorus nigripes* in Texas, Oklahoma and Kansas. Solid circles represent collections from native grasses; open circles represent collections from corn.

The manner in which *S. nigripes* established in the Texas High Plains is unknown. Gordon (1993) hypothesized that specimens were obtained from the California cultures of *S. nigripes* mentioned above, and were released "unofficially" in west Texas. It is unknown whether *S. nigripes* and its apparently expanding range is having a negative effect on native species of *Stethorus*. We were initially somewhat surprised at the low diversity of *Stethorus* spp. in the study area from the first summer of sampling; however, it seems likely that the diversity was probably low even before *S. nigripes* was introduced, at least according to the documented geographical ranges of *Stethorus* in Gordon (1985). According to Gordon and Chapin (1983) and Gordon (1985, Figs. 52, 55-56), there were no collection records for any species of *Stethorus* from the Texas panhandle. *Stethorus pinachi* Gordon and Chapin and *S. caseyi* Gordon and Chapin are known from south Texas, *S. utilis* Horn from east Texas, and *S. p. punctum* from Kansas and eastern Colorado. Therefore, other than the original specimens from Deaf Smith County, TX documented in Gordon (1993), these newly collected specimens represent the first records of *S. nigripes* from a large area of the Texas Panhandle and adjacent areas of Oklahoma and Kansas.

From the many collecting sites for *S. nigripes* in Texas, Oklahoma, and Kansas, it seems that the species has indeed established in this area. Also, S. *nigripes* may prove to be a very effective predator of *O. pratensis* and *T. urticae* on corn and other important crop plants. The fact that *S. nigripes* has been virtually unnoticed in Texas may be attributable to its small size (around 1 mm), although its numbers can be very conspicuous in heavily mite-infested corn fields. Clearly, additional research is needed on *S. nigripes*, specifically in the Texas corn belt, and the impact of this Australian introduction on the native species of *Stethorus* in the area.

We would like to thank Allison Funk, Shana Camarata, Matt Golliday, Dayna Dowdy, and Debi Owings, who have assisted in various parts of the *Stethorus* project. Robert Gordon, who originally identified the specimens from Deaf Smith County, confirmed the identifications of our newly collected material. The authors appreciate the constructive criticisms and suggestions of Drs. B. Payne and C.M. Rush, TAES-Bushland. Our thanks also go to the various producers who allowed us to sample *Stethorus* in their fields; we hope it has been a mutually beneficial learning experience! This project is funded, in part, by a grant from the Texas Higher Education Coordinating Board Advanced Research Program, Biological Sciences Research Area (0227-1999).

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A NOCTURNAL RAID OF *Nomamyrmex* ARMY ANTS[1] ON *Atta* LEAF-CUTTING ANTS[1] IN TAMAULIPAS, MEXICO

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Neotropical army ants (Hymenoptera: Formicidae, Ecitoninae), as a group are primarily predators of immature stages of ants, termites and some wasps (Rettenmeyer 1963, Schneirla 1971). Different species of army ants have marked preferences for attacking specific ant taxa (subfamilies or genera) (Rettenmeyer et al.1982, Franks and Bossert 1983, Franks and Norris 1987), displaying variable prey preference among all major subfamilies of ants with the exception of the Ecitoninae itself (Rettenmeyer 1963, Schneirla 1971, Rettenmeyer et al.1982, Gotwald 1995). However, predation by army ants on the often massive colonies of leaf-cutting ants, *Atta* and *Acromyrmex* (Hymenoptera: Formicidae, Myrmicinae, Attini) has very rarely been reported. *Atta* spp. and army ants in the genus *Eciton* usually avoid confrontation and ignore each other (Rettenmeyer 1963); *Neivamyrmex* army ants have even been reported as inquilines in the nest cavities of *Atta* (Schneirla 1971). On the other hand, the few observations on foraging by the uncommon, robust, heavily sclerotized ecitonine *Nomamyrmex esenbecki* (Westwood) suggest that these army ants seem to be rather specialized predators of the brood of species of *Atta* and *Acromyrmex*, and particularly of *Atta* spp. (Swartz 1998). All reports of prey of *N. esenbecki* mention leaf-cutting ants, and it appears that all reports of army ant raids on *Atta* and *Acromyrmex* nests involve *N. esenbecki* (Borgmeier 1955; Mariconi 1970; Rettenmeyer 1963; Rettenmeyer et al. 1982; Swartz 1998, and references therein; J. Longino, personal communication). In Costa Rica, *N. esenbecki* was the only army ant observed to attack mature *Atta* colonies (J. Longino, personal communication). Eighty to ninety percent of the diet of *N. esenbecki* consists of ant larvae and pupae (Rettenmeyer 1963). Working in the Panamanian rainforest of Barro Colorado, Schneirla (1971) reported that *N. esenbecki* is a subterranean species also capable of surface activity, raiding in those dense forests both day and night.

Here we describe a new distribution record for *N. esenbecki* and for its raids on *Atta*, namely, a nocturnal surface raid on an *Atta mexicana* (Smith) colony in Northeastern Mexico. This army ant raid took place at the northeastern fringe of the Neotropical zone, extending the known occurrence of *Nomamyrmex* raids on leaf-cutting ants more than 1,000 km to the north, from Jalisco, Mexico. It occurred in a disturbed subtropical habitat, on a clearing inside a village. Most reports of *Nomamyrmex* surface raids against *Atta* are from tropical rain forests in Central and South America. The previous northernmost raid observed, in a subtropical area (Jalisco), was subterranean (Rettenmeyer et al.1982).

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The locality of the raid is the town of Buenavista, in the municipality of Soto La Marina, Tamaulipas, Mexico (27°47' northern latitude; 90°12' western longitude; 20 meters altitude above sea level), about 200 km south of the United States border. Weather, in the classification of Koppen and modified by Garcia (INEGI 1983), is BS (h') KW (e): extremely variable, mean annual temperature 23° C; annual precipitation: 800-1000 mm in the summer. Natural vegetation is very disturbed in the village; native plants reflect the boundary of low tropical thorn forest and low deciduous tropical forest; native trees and shrubs are ebony (*Phitecellobium flexicaule* (Benth.) Coult), cornezuelo (*Acacia cornigera* (L.) Willd.), huisache (*Acacia farnesiana* (L.) Willd.), brasil (*Condalia sp.*), coma (*Bumelia* sp.), and *Randia* spp.

The raid occurred in a lawn of blue grass, *Poa pratensis* L., on sandy soil. The *A. mexicana* colony was under a small orange tree (2.5 m canopy diameter) at least 20 m from other tree or bush cover, inside a farmer's garden located in the town. The colony was small (mound less than 1 m^2) and had two entrance holes on the mound. From the mound and workers' size, this *Atta* nest was probably about two years old. *Nomamyrmex* workers were carrying *Atta* larvae and possibly pupae out from this small *Atta* colony. A column of polymorphic *Nomamyrmex* workers (up to 11 mm long) in a single line, were exiting one of the nest holes of the *Atta* mound at 21:00h on 15 June 2000. They were very swift runners; this made their capture at night difficult. The army ants were very photophobic and when illuminated by the flashlight within a meter from an entrance hole, immediately retreated back into the *Atta* nest, and exiting from the hole was interrupted. About twenty to thirty seconds after turning the light off, the ants resumed their activity and left the exit hole. Approximately one in every ten *Nomamyrmex* workers carried one ant larva, which were later identified as *Atta mexicana* by Dr. Ted Schultz, Smithsonian Institution; the *Nomamyrmex* could have been carrying their own larvae in addition to those of *Atta*. No aggressiveness was observed from the few *Atta* workers present on the mound; these workers stood still or slowly walked around the nest openings. Only minors and media *Atta* workers (no majors nor soldiers) were observed. The exodus of *Nomamyrmex* from the *Atta* nest continued for at least 3h, until midnight (24:00h) when observation was suspended. Rettenmeyer (1963) described similar swift column raids. The army ants left the *Atta* nest at a steady rate of no less than one worker every two seconds; therefore, this *Nomamyrmex* colony had at least 5400 workers.

Historically, *N. esenbecki* has been collected in the USA, from southern Texas (Rettenmeyer 1963), including Cameron county, in the Lower Rio Grande Valley (LRGV); *Atta texana* (Buckley) has been reported from adjacent counties in the Valley (O'Keefe et al. 2000). Currently, most of the LRGV supports very intensive agriculture and pesticide use (Howe et al. 1986). *Atta texana* has pest status there and possibly forages almost exclusively in human-disturbed areas since native vegetation cover in the LRGV has disappeared in more than 95% (Howe et al. 1986; TAMU 2001). Mature *Nomamyrmex* colonies are huge (Swartz 1998); estimates are > 700,000 workers (Rettenmeyer 1963). Such species of army ants are unable to survive in extensively disturbed areas (Swartz 1998). The recent association of *Atta* with man and the extirpation of natural habitats in the LRGV could imply that *Nomamyrmex* is being eliminated from the United States if not already extinct there.

As most *Atta* species of the Neotropical region, *A. mexicana* colonies are common in both natural and disturbed areas (Holldobler and Wilson 1990), including Tamaulipas where this raid occurred. Topics deserving further study are the adaptation and current status of *Nomamyrmex* in disturbed, subtropical areas, and more specifically its biology at the northern edge of its distribution; the impact of army ants as a mortality factor of young and mature *Atta* colonies; and the architectural, chemical and behavioral defenses of *Atta* ants against raids by these army ants.

We thank Dr. Ted Schultz (National Museum of Natural History, Smithsonian Institution) for confirming the identities of *Nomamyrmex* and *Atta*, and Dr. Monica Swartz (University of Texas at Austin) for comments and suggestions. This research was supported by grants DEB-9707209 and DEB-9983879 from the National Science Foundation.

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MINUTES OF THE 2002 ANNUAL MEETING OF THE EXECUTIVE COMMITTEE OF THE SOUTHWESTERN ENTOMOLOGICAL SOCIETY

The Executive Committee met at 6:30 a.m. Monday, February 25, 2002, at the Hotel Real de Minas, Guanajuato, Mexico, during a joint meeting of the Southwestern Branch of the Entomological Society of America and the Sociedad Mexicana de Entomologia. Present were President John Burd, President-Elect John Jackman, and Secretary-Treasurer Allen Knutson. The Editor's and Secretary-Treasurer's reports were reviewed and approved. The recent increase in membership dues and the need for increasing the fee for subscribers was discussed. The ballots for President-Elect were counted, and there being no further business, the meeting was adjourned.

MINUTES OF THE 2002 ANNUAL MEETING OF THE SOUTHWESTERN ENTOMOLOGICAL SOCIETY

The 25th Annual Meeting of the Southwestern Entomological Society was called to order by President John Burd at 7:00 a.m. on Monday, February 25, 2001, at the Hotel Real de Minas, Guanajuato, Mexico, during a joint meeting of the Southwestern Branch of the Entomological Society of America and the Sociedad Mexicana de Entomologia. The minutes of the 2001 Annual Meeting as published in the June issue of the Southwestern Entomologist were distributed. The Secretary-Treasurer's report and Editor's report were distributed, reviewed, and approved. The increase in printing and mailing costs and the decision by the Executive Committee to increase page charges and membership dues to meet these increasing expenses were discussed. President Burd reported that the decision to increase these charges was a unanimous decision of the Executive Board. An increase in the subscription rate from $20.00 to $30.00 to cover the cost of printing and mailing was discussed but no action was taken. The steady decline in Society membership was discussed. President Burd appointed Bart Drees, Scott Russel, and Allen Knutson to a committee to revise and print the brochure to be used in membership recruitment.

Julio Bernal, Associate Editor for Spanish-language manuscripts, reported that there is considerable interest among Mexican entomologist to publish in the Southwestern Entomologist. John Jackman, Electronic Publication Committee, reported that the new printer for the journal now scans the manuscripts while the previous printing used a photographic process to print the journal. Scanning the manuscripts will foster adoption of electronic publishing. The committee will continue to investigate how electronic publishing on the internet will impact membership in the Society, who will have access to the electronic journal, who will have responsibility for maintaining the web site, and how the related costs can be recovered.

President Burd reported that the Nominating Committee, consisting of Grant Kinzer and Tom Royer, had identified two candidates for President-Elect, and that Bart Drees had been elected President-Elect for 2002. Greg Cronholm, Society Archivist, reported that he had deposited the Society's records at the Texas A&M University Library. President-Elect John Jackman then presented a plaque to President Burd in recognition for his service to the Society. President Burd thanked the officers of the Society and its members for their support and passed the gravel to incoming President John Jackman. There being no other business a motion to adjourn was made and approved.

Respectfully submitted,
Allen Knutson, Secretary Treasurer

SECRETARY-TREASURER'S REPORT 2001
February 1, 2001-January 31, 2002

Balance on hand as of February 1, 2001		$ 6,570.36
Income:		
Memberships	$ 5,180.00	
Subscriptions	1,700.00	
Page Charges	19,065.59	
Back Issues	262.40	
Royalties	172.60	
Total Income		$ 26,380.59
Expenses:		
Journal:		
Editor's Fee	$ 2,000.00	
Printing	17,192.10	
Secretary Fee	2,000.00	
AdMail Mailing Service	868.45	
Postage	1,200.00	
Society:		
Secretary Fee	$ 1,000.00	
Supplies	334.68	
Postage	474.81	
President Plaque	110.00	
Secretary-Treasurer Fee	1,500.00	
Bank Charges	43.66	
Miscellaneous Expenese	37.04	
Total Expenses		$ 26,760.74
Balance on hand January 31, 2002		$ 6,190.21

As of January 31, 2002, there were 336 members paid for 2001 and 99 institutional subscribers in the Southwestern Entomological Society. There were 20 unpaid page charges totaling $ 7,520.00

Respectfully submitted.
Allen Knutson, Secretary-Treasurer

AUDIT COMMITTEE REPORT

I have examined the financial record of the Society beginning with the bank statement dated 1/31/2001. This record and the secretary-treasurer's report of February 1,2001 though January 31, 2002 were found to be in order.

Respectfully submitted
John Jackman

EDITOR'S REPORT

There were 42 manuscripts, totaling 386 pages, published in the four regular issues of Volume 26 of the Southwestern Entomologist during 2001 compared to 38 manuscripts and 314 pages in Volume 25 for 2000. Additionally, Supplement No. 24, consisting of 100 pages, also was published during the year.

A total of 55 manuscripts were received for consideration for publication, compared to 53 in 2000. A number of these are still in the review process; however, nine have been rejected as of this date. In addition, three manuscripts originally submitted in 2000 also were rejected during 2001. The total of twelve manuscripts rejected represents a decrease of four from the sixteen that were rejected during 2000.

Editor's Financial Report

Date	Description	Receipts/Expenditures	Balance
01/01/01	Balance Forward		$ 29.89
01/20/01	Postage	32.77	-2.88
02/21/01	Postage	17.53	-20.41
03/17/01	Postage	2.65	-23.06
04/17/01	Postage	19.45	-42.51
06/05/01	From Treasurer	200.00	157.49
06/12/01	Postage	4.37	153.12
06/22/01	Postage	31.27	121.85
07/10/01	Postage	5.26	116.59
07/21/01	Postage	10.76	105.83
08/18/01	Postage	16.51	89.32
09/01/01	Postage	3.32	86.00
10/05/01	Postage	11.39	74.61
10/27/01	Postage	20.59	54.02
11/21/01	Postage	5.73	48.29
12/05/01	Postage	15.52	32.77
12/22/01	Postage	17.83	14.94

Cash Summary

Balance Forward 01/01/01	$ 29.89
Receipts	200.00
Expenditures	214.95
	$ 14.94 Ending Balance

Respectfully submitted,
Darrell E. Bay, Editor

AUGMENTATION OF GREEN LACEWING, *CHRYSOPERLA RUFILABRIS*, IN COTTON IN TEXAS.

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ABSTRACT

Eggs of *Chrysoperla rufilabris* Burmeister (Neuroptera:Chrysopidae) were immersed in a liquid carrier and adhered to cotton foliage with a tractor-mounted applicator. In two trials, the release of 200,000 eggs per acre yielded 4,600 and 5,900 larvae per acre seven and nine days after application, respectively. The release of 400,000 eggs per acre yielded 8,100 larvae per acre nine days after application. Only the highest release rate of 400,000 eggs per acre significantly increased the number of lacewing larvae relative to plots not receiving lacewing eggs. Eclosion of *C. rufilabris* eggs applied with the mechanical applicator and collected from the cotton canopy was 39.5 %, while eclosion of extant eggs was 42.3 %. Densities of extant eggs in cotton averaged 2.7 eggs per 25 leaves but subsequent densities of lacewing larvae or pupae were 1,400 or less per acre where no lacewing eggs were released. Densities of cotton aphid, *Aphis gossyppi* Glover, were not significantly different in cotton five and eighteen days after the release of 100,000, 200,000 or 400,000 lacewing eggs per acre. Results confirm previous studies which suggest that augmentation of lacewing in cotton by the release of eggs is constrained by high mortality during the immature stages. Newly eclosed lacewing adults were released in three cotton fields at a rate of ten per acre on the first date and 20 per acre on two subsequent dates. During the three week study, densities of lacewing eggs were not significantly greater in fields in which adults were released relative to control fields. These results suggest that high larval mortality, adult dispersal and high cost are constraints to the use of lacewings for augmentation biological control of insect pests in cotton.

INTRODUCTION

The green lacewings, *Chrysoperla rufilabris* Burmeister and *Chrysoperla carnea* Stephens, are common generalist predators in cotton in the U.S. In central Texas, *C. rufilabris* is the most abundant Chrysopid species in cotton and is present throughout the growing season. *Chrysoperla carnea* is the second most common Chrysopid but densities decline, relative to *C. rufilabris*, in late summer in Texas cotton (Burke and Martin 1956, Agnew et al. 1981). *Chrysoperla* spp. prey on eggs and larvae of lepidopteran pests of cotton and the cotton aphid, *Aphis gossypii* Glover (Ridgeway and Jones 1969). In small field-cage experiments, larvae of *C. carnea* significantly reduced densities of cotton aphid on cotton in California (Rosenheim et al. 1993). The cotton aphid emerged as a major cotton pest in the early 1990s with widespread outbreaks occurring in California and Texas during 1991-93

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(Godfrey and Leser 1999). Coincident with these outbreaks was an increased tolerance of cotton aphid to conventional insecticides. Because of the difficulty of controlling aphids with insecticides, growers and IPM managers sought non-chemical control tactics, including biological control methods for cotton aphid (Godfrey and Leser 1999). Interest in augmentative biological control focused on green lacewings as they are an effective predator of cotton aphid (Ridgeway and Jones 1969), readily available from commercial suppliers, and some commercial insectaries advocate the release of lacewing for control of cotton aphid (Anonymous, no date).

A constraint to augmentative releases of *Chrysoperla* in field crops such as cotton has been the lack of an efficient and rapid means for field distribution. The USDA Agricultural Research Service developed a tractor-mounted ground sprayer for applying chrysopid eggs and other natural enemies suspended in a liquid carrier (Tedders and Blythe 1998). This system delivers and adheres the natural enemies to the target plant, thus improving natural enemy viability and discovery of the target pest by the natural enemy (Morrison et al. 1998). This application system and liquid carrier, termed herein as the ARS applicator, were further developed and are commercially available as the Biosprayer and BioCarrier,™ respectively (Smucker Manufacturing, Inc., Harrisburg, OR 97446).

While earlier studies (Ridgeway and Jones 1969, Lopez et al. 1976) and most commercial insectaries recommend releasing *Chrysoperla* as eggs or pre-fed larvae, some companies market adult *Chrysoperla* spp. for augmentative releases (Anonymous, no date). The lacewing adults are released by hand while moving through the field, thus eliminating the need for a specialized delivery system. The objective of this study was to evaluate augmentation biological control of cotton aphid using green lacewings eggs applied to cotton plants with the ARS applicator and hand release of green lacewing adults in cotton.

METHODS AND MATERIALS

Application of green lacewing eggs. Eggs of the green lacewing, *Chrysoperla rufilabris*, were obtained from a culture maintained at the USDA-ARS Southeastern Fruit and Tree Nut Research Laboratory at Byron, Georgia. Eggs less than 24 hrs old were packaged in styrofoam boxes with a cooled gel-pak and shipped via overnight freight to the Texas A&M Research and Extension Center at Dallas. Upon receipt, the boxes were refrigerated less than 24 hrs prior to use in the field trials. Egg viability was determined prior to application by placing two subsamples of 50 eggs each on a strip of masking tape. The tape adhesive prevented larvae from consuming nearby and uneclosed eggs. Eggs were held in plastic petri plates on moist filter paper in the laboratory at room temperature. The number of eclosed and uneclosed eggs were recorded after 10 days.

Lacewing eggs were applied to cotton plants with the ARS applicator mounted on a tractor. The applicator consisted of a 3-liter stainless steel tank, a gasoline powered blower, and an electric-powered air compressor mounted on a steel platform. A system of plastic tubing connected the spray tank to two flexible metal pipes each positioned above a row of cotton. The spray solution consisted of 250 ml of BioCarrier ™ (Smucker Manufacturing Inc., Harrisburg, OR 97446) adhesive mixed in 2,000 ml of distilled water (1:8 ratio as per manufacturer's directions). The spray solution was mixed for about one minute and allowed to set for 30 minutes. The solution and lacewing eggs were then added to the 3-liter stainless steel tank. The air compressor maintained a pressure of 0.8 pounds per square inch inside the tank. The compressor also bubbled air into the spray tank to maintain a uniform suspension of lacewing eggs in the liquid carrier solution. The spray solution was metered through a plastic tube that terminated inside the end of a two inch flexible pipe. Air flow from the

blower passed down the flexible pipe and carried the spray solution onto the cotton foliage. The end of the flexible pipe was positioned 3 to 4 inches above the canopy and was positioned at a 45 degree angle above each of two rows of cotton. The applicator was calibrated based on a tractor speed of 3.4 mph and by adjusting the pump pressure to apply 100 ml of solution per 100 row feet of cotton. Air speed exiting from the air tube was 30 to 35 mph which broke up the stream of solution into large drops and deposited them in the upper canopy of the row of cotton.

Field studies were conducted in 1994 at the Texas A&M Research and Extension Center at Dallas, Texas, and in two commercial cotton fields in Kaufman and Ellis Counties in North Central Texas. Field plots were each four rows wide by 100 feet long. Cotton was planted on 40-inch row spacings. Treatments were a single application of 100,000, 200,000 and 400,000 lacewing eggs per acre, except in the Ellis County trial when one-half of each of these rates was applied. The fourth treatment was no application of eggs. Lacewing eggs were applied to cotton within one hour of mixing the eggs with the liquid carrier. Treatments were arranged in a randomized, complete block design with four replications.

Treatments were evaluated by determining numbers of lacewing eggs, larvae and pupae collected from cotton following the application of eggs. Eggs were anticipated to eclose within 1-2 days following application to the field based on the time from collection from the laboratory colony to the time of application in the field. Sampling dates were selected to detect the third instar, pre-pupal and pupal stages due to the difficulty of finding small first and second instar on cotton plants. Mean development time for the larval, prepupal and pupal stages of *C. rufilabris* is 7.7, 2.7 and 6.5 days, respectively (Burke and Martin 1956).

At the Dallas location, lacewing eggs were applied 18 July at a rate of 100,000, 200,000 and 400,000 per acre. Immediately after application, a sample of 25 leaves with visible spray residue was collected from each plot. Each leaf was examined with a dissecting microscope and the number of applied eggs and naturally oviposited eggs were recorded. Applied eggs were identified by the lack of a complete pedicel, which is broken during the collection of eggs from the rearing containers. Also, applied eggs were adhered to the leaf surface, while naturally oviposited and therefore extant eggs were suspended on a pedicel above the leaf surface. A small disc of leaf with each egg was cut from the leaf and held in a plastic petri plate with moist filter paper in the laboratory. After ten days, each egg was examined to determine if it had eclosed, died due to parasitism as evidenced by the presence of adult parasites, or died from unknown causes. Nine days after the application of the eggs, ten plants in each plot were visually examined for lacewing larvae and pupae. The plant population in the study plot was 45,000 plants per acre.

The trials in Kaufman County and Ellis County were conducted in large commercial cotton fields. At Ellis County, lacewing eggs were applied 28 June at a rate of 50,000, 100,000 and 200,000 per acre. The plant population was 35,000 per acre. Densities of cotton aphids and lacewing eggs and larvae were estimated by visually searching ten plants in each plot prior to application of lacewing eggs. Seven days after application, six cotton plants were visually examined for lacewing larvae in each plot. At Kaufman County, lacewing eggs were applied 21 July at a rate of 100,000, 200,000 and 400,000 per acre. Immediately after application, leaves wet with the spray solution were marked with a pen and 25 marked leaves were collected from each plot. Marked leaves were examined with a microscope as described above to record the number of applied and extant eggs. The number of cotton aphids per leaf was counted on the third leaf from the terminal on five and ten plants in each plot at 5 and 18 days after application, respectively. Also, at 18 days after application (8 August), ten plants in each plot were visually examined for lacewing larvae, pupae and other predatory insects.

Release of green lacewing adults. The release of adult green lacewing (*C. rufilabris*)

was evaluated in six commercial fields in Hunt County in northeastern Texas. Lacewing adults were purchased from a commercial supplier at a cost of $125 for 500 adults and shipped overnight in a styrofoam box. Adults were provided moisture upon receipt and again prior to release via wet paper toweling placed on the screened end of the container. Lacewing adults were held in the container in a refrigerator overnight and released within 24 hr of receipt. Adults were released while walking across the field during the early morning, prior to 08:30 hours and again in the evening after 19:00 hours, as per recommendations from the supplier. Adults were released at a rate of ten per acre on 7 July, and 20 per acre on 14 July and 20 July 1993. These release rates were consistent with recommended release rates, per the supplier, of 10 adults/acre on cotton plants 12-18 inches tall and 20 adults/acre when cotton plants were 24-36 inches tall. The three fields in which lacewing adults were released were 20, 28 and 52 acres in size. Three cotton fields of 20, 25 and 32 acres and located at least one mile from a release field were selected as control fields and did not receive adult releases. Each field was divided into four quadrants and 12 cotton plants in each quadrant were visually examined for lacewing eggs, larvae and pupae and the presence of cotton aphids on five sample dates from 7-29 July. In addition, the number of cotton aphids was recorded for each sample plant on 22 July. The most recent insecticide application made to any of the six study fields was on 20 June, or 17 days prior to the first release and no insecticides were applied to the fields during the study period. Weather conditions were hot and dry, typical of midsummer conditions for the area, and the study fields received no rainfall during the study period.

Significant differences between treatment means were identified using Least Significant Difference and t - test at $a = 0.05$ following analysis of variance. Percentage data were transformed by arcsin prior to analysis.

RESULTS

Application of green lacewing eggs. Eclosion of lacewing eggs collected from shipments upon receipt on two sample dates averaged 76 % $\pm$ 1.4 and 71 % $\pm$ 7.8 when held in the laboratory. Eclosion of eggs applied with the ARS applicator and collected from cotton leaves was not significantly different from eclosion of extant (naturally oviposited) eggs collected from cotton leaves, averaging 39.5 % and 42.3 %, respectively ($F = 0.19$, d. f. = 1,6, $P = 0.68$) (Table 1). Parasitism was an important source of mortality of extant eggs, while none of the applied eggs were parasitized since they were collected from the field immediately after application. Unexplained mortality of applied eggs was significantly greater, 60.5%, than that for naturally oviposited eggs (29.8%)($F = 23$, d. f. = 1,6, $P = 0.003$), suggesting that application of lacewing eggs through the ARS applicator reduced egg eclosion in this trial. Morrison et al. (1998) reported immersion of *C. rufilabris* eggs in the BioCarrier ™ for 3 hrs did not reduce egg viability. Egg mortality could result from physical damage to the eggs during passage through the application system.

TABLE 1. Fate of Applied and Extant (Naturally Oviposited) Lacewing Eggs. Dallas, TX.[a]

Source	n	Mean percentage		
		Eclosed	Parasitized	Died
Applied	82	39.5 a	0 a	60.5 a
Field	100	42.3 a	27.8 b	29.8 b

[a]Means followed by the same letter are not significantly different, LSD, $a = 0.05$

Increasing the application rate of lacewing eggs increased the number of applied eggs recovered from leaves with visible spray immediately after collection in the Dallas trial. Also, significantly more applied eggs were recovered at the highest release rate than at the two lower release rates ($F = 12.0$, d. f. = 2, 9, $P = 0.003$) (Table 2). The highest release rate, 400,000 eggs per acre, resulted in an average of 6.3 applied eggs/25 sprayed leaves. Densities of extant lacewing eggs ranged from 4.8 to 6.8 eggs per 25 leaves, and treatment differences were not significant ($F = 1.75$, d. f. = 2, 9, $P = 0.23$). Surprisingly, the mean density of extant eggs was similar to that of the highest rate of applied eggs. Nine days after application of eggs, densities of lacewing larvae per plant were significantly greater than the check only at the highest release rate ($F = 2.68$, d. f. = 3,156, $P = 0.048$) (Table 2). Densities of larvae at this release rate averaged 8,100 per acre (0.18 larva per plant). No lacewing pupae were present in the samples.

In the Ellis County trial prior to application of eggs, the density of naturally oviposited lacewing eggs was 0.6 per plant (21,000 / acre) across all treatments. No lacewing larvae were present in the pre-treatment sample. Cotton aphids were present at the time of application and densities ranged from 5-30 aphids per terminal. Seven days after application of eggs, the density of lacewing larvae was 4,400 per acre (0.13 per plant) at the highest application rate of 200,000 eggs/acre (Table 2). However, differences in mean density of larvae between treatments in the Ellis County trial were not significant ($F = 0.60$, d.f., = 3, 12, $P = 0.63$) (Table 2). In the Ellis and Dallas County trials, the application of 200,000 eggs per acre resulted in a recovery of 4,400 to 5,900 lacewing larvae per acre 7 to 9 days after application, respectively.

In the Kaufman County trial, significantly more applied eggs were recovered from leaves with spray deposit at the highest release rate than at the two lower release rates ($F = 12.76$, d. f. = 2, 9, $P = 0.002$) (Table 3). As in the Dallas trial, densities of naturally oviposited eggs were also high, averaging 2.0 to 3.3 per leaf, but differences between treatments were not significantly different ($F = 0.36$, d. f. = 2, 9, $P = 0.71$). Application of lacewing eggs had no measurable effect on cotton aphid densities as differences among

TABLE 2. Mean Number of Lacewing Eggs on Leaves with Spray Deposit and Number of Larvae Following Application of Lacewing Eggs to Cotton at Two Locations.

	Dallas County Trial[ab]			Ellis County Trial[ac]	
Release rate	Eggs per 25 leaves		Larvae per	Release rate	Mean no.
eggs / acre	Applied	Extant	acre	eggs / acre	larvae / acre
0	–	---	0.0 b	0	1,400 a
100,000	2.3 a	4.8 a	2,300 ab	50,000	1,400 a
200,000	3.8 a	5.3 a	5,900 ab	100,000	1,400 a
400,000	6.3 b	6.8 a	8,100 a	200,000	4,400 a

[a]Means followed by the same letter within a column are not significantly different, LSD, $\alpha = 0.05$

[b]Nine days after release of eggs

[c]Seven days after release of eggs

TABLE 3. Average Number of Applied and Extant Lacewing Eggs Recovered per 25 Leaves with Spray Deposit. Kaufman, TX.[a]

Release rate, eggs/acre	Applied eggs	Extant eggs
100,000	1.0a	3.3a
200,000	1.8a	2.0a
400,000	7.3b	3.3a

[a]Means followed by the same letter are not significantly different, LSD, $a = 0.05$

TABLE 4. Mean Number of Cotton Aphids and Adult Coccinellidae 5 and 18 Days After Application of Lacewing Eggs to Cotton. Kaufman, TX.[a]

No. applied eggs/acre	Mean no aphids / leaf		Coccinellidae/10 plants
	5 days	18 days	at 18 days
0	69a	17a	3.3a
100,000	52a	20a	5.3a
200,000	49a	11a	6.0a
400,000	47a	11a	7.0a

[a]Means followed by the same letter are not significantly different, LSD, $a = 0.05$

treatment means were not significantly different at 5 and 18 days after application of lacewing eggs ($F = 1.52$, d. f. = 3, 76, $P = 0.22$ and $F = 1.12$, d. f. = 3, 76, $P = 0.35$, respectively) (Table 4). Eighteen days after application of eggs, no lacewing larvae were found in any treatment and only two lacewing pupae were recovered. Densities of Coccinellidae, primarily *Hippodamia convergens* Guerin-Meneville, were high in response to the large numbers of aphids present in the test plot but differences among treatments were not significantly different ($F = 1.08$, d. f. = 3, 8, $P = 0.41$) (Table 4).

Release of green lacewing adults. Cotton aphids were present in all of the release and control fields throughout the study period. On 7 July, prior to release of adults, cotton aphids were present on 21 % $\pm$ 6 and 34 % $\pm$ 25 of the cotton plants in the release and check fields, respectively. Aphid infestations reached a maximum on 16 to 20 July when cotton aphids were present on 49 % $\pm$ 16 and 68 % $\pm$ 34 of the plants in the release and control fields, respectively, and infestations then declined. Following the first release of lacewing adults, densities of lacewing eggs in release and control fields were significantly different only on the last sample date 29 July when densities of lacewing eggs were significantly greater in the control treatment (Table 5). These results suggest that the release of adult lacewings did not increase densities of lacewing eggs in cotton in the three release fields. The mean density of cotton aphids on 22 July was 3.1 and 13.3 per plant in the release and control fields, respectively, and the difference between means was not significantly different (t- test, $P = 0.001$).

TABLE 5. Mean Number of Green Lacewing Eggs in Release and Check Fields. Hunt County. 1993[a]

Sample Date	Lacewing eggs / plant: mean ± standard error		*P* - value
	Release fields	Control fields	
July 7	0.01 ± 0.01	0.04 ± 0.02	0.02
July 14	0.04 ± 0.01	0.06 ± 0.03	0.32
July 20	0.23 ± 0.03	0.21 ± 0.03	0.63
July 22	0.22 ± 0.03	0.17 ± 0.03	0.20
July 29	0.14 ± 0.03	0.39 ± 0.04	0.01

[a]Lacewing adults released July 8, July 14 and July 20. Means between release fields and control fields are compared with *t*- test at $a = 0.05$.

DISCUSSION

Lacewing populations in cotton often consist of large numbers of eggs with much fewer numbers of larvae, as reported by Whitcomb and Bell (1964) in Arkansas and Wilson and Gutierrez (1980) in California. Extant eggs were also common in the current study (Tables 2 and 3) yet lacewing larvae and pupae were rare (Table 3). Earlier efforts to augment lacewing densities confirmed that immature stages of lacewing suffer high mortality in cotton. Ridgway and Jones (1968) applied 800,000 *C. carnea* eggs per acre by hand to cotton plants and recovered 44,700 larvae per acre (5.6 %) ten days after egg eclosion. In this study, cotton plants had previously been treated with methyl parathion to kill other insects and infested with cotton aphids to provide food for eclosing lacewing larvae. In a second study, hand application of 200,000 *C. carnea* eggs per acre to cotton yielded 6,210 larvae per acre (3 %) nine days after egg eclosion (Ridgway and Jones 1969). The latter results are very similar to the recovery of 5,800 larvae per acre nine days after the application of 200,000 eggs per acre in the Dallas trial (Table 2).

Intraguild predation by *Zelus renardii* Kolenati, *Nabis* spp. and *Geocoris* spp. was a major source of mortality of larval *C. carnea* in cotton in California (Rosenheim et al. 1993) Intraguild predation by these generalist predators was demonstrated to release aphid populations from control by lacewing larvae (Roseheim et al. 1993). Although predator densities were not measured in the study fields reported herein, *Geocoris* spp. are present in north Texas cotton while *Zelus* spp and *Nabis* spp. are uncommon (Knutson, unpublished data). The most abundant generalist predators in cotton in this area are *Orius* spp., *Misumenops* spp. and other spiders, and the red imported fire ant, *Solenopsis invicta* Buren. The red imported fire ant preys on lacewing eggs, larvae and pupae in pecans and is considered a major constraint to the successful augmentation of lacewing in pecans for control of pecan aphids (Tedders et al. 1990). In the Kaumfan trial, *S. invicta* was present on 18 % ± 5.5 (mean ± S. D) of the plants sampled on 8 August. Also, lacewing eggs have been collected from *S. invicta* workers foraging in cotton (Knutson, unpublished data). Predation of extant and applied lacewing eggs and larvae by *S. invicta* in cotton may have contributed to the low survival of *C. carnea* reported herein.

Parasitism of lacewing eggs by Hymenoptera is also important, as shown from the results of collecting extant eggs in the field experiment at Dallas (Table 1). Parasitism of supplemental eggs could be avoided by applying eggs just before eclosion but subsequent

parasitism of eggs and larvae could reduce survival of succeeding in-field generations of lacewing. A large number of parasite species attack Chrysopid eggs and larvae (Clancy 1946). In cotton in Israel, parasitism of lacewing larvae is as high as 50 % and is important in suppressing population increase of lacewings (Gerling and Bar 1985).

In addition to predation, starvation of lacewing larvae, especially the first-instar larva, is an important source of mortality. However, lack of prey is not considered important in the tests herein. Cotton aphids were present in all of the current studies and densities in the Kaufman trial approached 50 aphids per leaf which is considered economically threatening (Moore et al. 2001).

The release of lacewing adults was based upon the assumption that adults would oviposit in the field in which they were released. The preoviposition period for *C. rufilabris* ranges from 4-14 days with a mean of 8.2 days (Burke and Martin 1956). During this time, released adults could have dispersed from the release field. Adult female *C. carnea* exhibit an obligatory migration flight during the first 2-3 nights after eclosion and prior to oviposition (Duelli 1980) Flight distance of newly eclosed adult females is estimated to be several kilometers in the absence of wind and up to 40 km per night depending upon wind velocity. Dispersal of recently eclosed adult females from the fields in which they were released could account for the absence of an increase in egg density relative to the check fields (Table 5).

As suggested by Morrison et al. (1998), the results of the current study indicate the ARS applicator is a significant improvement in technology for applying lacewing eggs to field crops. The system is tractor mounted and can be modified to rapidly treat multiple rows of cotton in large commercial fields. The liquid-carrier system adheres the lacewing eggs to the cotton plant as opposed to other mechanical application systems which result in some eggs falling to the ground where they are exposed to high soil temperatures and lack of prey. However, the very low recovery of lacewing larvae and pupae in this study following applications of high number of eggs suggest biological factors including predation and dispersal of adults may be significant constraints to augmentative biological control of lacewings in cotton. In addition, the expense of lacewing eggs, about $2.10 for 1,000 eggs, is cost prohibitive for cotton production at the release rates used in this study.

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FORAGING BEHAVIOR, HOST STAGE SELECTION AND GUT CONTENT ANALYSIS OF FIELD COLLECTED *DRAPETIS* NR. *DIVERGENS*[1]: A PREDATORY FLY OF *BEMISIA ARGENTIFOLII*[2]

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ABSTRACT

A laboratory investigation of the foraging behavior and host stage selection of field collected *Draptis* nr. *divergens* presented with a surfeit of silverleaf whitefly, *Bemisia argentifolii* Bellows and Perring, eggs, nymphs and adults was undertaken. The foraging behavior of *D.* nr. *divergens* resembled that of an ambush attack strategist, frequently exhibiting motionless behavior and feeding exclusively on mobile adults. A gut content evaluation of *D.* nr. *divergens* using a whitefly-specific enzyme-linked immunosorbent assay (ELISA) was conducted on field collected flies. The analysis revealed that 15% of the individuals collected contained whitefly remains in their guts.

INTRODUCTION

The silverleaf whitefly, *Bemisia argentifolii* Bellows and Perring [= *B. tabaci* (Gennadius), strain B] (Homoptera: Aleyrodidae) and other whitefly species are pests of many crops and ornamentals throughout the world (Mound and Halsey 1978, Bellows et al. 1994). An enormous research effort has been made toward identifying the whitefly's predaceous natural enemies. The most common whitefly predators identified thus far are various species of beetles, true bugs, lacewings and mites (e.g., Meyerdirk and Coudriet 1985; Gerling 1986, 1990, 1992; Butler and Henneberry 1988; Kapadia and Puri 1991; Breene et al. 1992, 1994; Hoelmer et al. 1993; Legaspi et al. 1994; Hagler and Naranjo 1994a,b; Heinz et al. 1994; Nordlund and Legaspi 1995). Several recent surveys of the predator complex in whitefly infested Arizona cotton revealed that a predatory fly, *Drapetis* nr. *divergens* Loew (Diptera: Empididae) is the most abundant predator species (Butler and Henneberry 1993; JRH, unpublished data). A thorough search for literature on *Drapetis* spp. revealed that very little is known about their feeding behavior, diet breadth, or life history. For example, the habitat of the egg, larval, and pupal stages are unknown, its diet breadth is unknown, and a complete taxonomic description of the *Drapetis* species found in Arizona does not exist.

This study was initiated to evaluate the behavior and host stage selection of field

[1] Diptera: Empididae.
[2] Homoptera: Aleyrodidae.
[3] This article reports the results of research only. Mention of a proprietary product does not constitute an endorsement or a recommendation for its use by the USDA.

collected adult *D.* nr. *divergens*. My primary objectives were to: (1) determine if adult flies preferentially feed on whitefly eggs, nymphs, or adults; (2) determine prey consumption rate; and (3) observe fundamental foraging behavior. To accomplish these objectives, I directly observed 22 individuals, for one hour each, in a feeding arena containing a surplus of whitefly eggs, nymphs, and adults. The host stage selection and the amount of time that each individual predator spent feeding on its prey were recorded. Additionally, an analysis of the predator foraging sequence behavior was conducted to identify and quantify general foraging patterns exhibited by *D.* nr. *divergens*. Following the laboratory experiment, I analyzed the gut contents of field collected individuals for whitefly remains using a whitefly-specific enzyme-linked immunosorbent assay (ELISA) (Hagler et al. 1993) to determine the proportion of *D.* nr. *divergens* preying on whiteflies in a cotton field.

MATERIALS AND METHODS

Laboratory Evaluation of Feeding Behavior. Adult *D.* nr. *divergens* were collected with a 36.0-cm diameter sweepnet throughout the summer of 1997 from cotton fields at the University of Arizona's Maricopa Agricultural Research Station (Maricopa, Arizona). The entire contents of the sweepnet samples were returned to the laboratory and placed in a 60-cm tall by 30-cm diameter communal rearing container containing a single, ≈55-cm tall, potted cotton plant (cv '*Delta Pine 5415*') and held at 27°C. The flies were allowed to feed *ad libitum* on any available prey collected in the sweepnets. The day before the feeding observations, individuals were randomly removed from the communal rearing container, placed individually into a clean petri dish (9.0-cm diameter) and provided with water only.

Cotton plants were grown in 15.2-cm diameter pots in a greenhouse. Four- to five-week old cotton plants were infested with adult whiteflies on a weekly basis. When the plants were approximately 8 to 9 weeks old, a single cotton leaf was removed from a plant and cut to fit exactly into the bottom of a 3.5-cm plastic petri dish (the feeding arena). The numbers of whitefly eggs and nymphs were counted on each leaf disk, after which each disk was placed abaxial side up into the bottom of the feeding arena. Adult whiteflies were introduced into the arena and the petri dish lid was placed over the top of the arena. A typical feeding arena contained a 3.5-cm diameter cotton leaf disk infested with an average of 982.0 ± 181.5 eggs, 634.4 ± 117.2 nymphs, and 42.6 ± 2.7 adults (males and females at a 1:1 sex ratio). A single fly that was held overnight without food was placed into the feeding arena and continuously monitored for 1 h under a dissecting microscope as described below. The observations were made between 0800 and 1200 h at 27°C and 20% RH. Subsequently, a behavioral transition matrix (Lehner 1998, Isaacs et al. 1999) was developed and its components were programmed into The Observer®, a software program designed specifically for animal behavior research (Noldus Information Systems, Ver. 3.0, 1996). Descriptions of the behaviors exhibited by *D.* nr. *divergens* are given in Table 1. After each 1 h observation (n=22 observations), the first predatory fly was removed from the arena and replaced with a second fly. No more than two flies were observed consecutively in the same arena. The feeding arenas were replaced daily with fresh plant and prey material.

Behavior transition matrices for *D.* nr. *divergens* were determined by transferring the observational data obtained from The Observer® from columns of sequences into matrices of preceding and succeeding behavioral elements as described by Lehner (1998) and Isaacs et al. (1999). Thereafter, each first-order transition (Slater 1973) from one behavior to another was analyzed by a χ^2 test to identify those transitional behaviors that were significantly greater than expected by chance. This was done only for those

TABLE 1. Description of the Behavioral Events Observed for 22 Individual *Drapetis* nr. *divergens* Observed for One Hour Each in a Feeding Arena Containing a Cotton Leaf Disk and All Lifestages of *Bemisia argentifolii.*

Observation	Prey	Description of predator behavior
Walking		Moving in a straight line across the leaf surface
Resting		Standing motionless
Grooming		Rapid movements with its fore and hind legs across its body surface and antennae
Orienting		Pivoting on the leaf without moving in any particular direction
Probing	Egg	Probing an egg, but doesn't insert its mouthparts
	Nymph	Probing a nymph, but doesn't insert its mouthparts
	Adult	Probing an adult, but doesn't insert its mouthparts
Feeding	Egg	Consuming an egg by inserting its mouthparts
	Nymph	Consuming a nymph by inserting its mouthparts
	Adult	Consuming an adult by inserting its mouthparts

transitions with a frequency greater than 1.0% of the total number of transitions in order to reduce the likelihood of making a type II statistical error (Isaacs et al. 1999). The critical P-value assigned to the χ^2 test was 0.001. The prey choice and mean ± SE amount of time that *D.* nr. *divergens* spent handling its prey was determined.

Gut Content Evaluation of Field Collected Drapetis nr. divergens. Adult *D.* nr. *divergens* were collected in a 36.0-cm diameter sweepnet on 15 August 2001 from a whitefly-infested cotton field located at the University of Arizona's Maricopa Agricultural Research Station. The entire contents of the sweepnet sample were put into a 3.8-liter waterproof plastic container and immediately placed on ice. Upon return to the laboratory, predators were removed and stored at -70°C. The flies were then assayed for the presence of whitefly remains in their gut using the whitefly-specific ELISA described below. Voucher specimens of *D.* nr. *divergens* used in this study were deposited at the United States National Collection, Systematic Entomology Laboratory, Beltsville, MD.

Negative control *D.* nr. *divergens* were collected from the field and provided with only water *ad libitum* for 72 h. This allowed the insects to digest any egg antigen in their guts at the time of their collection (Hagler and Naranjo 1997). Individuals were thoroughly ground in 250-µl tris buffered saline (TBS) and stored at -70°C until assay. Negative control *D.* nr. *divergens* were assayed for the presence of whitefly remains in their guts using the ELISA described below. Mean ± SD ELISA optical density values were calculated for the negative control predators.

The whitefly-specific ELISA described by Hagler et al. (1993) was used to determine the proportion of field collected *D.* nr. *divergens* containing whitefly remains in their guts. A field collected fly was scored positive for whitefly egg antigen if its ELISA optical density value exceeded the mean negative predator control reading by three standard deviations (Schoof et al. 1986, Sutula et al. 1986). The percentage of individuals (qualitative outcome) scoring positive for whitefly remains was tallied.

RESULTS AND DISCUSSION

Laboratory Evaluation of Feeding Behavior. Drapetis nr. *divergens* spent 75% of its time resting and grooming. Observation revealed that the percentage of time spent in

each behavioral event was grooming (38%) ≈ resting (37%) > feeding (12%) ≈ walking (11%) > orienting (1%) = probing (1%). *Drapetis* nr. *divergens* walking behavior was rapid and almost always in a direct line, as orienting in different directions was rarely observed. The analysis of transitional behavioral events showed that *D.* nr. *divergens* had a total of 584 behavioral transitions recorded during 22 h of observation (26.5 per hour) (Table 2). The only significant behavioral sequences were: (1) grooming to resting 21.9% of the time, (2) resting to grooming 17.8% of the time, and (3) adult probing to adult feeding 8.7% of the time.

Drapetis nr. *divergens* ignored whitefly eggs and nymphs and fed solely on adult whiteflies. This concurs with anecdotal observations reported by other researchers. For example, Sussmann (1988) noted *D. subaenescens* Collin preying on adult *B. tabaci* Gennadius in Israel and Butler and Henneberry (1993) observed that male *Drapetis* sp. frequently had whiteflies impaled on their mouthparts. They hypothesized that males presented their potential mates with a whitefly meal just prior to mating. It is generally accepted that most whitefly predators feed on every lifestage, but the immobile egg and nymphal lifestages are the most vulnerable to attack (Breene et al. 1994, Nordlund and Legaspi 1995). This is the first report of a whitefly predator preying exclusively on the adult lifestage. This finding is useful for refining the interpretations of *D.* nr. *divergens* gut content examinations using an established whitefly egg-specific ELISA. For example, a limitation of the whitefly-specific ELISA is that the MAb used can't differentiate between an egg and an egg-carrying (i.e., gravid) female meal (Hagler et al. 1993). Since most whitefly predators readily prey on both eggs and adults, I can't be certain whether a positive ELISA reaction is due to predation on whitefly eggs, adult females, or both lifestages (Hagler and Naranjo 1994a,b). However, since this study shows that *D.* nr. *divergens* feed exclusively on adult whiteflies, when all whitefly lifestages are available, a positive egg-specific ELISA reaction yielded by a field collected fly can be attributed to consuming an adult female.

Drapetis nr. *divergens* consumed an average of 1.9 ± 0.22 adult whiteflies per hour. This predation rate is less than that I have observed in identical feeding arenas for adult *Hippodamia convergens* Guèrin-Mèneville and *Collops vittatus* (Say) that consumed ≈ 10 adults per hour, *Geocoris punctipes* (Say) and *Lygus hesperus* Knight that consumed ≈ 6 adults per hour, and about the same as that observed for *Orius tristicolor* (Say) (unpublished data). The average prey handling time for *D.* nr. *divergens* on adult whiteflies was 246.8 ± 28.5 sec. This handling time is much longer than that of ≈ 15 sec observed for *H. convergens* and *C. vittatus* (unpublished data) and ≈ 110 sec observed for *G. punctipes*, but shorter than ≈ 455 sec observed for *O. tristicolor* (unpublished data).

The overall results from this laboratory study provide some insight into the hunting strategy used by *D.* nr. *divergens*. Insect predators are usually classified as either stalking or ambush predators (O'Brien et al. 1989, Sabelis 1992, Hagler 1999). Stalking predators walk in a stop-and-go manner constantly scanning their habitat for prey. When prey is detected, the predator rapidly consumes it and moves on toward the next meal. This hunting strategy is generally used by predators that feed on immobile prey such as insect eggs (Breene et al. 1994). Ambush predators place themselves in a strategic location and wait until prey wanders into their field of attack. Then, once a prey item is in range, they pounce and feed on it for an extended period of time. Generally, mobile insects are more susceptible to ambush predators (Cohen et al. 1995). The high frequency of resting and grooming, the mobile lifestage prey choice, and the relatively long prey handling time exhibited by *D.* nr. *divergens* indicate that it is an ambush predator. Although *D.* nr. *divergens* is the first predator described that feeds exclusively on adult whiteflies, it is likely that other ambush predators, such as *Zelus renardii* Kolenati, also prey exclusively on adult whiteflies (personal observation). However, Z.

TABLE 2. Transition Frequencies Between 10 Observed Behavioral Events of Adult *Drapetis* nr. *divergens* on Cotton Leaves Infested with Whitefly Eggs, Nymphs and Adults. Transitions that were Significantly more Common than Expected by Chance are Shown in Bold (Chi-Square, Adjusted $P < 0.001$).

Preceding Behavioral Event	Succeeding Behavioral Event										
	Feeding event			Probing Event			Other Behaviors				
	Egg	Nymph	Adult	Egg	Nymph	Adult	Walking	Resting	Grooming	Orienting	Total
Egg feeding	0	0	0	0	0	0	0	0	0	0	0
Nymph feeding	0	0	0	0	0	0	0	0	0	0	0
Adult feeding	0	0	0	0	0	0	2	11	23	5	41
Egg probing	0	0	0	0	0	0	0	0	0	0	0
Nymph probing	0	0	0	0	0	0	4	0	0	0	4
Adult probing	0	0	**51**	0	0	0	3	2	5	0	61
Walking	0	0	5	0	4	5	0	36	59	2	111
Resting	0	0	11	0	0	15	32	0	**104**	2	164
Grooming	0	0	11	0	0	4	45	**128**	0	3	191
Orienting	0	0	0	0	0	1	4	1	6	0	12
											584

renardii is also known to attack a wide variety of mobile stages of other insect species (Lingren et al. 1968, Ables 1978, Cohen 1993). Whether *D.* nr. *divergens* attacks other insect species is not known and requires further investigation.

Gut Content Evaluation of Field Collected Drapetis nr. divergens. None of the seven negative control *D.* nr. *divergens* yielded a positive response for the presence of whitefly remains in their guts. The critical threshold value (mean ± 3 SD) for a positive response was 0.15 (Fig. 1). These data indicate that starved flies do not contain any inherent antigens that cross react with the whitefly-specific ELISA. These results are similar to previous studies where this whitefly-specific ELISA was tested for cross reactivity against other predator species (Hagler et al. 1993, Hagler and Naranjo 1994a,b).

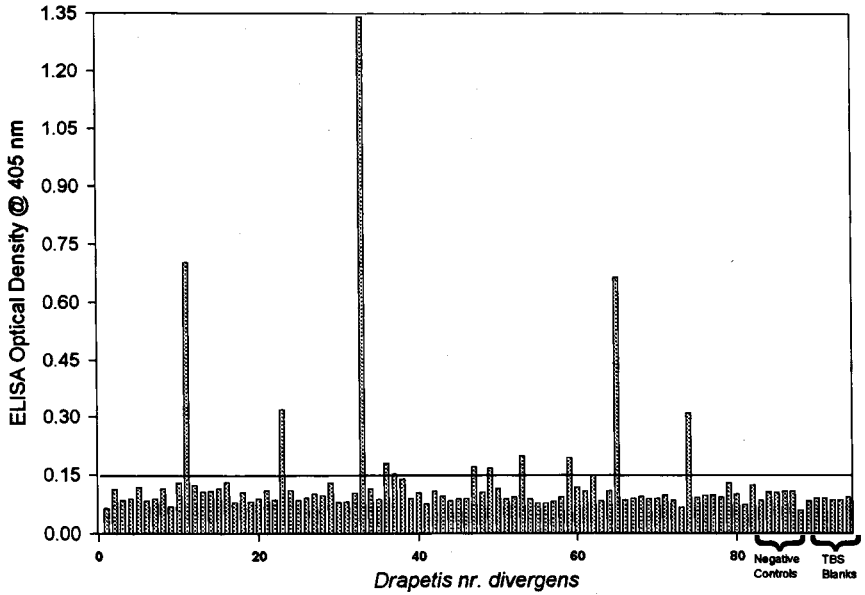

FIG. 1. The ELISA reaction yielded by individual field collected *Drapetis* nr. *divergens* examined for *Bemisia argentifolii* prey remains. The bold horizontal line parallel to the x-axis is the critical threshold value for a positive ELISA response. Individuals yielding an ELISA optical density value above the line scored positive for the presence of *B. argentifolii* remains in its gut.

The quantitative ELISA response yielded by 80 field collected flies examined in this study is given in Fig. 1. The ELISA optical density values yielded by those individuals scoring positive for whitefly remains ranged from just above the positive ELISA threshold value of 0.15 to as high as 1.34. Whitefly remains were detected in 12 of the 80 individuals examined (Fig. 1). The frequency of positive responses for *D.* nr. *divergens* is similar to the 14.3% and 12.2% yielded by the ambush predators, *Sinea*

confusa Caudell and *Z. renardii* (Hagler and Naranjo 1994a), but less than the 20 to 58% frequency of positive responses for the stalking predators, *C. vittatus*, *H. convergens*, *G. punctipes*, *G. pallens* Stål, *O. tristicolor*, and *L. hesperus* (Hagler and Naranjo 1994a,b). However, as mentioned above, the proportion of positive ELISA reactions for the stalking predators is likely due to these predators feeding on whitefly eggs as well as adult females.

From these studies, *D.* nr. *divergens* is an ambush-type predator that feeds specifically on adult whiteflies. Based on its relatively low prey consumption rate and long prey handling time, it is not an ideal single candidate for whitefly control. However, its abundance in Arizona cotton fields makes it a possible contributor to an overall program targeted for whitefly control.

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ATTRACTIVENESS AND EFFICACY OF FIPRONIL AND SULFLURAMID BAITS FOR CONTROL OF THE TEXAS LEAFCUTTING ANT[1]

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ABSTRACT

Citrus pulp baits containing fipronil or sulfluramid were evaluated for their attractiveness to the Texas leafcutting ant and effectiveness in halting ant activity. Texas leafcutting ants were more than twice as likely to retrieve the fipronil bait compared to the sulfluramid bait. Both citrus pulp baits were highly effective in halting ant activity within 8 weeks following treatment. However, the fipronil bait reduced ant activity at a faster rate.

RESUMEN

Cebos de pulpa de árboles cítricos que contenían fipronil o sulfluramid se usaron para evaluar la atracción de la hormiga cortadora de hojas de Texas y para ver la eficiencia en detener la actividad de esta hormiga. En comparación, las hormigas cortadoras de hojas de Texas sacaron el cebo de fipronil casi en doble proporción al cebo de sulfluramid. Ambos cebos de pulpa de árboles cítricos fueron extremadamente eficientes en detener la actividad de la hormiga en las ocho semanas despues del tratamiento. Sin embargo, el cebo de fipronil redujo la actividad de la hormiga con más rapidez.

INTRODUCTION

Leaf-cutting ants in the genus *Atta* (Hymenoptera: Formicidae) are among the most destructive insects in the tropical and subtropical Americas. Crops particularly susceptible to defoliation include citrus, cocoa, coffee, maize, cotton, eucalyptus, and pines (Cherrett 1986). The Texas leafcutting ant, *Atta texana* (Buckley), is the most northern representative of this genus and is a serious pest in first- and second-year plantations of loblolly pine, *Pinus taeda* L., in eastern Texas and west-central Louisiana (Moser 1984, Cameron and Riggs 1985). It also attacks citrus groves in southern Texas (V. French, Texas A&M Citrus Board, personal communication). This insect was rated third in relative pest importance (behind the southern pine beetle, *Dendroctonus frontalis* Zimmermann, and fusiform rust, *Cronartium quarcuum* f. sp. *fusiforme*) in a 1981 survey of major forest industries of eastern Texas (Texas Forest Service 1982). Pine seedling mortality due to the Texas leafcutting ant occurs on nearly 4,900 ha per year with control and seedling replacement costs averaging $2.3 million per year (Cameron and Riggs 1985). This insect also is a considerable pest to homeowners within its range. A recent survey of Texas Cooperative Extension county agents in 70 counties indicated that, on average, nearly 30 calls are received per county per year primarily from

[1] *Atta texana* (Buckley) (Hymenoptera; Formicidae)

homeowners with complaints about ants foraging on shrubs, rose bushes, and fruit trees or in vegetable gardens (B.M. Drees, Texas Cooperative Extension, personal communication).

Fumigation with methyl bromide (Brom-o-gas®) has been widely used during winter months for nearly 50 years to effectively control the Texas leafcutting ant. However, the potential contribution of methyl bromide to ozone depletion has led to the scheduled removal of its Environmental Protection Agency (EPA) registration by the year 2005. Due to the imminent methyl bromide registration withdrawal and the chemical's high toxicity to man, a citrus pulp bait containing sulfluramid (Volcano® leafcutter ant bait, Griffin L.L.C., Mexico, 0.5% ai) was registered in Texas and Louisiana in 1999 and 2000, respectively. The registered application rates are 4.0 g/m^2 for winter treatments and 10.0 g/m^2 for summer treatments. This sulfluramid bait is nearly 100% effective in halting ant activity year around with a single application (D. M. Grosman, unpublished data; Darwin Foster, Temple-Inland Forest Products, and Ken Addy, Louisiana Pacific, personal communications). However, due to EPA concerns about the potential health and ecological effects of perfluorooctyl sulfonate chemicals (including sulfluramid), products containing sulfluramid are scheduled to be phased out by 2011.

Fipronil, a phenyl pyrazole insecticide, is registered in the United States for several uses including turf pests, fleas, ticks, roaches, termites and ants. Another citrus pulp bait, containing fipronil (Blitz®, Bayer CropScience, Brazil, 0.03% ai), is registered in Brazil, Columbia, Bolivia and Paraguay for control of several leaf-cutting ant species including *Atta sexdens* L., *Atta laevigata* (Smith) and *Acromyrmex subterraneus subterraneus* Forel (K. Holmes, Aventis, personal communication). This formulation uses an orange peel-based matrix. The recommended application rate is 10.0 g/m^2 of central nest area. This formulation is not yet registered in the United States.

Field trials were conducted during the winter 2000/2001 and summer 2001 to evaluate the attractiveness of the fipronil bait to the Texas leafcutting ant and its effectiveness in halting ant activity in comparison to the sulfluramid bait (Volcano®).

MATERIALS AND METHODS

Preference and efficacy trials were conducted in Angelina, Cherokee, Jasper, Newton, Nacogdoches, Rusk, and Shelby counties in eastern Texas on land owned and/or managed by Temple Inland, Louisiana Pacific, International Paper, and the USDA Forest Service. Colonies larger than 30 m by 30 m, smaller than 3 m by 3 m, those adjacent to each other (within 100 m), or those lacking a distinct central nest area were excluded. The central nest area was defined as the above-ground portion of the nest, characterized by a concentration of entrance/exit holes (generally > 5 holes/m^2), surrounded by mounds of loose soil excavated by the ants (Cameron 1989). Scattered, peripheral entrance/exit and foraging holes (mounds) were not included in the central nest area.

Bait Preference. The trials were performed by placing 5.0 g portions of different formulations of citrus pulp baits (sulfluramid, fipronil, and blank citrus pulp) in plastic petri dishes (Cameron 1990, Della Lucia et al. 1992). Both the fipronil formulation and blank were comprised of an orange peel matrix; whereas, the sulfluramid formulation is reported be made of a mixture of citrus pulp types, i.e., orange, lemon, lime, and/or grapefruit (J. Whatley, Griffin L.L.C., personal communication). Five replicates, containing one dish for each treatment, were evaluated on one colony on September 23, 2001 and five more replicates were evaluated on a second colony on October 2, 2001. The replicates were distributed at random at about 10:00 hours CST within the central nest area or along active foraging trails of the two colonies. All dishes within each replicate were retrieved when the most attractive

bait was nearly gone or at the end of the test period (4 hours). The bait not removed by ants from each petri dish was weighed and mean weight of bait removed was computed.

Bait Efficacy. All trials were conducted using procedures developed by Cameron (1989). The sulfluramid bait was applied to the central nest area at 4 g/m^2 in winter and 10 g/m^2 in summer; whereas, the fipronil bait was applied at 10 g/m^2 for both seasons. Application rates were based on the area (length X width) of the central nest. Treatments were randomly assigned to the selected ant nests with 10 - 12 replicates per treatment per season. The baits were applied to central nests only. In all trials, applications were timed to avoid wet soil conditions and rain within 24 hr following application of baits.

For all efficacy trials, treatment effectiveness was evaluated by counting the number of active entrance/exit holes prior to treatment and at 2, 8, and 16 weeks following treatment. Ten untreated colonies were included each season in the efficacy trials as checks and monitored to account for possible seasonal changes in ant activity. In addition, observations were made on several colonies just after treatment with fipronil or sulfluramid baits to determine the length of time necessary for the ants to retrieve all of the applied bait. For each colony, the percentage of initial activity was calculated as the current number of active holes divided by the initial number of active holes at each post-treatment time period. Also, the percentage of colonies deemed to be totally inactive was calculated for each treatment at each post-treatment evaluation. Data were transformed using the arcsin $\sqrt{\%}$ transformation and analyzed by the GLM procedure. Fisher's Protected LSD test was used to detect significant differences among treatments at the $\alpha = 0.05$ probability level (StatView 1999).

RESULTS

Bait Preference. In preference trials (10 replicates) conducted in late September and early October 2001, the Texas leafcutting ant retrieved an average of 79% of the available fipronil bait compared to only 35% of the sulfluramid bait (Table 1). The ants retrieved 64% of a blank citrus pulp bait (no active ingredient), known to be composed of orange citrus pulp. Although the true citrus pulp composition of the sulfluramid bait is not known to the authors, communications with Griffin L.L.C. and the above results suggest that the Texas leafcutting ant prefers baits composed primarily of orange citrus pulp and are less attracted to baits composed of other types of citrus pulp (e.g., lemon, lime, grapefruit).

TABLE 1. Attractiveness of Fipronil and Sulfluramid Baits to the Texas Leafcutting Ant (*Atta texana*) - September and October 2001.

Treatment	N	Mean Percent Citrus Pulp Bait Removed from dishes ± SE [a]
Fipronil	10	79.1 ± 7.8 b
Sulfluramid	10	35.0 ± 5.3 a
Blank citrus pulp	10	63.5 ± 10.4 b

[a] Means followed by the same letter are not significantly different (Fisher's Protected LSD, $P > 0.05$).

Bait Efficacy. In the winter 2000/2001 trial, both fipronil and sulfluramid baits were 100% effective in completely halting ant activity within 8 weeks post-treatment (Table 2). However, a comparison of the proportion of colonies inactive and the level of remaining ant

TABLE 2. Efficacy of Fipronil and Sulfluramid Baits Applied by Spreader to Control the Texas Leafcutting Ant (*Atta texana*) in Eastern Texas (Winter 2000-2001 and Summer 2001).

Treatment	No. of Colonies Treated	Mean Nest Area (m^2)	Mean No. Active Holes Per Nest @ Trt.	Mean % initial activity [a] (% inactive colonies): 2 wk		8 wk		16 wk	
Winter 2000/2001									
Fipronil (10.0 g/m^2)	12	31	90	3.7 a	(50)	0.0 a	(100)	0.0 a	(100)
Sulfluramid (4.0 g/m^2)	12	45	130	14.9 b	(0)	0.0 a	(100)	0.0 a	(100)
Check (no treatment)	10	40	91	88.9 c	(0)	92.8 b	(10)	64.4 b	(0)
Summer 2001									
Fipronil (10.0 g/m^2)	10	56	221	0.5 a	(70)	0.0 a	(100)	0.0 a	(100)
Sulfluramid (10.0 g/m^2)	10	53	181	9.9 b	(10)	0.2 a	(90)	6.7 a	(90)
Check (no treatment)	10	36	169	82.3 c	(0)	100.0 b	(0)	87.4 b	(0)

[a] Means followed by the same letter within each season and column are not significantly different (Fisher's Protected LSD, $P > 0.05$).

activity at 2 weeks post-treatment for fipronil and sulfluramid bait treatments indicated that the fipronil bait was significantly faster in reducing ant activity.

The summer 2001 trial similarly indicated that the fipronil bait was highly effective during the summer months and can completely halt ant activity of most colonies within 2 weeks post-treatment (Table 2). Again, the fipronil bait significantly reduced ant activity faster than did the sulfluramid bait. However, there was no difference in efficacy between treatments after 8 weeks post treatment.

Field observations indicated that once ants become active (about 10:00 AM CST in the winter and 9:00 PM CST in the summer), worker ants readily found and retrieved both fipronil and sulfluramid baits. In August 2000, hourly observations made after the central nest areas of six colonies were treated with sulfluramid bait (10 g/m^2) revealed that the ants consistently retrieved all bait particles within 5 to 6 hours. In October 2001, observations made after the central nest areas of two colonies were treated with fipronil bait (10 g/m^2) found that the ants retrieved all bait particles in half the time (2.5 to 3 hours), compared to sulfluramid bait.

DISCUSSION

Overall, both the fipronil and sulfluramid baits were highly attractive to the Texas leafcutting ant and were highly effective in halting ant activity in both the winter and summer seasons. However, in side by side comparisons, the fipronil bait was noticeably more attractive to the ants and reduced ant activity at a more rapid rate than did the sulfluramid bait. Given the pending phase-out of both methyl bromide and Volcano®, a critical need for an effective alternative to control the Texas leafcutting ant exists, not only in pine plantations, but also in areas surrounding citrus groves and homes. The fipronil bait was a highly attractive and effective alternative for this purpose. As of August 2002, Bayer CropScience was pursuing EPA registration of this same fipronil bait formulation in the United States under the trade name BES 100.

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NEW ECTOPARASITE RECORDS FOR THE ROCK SQUIRREL, *SPERMOPHILUS VARIEGATUS GRAMMURUS*, IN SOCORRO COUNTY, NEW MEXICO.

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ABSTRACT

A small sample of rock squirrels, *Spermophilus varigatus grammurus* (Say), was examined in order to document their ectoparasitic fauna. The rock squirrels yielded three species of fleas [*Oropsylla montanus* (Baker), *Echidnophaga gallinacea* Westwood, and *Hoplopsyllus anomalus* Baker], two species of sucking lice [*Linognathoides laeviusculus* (Grube) and *Enderleinellus suturalis* Osborn], and one mite [*Androlaelaps fahrenholzi* (Ewing)]. All of these, except for the lice, are new county locality records for this host. *Linognathoides laeviusculus* represents a new state locality record and *Enderleinellus suturalis* represents a new host record for *Spermophilus varigatus grammurus*.

INTRODUCTION

The ectoparasites of ground squirrels have received limited attention in the southwestern United States (Whitaker and Wilson 1974), with the greatest emphasis placed in Utah (Allred and Beck 1966, Juelson 1970). Due to the geographic distribution of the eight known subspecies of *Spermophilus varigatus* (Erxleben), the majority of work for this species has focused on *S. v. utah* (Merriam) (Stark 1958, Allred and Beck 1966, Juelson 1970, Jenkins and Grundmann 1973). This has resulted in an underrepresentation of the ectoparasites from the other subspecies of *S. varigatus* throughout its range. Only four works, one from New Mexico (Holdenried and Morlan 1955) and three from Texas (Eads and Hightower 1950, Layton 1973, Richerson et al. 1992), have reported the ectoparasites of *S. v. grammurus*. This is surprising in that *S. v. grammurus* possesses the broadest distribution of all eight subspecies (Oaks et al. 1987). On this basis, an investigation into the ectoparasites associated with *S. v. grammurus* was conducted in the middle of its known range, southwestern New Mexico.

METHODS AND MATERIALS

Eight *S. v. grammurus* were collected by W. M. Shriner in Water Canyon, located in the Magdalena Mountains, 30 km W Soccorro, Socorro Co., New Mexico, during August 1986 as part of a study of spatial relationships and dispersal patterns (Shiner and Stacey 1991). These hosts were maintained at the Indiana State University Vertebrate Collection (ISUVC) as frozen skins in individual plastic bags until examined for ectoparasites.

Ectoparasites were recovered by first thawing the skins and processing them individually using the washing technique outlined by Whitaker et al. (1993). Filters were observed for

the presence of ectoparasites, which were removed using fine forceps and directly slide mounted in PVA medium (Bioquip Products, Gardena, CA). Hosts were then examined under magnification using an Olympus SZH zoom dissection scope while parting the fur with probes (Whitaker et al. 1993). This second technique was applied in an attempt to recover parasites clinging to the fur or embedded in the flesh, which would be missed by the washing technique alone. Specimens recovered from examining the fur with a dissection scope were also slide mounted in PVA medium for later identification. Specimens were identified using Kim et al. (1986) for the lice; Lewis et al (1988) and Pratt (1956) for fleas; and Allred and Beck (1966) for mites. Prevalence and mean intensity are reported for each parasite following Margolis et al. (1982), including whether the parasite represents a new host locality record, new host state locality record, or a new host county locality record. Voucher specimens of ectoparasites were deposited into the Indiana State University Vertebrate Collection Ectoparasite Depository (ISUVCED), and seven of the hosts were prepared as museum study skins and deposited into the ISUVC (ISUVC 6854-6860).

RESULTS AND DISCUSSION

We examined eight *S. v. grammusus* for ectoparasites, recovering three species of flea, two of lice, and one mite (Table 1). The prevalence and mean intensity of each parasite is reported, and new records are indicated.

TABLE 1. Ectoparasites of *Spermophilus variegatus grammurus* from Soccorro Co., New Mexico.

Parasite	# Recovered	Prevalence [a]	Mean Intensity [b]
Siphonaptera			
Echidnophaga gallinacea Westwood [c]	13	37.5	3.7
Hoplopsyllus anomalus Baker [c]	11	25.0	6.5
Oropsylla montanus (Baker) [c]	2	25.0	1.0
Anoplura			
Linognathoides laeviusculus (Grube) [d]	21	50.0	5.25
Enderleinellus suturalis Osborn [e]	5	12.5	5.0
Acarina			
Androlaelaps fahrenholzi (Ewing) [c]	4	25.0	2.0

[a] Prevalence reported as a percentage
[b] Mean Intensity as average number of parasites per infected host
[c] New County Host Locality Record
[d] New State Host Locality Record
[e] New Host Record

Anoplura: Haematopinidae: *Linognathoides laeviusculus* (Grube). This species of louse is found from across Eurasia, Canada, and south through the western United States and Mexico. It has been collected primarily from ground squirrels, including the following species in North America: *Spermophilus armatus* Kennicott, *S. beecheyi* (Richardson), *S. beldingi* Merriam, *S. brunneus* (Howell), *S. columbianus* (Ord), *S. dauricus* Brandt, *S. elegans* Kennicott, *S. franklinii* (Sabine), *S. lateralis* (Say), *S. parryii* (Richardson), *S. pygmaeus* (Pallas), *S. richardsonii* (Sabine), *S. townsendii* Bachman, *S. tridecemlineatus* (Mitchill), *S. undulatus* (Pallas), *S. variegatus, S. washingtoni* (Howell), *Ammospermophilus leucurus* (Merriam), *Marmota flaviventris* (Audubon and Bachman), *Tamias minimus* Bachman, *Perognathus parvus* (Peale), and *Peromyscus maniculatus* (Wagner) (Eads and Hightower 1950, Juelson 1970, Jenkins and Grundmann 1973, Kim

and Adler 1982, Kim et al. 1986, Yenson et al. 1996). These lice were commonly found throughout the body fur on the dorsal and lateral sides, while nits were concentrated about the junction of the torso and neck on the lateral surfaces. Although this louse is the most common species recovered from ground squirrels, previous records from the state report *Neohaematopinus citellinus* Ferris, making *S. v. grammurus* a new state host locality record.

Anoplura: Hoplopleuridae: *Enderleinellus suturalis* (Osborn). This louse is commonly found throughout the western United States and Canada. It has been found on numerous rodents, including the following species: *Spermophilus beldingi, S. franklini, S. lateralis, S. medrensis* (Merriam), *S. mexicanus* (Erxleben), *S. richardsonii, S. spilosoma* (Bennett), *S. tereticaudus* Baird, *S. townsendii, S. tridecemlineatus, Cynomys gunnisoni* (Baird), *C. leucurus* Merriam, *Ammospermophilus harrisii* (Audubon and Bachman), and *A. nelsoni* (Merriam) (Jenkins and Grundmann 1973, Kim 1966, Kim et al. 1986). This louse was found throughout the body fur on the dorsal and lateral surfaces. Due to the presence of *L. laeviusculus* on the same host on which *E. suturalis* was recorded, and the age and condition of the specimens, nits were unable to be differentiated between lice. All nits observed were located at the junction of the torso and neck on the lateral surfaces. Although it has been reported previously in the state (Kim et al. 1986), this is the first record from *S. v. grammurus*, making it a new host record.

Siphonaptera: Ceratophyllidae: *Oropsylla montana* (Baker). This flea, formerly included in the genus *Diamanus*, has been found from a variety of hosts across the southwest and Pacific Coast of the United States as far east as Montana. Common rodent hosts with which it is known to associate include *Peromyscus maniculatus, P. truei* (Shufeldt), *Neotoma cinerea* (Ord), *N. fuscipes* Baird, *Aplodontia rufa* (Rafinesque), *Thomomys bottae* (Eydoux and Gervais), *T. monticola* Allen, *Tamiasciurus douglasi* (Bachman), *Reithrodontomys megalotis* (Baird), *Spermophilus armatus, S. beecheyi, S. beldingi, S. columbianus, S. lateralis, S. townsendii, S. variegatus utah, S. v. grammurus, Ammospermophilus leucurus* (Eads and Hightower 1950, Holdenried and Morlan 1955, Stark 1958, Hubbard 1968, Jenkins and Grundmann 1973, Richerson et al. 1992). The collection of this flea from Soccorro County represents a new county host locality record on *S. v. grammurus*.

Siphonaptera: Pulicidae: *Echidnophaga gallinacea* Westwood. This flea, also known as the stick-tight flea, is known from Eurasia and across the breath of North America, ranging from New York to Oregon and southward into warmer regions. A common flea on domestic fowl, it now infests both domestic and wild mammals of North America. It has been recovered from over seventy species of birds and mammals (Costa Lima and Hathaway 1946), including the following rodents: *Chaetodipus hispidus* Baird, *Dipodomys merriami* Mearns, *Rattus norvegicus* (Erxleben), *R. rattus* (L.), *Peromyscus maniculatus, P. boylii* (Baird), *Neotoma albigula* Hartley, *N. lepida* Thomas, *N. micropus* Baird, *Microtus californicus* (Peale), *Mus musculus* L., *Sigmodon hispidus* Say and Ord, *Spermophilus beecheyi, S. spilosoma* Bennett, *S. tereticaudus* Baird, *Spermophilus varigatus grammurus, S. v. utah*, and *Ammospermophilus leucurus* (Eads and Hightower 1950, Pratt and Good 1954, Holdenried and Morlan 1955, Stark 1958, Hubbard 1968, Jenkins and Grundmann 1973, Richerson et al. 1992). This fleas were found anchored to the face around the mouth and extending downward toward the neck. Although this flea has been recovered from Santa Fe (Holdenreid and Morlan 1955) and Grant Counties (Hubbard 1968), New Mexico; it represents a new county host locality record on *S. v. grammurus* in Soccorro County.

Siphonaptera: Pulicidae: *Hoplopsylla anomalus* Baker. This species is known to occur from Utah and Colorado, west to the Pacific coast, and south into northern Mexico. It is primarily found on *S. beecheyi*, and *S. varigatus*, but has also been found on *Rattus norvegicus, Sylvilagus auduboni* (Baird), *Spermophilus armatus, S. lateralis, S. townsendii, Ammospermophilus leucurus, Cynomys gunnisoni*, and *Neotoma albigula* (Holdenried and

Morlan 1955, Stark 1958, Hubbard 1968, Jenkins and Grundmann 1973, Richerson et al. 1992). *Hoplopsylla anomalus* has been recovered previously from *S. v. grammurus* in New Mexico in Santa Fe County (Holdenreid and Morlan 1955) and Grant County (Hubbard 1968), making this collection a new county host locality record for Soccorro County.

Acarina: Laelapidae: *Androlaelaps fahrenholzi* (Berlese). This mite possesses a nearly cosmopolitan distribution, being found in Eurasia, North America (Whitaker and Wilson 1974), Central America (Strandtmann 1949), and South America (Furman 1972). Whitaker and Wilson (1974) reported 118 mammalian hosts for *A. fahrenholzi* in North America north of Mexico, including rodents, insectivores, marsupials, bats, lagomorphs, and carnivores. Since that time, Whitaker (pers. comm.) has compiled 42 additional species that have been found to harbor this generalist mite. Members of the genus *Spermophilus* that have hosted *A. fahrenholzi* are the following: *Spermophilus armatus, S. beecheyi, S. beldingi, S. brunneus, S. columbianus, S. franklinii, S. lateralis, S. mexicanus, S. mollis* Kennicott, *S. parryii, S. richardsonii, S. spilosoma, S. tereticaudus, S. townsendii, S. tridecemlineatus,* and *S. washingtoni* (Strandtmann 1949, Eads and Hightower 1950, Holdenried and Morlan 1955, Jenkins and Grundmann 1973, Whitaker and Wilson 1974, Yensen et al. 1996). This mite was recovered from throughout the body fur, as well a single specimen found on the hindfoot of a host. The records presented here represent new county host locality records for *A. fahrenholzi* on *S. v. grammurus* in Soccorro County, as it has been collected only in Santa Fe County to this point (Holdenreid and Morlan 1955).

GENERAL DISCUSSION

Previous studies of ectoparasites of ground squirrels have used either visual examination of the host (Yensen et al. 1996), or the washing technique (Jenkins and Grundmann 1973) to recover parasites. Individually, these techniques make accurate representation of abundance and species diversity difficult to determine. By combining the washing and dissection microscope techniques, we are confident that all parasites were observed and counted. The washing technique provided a count of the number of individuals present for species that could be easily removed from the host with disturbance. The microscope technique, however, was useful for recovering arthropods that were anchored to the host, such as *E. gallinacea* , and also in provided information about site preference for ectoparasites on the host. The dissection microscope technique is labor intensive, making a combination of both techniques valuable in ensuring that all ectoparasites are efficiently sampled. Although ticks and chiggers were recovered in other studies (Juelson 1970, Keirans and Clifford 1974), we failed to detect them in our study. Our techniques have proven successful at recovering both mites on the fur (such as Glycephagidae, Listophoridae, and Trombiculidae) and *Ixodes* ticks (Whitaker et al. 1993), suggesting that these parasites were not present on the animals that we sampled.

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LEAF SURFACE SELECTION BY *BEMISIA ARGENTIFOLII* [1] CRAWLERS

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ABSTRACT

The preference of *Bemisia argentifolii* Bellows & Perring crawlers for the adaxial or abaxial leaf surface was investigated in the laboratory using cheeseweed, *Malva parviflora* L., leaves as the substrate. In free choice experiments, crawlers exhibited a negative phototactic response, selecting the leaf surface opposite from the incoming light direction on which to settle. When crawlers were transferred directly to either the adaxial or abaxial surface, they remained and settled on the surface on which they were initially placed, regardless of leaf orientation or direction of incoming illumination. Crawlers maintained in total darkness failed to move from the location of their original placement.

INTRODUCTION

Silverleaf whitefly, *Bemisia argentifolii* Bellows & Perring, crawlers are capable of moving several centimeters along a plant stem or between plants in search of an acceptable leaf on which to settle and complete their development (Summers et al. 1996). Movement is upward on the plant with crawlers displaying a positive phototaxis rather than a negative geotaxis (Summers 1997). The only other mobile whitefly stage is the adult. Adult *Bemisia tabaci* (Gennadius) are positively phototactic during flight (van Lenteren and Noldus 1990, Blackmer and Byrne 1994, Byrne and Blackmer 1996) and adults of *B. argentifolii* are attracted to light under both field and laboratory conditions (Chu et al. 1995). Upon landing, however, adults of both species immediately move to the underside of the leaf to feed and oviposit (Chu et al. 1995). Chu et al. (1995) suggested this behavior was a positive geotactic response while van Lenteren and Noldus (1990) considered such movement a response to shade rather than positive geotaxis or negative phototaxis. The adults of both species, however, also oviposit on the upper leaf surface and immature feeding and development on the adaxial surface has been reported by Lynch and Simmons (1993), Simmons (1994, 1999) and Chu et al. (1995). Van Lenteren and Noldus (1990) summarized the suggestions of various authors regarding the preference of *B. tabaci* for leaf surface, but noted that insufficient experimental data were available at that time to determine the factors leading to selection of the abaxial surface by the adults as the preferred feeding and oviposition site.

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This paper reports the results of studies conducted to determine the selection of leaf surface by the crawler stage. Laboratory studies were conducted to determine if *B. argentifolii* crawlers preferred to settle on the adaxial or abaxial leaf surface and if that selection was a phototactic response.

MATERIALS AND METHODS

Free Choice Experiments. Cheeseweed, *Malva parviflora* L., leaves were used in all experiments. Leaves were cut from plants in the field, returned to the laboratory, and examined microscopically to be sure they were free of whitefly eggs and immatures. A 2.5cm foam rubber strip was wrapped around the petiole, 60cm below the leaf blade, and the petiole was then inserted into a vial of water. The foam rubber strip formed a tight seal with the neck of the vial, preventing water from leaking out. Ten newly emerged *B. argentifolii* crawlers, reared on greenhouse cotton, *Gossypium hirsutum* L., were individually transferred from a cotton leaf to each of 15 petioles at a point 50mm below the leaf blade. Crawlers were observed microscopically for 60sec after transfer and if they failed to move they were presumed to have been injured and were removed and replaced with another individual. Vials holding the cheeseweed leaves were placed in a styrofoam frame, which was in turn placed in a growth chamber and left undisturbed for 72h. Experiments were conducted in a Percival E 30-B growth chamber (Percival, Boone, IA) at 24 ± 1.0° C, 80 ± 15% RH, and a 24:0 L:D photoperiod. The photon flux density, measured with a LI-COR Quantum Sensor (LI-COR, Lincoln, NE), was 105.7 μmol m^{-2} s^{-1} at the level of the leaf blade. After 72h, leaf setups were returned to the laboratory and examined microscopically. The number of individuals reaching the leaf blade and their location (abaxial or adaxial surface) was recorded.

The following combinations of leaf orientation and light position were evaluated. In Experiment 1, leaves were placed in an upright position with the light source above the leaf blade; and, in Experiment 2, leaves were placed in an inverted position with the light source below the leaf blade. In both experiments, the light source was on the opposite end of the leaf from where the crawlers were placed. In Experiments 3 and 4, leaves were positioned as in Experiments 1 and 2, respectively, but were maintained in the growth chamber in total darkness.

Crawler Placement Experiments. To further test the hypothesis that crawlers exhibited a positive or negative geotaxis or phototaxis in the selection of a feeding site once on the leaf, an additional set of experiments was conducted. Ten crawlers were selected and placed 2cm from the leaf margin on either the abaxial or adaxial surface of a cheeseweed leaf whose petiole had been inserted into a vial of water as previously described. Fifteen leaves (150 crawlers) were used for each experiment. The leaf setups were placed in a Percival E 30-B growth chamber for 72h under temperature and humidity conditions described above, after which they were returned to the laboratory, examined under the microscope, and the location (abaxial or adaxial surface) and settling of all crawlers recorded.

The following combination of crawler placement on the leaf surface, leaf orientation, and light position were evaluated. In Experiment 5, crawlers were placed on the abaxial or adaxial surface, leaves were oriented upright, and light source was above the leaves. In Experiment 6, crawlers were placed on the abaxial or adaxial surface, leaves were oriented in an inverted position, and the light source was below the leaves. In Experiment 7, crawlers were placed on the abaxial or adaxial surface, and the setups were kept in the dark.

Crawler settling was assessed by Student's *t*-test or ANOVA (Abacus Concepts, 1989). All original percentage values were transformed to arcsine $\sqrt{}$ percentage before analysis and back transformed for presentation (Snedecor 1956).

RESULTS

Free Choice Experiments. In Experiments 1 and 2, the light was positioned to illuminate the adaxial leaf surface. In both experiments, approximately 96% of the crawlers settled on the surface facing away from the direct light source (Table 1). In Experiment 2, a significant ($P < 0.05$) majority of the crawlers settled on the true morphological abaxial surface away from the light even though that surface faced upward. When the leaf setups were maintained in total darkness (Experiments 3 and 4) crawlers failed to move from their point of origin to the leaf blade (Table 1).

TABLE 1. Mean Percentage (± SEM) of *B. argentifolii* Crawlers Under Different Leaf Orientation and Illumination Conditions Settling on the Morphological Abaxial or Adaxial Surface in a Free Choice Experiment.

Expt. No.	Leaf Orientation [a]	Light Position [b]	Percentage of Crawlers on Abaxial Surface	Percentage of Crawlers on Adaxial Surface	*t*
1	Upright	Above	96.1 ± 1.4	3.9 ± 1.4	5.02 [d]
2	Inverted	Below	95.6 ± 2.3	4.4 ± 1.2	8.21 [d]
3	Upright	Dark	– [c]	–	–
4	Inverted	Dark	–	–	–

[a] Upright = Normal leaf position with leaf blade pointing up. Inverted = leaf inverted with leaf blade pointing downward.

[b] Above = light source over head and above leaf setup. Below = light source underneath and below setup.

[c] No crawlers reached the leaf blade in either experiment 3 or 4.

[d] Means significantly different at $P < 0.05$.

TABLE 2. Mean (± SEM) Percentage of Crawlers Settling on the Abaxial or Adaxial Leaf Surface Following Their Direct Placement on the Leaf [a].

Crawler placement [b]	Leaf Orientation	Light Position [b]	Percentage of Crawlers on Abaxial Surface	Percentage of Crawlers on Adaxial Surface	*t*
Adaxial	Upright	Above	8.7 ± 2.2	91.3 ± 3.9	18.72 [c]
Abaxial	Upright	Above	98.6 ± 1.6	1.4 ± 0.9	44.89 [c]
Adaxial	Inverted	Below	8.0 ± 5.8	92.0 ± 5.4	21.28 [c]
Abaxial	Inverted	Below	96.5 ± 2.4	3.5 ± 1.3	25.90 [c]
Adaxial	Upright	Dark	0.0 ± 0.0	100.0 ± 0.0	35.31 [c]
Abaxial	Inverted	Dark	100.0 ± 0.0	0.0 ± 0.0	36.42 [c]

[a] See footnotes in Table 1 for description of upright, inverted, above and below.

[b] Leaf surface on which crawlers were initially placed.

[c] Means significantly different at $P < 0.01$.

Crawler Placement Experiments. When crawlers were placed directly on the abaxial or adaxial leaf surface, they displayed a significant ($P < 0.01$) tendency to settle and begin feeding on that surface (Table 2). Neither leaf orientation nor the direction of incoming light appeared to effect settling. While some movement to the opposite leaf surface occurred, it was not significant ($F = 0.70$; df = 3, 42; $P > 0.05$). When the leaf setups were maintained in darkness, no crawlers moved from the surface on which they were placed, regardless of leaf orientation (Table 2).

DISCUSSION

Bemisia argentifolii crawlers exhibited different behavioral characteristics in the free choice and the crawler placement experiments. They settled on the leaf surface opposite incoming light in the free choice experiment, but remained on the surface where they were originally positioned in the placement experiments, regardless of light orientation. Under free choice conditions, their behavior resembled that of the adult. Adult *B. argentifolii* and *B. tabaci* are positively phototactic in flight (Chu et al. 1995), but upon landing, move immediately to the underside of the leaf to begin feeding and ovipositing (van Lenteren and Noldus 1990, Chu et al. 1995). While searching for an acceptable leaf, *B. argentifolii* crawlers are also positively phototactic (Summers 1997). The current study suggests that once they find the leaf, however, they appear to exhibit a negative phototaxis, moving to the leaf surface away from the incoming light (Table 1). In a study on *B. argentifolii* crawler dispersal, Summers et al. (1996) found that 80 to 100% of the crawlers chose to settle on the abaxial surface of prickly lettuce, *Lactuca serriola* L., annual sowthistle, *Sonchus oleraceus* L., and broccoli, *Brassica oleracea* var. *botrytis* L., when incoming illumination was from above the plants.

When crawlers were transferred directly to either the adaxial or abaxial surface, as would be the case if the adult had oviposited there, a significant majority remained and settled on the surface where they were initially placed. Such crawlers failed to show either a negative phototaxis or positive geotaxis regardless of leaf orientation. The small percentage of individuals moving to the opposite leaf surface probably did so by random wandering during their search for an acceptable leaf vein on which to initiate feeding. The results from the direct placement experiments agree with those of Simmons (1999) who concluded that the impetus to move from the upper leaf surface appeared to be a response to feeding and tactile cues rather than a response to geotropic or phototropic stimuli. In studies in which adults oviposited on either the abaxial or adaxial leaf surface, Simmons (1999) found a strong host effect in the settling of *B. argentifolii* crawlers eclosing from the eggs. Crawler movement from the upper to the lower leaf surface was high (80%) on pepper, *Capsicum frutenscens* L., moderate (55%) on cantaloupe, *Cucumis melo* L. and cowpea, *Vigna unguiculata* (L.), and low (18 to 30%) on collard, *Brassica oleracea* L. *acephala* DC and tomato, *Lycopersicon esculentum* Mill., respectively. Cheeseweed leaves are highly vascularized with many small veins near the leaf surface on both the abaxial and adaxial surface.

The failure of the crawlers to disperse when maintained in darkness supports my earlier conclusions that some minimal light intensity is necessary to stimulate activity (Summers 1997). *B. argentifolii* crawlers maintained in total darkness moved only a few millimeters from their point of origin (Summers 1997), and in the current study, none moved from their initial placement on one leaf surface to the opposite leaf surface. Adult whiteflies apparently also require a minimal light intensity to promote activity. Bellows et al. (1988) reported that adult *B. tabaci* failed to fly at night even though temperatures were

high enough to promote flight activity. These results do differ, however, from those of Simmons (1999) who found that even under complete darkness, approximately 70% of the crawlers eclosing from eggs deposited on the upper surface of pepper leaves moved to the lower surface.

Both my studies and those of Simmons (1999) suggest a possible interaction between host influence, response to tactile stimuli, and phototaxis. Crawler behavior is difficult to evaluate and additional studies are necessary to accurately assess this interaction.

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BEETLE (COLEOPTERA) DIVERSITY IN MIXED PINE-HARDWOOD STANDS IN THE OUACHITA HIGHLANDS FIVE YEARS FOLLOWING TREE HARVESTS

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ABSTRACT

Beetles (Coleoptera) were trapped within forest stands that had received various even- aged (clearcuts and shelterwood cuts) or uneven-aged (group selection cuts and single-tree selection cuts) management strategies using semiochemical-baited Lindgren funnel traps, carrion-baited pitfall traps and non-baited malaise traps. A total of 6,322 beetles in 45 families were captured. Beetle abundance and family richness were highest in the Lindgren funnel traps. Family richness and family diversity were lowest in the non-harvested control plots and there was a significant negative correlation between family diversity and stand basal area. The results indicate that stand disturbance in the form of tree harvesting can increase some measures of biodiversity.

INTRODUCTION

Insects are abundant throughout terrestrial ecosystems (for examples see May 1989, Wheeler 1990, Gaston 1991) where they play a role in most ecosystem processes and have a major impact on energy flow through the systems (Samways 1994). Because of their abundance, species richness and functional importance, insects are useful indicators of ecosystem change (Rosenberg et al. 1986). In the past, forest stand management research that involved insects primarily focused on species of economic importance. However, stand management practices act as disturbances that can potentially impact other, less economically important insect species found within the forest. Studies that have used insects to examine environmental impacts have utilized a range of taxonomic groups from the total compliment of insects present (i.e., Stork 1991) to a single family of beetles such as the tiger beetles (Coleoptera: Cicindelidae) (Pearson and Cassola 1992). The choice of taxonomic group(s) for monitoring change should be related to the system under examination and the potential impact of the applied treatment on the selected taxonomic group(s). Therefore, a taxonomic group does not need to be rare to be useful as a bio-indicator of change within a system. In addition, many past investigations have focused on examining the impact on insect diversity either of intensive stand management practices such as clearcutting (McIver et al. 1992) or the use of potentially non-specific control tactics such as some insecticides (Martinat et al. 1988). Fewer studies have examined the impact of selective harvesting techniques that remove less total tree volume and result in uneven-age stands. One study that did examine such management practices reported that the species richness was higher on disturbed sites and that the abundance of braconid wasps (Hymenoptera: Braconidae) was not significantly different between disturbed and control plots (Lewis and Whitfield 1999). These authors

did report a change in the species complex for braconids among the stands that had received various management tactics.

The objective of this research was to compare the overall abundance of beetles, along with the family richness, diversity and evenness of three guilds of beetles present within forest stands five years after the stands had received various harvesting treatments. The beetles that were examined during discrete time intervals included the guild of carrion-feeding beetles, the pine-infesting bark beetles (Scolytidae) and their associates and a general survey of beetles flying through the stands. The tree-harvesting treatments ranged from the total removal of trees from a stand to the selection and removal of individual trees.

MATERIALS AND METHODS

Fifteen plots, each approximately 16 hectares in size, were used in this study. These 15 plots represent a sub-sample of 52 plots that were established by the USDA-Forest Service to examine stand-level forest management strategies in the Ouachita Highlands (for an overview of the experimental design, see Baker 1994). Overstory trees were over 70 years old in all of the plots. All plots had a southern to southwestern aspect and were located in the Ouachita Highlands of western Arkansas and eastern Oklahoma. Thirteen of the plots were in Ouachita National Forest and the other two plots were in Ozark National Forest. Overall, shortleaf pine, *Pinus echinata* Mill., was the dominant tree species in the plots, but various hardwood species were also important components (Guldin et al. 1994).

Each plot received one of five tree-harvesting treatments in (May-September, 1993). Each treatment was replicated three times. The five treatments were control, single-tree selection cut, group-selection cut, pine shelterwood cut and clearcut. No tree harvesting occurred in the control plots. Some trees were harvested in the single-tree selection plots, but a residual basal area of 10.5-15.1 m^2 per hectare was retained. Within the group-selection cuts, trees were harvested to create openings that ranged from 0.04 to 0.4 hectares. Outside of these openings, pines were thinned to a basal area of 16.3-18.6 m^2 per hectare. The single-tree selection cuts and group-selection cuts are referred to as uneven-aged stand management because the resulting stand will consist of trees in a mixture of age classes. The pine shelterwood cuts had most of the trees removed from the plots, but retained a total of 50-100 of the largest pines per hectare (7.0-9.3 m^2 per hectare) on each site. All trees (except 0.5-1.2 m^2 per hectare of hardwoods that were retained as den trees) were removed from the plots that received the clearcut treatment. The shelterwood cuts and clearcuts are referred to as even-age stand management because the resulting stands of trees will be dominated by a single age class. For a more thorough description of the treatments see Baker (1994).

Within the plots, a variety of trapping techniques were used to identify beetle populations. Malaise traps were used to sample the beetles flying in the plots. These traps were placed in the plots during (26 May-5 June, 1998) and beetles were collected after a three-day trapping period. Carrion-baited pitfall traps (Lomolino et al. 1995) were used on ten of the plots during this same time period. An eight-trap transect with traps being approximately 25 m apart was established in each plot and beetles collected from the traps for three consecutive days. The carrion was replaced as needed. Two Lindgren funnel traps (1.15 m long) were placed in each of ten plots during 1999. One trap per plot was baited with ethanol and α-pinene, and the other trap was baited with these two compounds plus frontalin, the aggregation pheromone of the southern pine beetle, *Dendroctonus frontalis* Zimmermann. These traps were placed in the study plots on 5-6

May and beetles were collected on 26-27 May. All of the respective traps or trap transects were placed near the middle of the 16-hectare stands. This placement was chosen to minimize any potential edge effects.

All of the beetles collected in the various traps were identified to family using available keys (Borror et al. 1976, Arnett 1963). The abundance (number of beetles captured), family richness (number of families of Coleoptera) and family diversity were calculated for each plot based on the individual trapping techniques and by combining all of the trapping data. These same measurements were calculated for all plots that had received each of the five tree harvesting treatments. The Shannon-Weaver diversity index was used to calculate family diversity (Price 1975). The equation for the Shannon-Weaver diversity index is $H' = -\Sigma(p_i \log_e p_i)$, where p_i is the number of beetles in family 'i' divided by the total number of beetles trapped.

Beetle abundance, family richness and family diversity were compared among treatments and with the data collected on some of these sites in 1993 (Carlton et al. 1994). Differences in these three parameters were compared among management schemes (control, uneven-age management and even-age management) using a distribution-free Kruskal-Wallis rank sums test with a Chi-square distribution-free multiple comparisons test (Hollander and Wolfe 1973) performed when the *p*-value associated with the individual Kruskal-Wallis tests was less then 0.15. The *p*-value for rejection of the null hypothesis was set relatively high because of the small sample size for the data set. Additionally, for the seven plots that had all three types of traps, a Pearson's correlation analysis (Ott 1977) was conducted to determine if there were trends in beetle abundance, family richness or family diversity related to stand basal areas reported in Baker (1994). All statistical tests were conducted using STATISTIX analytical software (Analytical Software 1998).

RESULTS AND DISCUSSION

A combined total of 6,322 individual beetles in 45 families were captured during this study (Table 1). Greater numbers of beetles were consistently captured in the Lindgren funnel traps and the pitfall traps versus the malaise traps. Within each treatment, the total family richness (number of families) was highest for beetles captured in the Lindgren funnel traps followed by the malaise traps and then the pitfall traps. The three families that were most frequently captured (Staphylinidae, Scolytidae and Histeridae) comprised 49% (n = 3,090) of the total number of beetles captured during this study.

Family richness was lowest in the control plots followed by the plots that had received even-age management treatments (clearcuts and shelterwood cuts) and the plots that had received uneven-age management treatments (group selection cuts and single-tree selection cuts) (Table 1). Of the 45 families of beetles captured during this study, 26 (58%) were captured in plots from each treatment; 14 families (31%) were captured only in plots that had received some type of harvesting disturbance, and 2 families (4%) were captured only in control plots.

Family diversity, as measured by the Shannon-Weaver diversity index, was also lowest in the control plots (Table 1). However, the next lowest diversity was measured in the uneven-age management plots (group selection cuts and single-tree selection cuts) and the highest measurements of family diversity were in the plots that had received the even-age management treatments (clearcuts and shelterwood cuts).

TABLE 1: The Abundance of Beetle Families and Summary Data for Beetles Collected During 1998 and 1999 Using all Three Types of Traps (Malaise Traps, Lindgren Funnel Traps and Carrion-Baited Pitfall Traps) in Plots Treated in 1993 with Various Tree-Harvesting Strategies.

	Treatment Category				
Family	Control	Single-tree	Group-Select	Shelterwood	Clearcut
Carabidae	23	20	36	8	9
Hydrophilidae	21	7	30	17	0
Staphylinidae	255	277	305	190	170
Pselaphidae	2	3	2	3	4
Silphidae	52	120	162	86	114
Leiodidae	3	2	15	24	2
Histeridae	29	191	425	28	247
Scarabaeidae	67	135	191	76	109
Eucinetidae	0	8	1	6	0
Byrrhidae	0	1	0	0	0
Ptilodactylidae	0	0	0	1	0
Buprestidae	6	4	2	2	6
Elateridae	64	79	125	95	30
Throscidae	5	0	0	0	0
Eucnemidae	0	6	7	1	1
Phengodidae	1	1	1	0	3
Cantharidae	4	2	3	7	55
Lycidae	4	6	12	1	6
Lampyridae	0	1	0	0	3
Dermestidae	6	10	14	9	4
Anobiidae	241	18	77	51	16
Bostrichidae	0	3	1	1	0
Lyctidae	0	8	8	1	1
Trogositidae	49	16	11	4	2
Cleridae	7	12	14	10	8
Melyridae	0	3	0	5	0
Mordellidae	3	36	12	10	15
Tenebrionidae	6	10	6	19	7
Melandryidae	1	9	1	5	77
Oedemeridae	1	2	4	5	11
Pedilidae	1	0	0	0	0
Nitidulidae	0	0	1	1	6
Cucujidae	7	9	5	11	8
Endomychidae	0	0	0	0	1
Coccinellidae	1	12	4	10	10
Erotylidae	3	1	1	1	0
Phalacridae	0	0	1	3	0
Colydiidae	0	1	3	0	2
Cerambycidae	18	8	20	19	22
Chrysomelidae	9	12	14	10	39
Bruchidae	0	1	2	1	2

Table 1: continued

Anthribidae	1	0	1	0	0
Curculionidae	40	83	31	28	15
Platypodidae	0	1	0	0	0
Scolytidae	270	356	241	88	18
Beetle Abundance	1200	1474	1789	837	1023
Family Richness	31	38	37	36	33
Family Diversity	2.3107	2.4344	2.3993	2.6970	2.5150

Beetle diversity was sampled directly following harvesting in 1993 on three of the current study sites (Carlton et al. 1994). The plots sampled in 1993 included a control plot, one group-selection cut plot and one shelterwood cut plot. There was also one additional plot sampled that was dominated by young saplings that was not part of the original USDA-Forest Service project design. Beetles representing 51 families were captured on these four plots in 1993 (Carlton et al. 1994). The number of families captured (51) is comparable to the 45 families reported here. Other investigations at other sites have reported that various insect groups including ground beetles (Coleoptera: Carabidae) and braconid wasps have similar to increased measures of abundance and/or richness following disturbance (Beaudry et al. 1997, Lewis and Whitfield 1999). These two prior studies and others (Coyle 1981, McIver et al. 1992) also demonstrated a difference in the species complex among disturbed and undisturbed stands. Differences in species composition among the various types of stand management treatments have also been reported for three families of beetles from the current study plots (the Pselaphidae [Carlton et al. 1994], Scarabaeidae and Silphidae [Cook in press]).

There were no significant differences in beetle abundance in the carrion-baited pitfall traps or the semiochemical-baited Lindgren funnel traps among the three management schemes (Table 2). However, there were significantly more beetles captured by the malaise traps in the even-age management plots compared with the control plots. Beetle abundance in the malaise traps from the uneven-age management plots did not differ significantly from the other two treatments.

TABLE 2: Abundance (Mean ± SEM) of Beetles Captured in the Three Types of Traps (Semiochemical-Baited Lindgren Funnel Traps, Carrion-Baited Pitfall Traps and Non-Baited Malaise Traps) Placed in Plots That Had Received Various Stand Management Treatments (Control = No Harvesting, Uneven-Age Management = Single-Tree Selection Cuts or Group-Selection Cuts and Even-Age Management = Shelterwood Cuts or Clearcuts).

Management	Abundance by trap type[a]		
Scheme	Funnel	Pitfall	Malaise
Control	420.5 ± 57.5 a	172.5 ± 13.5 a	4.7 ± 3.2 a
Uneven-age	369.8 ± 46.1 a	415.0 ± 92.2 a	20.7 ± 6.9 ab
Even-age	210.3 + 34.9 a	212.3 + 81.2 a	34.5 + 14.8 b

[a] Within a column, means followed by the same letter are not significantly different (distribution-free multiple comparisons test based on Kruskal-Wallis rank sums statistic).

The only significant difference in family richness was observed in beetles captured in the malaise traps (Table 3). Family diversity of beetles captured in the Lindgren funnel traps was significantly higher in the stands that had received even-aged

management treatments versus the untreated controls, with the stands that had received uneven-age management treatments being intermediate (Table 4).

TABLE 3: Richness (Mean ± SEM) of Beetle Families Captured in the Three Types of Traps (Semiochemical-Baited Lindgren Funnel Traps, Carrion-Baited Pitfall Traps and Non-Baited Malaise Traps) Placed in Plots That Had Received Various Stand Management Treatments (Control = No Harvesting, Uneven-Age Management = Single-Tree Selection Cuts or Group-Selection Cuts and Even-Age Management = Shelterwood Cuts or Clearcuts).

Management Scheme	Family richness by trap type[a]		
	Funnel	Pitfall	Malaise
Control	22.5 ± 2.5 a	5.5 ± 0.5 a	3.0 ± 2.0 a
Uneven-age	24.5 ± 2.6 a	6.8 ± 0.5 a	6.7 ± 1.5 ab
Even-age	24.7 + 0.3 a	6.3 + 0.8 a	8.7 + 1.0 b

[a] Within a column, means followed by the same letter are not significantly different (distribution-free multiple comparisons test based on Kruskal-Wallis rank sums statistic).

TABLE 4: Diversity (Mean ± SEM) of Beetles Families as Measured by the Shannon-Weaver Diversity Index Captured in the Three Types of Traps (Semiochemical-Baited Lindgren Funnel Traps, Carrion-Baited Pitfall Traps and Non-Baited Malaise Traps) Placed in Plots That Had Received Various Stand Management Treatments (Control = No Harvesting, Uneven-Age Management = Single-Tree Selection Cuts or Group-Selection Cuts and Even-Age Management = Shelterwood Cuts or Clearcuts).

Management Scheme	Family diversity by trap type[a]		
	Funnel	Pitfall	Malaise
Control	1.9048 ± 0.0324 a	0.9909 ± 0.3704 a	1.8461 ± -------- a
Uneven-age	2.0312 ± 0.2342 ab	1.3934 ± 0.0105 a	1.5160 ± 0.2145 a
Even-age	2.4880 + 0.0207 b	1.3414 + 0.0663 a	1.7253 + 0.1416 a

[a] Within a column, means followed by the same letter are not significantly different (distribution-free multiple comparisons test based on Kruskal-Wallis rank sums statistic).

There was also a significant negative correlation between basal area and family diversity ($r = -0.7664$; $p = 0.0445$) when the data from the three trap types were combined. However, there were not significant correlations between basal area and either beetle abundance ($r = 0.4636$; $p = 0.2947$) or family richness ($r = -0.3161$; $p = 0.4898$). The significant correlation indicates that as the disturbance increased in size (lower basal area) the measure of beetle family diversity increased so that even-age management practices such as clearcutting or shelterwood cuts tended to increase family diversity. Similar results have been reported for braconid wasps (Lewis and Whitfield 1999) and spiders (Coyle 1981).

Results from studies conducted in old fields suggest that small-scale habitat fragmentation is detectable at the community, guild and individual species levels (Crist and Ahern 1999, Golden and Crist 1999). Another report also indicated an increase in dung beetle (Coleoptera: Scarabaeidae) species richness and diversity in logged forest stands using reduced-impact techniques and suggested that such techniques better preserved the species assemblages from the primary forest (Davis 2000). Further, disturbed stands can have similar to higher abundance, species richness and diversity within individual families when compared with undisturbed stands (Beaudry et al. 1997,

Humphrey et al. 1999, Lewis and Whitfield 1999). The typical pattern in the current study was for the uneven-age stand management practices (single-tree harvesting and group-selection harvesting) to have measures of beetle abundance, family richness and family diversity that were intermediate between the control plots and the more disturbed even-age management plots. This may be the result of beetles that require openings being able to utilize these disturbed stands but maintaining the species complex that was present in the primary forest prior to the harvesting disturbance.

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APPLE SAMPLING IN PACKING HOUSES SUPPORTS THE SYSTEMS APPROACH FOR QUARANTINE CONTROL OF CODLING MOTH

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ABSTRACT

Sorting efficacy to eliminate arthropod-infested apples was studied in 17 grower lots at six packing houses from the beginning of the packing line to the final pack. The number of fruits examined include 28,000 before sorting, 14,376 from the cull bin, and 12,539 in the final pack. Also, an additional 10% of these totals were examined using a 30x microscope. In the larger survey, only one live codling moth, *Cydia pomonella* (L.) (Lepidoptera: Tortricidae), larva was found among the unsorted fruits, 12 live codling moth larvae were found in the culls, and none were found in the final pack. Codling moth damage was found in about 0.1% of the pre-sort, 1.9% in the culls, and only one fruit in the final pack. In the microscopic examinations, only one dead codling moth larva was found among the unsorted fruits, four dead codling moth larvae were found in the culls, and none were found in the final pack; codling moth damage occurred in about 0.5% of the presort, 1.8% in culls, and none in the final pack. The most prevalent arthropod collected was codling moth, followed by spiders. Microscopic examination increased efficacy of detecting codling moth from 0.01% to 0.03% in the presort, but only from 0.17% to 0.26% in the culls. Observations of apparent codling moth damage increased from 0.12% in the large survey to 0.98% in the microscope examination, but declined with the culls from 1.90% to 1.77%, respectively. Overall, observations of codling moth damage in the same lots were similar between visual inspection of the large survey and the microscopic examination. In both the large survey and in the microscope examinations, culling efficacy was found to be not directly influenced by packing line speed.

INTRODUCTION

Japan requires domestic apples to be treated for codling moth, *Cydia pomonella* (L.) (Lepidoptera: Tortricidae) (MAFF-Japan 1950). The current accepted procedure is a two-component quarantine treatment consisting of 55 days cold storage at 2.2°C, followed by a two-hour fumigation 56 g/m^3 of methyl bromide at 10°C. However, methyl bromide has been identified by the U.S. Environmental Protection Agency (EPA), under the Federal Clean Air Act (Anonymous 1990) and by the Montreal Protocol (Anonymous 1995), as an ozone depleter. The EPA has mandated the removal of this fumigant from the chemical register and the phase out of its production and import into the United States by 31 December 2005. Although methyl

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bromide fumigation has been exempted for postharvest quarantine, an alternative protocol will be needed eventually because the future of methyl bromide use is vulnerable due to price increases with reduced production, reduced availability from unreliable sources, or future restrictions under international agreements (Anonymous 1998).

The systems approach is an alternative to maintaining quarantine security in international commerce of fresh fruits (Jang and Moffitt 1994). This involves use of insect pest management in the orchard; reduction in the incidence of infestation at harvest and upon arrival at the packing house; removal of infested fruits by postharvest grading, sorting, and packing; pest mortality from prepacking storage; and inspection and certification of packed fruits for export (Moffitt 1997). This is not a recent concept, with origins arising from Baker (1939) who recognized that quarantine security is not breached until there is a mating pair from a consignment. Previous studies examining efficacy of packing house culling indicated the incidence of codling moth was extremely low (Moffitt 1990, Knight and Moffitt 1991). However, pest field control, types of cultivars grown, and computerization in commercial packing operations have all progressed since those studies were conducted.

Hence, the application of the systems approach would be advanced by a comprehensive survey of current packing house operations. Because culling on the packing line is based on visual observations, validation by microscopic examination would demonstrate culling efficacy. Furthermore, inspections on large numbers of fruits would indicate if cold storage contributes significantly to pest mortality. Finally, a thorough survey of different cultivars processed at various packing houses would indicate if fruit inspections are feasible in detecting the presence of pests in grower lots.

The objectives of our study were: 1) to measure the efficacy of culling in the packing house to eliminate codling moth infested and damaged fruits by following an examined lot from the dump tank to the packed cartons; 2) to measure mortality of codling moth larvae in culls after standard cold storage and 90-day controlled atmospheres-cold storage; 3) to compare reliability of visual inspections with microscope examinations for identifying infested damaged fruits; and 4) to evaluate the effectiveness of the proposed inspections that would be used in the systems approach to maintain quarantine security.

MATERIALS AND METHODS

At six packing houses and among 17 grower lots, fruits were sampled from harvested apples and apples in controlled atmosphere-cold storage. Samples were organized from a single grower's lot and included export quality fruits of major cultivars ('Red Delicious,' 'Golden Delicious,' 'Fuji,' and 'Gala'). In the large survey, each lot was randomly sampled ($n \simeq 1{,}800$) at the dump tank where the fruits entered the packing house. The fruits were examined visually and another group of apples (10% of the sample size) were inspected using 30x stereomicroscopes (StereoZoom 4, Bausch & Lomb, Rochester, NY). In both the large survey and the microscopic examinations, physical parameters (e.g., size, quality, damage, etc.) and pest information (e.g., species, viability, number and life stage, and type of damage) were recorded for each fruit, and all samples were returned that were not dissected for pest determination. Identical observations were made at the cull station and at the final pack using about the same number of fruits at both stations ($n \simeq 1{,}000$). Culling efficacy was determined by comparing fruit quality and infestation rate from three sampling locations. Larval survival was grouped by life stage and prior cold storage.

Routine inspection reports by the Washington State Department of Agriculture (WSDA) were reviewed for the same lots used in the study. These reports indicate number of fruits examined from the final pack and the number of codling moth larvae intercepted. This is standard procedure for packing high grade fruits intended for export.

RESULTS AND DISCUSSION

The large survey emphasized sampling fruits at the beginning of the packing line where nearly 10,800 'Fuji' and 8,500 'Golden Delicious' were examined (Table 1). Very few codling moths were found at this sample station (0.007% of all presort samples). Even in the cull station, < 0.2% of all fruits had codling moth. Codling moth damage was also low at the cull station (1.9% of these fruits). No codling moths were found among the 12,539 fruits in the final pack, and only one fruit was found with codling moth damage.

The microscope examinations were intended to verify the visual observations of the large survey. A subsample of 10% of the observed grower's lot was taken independently of the fruits used in the visual observations (Table 2). With increased magnification, greater detail can be observed than in the visual survey, such as for eggs or larval feeding sites by early instars, which would presumably result in more records of codling moth. Yet, the fruits examined before sorting still had very low incidence of codling moth (0.03%). Frequency of codling moths in cull fruits was higher (0.26%), but none were found in the final pack. Frequency of codling moth damage increased eightfold for the presorted fruits (0.98% of sample) over that of the large survey, but remained about the same for the cull fruits (1.77%). Microscope examination found no codling moth damage in the final pack. Similar inspections were conducted by the WSDA where 70,275 fruits were inspected, but none had codling moth.

No codling moth larvae exposed to cold storage longer than a month survived. Previous studies indicated that codling moth larvae would not survive standard cold (-0.6°C) or controlled atmosphere (2 - 3% O_2, -0.6°C) storage. Moffitt (1971) found $\simeq$ 60% mortality after a month in standard cold storage (near freezing) and no live larvae in controlled atmosphere storage after 90 days. Moffitt and Albano (1972) reported no live nondiapausing codling moth larvae, which is the condition of the larvae infesting fruits, after 60 days of standard cold storage. No codling moth larvae survived beyond 91 days of controlled atmosphere storage (Toba and Moffitt 1991).

In our survey, we tried to observe as many fruits as possible produced from different growers and packed under different conditions. The mean (± SEM) incidence of codling moth among grower lots was 0.01(± 0.01)% for presorted fruits and 0.22 (± 0.08)% for culled fruits. These data suggest that growers are adept in controlling codling moth in the orchards and that packing houses are efficient in removing infested fruits. Although the market destinations varied from Mexican and Asian exports to domestic, all the packing houses were very effective in processing fruits free of codling moth eggs and larvae. Furthermore, even codling moth damaged fruits were removed before the final pack. The microscope examinations verified the results of the large survey; slightly more infested fruits were observed entering the packing line (mean ± SEM = 0.04 ± 0.04%), but the culling efficiency remained about the same (mean ± SEM = 0.27 ± 0.15%). Line speeds, measured between 0.08 and 0.22 m/sec., were not related to changes in culling efficacy. The WSDA reports provide further corroboration because these contained no records of codling moth observations.

Codling moth, although not abundant, was dominant in the large survey. Spiders were also found in the presorted fruits (0.05%). However, microscope examinations also revealed European red mite [*Panonychus ulmi* (Koch) (Acari: Tetranychidae)] eggs (0.5% of presort and 2.5% of culls), which were carried to the final pack (4.4%). Most of the pest damage was caused by leafrollers (Lepidoptera: Tortricidae), which produced distinctive feeding marks. However, the microscope examinations did not improve pest damage detection (Table 3). Neither oriental fruit moths [*Cydia molesta* (Busck) (Lepidoptera: Tortricidae)] nor apple maggots [*Rhagoletis pomonella* (Walsh) (Diptera: Tephritidae), both quarantine pests for Mexico, were detected.

TABLE 1. Occurrence of Codling Moth Larvae and Larval Damage, by Cultivar, from Three Visual inspection Stations Pooled from Six Commercial Packing Houses.

	No. of fruits			% codling moth			% codling moth damage		
Cultivar	Presort	Culls	Final	Presort	Culls	Final	Presort	Culls	Final
'Fuji'	10,795	5,400	5,390	0.009	0.093	0.000	0.093	2.537	0.000
'Gala'	2,925	1,499	1,500	0.000	0.330	0.000	0.137	3.869	0.000
'Golden Delicious'	8,542	4,522	4,400	0.000	0.221	0.000	0.129	0.486	0.000
'Red Delicious'	5,737	2,955	1,249	0.017	0.203	0.000	0.176	1.930	0.000

TABLE 2. Occurrence of Codling Moth Larvae and Larval Damage, by Cultivar, from Microscopic Examinations at Three Stations Pooled from Six Commercial Packing Houses.

	No. of fruits			% codling moth			% codling moth damage		
Cultivar	Presort	Culls	Final	Presort	Culls	Final	Presort	Culls	Final
'Fuji'	1,192	600	600	0.00	0.33	0.00	1.01	2.67	0.00
'Gala'	300	100	160	0.00	1.00	0.00	1.00	2.00	0.00
'Golden Delicious'	947	496	509	0.11	0.20	0.00	0.00	1.81	0.00
'Red Delicious'	434	333	135	0.00	0.00	0.00	0.00	1.50	0.00

TABLE 3. Occurrence of Leafroller Damage from Three Visual Inspection and Microscope Examination Stations Pooled from Six Commercial Packing Houses.

Inspection method	% leafroller damage		
	Presort	Culls	Final
Visual	0.7	4.2	0.6
Microscope	0.6	4.6	0.6

These results are similar to earlier observations. Moffitt (1990) reported that only 33 codling moth larvae were found among 41,397,020 apples inspected by the Washington State Department of Agriculture over a five-year period. Moffitt (1990) found only 10 codling moth larvae in 171,488 culled 'Delicious' and 'Golden Delicious' apples and no codling moth larvae in 501,537 apples from packed boxes. In an indepenedent survey in Washington state, only one live codling moth larva was found among 4,800 presorted apples and no live larvae among 5,500 packed apples (Simmons et al. 2000). Recently, USDA-APHIS and Mexican regulatory officials inspected 3,625,867 culled apples and found only nine live codling moth larvae (Barbara Chambers, unpublished data). Knight and Moffitt (1990) found only dead larvae after controlled atmosphere storage or regular cold storage for 100 days. Hence, these observations indicate that not only is the incidence of codling moth infestation rare in commercially harvested and packed apples, but improved pest management practices have achieved higher quality fruit delivered to the packing house.

Examinations have been used to predict the probability of risk in other codling moth infested fruits. Curtis et al. (1991) found only one live codling moth larva in 37,908 culled nectarines, *Prunus persica* (L.), and argued that inspections of packed fruit can provide quarantine security. Curtis et al. (1992), in examining 326,625 packed nectarines over three years, collected only three live codling moth larvae and recommended that this fruit should be considered as a nonpreferred host. Yokoyama and Miller (1999) inspected about 1,300 kg of culled fresh prune, *Prunus domestica* L., and concluded that the occurrence of codling moth is so low that exported fruits are no risk for spreading infestations.

Our data indicate very low risk for the importing country. No potential codling moth introductions can be projected, such as proposed by Yamamura and Sugimoto (1995), because no codling moths were found in the packed boxes. Furthermore, the technique to estimate the number of codling moth infested fruits based on injury (Yamamura and Katsumata 1999) cannot be used because there was no larva associated with the lone damage found.

Important information was obtained from this study that demonstrated the effectiveness of field control and sorting in eliminating codling moth infestations in commercial apples. Replacing methyl bromide fumigation with the systems approach will result with the same quarantine security level, but with high fruit quality, reduced costs, lower environmental hazards, and improved worker safety.

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EFFECT OF SUBLETHAL CONCENTRATIONS OF AVERMECTIN ON POPULATION PARAMETERS OF *TETRANYCHUS URTICAE*[1] ON STRAWBERRY

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ABSTRACT

Experiments were carried out under laboratory conditions to determine the effect of sublethal concentrations of avermectin on population parameters of the two-spotted spider mite *Tetranychus urticae* Koch. Initially, a series of bioassays were developed to determine the lethal concentration of avermectin. Bioassays were performed using the immersion technique exposing mites to seven different concentrations of the product. Once the mortality-concentration curve was obtained, concentrations of 0.04 and 0.18 ppm were selected corresponding to the LC_{10} and LC_{35}, respectively. With these concentrations, the second stage of the experiment, which consisted of analyzing populations of mites exposed to these concentrations and comparing them to a control population, was performed. To achieve this, mites were exposed to strawberry leaves treated with avermectin. The results indicated that concentrations of 0.04 and 0.18 ppm avermectin had a significant effect on population parameters of *T. urticae* compared with the control. With the concentration of 0.04 ppm, the intrinsic rate of increase (r_m) was 0.3001, the net reproductive rate (Ro) 31.6430, the generation time (T_G) 11.510 days and the duplication time (t_2) 2.3095 days, while the control population showed values of 0.2816, 30.150, 12.094 and 2.4611, respectively, for these same parameters. The above results indicate that more vigorous populations emerged after avermectin treatment at 0.04 ppm compared to those derived from non treated individuals. At the higher concentration (0.18 ppm), avermectin caused a considerable decrease in all of the population parameters with 0.1581 for r_m, 7.20 for Ro, 12.4880 for T_G and 4.3847 for t_2.

INTRODUCTION

Resistance of the two-spotted spider mite, *Tetranychus urticae* Koch, to acaricides has become a critical problem in numerous agricultural production systems (Ferguson et al. 1991). The problem is further complicated by the presence of hormoligosis (the biological alteration of an individual as a response to the effects of sublethal dosages of a toxicant) which may induce the abnormal increase of the reproduction rate of the pest (Luckey 1968). Other research studies have been carried out to determine changes in the population behavior of this species when exposed to certain acaricides. Ibrahim and Knowles (1986) published a study on the influence of 105 formamidines on *T. urticae* reproduction and reported that the most common effects were stimulation of fecundity, delay of oviposition, and inhibition and delay of hatching. These responses varied according to the compound, concentration, and interval after the treatment. In

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another investigation conducted by Ahmadi (1983), individuals were exposed to different concentrations of dicofol on cotton *Gossypium hirsutum* L. leaf discs. Generation time did not vary between the treated individuals and the control; however, a decrease in the gross and net reproduction rate was observed. Flores et al. (1996) determined that one of the effects of sublethal concentrations of dicofol on *Eutetranychus banksi* (McGregor) was a decrease in the sex ratio to 2.143:1 (female: male) in a dicofol susceptible strain compared with 3.048:1 in a tolerant population. More recently, Flores et al. (2000) found that concentrations of 0.01, 0.05 and 0.1 ppm avermectin on bean leaves had a significant effect on the mean time of development of two-spotted spider mite females. Also found was a significant difference among proportions of age-specific survival and fecundity between each sublethal concentration and the control. Fecundity was lower for 0.5 and 0.1 ppm treatments compared to 0.01 ppm and the control. In general, the authors found that life parameters were affected inversely and significantly at doses of 0.05 and 0.1 ppm avermectin.

Considering all of the above facts, this study was undertaken to determine the effect of sublethal concentrations of avermectin on *T. urticae* using strawberry leaves as a substrate.

MATERIALS AND METHODS

In order to establish a laboratory colony of *T. urticae*, collections were made from various crops in the area of Saltillo, Coahuila, Mexico, and maintained on bean leaves using a Biotronette® environmental chamber at 25±2°C, 60-70 RH, and 12:12 L:D conditions.

The biological material was handled according to the method of Abou-Setta and Childers (1987), known as the "open leaf arenas." The mites used in the bioassays were transferred from the colony using a moistened 000 camel's hair brush to fresh strawberry leaf discs made with a 25-mm diameter cork borer. Discs were maintained with the ventral side up in trays with water-saturated cotton pads.

Two-day-old females were transferred to clean discs and maintained for 24 h for oviposition. Mites from these eggs were maintained under the same environmental conditions as in the main colony until they reached the adult stage. All of the biological material obtained in this manner was used to perform the bioassays.

Initially, a test was undertaken to determine the dosage-mortality range of avermectin. Seven concentrations were used ranging from 0 to 10 ppm selecting five-day-old female mites. Dosages of avermectin were prepared with distilled water from the commercial product Agrimec® (1.8 C.E) and the commercial adhesive Penatrex® at a concentration of 0.1%. Fresh strawberry leaf discs were individually treated by dipping them for about 5 sec in the acaricide solutions. Once dried, they were placed in rearing trays and five mites were transferred to individual discs, with a total of 100 mites per concentration.

Mortality records were taken 72 h after the beginning of the experiment. Mites that showed ataxia (uncoordinated active movement) were considered dead, as were those that remained with legs up and /or totally motionless. Mortality data were analyzed statistically (probit analysis) using the method of maximum likelihood (Finney 1971). Abbott's correction formula was applied when necessary (Abbott. 1925).

To carry out the main experiment, two concentrations of avermectin (0.04 and 0.18 ppm) were used and compared with a control treatment consisting of distilled water. Females that survived after 72-h exposure to the acaricide during the previous set of bioassays to determine the LC_{50} were transferred and kept for 24 h on discs free of avermectin. Once the mites oviposited, they were removed from the discs and only the eggs were retained so they would develop and eventually produce new individuals. At the time that these individuals reached the adult stage, 90 pairs (males and females) were selected at each toxic level of avermectin. Specimens were placed separately on treated discs and once oviposition started,

the male of each pair was discarded leaving only one female mite per disc. Eggs laid by these females were maintained on the same leaf disc until the emergence of the larvae, which were then placed singly on an untreated leaf disc. Monitoring was carried out until the last female died. Population parameters were determined and compared according to the standard life table analysis (Birch 1948). It is important to mention that in all cases the initial population of female breeders was reduced since some of them disappeared during the experiment; therefore, they were discarded from the data of the progeny resulting from them. Survivorship curves were compared based on the Log-rank test (Mendez et al. 1984).

RESULTS AND DISCUSSION

Fig. 1 shows the concentration-mortality relation for *T. urticae* after 72 h of exposure to avermectin. According to the probit analysis, the LC_{50} was 0.35 ppm with a confidence interval (CI) of 0.3465 to 0.3535 ($p \leq 0.05$) while 100% mortality, assessed at 72 h of exposure, occurred at a concentration of 10 ppm. These results are higher than those reported by Flores et al. (2000) who obtained 100% mortality for the two-spotted mite at 2 ppm of avermectin. Once the dosage-mortality curve was obtained, the concentrations corresponding to the LC_{10} and LC_{35}, 0.04 and 0.18 ppm respectively, were selected to assess the effect of avermectin on population parameters of *T. urticae*.

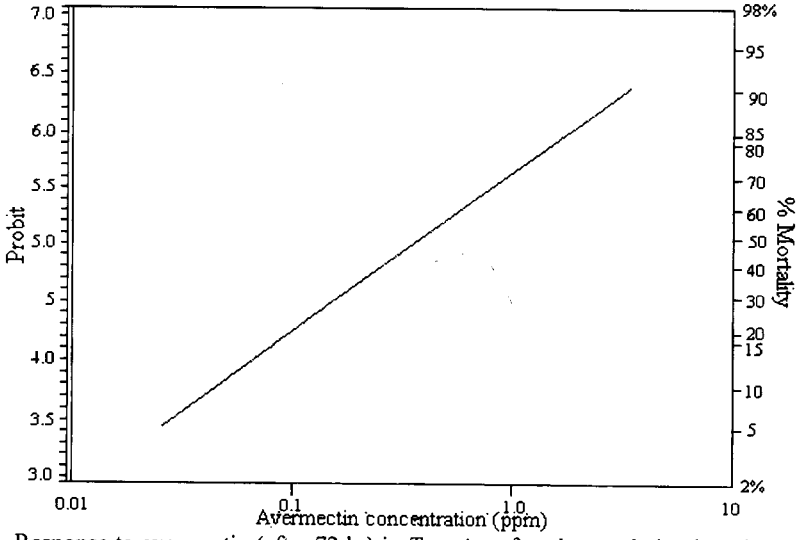

FIG.1. Response to avermectin (after 72 hr) in *T. urticae* female population based on the maximum likelihood method.

Survival and Fecundity. As far as survival is concerned very few variations were observed, and the rate of live females was reduced in the three treatments in a similar fashion. There was no significant difference (log-rank test, $p \leq 0.05$) among the proportions of the age-specific survival between each toxic level and the control (Fig. 2). Age-specific fecundity was markedly lower at 0.18 ppm compared with 0.04 ppm and the control (Fig. 3).

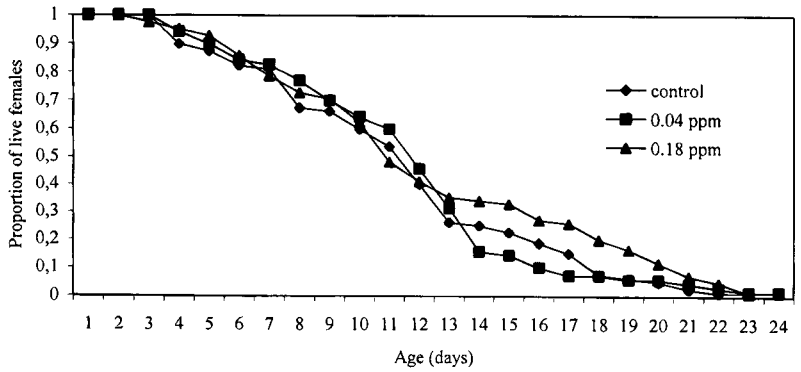

FIG. 2. Survivorship curves for *T. urticae* exposed to different concentrations of avermectin on strawberry leaves.

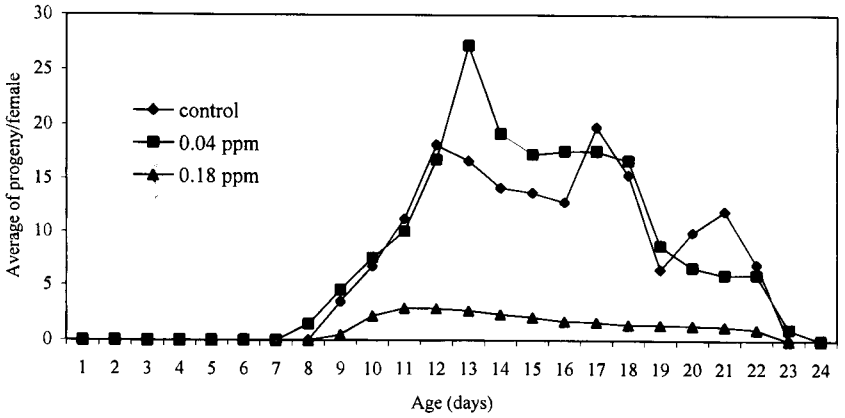

FIG. 3. Age-specific fecundity for *T. urticae* exposed to different concentrations of avermectin on strawberry leaves.

The gross reproduction rate (GRR). The gross reproductive rate (GRR), or number of females produced by a female parent for all ages, at a concentration of 0.04 ppm avermectin was higher than the control; however, at 0.18 ppm the GRR was noticeably lower (Table 1). This indicates that there would be an increase in the population at the 0.04 ppm dosage as time elapsed; the increase of the GRR at this concentration in relation to the control was of 8.99%.

The GRR values obtained in this investigation were higher compared with those in other studies. Ahmadi (1983) obtained a GRR for the same mite species of 22.19 in the control using cotton leaf discs and 6.10, 2.36 and 0.38 for treatments with concentrations of 1.77, 3.16 and 5.62 ppm dicofol, respectively. In the same investigation, Ahmadi described a direct relationship between the GRR value and the concentration of the

toxicant. On the other hand, Maggi and Leigh (1983) reported a GRR of 91.26 for the control, 76.92 for mites treated with phosphoric acid, and 96.07 for those treated with methyl parathion. Flores et al. (2000), using avermectin on bean leaf discs, reported a GRR of 218.22 for the control and 197.47, 29.30 and 95.54 for treatments with concentrations of 0.01, 0.05 and 0.1 ppm, respectively.

TABLE 1. Population Parameters of *Tetranychus urticae* after Exposure to Different Concentrations of Avermectin on Strawberry Leaves.

Parameter	control	avermectin (ppm)	
		0.04	0.18
Gross reproductive rate (GRR)	167.9000	184.4900	25.4440
Net reproductive rate (Ro)	30.1500	31.6430	7.2000
Capacity to increase (r_c)	0.2637	0.2778	0.1517
Intrinsic rate of natural increase (r_m)	0.2816	0.3001	0.1581
Finite rate of increase (λ)	1.3253	1.3500	1.1713
Cohort duration (T_C)	12.9150	12.4360	13.0150
Generation time (days) (T_G)	12.0940	11.5100	12.4880
Duplication time (days)	2.4611	2.3095	4.3847

Net Reproduction Rate (Ro). The number of female offspring that makes up the percentage of parent females during one generation of treated mites was affected by avermectin (Table 1). This population parameter showed the same trend as with GRR. That is, the lower concentration of 0.04 ppm showed a higher Ro (4.7%) than the control. The concentration of 0.18 ppm resulted in a substantial reduction of 76.1% in relation to the control. An induced increase of Ro at low concentrations were reported by Maggi and Leigh (1983) who noted that phosphoric acid and methyl parathion increased the net reproductive rate of *T. urticae* from 53.36 for the control to 56.49 and 65.11 for each of the toxicants under field conditions. On the other hand, in greenhouses, the values of Ro were 80.19 for the control and 69.90 and 88.11 for the acaricides. Boykin and Campell (1982) reported that *T. urticae* had a Ro of 141.35 for the control using peanut leaves (*Arachis hypogea*) as a substrate. This value increased when carbaryl was used and it was reduced with chemicals such as mancozeb, fentin hydroxide, benomyl, ammonical copper, and a mixture of benomyl, mancozeb and carbaryl.

Intrinsic rate of increase (r_m). The r_m reached a value slightly higher than the control at 0.04 ppm avermectin (Table 1) but not with treatment at 0.18 ppm, the latter resulting in a considerably lower value. The control value was 6.16% lower than that for mites treated with the 0.04 ppm dosage, while the treatment with 0.18 ppm resulted in a 43.85% reduction in relation to the control. This means that the colonies of mites that were exposed to the lower concentration of avermectin (0.04 ppm) responded by increasing the intrinsic rate of increase of the population, while the higher concentration lowered it.

The results obtained concerning this parameter are supported by other investigations. Wrensch (1985) reported that the quality, quantity and the time of exposure to a pesticide are some of the intrinsic factors that may influence the r_m value. Moreover, he cited Chaboussou (1966) who reported that this direct stimulation can occur as trophobiosis, where the acaricide conveys to the host plant certain favorable conditions for the mites. On the other hand, Luckey (1968) commented that the toxicant may directly stimulate development rate and fecundity (hormoligosis). The data gathered

in this investigation does not allow us to distinguish the effect of trophobiosis and the effect of avermectin per se.

Results that may compare with those of this investigation have been reported by Boykin and Campbell (1982). These investigators who worked with *T. urticae* reported that mancozeb and carbaryl, as well as the mixture of both chemicals, may increase the r_m of the treated mites in relation to the control. On the other hand, mites treated with ammoniated copper, fentin and benomyl, as well as a mixture of benomyl, mancozeb and carbaryl showed a reduced r_m.

Generation time (T_G). The T_G for the control was 12.0940 days, where the population increased daily by a factor of 1.3253. The treatment using the lower concentration of avermectin showed a generation time of 11.5100 days, with a daily population increase rate of 1.3500. The generation time for treatment with 0.18 ppm was 12.4880 days, with a daily population increase of 1.1713. All these data lead us to infer that avermectin at a concentration lower than the LC_{50} may produce shorter generation times resulting in a greater potential for population increase compared with mites not exposed to the acaricide. As the concentration of the acaricide was increased, the population began to be severely harmed and the generation time increased.

The duplication time for those individuals exposed to the acaricide concentration of 0.04 ppm was slightly lower than for the control, 2.3095 versus 2.4611. However, there was a very notable difference between acaricide treatment and control with the higher concentration, the data showing that the treatment with 0.18 ppm requires 43.8% more time compared to the control for the population to double.

In conclusion, populations of *Tetranychus urticae* exposed to sublethal dosages of avermectin undergo changes in population parameters. In relation to the control treatment, a concentration of 0.04 ppm produces favorable modifications in the population parameters assessed, while at a concentration of 0.18 ppm, the effect was adverse.

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VOL. 27 NO. 3/4 SOUTHWESTERN ENTOMOLOGIST SEPT/DEC 2002
SCIENTIFIC NOTE

HEAD CAPSULE WIDTHS OF LARVAL INSTARS OF THE BOLL WEEVIL[1]

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Studies of the ecology or population dynamics of the boll weevil, *Anthonomus grandis grandis* Boheman, often require the ability to accurately distinguish between the larval instars. Parrott et al. (1970) provided guidelines for distinguishing instars based on head capsule widths, but did not provide estimates of the variation within instars. Roach (1973) provided mean head capsule widths similar to those of Parrott et al. (1970) complete with estimates of variance (presumably standard deviations), but did not provide the ranges of head capsule widths associated with each instar. During ecological studies of boll weevil larvae, we frequently observed head capsule widths that were directly between the reported means. Consequently, instars of these individuals could not be determined with confidence. Therefore, we examined the range of head capsules widths within instars to establish additional guidelines for identification of larval instars.

Boll weevil-infested squares were collected directly off cotton plants, *Gossypium hirsutum* L., from commercial fields in Brazos and Burleson Counties, Texas. Squares were collected between mid-July and late-September 2000. Each collection was placed in a screened plexiglass cage (20x20x20 cm) and held in an environmental chamber (Model I-30BLL, Percival Manufacturing, Boone, IA) at 29.4 ± 1°C and a photoperiod of 13:11 (L:D) h. Approximately ten squares were removed daily and opened to assess larval development. When >50% of the squares contained the desired instar, larvae were removed from the squares for head capsule measurements.

Head capsule widths were determined at a magnification of 50x using a dissecting microscope equipped with an ocular micrometer (2.0-cm scale in 0.02-mm divisions; Model GSWH10x-H-2, Olympus America, Melville, NY). Each larva was placed on a glass slide so that the anterior portion of the head capsule faced upward. The greatest width across the head capsule of each larva was measured twice, and the mean was calculated.

To confirm instar classification after measurement, each larva was tentatively assigned an instar then placed in a plastic cup (PO75S-3/4 oz. plastic souffles, SOLO Cup, Urbana, IL) and held until it molted or died. Tentative classifications were assigned based on head capsule widths of instars as follows: first instar, <0.50 mm; second instar, 0.50 to 0.75 mm; third instar, >0.75 mm. The bottom of each plastic cup was lined with a filter paper disk moistened with de-ionized water to prevent larval desiccation. Each day larvae were provided fresh anthers dissected from one-third grown squares. Larvae were held in the same environmental chamber as square collections and were examined at least twice daily for the presence of exuvia. Larvae that molted were decapitated to facilitate final head capsule measurements. Pupation indicated that a larva was a third instar at the time of the initial head capsule measurement.

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[1] Coleoptera: Curculionidae

The data were examined for differences in head capsule widths among instars using a one-way ANOVA (PROC GLM, SAS Institute 1988). Means corresponding to the larval instars were compared using the Tukey test (TUKEY option of the MEANS statement of PROC GLM, SAS Institute 1988).

Head capsule widths differed significantly among larval instars ($F = 930.65$; df = 2, 112; $P < 0.001$; Table 1). Range of head capsule widths was greatest for third instars (0.32 mm), followed by second (0.16 mm) and first instars (0.08 mm). Overlap of the ranges of head capsule widths among larval instars was not observed.

TABLE 1. Means and Ranges of Head Capsule Widths of Boll Weevil Larvae.

		Head capsule width (mm)	
Instar	*n*	Mean ± SD[a]	Range
1	26	0.40 ± 0.02 a	0.36 – 0.44
2	39	0.64 ± 0.05 b	0.56 – 0.72
3	50	0.99 ± 0.08 c	0.76 – 1.08

[a] Values followed by different letters are significantly different ($\alpha = 0.05$; Tukey test).

Although our mean values of head capsule widths for instars were similar to previous reports, the variations in head capsule widths within instars were substantially greater than those previously reported by Roach (1973). These differences may be partially attributed to the sources of weevils used in the respective studies. Previous studies examined weevils from an established laboratory colony reared on artificial diet whereas we examined field-collected larvae reared on anthers dissected from squares. The limited genetic diversity and relative constancy of environmental and dietary conditions associated with laboratory culture may have reduced variation in head capsule width compared with those we observed. We observed that first-instar head capsule widths were ≤0.44 mm, second-instar head capsule widths were between 0.56 and 0.72 mm, and third-instar head capsule widths were ≥0.76 mm. These observations should provide a more thorough guide for determining instar of boll weevil larvae than was previously available.

Mention of trade names or commercial products in this article is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the U.S. Department of Agriculture. Research reported herein was conducted in partial fulfillment of the requirements of a Master of Science degree (B. J. R.) in Entomology, Texas A&M University, College Station, TX.

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SCIENTIFIC NOTE

ARTHROPODS FROM A ZONE-TAILED HAWK NEST IN TEXAS

James R. Philips[1], G. W. Hunt[2], and S. W. Matteson[3]

Raptor nests are a microhabitat with a complex community of arthropod parasites, predators, and saprovores (Philips and Dindal 1977). However, the fauna of zone-tailed hawk (*Buteo albonotatus* Kaup) nests is completely unknown. The objective of this study was to investigate the fauna of a zone-tailed hawk nest.

A sample of nest lining material was collected from a zone-tailed hawk nest of oak and pine twigs and feathers 30-m high in a ponderosa pine (*Pinus ponderosa* Dougl. ex Laws.) in Limpia Canyon, Davis Mountains, Texas, on July 1976, while the nestlings were still in the nest. The sample was placed in a modified Tullgren funnel for extraction of invertebrates.

Three hundred and fifty-seven arthropods of 23 species were found (Table 1). Fly larvae were numerically dominant (over 78% of the individuals) while mite populations were very low. Carrion scavenging flies, such as *Leptometopa*, probably have peak populations during the nestling period, when prey remains are fresh and abundant. The nestlings' excrement provides breeding habitat for flies such as *Coboldia fuscipes* (Mg.). Fly larvae like the Sciaridae, Phoridae, and Cecidomyiidae, living on decaying plant matter in the nest were few. This high stick nest in an arid site was also not favorable for many hygrophilic acarines which are abundant in New York raptor nests (Philips and Dindal 1990). Scavenging dermestid beetles are more tolerant of dry conditions, but only one was present.

The only parasite found was the chigger *Eutrombicula alfreddugesi* (Oudemans). This is the most common and widespread chigger which attacks man in the Western Hemisphere, and it also parasitizes many warm- and cold-blooded vertebrates (Baker et al. 1956). The single engorged specimen may have been parasitizing prey or the hawk. The predatory mite *Macrocheles rodriguezi* Oliver and Krantz is an avid feeder on acarid mites (Oliver and Krantz 1963), but none of these mites occurred in the sample. Other food possibilities include fly larvae and nematodes. *Dendrolaelaps* is a similar predator, as are the histerid larvae. Koskela and Hanski (1977) consider aleocharine staphylinid beetles to be predators, but Pirone (1974) classified them as fungivores. The oribatid mites *Scapheremaeus* and *Eporibatula are* fungivores. *Comyianoetus denticulatus* Fain and Philips is a scavenging nidicole which has also been found in nests of the great horned owl (*Bubo virginianus* (Gmelin)) in New York and Tengmalm's owl (*Aegolius funereus* L.) in Norway (Fain and Philips 1979). Its deutonymph stage is nonfeeding and phoretic, but the insect host it uses for transportation remains unknown. This species has not been previously reported in Texas. The larvae of the noctuid moth *Epizeuxis americalis* (Guen.) feed on dried leaves (Holland 1968). Adult lyonetiid moths such as *Bucculatrix* oviposit on leaves and the larvae are leaf miners (Borror et al. 1981). Another phytophagous insect was the thrips larva. The nitidulid larvae represent undescribed forms. More study of hawk nest fauna is needed to distinguish the effects of local climate from that of ecological

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sucession in the nest's incubation, nestling, and postfledging stages, and to elucidate microcommunity interrelationships.

TABLE 1. Arthropods from a Zone-tailed Hawk Nest in Texas

Taxon	Number
Class Arachnida, Order Acarina	
Cohort Gamasina sp.	2
Family Cymbaeremaeidae, *Scapheremaeus* sp.	1
Family Digamasellidae, *Dendrolaelaps* sp. nr. *presepum* (Berlese)	11
Family Histiostomatidae, *Comyianoetus denticulatus* Fain and Philips	16
Family Macrochelidae, *Macrocheles rodriguezi* Oliver and Krantz	8
Family Oribatulidae, *Eporibatula sp.*	1
Family Trombiculidae, *Eutrombicula alfreddugesi alfreddugesi* (Oud.)	1
Class Insecta, Order Coleoptera	
Family Dermestidae, *Dermestes* sp.	1
Family Histeridae, 2 genera	12
Family Nitidulidae	2
Family Staphylinidae, Subfamily Aleocharinae sp.	3
Order Diptera	
Family Cecidomyiidae, Subfamily Cecidomyiinae sp.	2
Anarete sp.	2
Family Milichiidae, *Leptometopa sp.*	182
Family Muscidae sp.	9
Fannia sp.	30
Family Phoridae, *Megaselia* sp	1
Family Scatopsidae, *Coboldia fuscipes* (Mg.)	4
Family Sciaridae, *Bradysia* sp.	11
Section Acalyptratae sp.	55
Order Lepidoptera	
Family Lyonetiidae, *Bucculatrix* sp	1
Family Noctuidae, *Epizeuxis americalis* (Guenee)	1
Order Thysanoptera, Suborder Terebrantia	1

We are very grateful to the following taxonomic specialists for assisting us with the identification of the arthropods: E. F. Cook (Scatopsidae); D. R. Davis (Lyonetiidae); R. J. Gagne (Cecidomyiidae, Sciaridae); E. E. Lindquist (Digamasellidae); J. F. McAlpine and H. J. Teskey (Milichiidae); A. F. Newton (Histeridae); R. A. Norton (Oribatida); W. H. Robinson and W. W. Wirth (Phoridae); M. K. Thayer (Staphylinidae) and E. L. Todd and D. M. Weisman (Noctuidae).

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SCIENTIFIC NOTE

CHEMICALS USEFUL FOR SEPARATING EGG MASSES OF THE SCREWWORM[1]

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Screwworms, *Cochliomyia hominivorax* (Coquerel), are serious economic pests that cause myiasis of warm-blooded animals in the tropical-subtropical regions of the Western Hemisphere. A successful eradication program has eliminated screwworms from the United States, Mexico and most of Central America using the sterile insect technique (SIT) (Galvin and Wyss 1996). The success of SIT depends upon the production of sufficient quantities of flies. Because of the large number of flies produced, small changes resulting in more efficient rearing can lead to significant cost savings for the eradication program.

Currently at the mass rearing facility, female screwworms lay their eggs in sheets on an egging board; eggs are removed with a spatula, and they are then divided and weighed before they are placed in the larval rearing medium (Brown 1984). Efficiency would be increased if eggs could be volumetrically, mechanically added to the medium (using a 'dosifier') but this could be accomplished only if a method was available for separating the egg masses without loss of viability.

Previous reports (Fredeen 1959) found sodium hydroxide (NaOH) useful for separating the egg masses of Simuliidae. LaChance et al. (1964) reported on separating egg masses in 1% NaOH to determine the number and hatchability of eggs laid by individual females; Rawlins et al. (1983) separated screwworm egg masses for an insecticide study by soaking them in a 1% NaOH solution for 15 to 25 minutes. Although NaOH was reported useful for separating screwworm egg masses, no data were published demonstrating the optimum concentration, time of exposure, or effect on survival of the embryo. Our objectives were to determine the effects of NaOH exposure on screwworm embryos and compare the efficacy of NaOH, sodium carbonate (Na_2C0_3) and potassium hydroxide (KOH) for separating egg masses from three screwworm strains.

Costa Rica 92 (CR92), the strain previously used in the mass rearing facility, was used in the NaOH studies while the strains CR92, J2 (developed from collections in Jamaica) and Linc01 (resulting from crosses of six wild-type screwworm strains) were used in the studies comparing NaOH, Na_2C0_3 and KOH. Adults were kept at 25°C, 50% RH and 12:12 photoperiod. Eggs were collected for ~30 min from 7-10 days-old females.

First, about 100 mg of eggs were placed in either 1 or 2 % (weight/volume) NaOH, occasionally agitated for 30 sec and sampled after 10 min. Thereafter, samples were taken at ~ 15-min intervals with the final sample taken after 100 min. Two samples of 100 eggs

1. Diptera: Calliphoridae.

2. This work was done in cooperation with the Institute of Agriculture and Natural Resources, University of Nebraska, Lincoln, NE and published as paper No. 13285, Journal Series, Nebraska Agricultural Research Division. Mention of a proprietary product does not constitute endorsement or a recommendation for its use by the USDA.

were taken at each time interval, incubated and categorized as hatched (only the chorion remained), sclerotized (mouth-hooks and/or setae were visible through the chorion), or undeveloped (no structures were obvious). This was replicated four times. The time the eggs remained in the NaOH solutions had a significant effect on the embryo survival but the concentration of NaOH had no significant effect. Survival was ~71.9% for both concentrations at 10 min and progressively decreased to ~27% at 100 min. From these data, the optimum length of time egg masses should be left in NaOH is 10 min or less. Similar mortality occurred in a preliminary study where, for removal of the NaOH-treatment, eggs were transferred to and submerged (for 5 to 15 min.) in distilled water after soaking in NaOH for 10 min. This indicates that the mortality was due to lack of oxygen rather than to effects of the NaOH. To further support the importance of oxygen for the survival of screwworm embryos, if spacers are not used to keep a seal from forming between the top and the bottom of the petri dish, egg hatch does not occur (using spacers is standard practice in our laboratory).

About 100 mg of eggs were then placed in each of three scintillation vials containing 6 ml of 0.5%, 1% or 2% (w/v) solution of NaOH. The eggs were soaked in the solutions, with an occasional 30 sec agitation, for 10 min. Eggs were then pipetted onto moistened filter paper in a Buchner funnel and rinsed with distilled water. Two samples of 100 eggs from each treatment were placed onto moist filter paper in 100 mm petri dishes and incubated at 37° C. Two petri dishes (subsamples) for each concentration were set up each day (replicated 14 times). After about 18 hr, the eggs were examined and categorized as above. The mean percentages of egg hatch (±S.E.) for the three concentrations of NaOH (79.7±1.3 for 0.5%; 79.5±1.7 for 1%; 80.3±1.8 for 2%) were not significantly different. Egg mass separation was noticeably slower and less efficient in the 0.5% solution.

Egg masses were then divided into five portions; each portion was placed on moist filter paper in a petri dish and incubated for 0, 1, 2, 4, or 6 h at 37° C. After incubation, egg masses were divided in half and placed in either 1 or 2% NaOH with periodic agitation for 10 min before sampling, further incubated at 37° C, and then categorized as above. This was replicated five times. Neither age of the embryos at the time of placement into the NaOH nor concentration of NaOH had a significant effect on the percentage of eggs hatching. The mean percentage (±S.E.) of hatching ranged from 74.5±2.6 for eggs 0 h old in 2% NaOH to 81.2±1.2 for eggs 4 h old in 1% NaOH.

About 10 mg (~ 220 eggs) of freshly laid, untreated eggs were then placed in a 100 mm petri dish containing moist filter paper for comparison to NaOH treatments. A sample of ~100 mg of eggs was treated with 1% or 2% NaOH, with occasional agitation, for 10 min. Two samples of 100 eggs from each NaOH concentration were transferred to 100 mm petri dishes with moist filter paper. Egg hatch was categorized as before after incubation for 18-20 h at 37° C. This was replicated six times. There was no significant difference between the mean egg hatch of the treated and untreated eggs.

Finally, NaOH, Na_2C0_3 and KOH were mixed as 1%, 4%, and 2% solutions (w/v), respectively. A small mass of eggs was placed in each solution and mildly agitated for 5 min. Each solution was then poured though filter paper and the eggs were rinsed with distilled water. One hundred eggs were counted onto moist filter paper, incubated and the hatch was categorized as before. This was replicated three times with each of the three strains (CR92, J2 and Linc01). No significant differences were found between treatments of eggs from the J2 and Linc01 screwworm strains. KOH and NaOH were superior to Na_2CO_3 for CR92 (Table 1). There also was a trend, although not statistically significant, that KOH was generally less detrimental to the survival of the embryos.

We have shown that NaOH, KOH or Na_2CO_3 can be used to separate egg masses of the screwworm. NaOH is currently used in our laboratory to simplify our routine monitoring of egg hatch for the 15 strains currently maintained for research. Berkebile et al. (2000) used NaOH to separate egg masses and prepare them for permeabilization and further

research with cryopreservation; the technique will be useful in genetic research requiring work with individual eggs.

TABLE 1. Effect of Sodium Hydroxide (NaOH), Sodium Carbonate (Na_2CO_3) and Potassium Hydroxide (KOH) on the Survival of Embryos from Three Strains of Screwworm

Strain	Treatment	Survival X±SE[a]
CR92	NaOH	73.55±1.35a
	Na_2CO_3	61.38±1.46b
	KOH	79.27±4.79a
J2	NaOH	64.52±4.72a
	Na_2CO_3	76.07±2.12a
	KOH	74.45±5.61a
Linc01	NaOH	76.89±5.93a
	Na_2CO_3	76.09±4.58a
	KOH	84.99±2.82a

[a] Values for the same strain followed by different letters are significantly different at $\alpha = 0.05$.

Separating egg masses could also be useful in the eradication program's mass rearing facility. This would allow eggs to be applied to the developmental medium by a volumetric 'dosifier'. The speed and accuracy at which the eggs could be applied to the media would reduce labor costs currently required to handle eggs and could enhance the efficiency of mass rearing.

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