WITHIN-FIELD DISTRIBUTION OF THREE HOMOPTERAN SPECIES IN TEXAS SUGARCANE

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ABSTRACT

Sugarcane fields composed of either 'CP 70-321' or 'NCo 310' were sampled during 1993 and 1994 for three species of homopteran insects. The West Indian canefly, *Saccharosydne saccharivora* (Westwood), was the most abundant species collected with densities reaching over 40 per shoot. Population densities varied significantly throughout the season in 1993 but were similar in 1994. No difference in densities were found between sugarcane cultivars. Both the sugarcane delphacid, *Perkinsiella saccharicida* Kirkaldy and the leafhopper *Draeculacephala portola* Ball, were found in low numbers (< 1.0 per shoot). *P. saccharicida* reached its highest levels later in the season, and 'CP 70-321' shoots harbored more individuals than 'NCo 310' shoots. Although *D. portola* densities were low, differences among fields and between cultivars were found. Aggregation, as measured using Taylor *a* and *b* coefficients, was shown to be greater for *S. saccharivora* than the other species.

INTRODUCTION

Sugarcane (interspecific hybrids of *Saccharum*) has been commercially grown in a three-county area of southern Texas since 1972. Stemboring pyralids, Mexican rice borer, *Eoreuma loftini* (Dyar), and sugarcane borer, *Diatraea saccharalis* (F.), have been the most serious insect pests of the industry (Meagher et al. 1994); however, other insects are active in the Texas sugarcane agroecosystem. The homopteran fauna was described in two earlier reports (Meagher et al. 1991, Meagher et al. 1993), although only basic survey information was noted and sampling was conducted using a large suction device that did not estimate insects per shoot. Three Auchenorrhyncha homopteran species, *Saccharosydne saccharivora* (Westwood) (Delphacidae), *Perkinsiella saccharicida* Kirkaldy (Delphacidae), and *Draeculacephala portola* Ball (Cicadellidae), were relatively abundant in these earlier surveys.

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The West Indian canefly, *Saccharosydne saccharivora*, has been associated with sugarcane in the Caribbean and in North, South, and Central America for centuries (Metcalfe 1969). This delphacid has historically caused varying degrees of economic damage (Charpentier 1970), and has been documented in all continental sugarcane-producing states (Charpentier 1970, Hall 1988, Meagher et al. 1993). The sugarcane delphacid, *P. saccharicida*, first noted in the continental United States in Florida in 1982 (Sosa 1985), was documented in Texas in 1989 (Meagher et al. 1991) and was recently discovered in Louisiana in 1994 (White et al. 1995). This insect can cause economic damage (Wilson 1987) and is a vector of *Fijivirus* sp., the causal agent of Fiji disease of sugarcane (Francki and Grivell 1972, Egan et al. 1989). *D. portola* is commonly collected in sugarcane (Hall 1988, Meagher et al. 1993) and is sporadically captured in other grass crops (Wilson et al. 1973, Hawkins et al. 1979). It was reported to be a vector of chlorotic streak in sugarcane (Abbott and Ingram 1942) until further investigation proved otherwise (Abbott et al. 1961).

An understanding of the spatial patterns of an insect can aid in developing sampling plans (Southwood 1978). Spatial patterns are affected by intrinsic factors such as oviposition pattern and immature dispersal, extrinsic factors such as host quality and environmental toxicants, and estimation procedures such as sampling plan parameters, sample unit size, number of samples, and sampler bias (Wilson 1994). Spatial patterns can depend on population density, with low densities tending to be indistinguishable from uniform or random, and high densities of most insects tending to be aggregated. The efficiency of a resulting sampling plan must balance cost considerations against the quality of the information provided. The objective of the present research was to describe the within-field distributions of *S. saccharivora*, *P. saccharicida*, and *D. portola* adults and nymphs in two sugarcane cultivars and calculate sample sizes based on a defined level of reliability.

MATERIALS AND METHODS

Fields and Sampling. Commercial sugarcane fields of either 'CP 70-321' or 'NCo 310' in different Lower Rio Grande Valley locations were sampled in 1993 and 1994. Field size ranged from 6.3 to 15.7 ha. Sampling was initiated during February or March and terminated in late August due to sugarcane harvesting. Every effort was taken to maintain a biweekly timetable; however, weather conditions or irrigation schedules occasionally delayed sampling in individual fields. Each field was sampled a total of 8 to 10 times per year. Sampling was conducted during mid- to late morning by randomly selecting 30 shoots (randomized by selecting sugarcane row and number of paces within row) per field. Starting at the base of the shoot, leaves were gently pulled back and the number of adults and nymphs present was recorded.

Statistical Analysis. Means per shoot for each species were calculated for each weekly sample taken in each field. Numbers were compared among weeks, among fields and between cultivars using analysis of variance (PROC GLM, SAS Institute 1996). Counts were square root $(y + 0.5)$ transformed before analysis, but untransformed means are shown in tables and figures.

Spatial dispersion was investigated using Taylor coefficients, $S^2 = a\,\bar{x}^{\,b}$ (Taylor 1961, 1984). Taylor coefficients were estimated by fitting $\ln(S^2) = \ln a + b \ln(\bar{x})$ (PROC REG, SAS Institute 1996). Estimates for a and b were compared using analyses of variance (PROC GLM, SAS Institute 1996) to examine the effects of cultivar.

Wilson and Room (1982) presented the following equation for estimating sample size where the goal was to estimate population density with a defined level of reliability:

$$n = (t_{\alpha/2} / D_{\bar{x}})^2 \, a_{\bar{x}}^{\,(b-2)},$$

where $t_{\alpha/2}$ is the standard normal variate for a two-tailed confidence interval, $D_{\bar{x}}$ is a proportion defined as the ratio of half the desired CI to the mean ($D_{\bar{x}}$ = [CI/2] / $\bar{x}$ for enumerative sampling), and a and b are Taylor coefficients. This equation permits one to estimate required sample size (n) over a range of densities for any species and sample unit whose Taylor

coefficients are known. Required sample size was calculated using $t_{\alpha/2} = 1.645$ and $D_{\bar{x}} = 0.2$.

RESULTS AND DISCUSSION

Higher numbers of *S. saccharivora* were collected in 1993 than 1994, with seasonal means of 23.7 and 3.0 individuals per shoot, respectively. Populations varied significantly throughout the season in 1993 ($F = 3.2$; df = 23, 42; $P = 0.0005$), with peak populations occurring in May (Fig. 1a). Meagher et al. (1993) also found more *S. saccharivora* during May-June than March-April. Migrating adults into young or ratoon sugarcane fields can boost populations to over 30 per shoot during the initial phases of colonization (Metcalfe 1969), and this was exemplified in one field with a mean of 44.7 ± 7.8 (SE) West Indian canefly per shoot. These large populations in a 'NCo 310' field near Elsa contributed to significant among field variation ($F = 6.9$; df = 3, 42; $P = 0.0007$). However, *S. saccharivora* were found in similar numbers between cultivars ($F = 1.8$; df = 1, 42; $P = 0.1898$). In 1994, only among field variation was significant ($F = 2.9$; df = 4, 31; $P = 0.0403$) (Fig. 1b). *S. saccharivora* adults and nymphs were the least mobile and were easy to locate because they were usually present on the underside of leaf blades.

Perkinsiella saccharicida populations were low in both years, not exceeding 1.0 per shoot. Low numbers of *P. saccharicida* were found in our previous study; however, sampling was conducted using a large suction sampling method that probably wasn't appropriate for this insect (Meagher et al. 1993). In 1993, populations varied significantly throughout the season ($F = 3.0$; df = 23, 42; $P = 0.001$), with peak populations occurring in July and August (Fig. 2a). Among field and cultivar differences were not significant ($P > 0.05$). Seasonal abundance studies in Florida showed that sugarcane delphacid population densities ranged widely field-to-field (Sosa 1985) and increased during the summer months with peak densities approaching 7.5 per shoot in October and November (Sosa et al. 1986). However, maximum population densities in Louisiana sugarcane were found to be 0.3 per shoot (White et al. 1995). We were not able to sample during fall and winter due to sugarcane harvesting. Peak population densities of this insect in southeastern Queensland, Australia were as high as 96 adults and 335 nymphs per shoot (Allsopp and Bull 1990).

In 1994, seasonal, among field, and cultivar variation were all significant ($P < 0.05$). Population densities were low from February through April, but were higher from May through August (Fig. 2b). 'CP 70-321' shoots contained more sugarcane delphacid adults and nymphs than 'NCo 310' shoots ($F = 5.6$; df = 1, 31; $P = 0.0242$) (Fig. 2c). Cultivar preference and population development differences have been shown experimentally and in field studies (Chang and Ota 1978, Taniguchi et al. 1980, Allsopp and Bull 1990).

Densities for *D. portola* were also low, never exceeding 1.0 per shoot. Earlier research showed this species to be the most commonly collected (Meagher et al. 1993). However, samples in that study were taken from sugarcane and surrounding grasses using a large suction sampler, and it is possible that most of the *D. portola* population was collected from non-sugarcane hosts. Population densities in both years were not different across weeks ($P > 0.14$) but were different among fields and between cultivars ($P < 0.05$). In 1993, densities were higher in 'NCo 310' than 'CP 70-321' shoots (0.2 ± 0.01 vs.0.12 ± 0.01, respectively; $F = 14.5$; df = 1, 42; $P = 0.0004$) (Figs. 3a and 3b), while in 1994, 'CP 70-321' shoots harbored more leafhoppers than 'NCo 310' shoots (0.37 ± 0.02 vs.0.27 ± 0.02, respectively; $F = 4.4$; df = 1, 31; $P = 0.0444$) (Figs. 4a and 4b). *D. portola* were the most active species collected, with nymphs present in the whorls and adults in whorls and on leaf blades.

Taylor *a* coefficient ranged from 0.85 for *D. portola* to 4.15 for *S. saccharivora*, and *b* ranged from 0.95 for *D. portola* to 1.55 for *S. saccharivora* (Table 1). Taylor coefficients for *P. saccharicida* were in between those values and were similar to coefficients calculated for *P. saccharicida* nymphs and adults sampled in Australian sugarcane fields (Allsopp and Bull 1990). Taylor *b* coefficient was significantly > 1.0 for *S. saccharivora* in both years and *P. saccharicida*

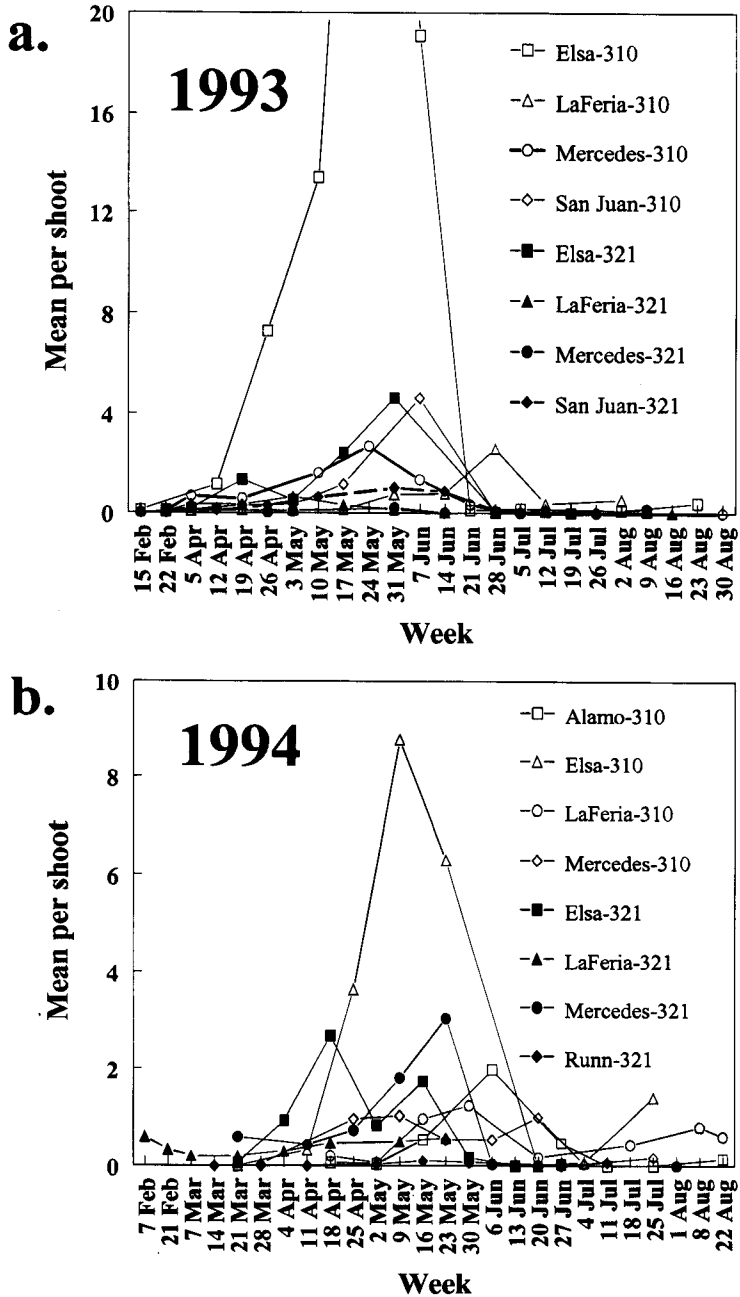

FIG. 1. Seasonal density of *Saccharosydne saccharivora* in eight sugarcane fields, lower Rio Grande Valley, Texas, 1993 and 1994. The density on 23 May 1993 was 44.7 per shoot. '310' refers to cultivar NCo 310, '321' refers to cultivar CP 70-321.

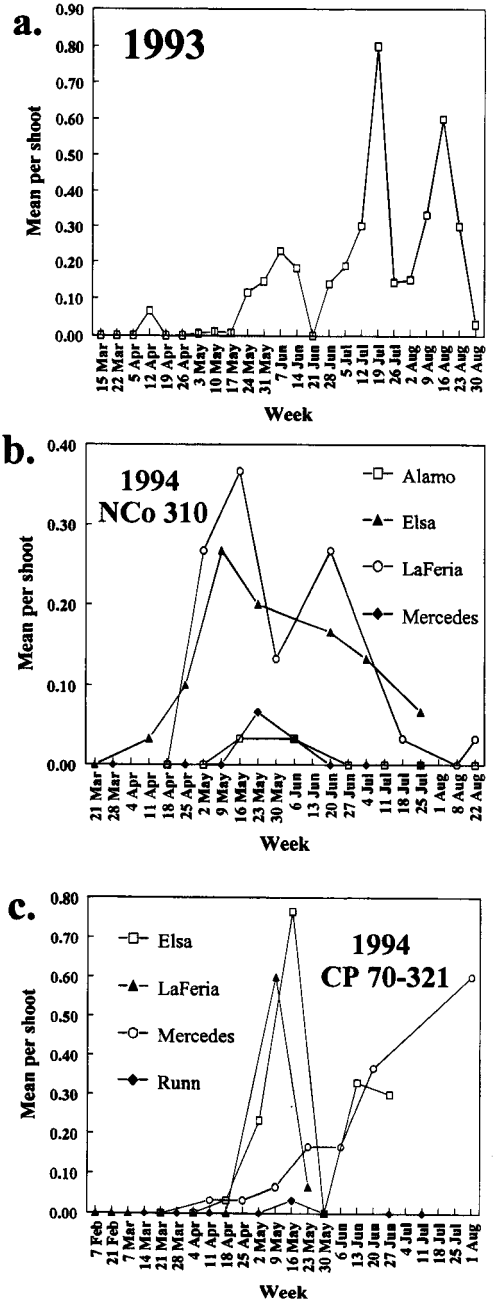

FIG. 2. Seasonal density of *Perkinsiella saccharicida* in sugarcane for 1993 and 1994, lower Rio Grande Valley, Texas. Means in 1993 are across both cultivars and all eight fields. Means in 1994 are for four fields for each of the cultivars NCo 310 and CP 70-321.

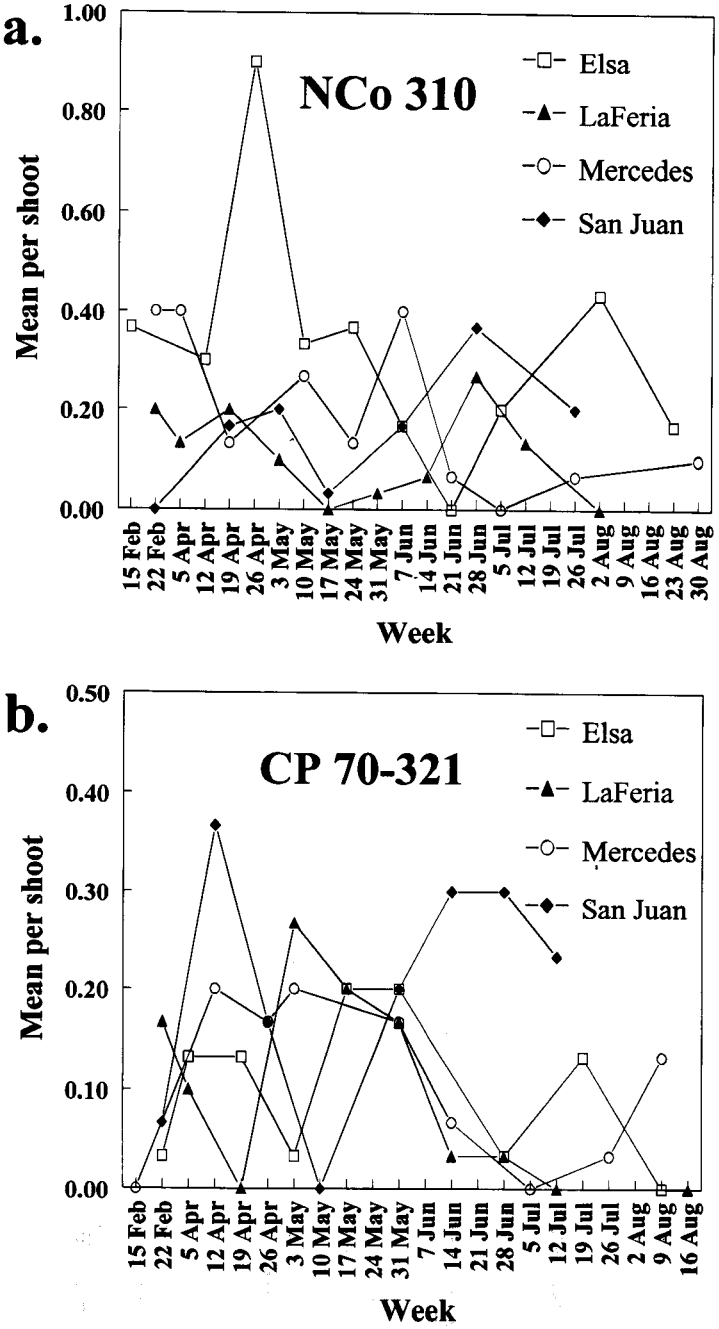

FIG. 3. Seasonal density of *Draeculacephala portola* in sugarcane for 1993, lower Rio Grande Valley, Texas. Means are for four fields for each of the cultivars NCo 310 and CP 70-321.

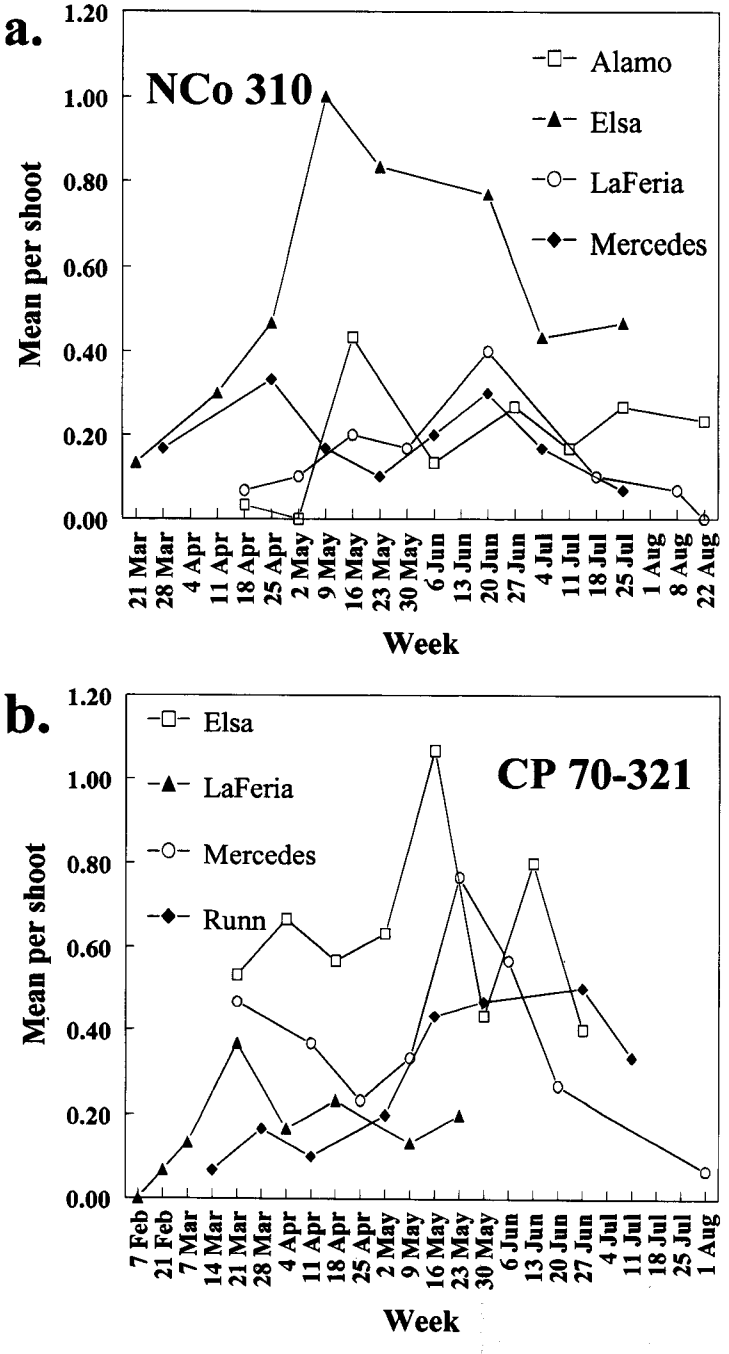

FIG. 4. Seasonal density of *Draeculacephala portola* in sugarcane for 1994, lower Rio Grande Valley, Texas. Means are for four fields for each of the cultivars NCo 310 and CP 70-321.

in 1993. For each species, there were no differences in Taylor *a* or *b* between sugarcane cultivars ($P > 0.15$). These results indicated that under Texas sugarcane growing conditions and for commonly encountered densities, *S. saccharivora* has a more aggregated spatial pattern than the other two species.

TABLE 1. Taylor Coefficients for Three Species of Homoptera in Texas Sugarcane, 1993-1994.

Species	Year	*a* (± SEM)	*b* (± SEM)	r^2	*n*
Saccharosydne saccharivora	1993	4.15 (1.07)	1.55 (0.03)[a]	0.974	58
	1994	3.16 (1.12)	1.43 (0.06)[a]	0.907	53
Perkinsiella saccharicida	1993	1.48 (1.11)	1.13 (0.04)[b]	0.953	34
	1994	1.10 (1.12)	1.02 (0.05)	0.935	32
Draeculacephala portola	1993	0.85 (1.07)	0.95 (0.03)	0.934	61
	1994	0.87 (1.11)	0.95 (0.06)	0.794	61

a, b, Slope significantly different from 1.0, *F*-test, $P < 0.01$, 0.001, respectively.

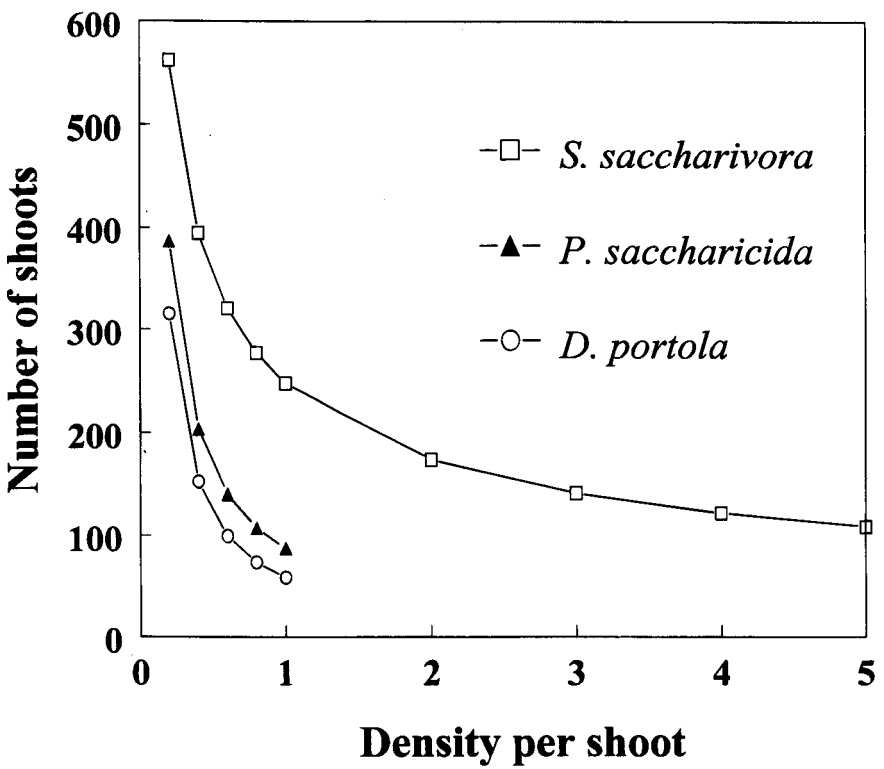

FIG. 5. Estimated number of shoots required to estimate *Saccharosydne saccharivora*, *Perkinsiella saccharicida*, and *Draeculacephala portola* densities in Texas sugarcane.

Due to this aggregation, larger sample sizes would be needed to estimate mean number of *S. saccharivora* per shoot than the other two insects. For example, the number of shoots required to obtain 90% confidence limits which are ± 20% of the mean of 5.0 *S. saccharivora* would be ca. 100 shoots (Fig. 5). The results presented here provide the first within-field distribution sampling data for these homopteran species in Texas sugarcane and form a baseline of information for growers, consultants, and researchers.

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RELEASE OF *TRICHOGRAMMA PRETIOSUM* IN COTTON WITH A NOVEL GROUND SPRAYER

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ABSTRACT

A mechanical system for applying insect natural enemies in a liquid suspension and adhering the natural enemies to foliage was evaluated for the release of *Trichogramma pretiosum* Riley in cotton. Immersion of host eggs containing *T. pretiosum* pupae in a mixture of commercial carrier (BioCarrier™) and water for up to four hours did not significantly reduce emergence of adults from host eggs. However, following application of host eggs with a novel sprayer, the BioSprayer™, emergence of adult *T. pretiosum* was significantly reduced by 22-30%. Significantly more of the host eggs with *T. pretiosum* pupae (78%) recovered from the cotton canopy after application with the BioSprayer™ were present in the upper plant canopy and 14% and 8% were recovered in the middle and lower portion of the canopy, respectively. The rate of loss of applied host eggs from cotton leaves was 13.6% per day and was attributed to weathering of the adhesive, abrasion from leaves and insect predation.

INTRODUCTION

Trichogramma spp. are mass reared and released for augmentative biological control of lepidopterous pests of crops and forests in many countries (Li-Ying 1994). *Trichogramma* are released manually in many of these areas and the lack of an efficient mechanical release method is one of several constraints to the commercial development of *Trichogramma* for pest control in the U.S. (King et al. 1985). Bouse and Morrison (1985) developed an aerial application system for *T. pretiosum* Riley which maintained pharate adults inside parasitized *Sitotroga cerealella* (Olivier) eggs at 13 °C until released from the airplane. Adult emergence occurred within four hours of release. This method reduced mortality of pupae resulting from prolonged exposure to high soil surface temperatures in cotton fields. However, the need for aircraft to be equipped with a refrigeration unit, the additional rearing costs to produce temperature-programmed eggs and a reduction in vigor in *T. pretiosum* following exposure to cold have limited commercial interest in this method (Morrison et al. 1998, Stinner et al. 1974). In Europe, aerial release of cardboard capsules containing pupae of *T. brassicae* Bezdenko has been commercially successful for control of *Ostrinia nubilalis* (Hubner) in corn, but this technology is not currently available in the U.S. (Bigler 1994, Suh et al. 2000). Gardner and Giles (1997) discussed the benefits of sprayer technology for releasing biological control agents and demonstrated that submergence of *T. pretiosum* pupae inside *E. kuhniella* (Zeller) eggs in water for up to three hours and passage through a large-orifice fan nozzle did not significantly reduce adult parasite emergence.

The USDA-ARS developed a low pressure, liquid system for applying and adhering eggs of *Chrysoperla* spp. to pecan tree foliage (Tedders and Blythe 1998) and this system

has been used to apply *C. rufilabris* eggs to cotton (Knutson and Tedders 2002). This sprayer system (BioSprayer™) and adhesive carrier (BioCarrier™) were commercially developed and marketed by Smucker Manufacturing, Inc., Harrisburg, OR 97446. Although designed to apply chrysopid eggs, the system was believed to have potential for applying pupae of *Trichogramma* spp. due to their small size and durability resulting from their protection inside the host egg.

The purpose of this study was to evaluate the BioSprayer™ system for releasing *T. pretiosum* in cotton plants for augmentative biological control of lepidopterous pests. The specific objectives were to determine the effect of exposure to the spray solution and application through the spray system on emergence of *Trichogramma* adults from pupae in host eggs, the retention time of parasitized host eggs on cotton leaves and the distribution of host eggs in the cotton canopy following application.

METHODS AND MATERIALS

The BioSprayer™ system consisted of a 37.9-liter tank pressurized to 0.7 pounds per square inch by a 12-volt DC air compressor and a variable pressure control valve. The spray solution was metered through a plastic tube that terminated inside the end of a two-inch diameter flexible metal pipe. Air flow from a 44-cc variable speed blower passed down the flexible pipe and carried the spray solution onto the cotton foliage. The end of the flexible pipe was positioned 10-15 cm above the canopy of a row of cotton. Air speed from the blower was maintained at 30-35 mph which broke up the stream of solution into large drops and deposited them in the canopy. The BioSprayer™ was mounted behind a tractor, powered by the tractor's 12-volt battery system and equipped with two spray lines and air hoses allowing application of *Trichogramma* to two rows. Calibration was accomplished by adjusting the pump pressure and tractor speed.

The spray solution consisted of a 1:8 ratio of a non-toxic solution marketed as BioCarrier™ (Smucker Manufacturing, Harrisburg, OR) and distilled water. The BioCarrier™ was designed to maintain the host eggs in solution and also to adhere the pupae to the foliage after the spray deposit dried. The spray solution and host eggs containing *T. pretiosum* pupae were agitated with a large paddle centered in the spray tank and rotated by an electric motor mounted in the top. The compressor also bubbled air into the spray tank to maintain a uniform suspension of host eggs in the liquid carrier solution. Eggs of *Ephestia kuhniella* (Zeller) parasitized by *T. pretiosum* were purchased from a commercial insectary (Beneficial Insectary, Oak Run, CA). The number of parasitized eggs in ten subsamples of 0.003 g each was counted to determine application rates.

Emergence of T. pretiosum after Immersion in Spray Solution. Host eggs, ca. 250, containing *T. pretiosum* pupae, were placed in each of 40 one-dram (4.5 ml), screw-top glass vials filled with the spray solution (1:8 BioCarrier™ and distilled water). Ten vials were selected at random after 1, 2, and 4 hrs and the solution was filtered through a Buchner funnel to recover the pupae. A subsample of 25 pupae was removed from the filter paper with a fine, moist brush and placed individually into cells of a tissue culture plate and incubated at 78°F and 80% R. H. After one week, host eggs were examined to determine if an adult wasp had emerged. Each time period was replicated ten times with a replicate equal to a subsample of 25 pupae per vial. Host eggs not immersed in the spray solution served as a control.

Emergence after Discharge from the BioSprayer™. To measure the effect of passage through the BioSprayer™ on adult emergence, two gallons of spray solution and ca 38,000 *T. pretiosum* pupae were added to the spray tank of the BioSprayer™. The large paddles inside the tank provided continual agitation to keep the pupae in suspension. The sprayer was

calibrated to deliver 160 ml per minute at a pump pressure of 0.7 psi and a blower speed of 31 mph. The spray stream from the BioSprayer™ was collected into a 500 ml-graduated cylinder. *T. pretiosum* pupae were removed from the spray solution by filtering it through a Buchner funnel. Pupae were removed from the filter paper with a fine brush and placed in individual cells of a tissue culture plate. Four subsamples of 40-50 host eggs were collected and held for adult emergence as described above. Host eggs not exposed to the spray solution served as the control. The experiment was repeated on three dates.

Distribution of Host Eggs in the Cotton Canopy. Field experiments were conducted to determine the distribution of host eggs in the cotton canopy when applied with the BioSprayer™. White, paper index cards (7.8 X 12.7 cm) were placed at 25, 50 and 75 cm above the soil in the cotton canopy. The average plant height was 80 cm, so the card at 75 cm was placed on a fully expanded leaf in the plant terminal. Host eggs applied with the BioSprayer™ adhered to the cards and were readily visible for counting. Cards were positioned flat against the upper leaf surface and stapled to the leaf at four corners. A single card was placed at the three heights on each of five plants in the center of each of ten 90 foot rows of cotton within a 0.5-acre plot. The plot was planted to 'Delta Pine 50' cotton on 40-inch row spacings at the Texas A&M University Research and Extension Center, Dallas, TX. Pupae were applied at a high rate, 1.6 million per acre, to increase the recovery rate of eggs on the cards. The BioSprayer™ was equipped with two spray tubes, each centered above a single row. Each spray tube was 10-15 cm above the center of the cotton row and directed back at a 45° angle to the top of the canopy. Tractor speed was 3.4 mph, air speed exiting the spray tube was 35 mph, and the spray output was 80 ml per 100 feet of row, or 2.4 gallons per acre. Pupae were applied to the cotton canopy on 11 August 1995. Air temperature was 95°F and wind speed was 5 mph. Fifteen minutes after application, all of the cards were recovered and the number of host eggs per card was recorded.

Retention of Trichogramma Pupae on Cotton Leaves. Host eggs containing *Trichogramma* pupae were applied with the BioSprayer™ on 9 August 1995 as described above to a 0.5-acre block of 'Delta Pine 50' cotton planted at the Texas A&M Research and Extension Center at Dallas, TX. Immediately after application, the terminal foliage was searched for host eggs. Once an egg was found, a small arrow was drawn on the leaf pointing to the egg and a red ribbon was tied to the leaf petiole and numbered to assist in relocating the egg. Each egg was examined daily for six days. The experiment was replicated twice with each replicate a single row each with 33 marked pupae.

In the second experiment, host eggs were applied through the BioSprayer™ as described above and were collected in a petri plate. Eggs with a *Trichogramma* pupae were removed from the spray solution with a fine brush and placed individually on the upper surface of a terminal cotton leaf. Spray solution on the egg adhered the egg to the cotton leaf. A circle was drawn around each egg with a marking pen to identify the position of each egg on the leaf. The petiole of the leaf was circled with a 4-cm band of Tanglefoot™ (Tanglefoot Company, Grand Rapids MI) to prevent access to the eggs by fire ants and other predators. Eggs were examined 1, 3, 4 and 5 days after placement on the leaf. The experiment was replicated five times with each replicate a single leaf with 25 pupae. Tests were conducted in mid-August when maximum temperatures were 95-100°F. No rainfall or dew occurred during the experiments.

Data on percentage emergence of adult *Trichogramma* from host eggs were transformed by arcsin before analysis of variance and untransformed values are reported. Differences in mean values were compared using Student's "*t*" test for paired treatment comparisons and Fisher's Least Significant Difference (LSD) at $a = 0.05$.

RESULTS AND DISCUSSION

Emergence of T. pretiosum after Immersion in Spray Solution. The mean and standard error for emergence of adult *T. pretiosum* from pupae not immersed in the BioCarrier™ was 97.3% ± 0.9. These values for percentage emergence for pupae immersed in the BioCarrier™ for 1, 2 and 4 hours were 92.9% ± 2.1, 90.9% ± 2.8 and 92.4% ± 1.7, respectively, and were not significantly different from emergence in the control ($F = 1.71$, d. f. = 3,36, $P = 0.18$). Results suggest that immersion of host eggs containing *T. pretiosum* pupae in the BioCarrier™ for up to four hours did not significantly reduce percentage emergence of adults. Morrison et al. (1998) concluded that the emergence of *T. pretiosum* following immersion in the BioCarrier™ for three hours was similar to that following immersion in water. However, emergence was reduced by 6-11% following immersion for 1-3 hours, respectively, and was significantly different from that in the non-immersed control.

Emergence after Discharge from the BioSprayer™. Mean percentage emergence of adults from host eggs passing through the BioSprayer™ was significantly less than adult emergence from the control group on each of the three sample dates (Table 1). These results indicate that some *T. pretiosum* pupae suffered injury during agitation in the spray tank and passage through the BioSprayer™, resulting in a decrease of 22-30% in subsequent emergence of adults from host eggs. Modifications to the tubing alignment and tubing attachment to solenoids and couplers have since been made to reduce constrictions to the flow and potential injury to the pupae (R. Morrison, personal communication). These studies will need to be repeated on the modified BioSprayer™ to determine the need, if any, for increases in release rates to compensate for reduced adult emergence resulting from passage through the BioSprayer™.

TABLE 1. Percentage Emergence (Mean ± S.E.) of Adult *T. pretiosum* Following Application of Parasitized Host Eggs With the BioSprayer™.

Date	Control[a]	BioSprayer™[a]	*F*	d.f.	*P*-value
20 July	81 ± 3 b	59 ± 5 a	14.9	1,6	0.008
4 August	88 ± 1 b	66 ± 2 a	177.4	1,2	0.006
8 August	86 ± 2 b	56 ± 7 a	49.3	1,6	0.004

[a] Means followed by the same letter within a row are not significantly different; t- test, $a = 0.05$.

Distribution of Host Eggs in the Cotton Canopy. A total of 339 parasitized host eggs were recovered from the cards positioned in the cotton canopy (Table 2). Significantly more of the recovered eggs, 78%, were found in the top canopy than at the two lower levels ($F = 11.6$, d. f. = 2, 147, $P = 0.001$). Also, the mean number of eggs per card was significantly greater in the top canopy than in the middle or bottom canopy ($F = 11.63$, d. f. = 2, 147, $P = 0.001$) and the number of cards with one or more eggs was significantly greater in the top canopy than in the bottom canopy ($F = 6.14$, d. f. = 2, 27, $P = 0.006$). These results suggest the majority (78%) of the host eggs recovered from the canopy were placed in the terminal or upper 25 cm of the cotton plant. Variables such as height of the cotton plant, leaf surface area and blower speed could potentially influence the relative distribution of pupae in the canopy but were not evaluated in these trails.

Retention of Applied Pupae on Cotton Leaves. Retention of host eggs on cotton foliage over time when applied with the BioSprayer™ was described by the relationship $Y = 88.67$ -

TABLE 2. Distribution of Parasitized Host Eggs in the Cotton Canopy Following Application with the BioSprayer™.

Canopy zone	Total eggs recovered (percentage)	Mean number eggs per card[a]	Mean number of cards with ≥ 1 egg[a]
Top	264 (78) a	5.3 a	2.8 a
Middle	48 (14) b	0.9 b	1.8 ab
Bottom	27 (8) b	0.5 b	1.1 b

[a] Means followed by same letter within a column are not significantly different. LSD, a = 0.05

13.64 X where Y is the percentage of pupae present and X is the number of days after application (Fig.1). This relationship predicted a loss rate of 13.6% of the host eggs per day.

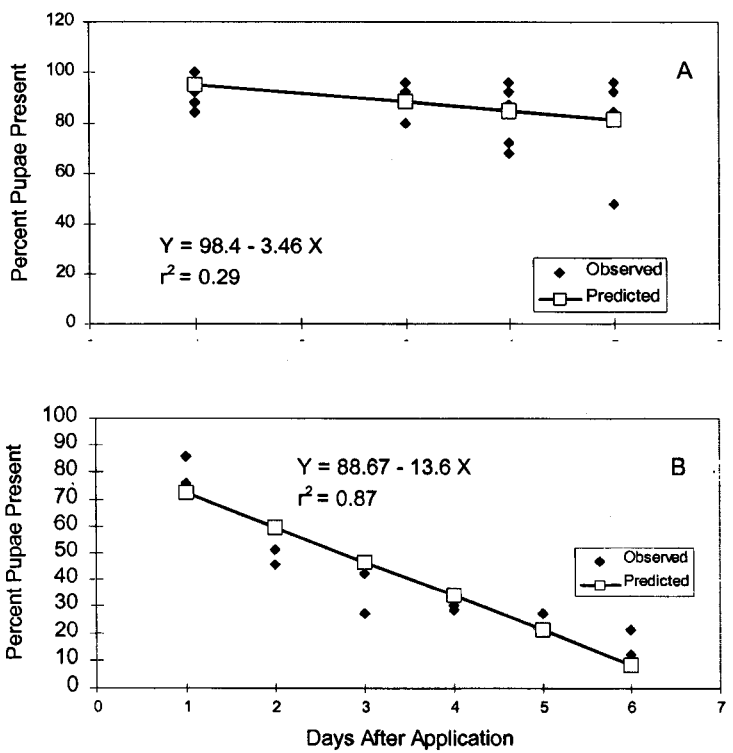

FIG. 1. Relationship between retention of parasitized host eggs on cotton leaves and days after application with the BioSprayer™ and with predators excluded (A) or predators with access to host eggs on leaves (B).

When predators were excluded from access to the eggs, this relationship was $Y = 98.4 - 3.46X$ and the rate of loss of eggs from the leaf was only 3.5% per day (Fig.1). These results suggest that removal of host eggs by predators was an important source of pupal disappearance. The red imported fire ant, *Solenopsis invicta* Buren, preys on lepidopteran eggs in the cotton canopy and was common in the study field. In earlier trials in this field, fire ants were observed to remove up to 50% of the *E. kuehniella* eggs parasitized by *Trichogramma* within 24 hours when eggs were glued to cards and placed in cotton terminals. In addition to predation, variable wind effects between experiments could also have contributed to the differences in rate of loss of host eggs.

No rainfall or dew occurred during these studies, both of which would be expected to increase loss of pupae as the BioCarrier™ is water soluble. Leaves moving across the surface of other leaves during windy periods may have dislodged some of the pupae. Removal of pupae by fire ants and other predators may also have been important. These results demonstrate that application of pupae should be timed such that the *Trichogramma* adults will emerge within 1-2 days following release. Also, release rates can be increased to compensate for the expected loss if detachment and predation rates are known.

These evaluations indicate the BioSprayer™ system can efficiently apply *Trichogramma* pupae inside host eggs to the cotton canopy. While there is some pupal mortality due to exposure to the BioCarrier™ solution and passage through the spray system, the application rate of host eggs can be increased to compensate for this loss and therefore achieve the desired release rate of *Trichogramma* adults. The BioSprayer™ deposits a majority of the host eggs in the upper canopy where *Heliothine* eggs, a potential target pest for augmentation of *Trichogramma*, are typically deposited. Efficacy of released *Trichogramma* would presumably be increased by placing pupae close to the target pest, thus reducing time for the parasite to search for the host. Also, adhering the pupae to the foliage avoids exposure of pupae to fatally high temperatures on the soil surface. Release of *Trichogramma* should be timed to ensure adult emergence occurs within one to two days of release to minimize loss of pupae due to predation and detachment from the foliage.

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ARTIFICIAL MATURATION OF FEMALE ALATES FOR THE PURPOSE OF THE PRODUCTION OF ONLY MALE *SOLENOPSIS INVICTA* (HYMENOPTERA: FORMICIDAE)

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ABSTRACT

Herein we describe a method of artificial maturation of 1-3 - day old, post-eclosion female imported fire ants, *Solenopsis invicta* Buren, through the use of virgin queen satellite colonies maintained by a parent colony that produces only haploid eggs and male brood on a sustained basis. Production of large numbers of such eggs with a predictable outcome will provide consistent material that can be used to focus on male biology and allow for various genetic manipulations that assist the production of transgenic fire ants.

INTRODUCTION

Ant colonies primarily consist of females that include queen(s), workers that are usually sterile, and female alates that are reproductively inactive; males are generally present for a limited period within a colony (Wilson 1975). The occurrence of males is regulated in part by the queen and in part by the workers resulting in queen-worker conflict over sex allocation (Trivers and Hare 1976) and is a subject of great interest (Bourk and Franks 1995). Brian (1969) showed that workers of *Myrmica rubra* L. laid haploid eggs, but these eggs often serve as food for developing queen-laid eggs, although he was able to rear males. Passera et al. (1988) working with the Argentine ant, *Linepithema humile* formally *Iridomyrmex humilis* (Mayr), reported that queens laid male eggs throughout the year, but most failed to reach the pupal stage. This failure was influenced by the ratio of workers to larvae. If there was plenty of protein available and a high worker to larva ratio, more males reached the adult stage (Passera et al. 1988).

Imported fire ant, *Solenopsis invicta* Buren (Hymenoptera: Formicidae) workers are sterile and reproduction is by the queen. This process begins following a mating flight that result in insemination, dealation, and her transformation into a queen that produces eggs for the remainder of her life. The eggs she produces have different fates: haploid, non-fertilized eggs develop into males; diploid, fertilized eggs develop either into fertile females or sterile workers; and trophic (non-embryonated) eggs are used as food. A colony is always in a dynamic state consisting of different classes of progeny such as workers, males, and alate females in various proportions at different times depending on a number of environmental conditions (Vargo and Fletcher 1986, 1987). However, a majority (about 97%) of queens' eggs develop into sterile workers. Less than 3-4% develop into fertile males and females. However, in polygyne fire ant colonies, some queens produce diploid males with various

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degrees of sterility (Hung and Vinson 1976, Ross and Fletcher 1986). As a result, males are not consistently produced and not all males produced are fertile.

Genetic manipulation of the fire ant will require consistent production of a large number of reproductively competent adults that can be manipulated genetically and still generate offspring. The preferred approach is to genetically manipulate the egg stage of the reproductives. Unfortunately, the social nature of the ant colony presents obstacles for using eggs for genetic manipulation. These obstacles include an inability to non-destructively differentiate between haploid, diploid, or trophic eggs immediately after oviposition and the inability to predict whether a fertilized diploid egg will develop into a fertile female, a sterile worker, or a sterile male. Moreover, manipulation of eggs by biolistics or microinjection can result in significant mortality. Thus, for efficient production of transgenic *S. invicta*, large numbers of 1-2-h old eggs that can be manipulated to develop into reproductive forms of propagation of a transgene are essential. Also it will be important that all eggs consistently have one fate. Because the fate of fire ant diploid eggs are influenced by external factors (Robeau and Vinson 1976), or genetic factors in the case of sterile males (Ross and Fletcher 1985), diploid eggs do not appear to be a good option for manipulation. Haploid eggs may offer a solution.

In a normal fire ant colony, the queen influences the overall behavior of the colony with a battery of pheromones (Vargo 1999, Obin and Vander Meer 1989). Some of these pheromones prevent dealation and the reproductive development of virgin female alates (Fletcher and Blum 1981, Sorensen et al. 1985, Vargo and Porter 1993, Vargo and Laurel 1994). Both mechanically dealated virgin female alates and virgin female alates removed from the inhibitory influence of a queen pheromone will initiate ovary development (Glancey et al. 1981, Fletcher et al. 1983). But, virgin female alates dealate more readily in the presence of workers (Fletcher et al. 1983) and would presumably initiate ovary development and lay haploid eggs sooner. However, these studies have been limited to addressing questions concerning the attraction of workers, dealation, wing muscle histolysis, ovary development, and egg laying by virgin female alates. Stringer et al. (1976) reported that individual dealates from multiple queen colonies were incapable of founding colonies and died within 12 wk when separated; however, these authors found that dealate survival was enhanced with the addition of workers. Further, 98% of over-wintered and spring-reared virgin queens that were placed separately in laboratory rearing conditions initiated oviposition and colony-founding behavior, but only 73% dealated. Their eggs were larger than normal and less than 1% were viable (Fletcher and Blum 1983). Field monogyne colonies of *S. invicta* orphaned by removal of a functional queen develop replacement queens from dealates already present in the colony. A proportion of these replacement queens were not inseminated and produced only male brood (Tschinkel and Howard 1978). Although male pupae were observed, no male production was reported. None of these studies demonstrate the production of adult males, and no studies have maintained queens with a goal of producing numerous males or only producing males. Further, such colonies were not sustained because workers were not replaced.

There have been a number of studies using virgin female alates to determine whether factors, such as juvenile hormone (JH) or carbon dioxide, initiate dealation, subsequent ovary development, and egg production (Backer 1979; Fletcher and Blum 1981, 1983; Fletcher et al. 1983; Kearney et al. 1977; Jones et al. 1978). But again, the goal of these studies was not male production. Since virgin females produce only haploid eggs, we examined the potential to use such females to produce adult males. We describe a method of artificial maturation of 1-3-day old post-eclosion virgin female alates without mating and establishment of satellite colonies that produce only haploid eggs, and, hence, only fertile males on a sustained basis. The eggs from such satellite colonies can be pooled together, and a foreign gene can be introduced by various methods to produce transgenic males that may be used to propagate the

foreign gene by natural mating. Alternately, the production of males will also allow for the planning of studies that can focus on male biology.

MATERIALS AND METHODS

Field colonies were excavated with shovels, transferred to buckets previously coated inside with Talc (Sigma, St. Louis, MO) to retain the ants, transported to the laboratory and allowed 2 days to reestablish a tunnel system by the workers. Colonies were separated from soil by floating out the ants and brood (Jouvenaz et al. 1977); ants and brood were transferred with a spaghetti strainer to a plastic shoebox (27x40x9cm) coated with ADI Fluon (ICI Fluropolymers, Bayonne, NJ) along the top edges to prevent ant escape. The box contained two Castone-floor (Densply, York, PA) petri dishes (150x15mm) that served as artificial nests. Each nest had a 15mm hole through the lid to allow queens and workers access. One filter paper (150mm diameter) was placed on the top of each nest chamber to reduce light disturbance to ants. These laboratory colonies (referred to a parent colonies) were housed in an environmental room at 28±1.2°C, 44-66% RH, and a photoperiod of 14:10 (L:D). Colonies were maintained on a daily diet of water, 25% honey water, yellow mealworms (*Tenebrio molitor* L.) and artificial diet (Kuriachan and Vinson 2000) as needed.

Maturation of virgin female alates by various chemical and physical treatments was evaluated by a series of tests in which ten small nests with 30 workers were set up. These nests consisted of a Castone-floor petri dish (95x15mm) with a 15mm hole through the lid that served as an artificial nest. These were placed in a shoebox and maintained as described above. In each case, a treatment female alate was placed in each nest and monitored daily for loss of wings, occurrence of egg-laying, and subsequent embryonic development. There were four female alate treatments. A juvenile hormone treatment evaluated the effect on egg production of the JH analog (methoprene) in acetone (100ng/ul) applied to 1-3-day old, post-eclosion virgin female alates removed from a polygyne colony. Five ul was applied to the bottom of a 3ml plastic vial to which the female alate was introduced one hour later. After three days, the JH exposed female was placed in one of the small 30 worker nests. In a second treatment, one to three day-old, post-eclosion virgin female alates were placed into a chamber and exposed to 100% carbon dioxide (CO_2) for 10 minutes. The chamber consisted of a 60mm plastic petri dish connected to a carbon CO_2 via plastic tubing. After recovering from CO_2 anesthesia, the CO_2 exposed females were transferred individually to 3ml plastic vials. After three days, these treated females were placed individually into one of the small 30 worker nests. A third test consisted of 1-3-day old, post-eclosion virgin female alates placed in a 60mm petri dish and transferred to a refrigerator at 4°C. After 48h, the petri dish was removed from the refrigerator. Females were transferred individually to 3ml plastic vials and held at room temperature. One day later, treated females were placed individually into one of the small 30 worker nests. A control consisted of a 1-3-day old, post-eclosion virgin female alate removed from a polygyne colony, held one day, and then placed into one of the small 30 worker nests.

One additional test consisted of placing virgin female alates into large queenless satellite colonies to increase access to resources through workers from a parent satellite colony. These colonies were initially set up with two virgin female alates (ages 1-3-day post-eclosion) removed from a large colony and placed in separate nests (150x15mm as described above) with about 1000 workers from their parent colony. These satellite colonies were maintained for 15 days and then the dominant female, the female producing the greatest number of eggs and attracting the greatest number of workers (Chen and Vinson 2000), was determined and retained. The other female was removed. Because these satellite colonies were unable to replenish lost workers, workers were added from the parent colony on a weekly basis to maintain approximately 1,000 workers per queen. These parent and satellite

colonies were housed in an incubator at 27°C, 50% RH, and a photoperiod of 14:10 (L:D). Colonies were maintained on a daily diet of water, 25% honey water, yellow mealworms (*T. molitor*) and frozen crickets. Four satellite colonies were set up from both monogyne (single queen) and polygyne (multiple queen) colonies (8 total) and were maintained over 6 months.

RESULTS AND DISCUSSION

All treatments were successful in inducing wing loss and egg laying, but few eggs hatched and none reached the pupal stage. Methoprene accelerated wing loss and egg laying compared to other treatments, but there was no further development of these eggs. Most likely these eggs were trophic (Glancey et al. 1973, Voss 1981, Voss and Blum 1987). Both the control group and the satellite colonies were slower to dealate (Table 1) and began to lay eggs at about the same time. Although some of the eggs in the control group hatched, none of these larvae reached second instar and the queen's oviposition rate declined.

TABLE 1: Effect of Various Treatments on the Maturation of Virgin Female Fire Ant Alates.

	Days after treatment[a]						
Treatments	1	5	10	15	30	60	N
Control	-	-	A+B	B	B	B	10
Methoprene	A	B	B	B	B	B	10
Carbon dioxide	-	A	B	B	B	B	10
4°C exposure	-	A	B	B	B	B	10
Satellite colonies	-	-	A+B	C	D	E+F	8

[a]Letters in the columns (A=Lost wings; B=Egg-laying; C=Egg development; D=Larvae; E=Pupae; F=Adult.) refer to the first appearance of the characteristic found in a majority of the nests.

In contrast, queens in satellite colonies had larvae by 30 days. Further, with one exception, larvae were continually found and, by 60 days, pupae and some adult males were found (Table 1). We found that male production increased over time, increasing three to four times over the third month compared to the first two months (Table 2). Colony #2185(b) was removed from the study after 60 days as this female dealated but never laid eggs for reasons that are not clear. By the fifth and sixth month (data not shown), production in the remaining nests increased by another 1.5X, but then leveled off (data not shown) to an approximate average of 100 to 200 males/month. There were no differences in male production using virgin female alates from either polygyne or monogyne colonies. At the end of six months, we were still successful in adding workers from the parent colony to the satellite colony that continued to produce males for several additional months at which time they were terminated.

TABLE 2: Male Production by Satellite Fire Ant Colonies.

Parent colony	Colony type	Satellite colony	No. males produced 0-60 days	62-120 days	121-180 a days	Total
#2504	Polygyne	a	30	120	170	320
		b	37	129	205	371
#2400	Polygyne	a	28	151	212	391
		b	39	113	207	359
#2509	Monogyne	a	41	105	161	307
		b	32	137	210	379
#2185	Monogyne	a	45	114	131	290
Mean ± S.D[a]			36±6	124±15	185±31	345±39

[a] Statistical analysis by ANOVA shows a significant increase in male production over time ($P<0.001$)

Removal of virgin female alates from colonies and stimulating dealation, subsequent ovarial maturation, and oviposition did not produce viable eggs. Such queens may not be physiologically prepared to become reproductively competent. Virgin female alates, under the influence of a resident queen and environmental factors, become sexually mature and wait for an environmental trigger that will initiate a mating flight. Environmental triggers (Morril 1974, McCluskey and McCluskey 1984, Hooper and Rust 1998) initiate changes in the behavior of the members of the colony, more specifically in the female reproductives (Obin and Vander Meer 1994, Alonso and Vander Meer 1997). Vinson et al. (1980) reported that prior to the mating flight, lipids in the digestive system of virgin female alates increased, suggesting that females were stimulated to maximize their nutritional reserves just prior to their mating flight. As a result, we speculate that virgin female alates removed from a colony prior to exposure to environmental conditions that trigger a mating flight do not have the nutritional resources needed for production of viable eggs. As a result, viable eggs were never produced by any of the treatments applied to the virgin female alates or control. Although removal of the virgin female alates from the queen's dealation and reproductive inhibitory pheromones (Obin et al. 1988; Vargo 1999) is an important step in initiating reproduction, another factor is providing nutrition. As shown by Sorenson et al. (1981, 1983), larvae are important to the nutritional needs of the colony through the digestion and processing of proteins. These nutritional resources are transferred through the workers to the queen. Virgin female alates in control nests, once separated from the parent colony, not only lacked the resources, but also probably received little additional nutritional support from the limited number of workers (just 30) that were added. This problem was overcome by providing virgin female alates with approximately 1,000 workers that collectively provided the needed resources, some of which may be important to the early development of larvae. Further male production increased with time in these satellite virgin queen colonies. This may be due to additional nutritional resources provided by the presence of the male brood itself and by the weekly addition of workers from the parent colony. It is not clear why one satellite colony failed to produce eggs.

The satellite method of male production was not only successful, but may provide some benefits. One advantage includes access to a large number of virgin female alates over an extended period of time that can be used to form a number of male producing satellite colonies supported by one parent colony. Another benefit is the opportunity to maintain the male-only production over a long period of time through periodic worker addition. This

greatly increases the numbers of haploid eggs that are available for various transgenic manipulations thus facilitating the production of transgenic males. Further, future studies concerned with male biology also may benefit from the consistent production of males. This method of male production may be applicable to other social insects with similar social and reproductive behaviors.

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RELATEDNESS AMONG CO-EXISTING QUEENS WITHIN POLYGYNE COLONIES OF A TEXAS POPULATION OF THE FIRE ANT, *SOLENOPSIS*[1] *INVICTA*

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ABSTRACT

Five microsatellite markers were isolated, characterized, and used as genetic markers to estimate the genetic relatedness among fire ant, *Solenopsis invicta* Buren, queens within polygynous colonies and among queens within aggregated groups in colonies maintained for a short time in the laboratory. Estimates of genetic relatedness using microsatellite markers showed that co-existing queens in the same colonies were not closely related, which is in agreement with previous findings using mitochondrial and nuclear markers. Queens also randomly aggregated into groups, and queen aggregation was not based on their relatedness. The F_{st}-value suggested that polygynous colonies from the Brazos Valley, Texas, can be considered as a single population and demonstrated that gene flow occurs frequently in this area, probably by dispersion caused by mating flights, transportation and wind. The near zero F_{is} value indicated that polygynous queens in this area were randomly mating.

INTRODUCTION

Polygyny is characterized by coexistence of more than one inseminated, egg-laying female in a colony. Polygyny within a colony can be primary or secondary. Primary polygyny arises when several queens work together to initiate a new colony (pleometrosis). However, as the colony begins to grow, workers kill or expel all but one of the queens to make the colony monogynynous. Pleometrosis is believed to rarely lead to permanent polygyny (Hölldobler and Wilson 1977, Rissing and Pollock 1988). Secondary polygyny develops when extra queens are adopted or fused into an established colony that was initiated by a single queen (haplometrosis). Primary polygyny is usually thought to be selectively advantageous because co-operative queens may have a higher survival rate during the early founding period; however, the existence of secondary polygyne presents a dilemma for Hamilton's kin selection theory (Hamilton 1964 a,b). As more queens are added into the colony, there will be a decrease in the overall nestmate relatedness and in the individual queens' relative fitness resulting from raising the progeny of adopted queens. Why should a resident queen and workers in an established colony accept additional queens? Why don't workers on one matriline kill other queens so that their mother will be able to maximize reproductive success? The behavioral interactions and genetic relatedness

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of co-existing queens within a colony must be examined to help explain the evolution and maintenance of polygynous social organization.

During the last two decades, considerable effort has been made to study relatedness patterns in polygynous colonies. In some ant species, polygynous queens within a colony have been reported to be closely related (Craig and Crozier 1979, Herbers 1986, Douwes et al. 1987, Pamilo 1991). In these cases, established queens might increase their genetic output by accepting related individuals such as daughters, granddaughters and cousins of the original queens back into their colony, compensating for the decrease in their individual output by proportionally increasing their relative output. High queen relatedness within a colony would be consistent with the expectation that the extraordinary level of altruism found in ants evolved through kin selection. However, in some other ant species, studies of kin selection have failed to demonstrate significant relatedness among individuals within a colony (Ross and Fletcher 1985; Hölldobler and Wilson 1990; Kaufmann et al. 1992; Herbers 1993; Bourke and Franks 1995; Ross and Keller 1995; Crozier and Pamilo 1996; DeHeer and Ross 1997; Goodisman and Ross 1997, 1998) and appear to challenge the kin selection theory (Hamilton 1964a,b). Several factors might account for the decrease in genetic relatedness among individuals. If unrelated queens were adopted into a colony such that there are multiple queens laying eggs, the genetic relatedness among the progeny should be low because of multiple matrillines among the progeny. If one queen mates with multiple males, the genetic variability of progeny would increase, and concomitantly, the genetic relatedness would decrease.

For many years, the red imported fire ant, *Solenopsis invicta* Buren (Hymenoptera: Formicidae), was described as exclusively monogynous. In 1973, Glancey et al. first reported the discovery of a polygynous form of *S. invicta* in Mississippi. Since that time, the polygynous form has been reported in many of the infested states (Hung et al. 1974; Morrill 1974; Mirenda and Vinson 1982; Fletcher 1983; Glancey et al. 1987, 1988; Porter et al. 1991). Since its recognition, polygynous *S. invicta* has received increased attention, and a considerable amount of information is now available on its colony life history, social behavior, and genetic structure (Ross and Fletcher 1985; Ross 1993; Ross et al. 1996; Goodisman and Ross 1997,1998; Krieger and Keller 1997; Chen and Vinson 1999,2000). Ross and Fletcher (1985) examined the relatedness of polygynous *S. invicta* queens using allozyme data and concluded that nestmate queens were no more closely related to one another than they were to any other population. A recent model for queen recruitment has revealed that co-existing queens display low levels of mitochondrial and nuclear relatedness and suggested that foreign queen recruitment occurs at a high frequency in the polygynous *S. invicta* (Goodisman and Ross 1998). Our previous study on polygynous queen interactions found that co-existing queens usually aggregated peacefully into one or more groups in the same nest, and workers frequently were observed to pull and push queens back into the queen aggregation when queens began to move away (Chen and Vinson 1999). This led us to hypothesize that separate queen aggregations within a colony may have a bearing on relatedness, even though overall genetic relatedness within the colony is low.

Recent advances, such as neutral allozymes, co-dominant random amplified polymorphic DNAs (RAPDs), dominant random amplified polymorphic DNA (RAPDs), microsatellites, mitochondrial DNA (mtDNA), and selected protein loci, have led to the increased availability of genetic markers. Among these, microsatellites have been reported to give more precise estimates of relatedness compared with allozyme electrophoresis (Kaufmann et al. 1992) and are the most effective markers for detecting genetic structure (Ross et al. 1999). Because they are co-dominant, selectively neutral, and highly polymorphic, microsatellite markers are ideal for the study of variation in social organizations, the evolution of individual phenotypes, patterns of gene flow, and relatedness among individuals (Ross and Keller 1995; Krieger and Keller 1997; Goodisman

and Ross 1998; Ross et al. 1997,1999). Although they have been widely applied to genetic analysis of social insects, including *S. invicta*, microsatellite markers have not been used previously to ex ine co-existing queens' relatedness within the introduced population of *S. invicta*. In the present study, we investigated the relatedness among aggregated dealate and queen groups within the same colonies of a Texas population of *S. invicta*. We also estimated relatedness of functional queens within polygyne colonies using isolated microsatellite markers in hopes of adding a different dimension to the genetic studies of polygynous queens in the *S. invicta*.

MATERIALS AND METHODS

Ten monogynous and 24 polygynous colonies of red imported fire ants were collected in College Station, Texas. The monogynous queen, which was readily identified by her physogastric condition and the worker size from her colony (Greenberg et al. 1985), 15 workers from each monogynous colony, and all the dealates (dealate numbers ranged from 7-56) in polygyne colonies were used to score the microsatellite loci.

Twenty-three polygynous colonies were collected from six sites in the Brazos Valley Area, Texas, for queen relatedness. Only nests that were separated from other nests by more than 2.0m at any one site were collected to avoid sampling a colony fra ent, as described by Goodisman and Ross (1997). Colonies were drip-floated and transferred to plastic trays containing two or more 150 x 15- petri dishes with a 3-mm-thick castone floor (Pemaco, St. Louis, MO). Colonies were left undisturbed for the 4 weeks following their collection to allow dealates to separate into groups. Dealates were removed from the dealate groups within the nest. The head and thorax of each dealate were removed and stored at -80°C for later DNA extraction. Abdomens of dealates from colonies were dissected to dete ine the mating status of each dealate and to confirm the polygynous condition of the colonies: when the dealate is inseminated, the spe atheca is opaque and milk-white; when the dealate is uninseminated, the spe atheca is transparent (Tschinkel 1987). In the text, inseminated dealates are referred to as queens.

Genomic DNA was extracted from 1.0 g of *S. invicta* brood (eggs, larvae, and pupae) from both monogynous and polygynous colonies individually using equilibrium centrifugation in a CsCl-ethidium bromide adient (Maniatis et al. 1982). *Mbol* fra ents of *S. invicta* genome were cloned into the *Bam*HI cut plasmid vector, pUC19, by standard procedures (Maniatis et al. 1982). The genomic library was screened by colony hybridization using ^{32}P-labelled synthetic (dA-dC)n oligonucleotides as a probe. Hybridization was conducted overnight at 42°C. Membranes were washed twice in a low stringency solution (1X SSPE, 0.1%SDS) for 15 minutes at room temperature and autoradio aphed on an intensifying screen overnight. Positive colonies were picked with a Pasteur pipette. Well-isolated colonies were grown in LB-amp media overnight and plasmid DNAs were extracted by standard plasmid mini-prep techniques (Maniatis et al. 1982). DNA sequence of the insert was dete ined by Sanger dideoxy sequencing on an ABI Prism 377 DNA sequencer. Insert DNA sequence was determined in both directions using forward and reverse vector PUC/M13 primers. Additional primers were synthesized, as needed, for primer walking across large DNA fra ents. Sequences were analyzed using MacVector Version 5.0 software (IBI, New Haven, CT, USA).

PCR primers complementary to unique sequences flanking each microsatellite locus were desi ed using the program OLIGOv4.0 and synthesized by Genosys Company (Bio-Synthesis Inc., P.O. Box 28, Lewisville, TX 75067-0028).

Template DNAs from monogynous queens and workers were prepared using the QIA amp tissue kit (QIAGEN, 28159 Avenue Stanford, Santa Clarita, CA 91355), following the manufacturer's instructions. The PCR reactions were carried out in a 25-µl reaction mixture containing 20mM dNTPs, 1X Taq buffer containing 1.5mM $MgCl_2$, 5µM

reverse primer, 0.625 unit of Taq polymerase, 5μM ^{32}p-dATP end-labeled forward primer, and 50ng template DNA. Thermal cycling parameters were standardized for all primers and template combinations with specific annealing temperatures: two minutes initial denaturation at 95°C followed by 35 cycles of 95°C for 45 seconds, 45 seconds at the primer specific annealing temperature and 72°C for 45 seconds, the last cycle was 10 minutes extension at 72°C. Aliquots of 5μl were taken from the reactions and electrophoresed on a 6% polyacrylamide gel, followed by autoradiography.

DNA extracted from the queen and 15 worker pupae from each of the ten monogynous colonies were used to verify the inheritance of the microsatellite markers, and about 500 dealates from polygynous colonies were used to evaluate the level of polymorphism. Microsatellite markers that were polymorphic with four or more alleles were selected for further estimation of genetic relatedness of polygynous queens. Tests for conformity of the observed nuclear genotype frequencies to those expected under Hardy-Weinberg equilibrium (HWE) were conducted using chi-square tests at the critical value for $\alpha = 0.05$.

Five hundred and seven polygynous queens were genotyped at four loci, and about 150 samples were genotyped for the microsatellite M-I locus by PCR using radioactive labeled primers. Template DNAs were prepared from samples of head and thorax using the QIAamp tissue kit, and the PCR reactions were performed as described above.

Genetic relatedness and F-statistics were estimated using the software program RELATEDNESS 4.2C (Goodnight and Queller 1994). Colonies were weighted equally when calculating the relatedness among the dealates and queens in the same colony. All available individuals were used to estimate the average allele frequency in the reference population. The standard errors for estimates were obtained by jackknifing over colonies (or groups). At t-test was used to determine whether the point estimate differed from zero.

RESULTS

About 2,000 recombinant clones were screened using a $(dA\text{-}dC)_n$ radiolabeled probe, and 30 positive clones were isolated. Among these, 18 microsatellites were identified. Potential primer pairs for 12 of the microsatellite repeats were designed using the Macvector program. Five of the microsatellites that were able to be amplified in fire ant DNA and were polymorphic with numbers of alleles from four to seven were selected for further analysis. Primer sequences, annealing temperatures, predicted lengths of products, and allele frequencies of each locus are presented in Table 1.

Analysis of the expected and observed levels of heterozygosity (He, Ho) for each locus among polygynous queens is given in Table 2. The sample size of N=150 was used for analyzing microsatellites M-I and M-II loci and N=517 for the other microsatellites. Expected heterozygosity for the five polymorphic loci ranged between 0.53 and 0.82. The allele frequencies for polygynous queens, monogynous queens and males are also listed in Table 2. The genotypes of males were inferred from the genotypes of workers in each monogynous colony. Because only ten monogynous queens were used in this study, the sample size is insufficient to test for genetic differences between polygynous and monogynous queens. Genotypic frequencies at each polymorphic microsatellite locus in polygynous queens fit Hardy-Weinberg expectations at a 5% level, consistent with Mendelian inheritance of variation in microsatellites (Litte and Luty 1989, Tautz 1989, Weber and May 1989). Progeny studies of ten monogynous colonies revealed that all worker pupae in a monogynous colony had a common allele from the presumed father and another allele from their mother in expected Mendelian ratios. Microsatellite genotypes of worker pupae in each monogynous colony indicated that each monogynous queen was inseminated by a single male, and that the workers were all offspring of the queen and her mate.

TABLE 1. PCR Primer Sequences and Repeat Motifs for Eleven $(dA\text{-}dC)_n$ Microsatellite Sequences from the Red Imported Fire Ant *Solenopsis invicta* Buren.

Locus	Primers	Core Sequence	Size	Annealing Temp.	No. of Alleles
M-I	F:AACAAGGGTTGATCGCAAAAC R:TCATTCGCTGACAATCGACAG	$(TG)_{23}$	181bp	53°C	5
M-II	F: CAATTACGTCTCGGTTATCGACTC R: GGGTTGATCGCAAAACGATTG	$(TG)_{17}$	249bp	52°C	4
M-III	F: ATTGTACGAGCGGGAGAAGCAC R: TCGAGATCGACCAGGTAGGAACAC	$(AG)_9$	211bp	57°C	4
M-IV	F: CGAGTCTTCGAGCTTGGAAAAG R: CTCATCCCCACAAAGTTTCATTG	$(TG)_{17}(AG)_{25}$	278bp	52°C	5
M-V	F: GTGGAATGCACATCTAAAC R: GGAAACGCTGACTTTTCGTC	100(AC)(GC) Repeat	397bp	56°C	7

TABLE 2. Allele Frequencies, Heterozygosity, and chi-square χ^2 test for Genotype Frequencies in Each Microsatellite Loci in *Solenopsis invicta*. (N=Sample Size).

	Allele Frequencies							Heterozygosity			
Locus	A	B	C	D	E	F	G	He	Ho	χ^2	P
M-I											
P queens (N=150)	0.308	0.043	0.052	0.396	0.201			0.69	0.71	3.93	0.70
M-II											
P queens (N=490)	0.315	0.038	0.317	0.331				0.69	0.67	11.80	0.07
M queens (N=10)	0.100	0.150	0.350	0.400							
Males[a] (N=10)	0.400	0.000	0.300	0.300							
M-III											
P queens (N=490)	0.586	0.087	0.343	0.002				0.55	0.53	3.91	0.70
M queens (N=10)	0.250	0.100	0.650	0.000							
Males[a] (N=10)	0.100	0.100	0.800	0.000							
M-IV											
P queens (N=490)	0.018	0.643	0.088	0.233	0.018			0.53	0.52	12.56	0.32
M queens (N=10)	0.400	0.550	0.000	0.050	0.000						
Males[a] (N=10)	0.600	0.100	0.200	0.000	0.100						
M-V											
P queens (N=490)	0.106	0.187	0.135	0.272	0.071	0.221	0.009	0.82	0.79	12.02	0.30
M queens (N=10)	0.050	0.250	0.400	0.050	0.150	0.100					
Males[a] (N=10)	0.000	0.600	0.200	0.000	0.100	0.100					

[a] The genotype of male was inferred from the genotype of workers and queen in a colony

Ten colonies contained separate dealate groups in which the dealate number ranged from 4 to 35; 25% of the dealates on average were uninseminated over all samples studied. The correlation coefficient between the number of mated queens and unmated dealates was 0.703 ($P < 0.001$, $n = 20$). The genetic relatedness of dealates and mated queens within a colony were 0.0245 $\pm$ 0.021 (SE) (sample size = 490) and 0.0203 $\pm$ 0.0153 (SE) (sample

size = 350), respectively, not significantly different from zero. Relatedness of dealate females and mated queens within the aggregated groups also was not significantly different from zero. The relatedness of queens between aggregated groups, correlation ooefficients for relatedness of dealates, and mated queens between aggregated groups within a colony ($r = -0.373$, $P = 0.289$, $n = 10$ for dealates and $r = -0.0975$, $P = 0.86$, $n = 10$ for mated queens) confirmed that there was no significant relationship between and within the aggregated group dealates or queens. A relatively high estimate of relatedness in one group did not imply a low estimate of relatedness in another (Table 3).

TABLE 3. Relatedness of Dealates or Mated Queens within a Colony or within and between Aggregated Groups in a Colony.

	R±S.E.	No. of Colonies	No. of Individuals
Dealates	0.0245±0.0021	23	490
Mated Queens	0.0203±0.0153	20	350
Dealates Within Groups	0.0043±0.0367	10	164
	0.0189±0.0249	10	139
Mated Queens Within Groups	0.0108±0.0421	10	120
	0.0330±0.0289	10	101
Dealates Between Groups	-0.0243±0.0234	10	164:139
Mated Queens Between Groups	0.0342±0.0244	10	120:101

All the twenty-three colonies were collected from the Brazos Valley Area. The overall F_{st} was 0.0244 ± 0.0149 (SE) (sample size = 491), not significantly different from zero at $\alpha = 0.05$ level, indicating that fire ants in polygyne colonies in the Brazos Valley area can be treated as a single population. There is no population structure among the six research sites. F_{is} was 0.0774 ± 0.0691 (SE) (sample size = 491), and was not significantly different from zero when estimated over all loci and from all the studied individuals.

TABLE 4. Correlation Coefficients between Number of Queens and Relatedness.

Relatedness	Correlation Coefficient(r)	p-value	No.of comparisons
Between number of mated queens and unmated queens	0.703	<0.001	20
Between number of dealates and relatedness of dealates in a colony	-0.136	0.22	24
Between number of mated queens and relatedness of mated queens	-0.0145	0.21	22
Relatedness of dealates between two aggregated groups in a colony	-0.373	0.29	10
Relatedness of mated queens between two aggregated groups in a colony	-0.0975	0.86	10
Between relatedness of mated queens and relatedness of unmated ealates	0.32	0.32	18

The correlation coefficient between colony queen number and relatedness was – 0.136 (P=0.22; n=24), not significantly different from zero, indicating that relatedness of dealates within a colony did not change as dealate number changed (Table 4). Tests for correlation between mated queen number and relatedness of mated queens within a colony also showed that there was no significant correlation between mated queen number and relatedness of mated queens (r=-0.0145; P=0.21; n=22). The results confirmed that relatedness of dealates or mated queens did not change as the number of dealates or mated queens changed (Table 4).

The correlation coefficient between relatedness of mated queens and relatedness of unmated dealates within a colony was 0.32 (P = 0.32; n = 18). There was no significant association between these two values, indicating that a relatively high estimate of relatedness among mated queens did not imply a low estimate for unmated dealates (Table 4).

DISCUSSION

Microsatellites are promising genetic markers for *S. invicta* genetic studies. Ross et al. (1999) compared six classes of genetic markers in describing the genetic structure of the imported fire ant, *S. invicta*, and concluded that microsatellites had the greatest proportion of total variance and were the most effective markers for detecting genetic differentiation. Our progeny study of single queen colonies confirmed that microsatellites in *S. invicta* are codominantly inherited and selectively neutral. Analysis of the genotypic frequencies of each polymorphic microsatellite locus indicated that the observed allele frequencies fit the Hardy-Weinberg expectation for autosomal alleles.

Our earlier studies (Chen and Vinson 1999) showed that co-existing queens in polygynous colonies usually aggregated peacefully to one or more groups. Workers were observed frequently to pull and push queens back into the aggregation when a queen began to move away. The studies (Chen and Vinson 2000) also showed that the co-existing queens were mutually tolerant but could exhibit a form of competitiveness. This competition was characterized by the formation of linear attractive ranking to workers. The queen that was most attractive to workers was the queen that had a high frequency of trophallaxis. The trophic advantage resulted in a greater reproductive success of the dominant queen as evidenced by her higher ovipositional rate. Previous studies posed an interesting question about relatedness within a queen aggregation and between the queen aggregations in the same nest. We hypothesized that queens within the same aggregation were more related than queens from different aggregations. This study confirmed the occurrence of queen aggregations, and among the studies samples of polygynous colonies, ten of 24 colonies (41.7%) yielded more than one dealate female group, which remained stable in undisturbed colonies. Nevertheless, analysis of relatedness within aggregated groups showed that the aggregation of dealate females or queens to a group was not based on their relatedness. This study showed a near zero relatedness among dealate females and queens within and between groups, as well as zero relatedness of queens within colonies. The results of this study suggest that queen aggregation might be a mutualism among the nestmates for maintaining a stable colony association and a high colony efficiency. It is unlikely that kin selection plays a major role in mediating the queen aggregation in *S. invicta*. However, competition among co-existing queens for worker attention could result in workers rearing unrelated queens, which could promote the evolution of polygynous social organization.

Relatedness of dealate females or queens within colonies in the Brazos Valley Area was not significantly different from zero. Hence, this indicates that queens moved randomly to form the permanent polygynous colonies, and that colonies frequently accepted unrelated queens from outside colonies. This result is consistent with previous findings that

nestmate queens in introduced polygynous *S. invicta* are not closely related (Ross and Fletcher 1985, Ross et al. 1996, Goodisman and Ross 1998). Polygyny may occur through adoption of reproductive queens into established colonies that originated with a few closely related queens (Keller 1995). As more newly mated queens are adopted into a colony, queen relatedness will decrease due to the introduction of new alleles from non-nestmate males. However, our results did not show a direct relationship between either dealate female or queen number and relatedness, as relatedness was zero even in the smallest colony.

Studies of the formation of polygynous colonies in the U.S. suggested that most new polygynous colonies may begin as buds of pre-existing polygynous colonies (Vargo and Porter 1989). Because the pre-existing queens were unrelated, the queens forming a new bud must also be unrelated. If the newly formed colonies accepted newly mated queens from outside colonies, then the near zero relatedness of queens within colonies would be retained. The results of the near zero F_{st} values of this study indicated that queens in polygynous colonies in the Brazos Valley Area could be considered as a single population with high gene flow among colonies. The near zero F_s also suggested that colonies accepted queens from outside colonies. Nonacs (1993) explained that the low genetic relatedness of queens within social insect colonies may be due to the change of biological environment. He suggested that newly mated queens choose to remain in their natal nest or enter another nest, rather than through independent founding due to the pressure of high population density. Initially, the mated queens had widely available open habitat, so independent-founding of the monogyne was common. As the appropriate nesting sites become filled, the ability for initiating a new colony is limited. As a result, newly mated queens reentered their natal nests or searched for other nearby nests. Workers initially are expected to accept only new queens originating from their own nest, but their nestmate discrimination abilities weaken as queen number increases (Ross et al. 1996). The loss of nestmate discrimination would presumably cause workers to rear unrelated queens thereby causing the relatedness within the colonies to decrease. Nonacs's suggestion may help to explain the rapid increase of polygynous forms in Texas population. Although our results and previous works (Ross and Fletcher 1985, Ross et al. 1996, Goodisman and Ross 1998) concluded that queens within the polygynous colonies are not related and that unrelated queens were frequently accepted into the colonies, direct evidence for genetic factors operating in polygyne evolution were not provided. The loss of genetic variation in the introduced *S. invicta* inferred that genetic factors, as well as behavioral and ecological factors, might be involved in *S. invicta* evolution. Further experiments in specific gene mapping or relatedness analysis from different populations will be helpful.

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DECOMPOSITION STUDIES, WITH A CATALOG AND DESCRIPTIONS OF FORENSICALLY IMPORTANT BLOW FLIES (DIPTERA:CALLIPHORIDAE) IN CENTRAL TEXAS

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ABSTRACT

This study was conducted in Brazos and Burleson Counties of Central Texas using 20-25 lb swine carcasses as bait. The pig carcasses were allowed to decompose to skeletonization, and the blow flies and other arthropods associating with the carcasses at the various stages of decomposition were collected and identified in order to catalog their occurrence. Twenty-three families of insects and mites were identified. The species of blow flies associating with the carcasses were studied closely in order to make preliminary predictions about the seasons in which they occur in this area of Texas.

INTRODUCTION

Forensic entomology makes use of insects' predictable life cycles to help provide clues in death investigations. Insects that feed on carrion are so specialized that they occur only when environmental and biochemical conditions are perfect. The mere presence of some species can provide detailed information about the location, time, and conditions of a corpse or carcass when the collection was made.

Blow flies (Diptera:Calliphoridae) are the insects of most importance to medicolegal entomology because they are usually the first to colonize a carcass, often within minutes of exposure (Catts 1992, Greenberg 1991). The species of blow flies present in a given area can be used in a court of law to help establish such things as geographic location, time of year, and postmortem interval (PMI).

Presently, Hall's Blow Flies of North America (Hall 1948) and the chapter on Calliphoridae in A Manual of Nearctic Diptera (McAlpine 1993) are among the best sources of information about the distribution of many forensically important blow fly species in the U.S. However, neither of these sources is specific to Central Texas. The importance of cataloging the most common carrion-associating species and succession patterns of these species in Central Texas was stressed most recently by Byrd and Castner (2001). They state that the biogeoclimatic zone in which a set of remains are found will have a major impact on the types and species of insects present as well as the seasonal availability of those species.

The project described herein was intended to provide information about the blow fly species present in Brazos and Burleson Counties of Central Texas during different seasons of the year, and to catalog the species of carrion-associating arthropods in the area.

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MATERIALS AND METHODS

In an effort to represent actual death scene settings, two sites were chosen to conduct this study. The first site was on the West Campus of Texas A&M University in College Station (Brazos Co.), Texas, adjacent to the TAMU Mosquito Research Laboratory. The site was a grassy area with only one tree in the immediate vicinity and otherwise surrounded and partially shaded by buildings. This site was chosen as the urban site for the study. The second site was located approximately 2.5 kilometers south, southwest of Snook (Burleson Co.), Texas, and represented a more rural setting common to Central Texas. This site was in a wooded area and shaded for most of the day. The two sites were approximately 25 kilometers apart.

Wild adult and larval calliphorid fly populations were surveyed using swine carcasses as bait. Carcasses were provided by the Texas A&M University Medical School under TAMU Laboratory Animal Use protocols allowing for tissue sharing between research projects. The pigs were freshly killed and not chilled prior to being set out in the late morning or early afternoon hours (between 10:00 and 14:00 hours). Each carcass was covered with a wire cage to prevent predation by vertebrate scavengers and was monitored at the site over a period of time to allow for complete decomposition to skeletal remains.

The possibility that some of the arthropods collected were the result of previous carcasses at that particular site was taken into consideration, and an attempt was made to prevent this from happening by allowing each carcass to reach the point of skeletonization. Each time, the area was allowed to rest for at least 5-7 days before another carcass was placed in the same location.

Adult flies were collected by using a short-handled sweep net, a portable inverted cone fly trap, sticky traps, and yellow pan traps. Flies collected using the sweep net and the cone trap were killed, pinned and identified. Flies collected on the sticky traps and in yellow pan traps were counted and identified.

The inverted cone trap, sticky traps, and yellow pan traps were passive methods of collection and used mainly to trap any flies and other arthropods that were not caught with the sweep net. Sticky traps were used when fly activity was the greatest. The cone trap was baited with rotten beef heart, and the yellow pan traps were set out next to and around the carcasses overnight and filled with soapy water to trap the insects.

Fly eggs, larvae, and pupae were collected on days when they were noted to be present using a microspatula or forceps. The microspatula was used to scoop up samples of egg masses and first- and second-instar larvae. The forceps were used to collect third-instar larvae and pupae.

Only small representative samples of each life stage were taken from the carcasses. These samples were immediately placed on rearing media, Friskies Fancy Feast® Sliced Chicken Hearts and Liver Feast™ moist cat food (Friskies Petcare Company Inc., Glendale, CA 91203) and kept at 22°C (±1°C) in BioQuip® (Gardena, CA 90248-3602) mosquito breeder containers filled with 1.5 inches of vermiculite to facilitate pupation. Samples of larvae were treated in the same manner with approximately half of each sample killed in near boiling water. Pupae, harvested after all larvae migrated from the carcass, were treated in the same manner.

Each specimen was identified using morphological characteristics when the characters were viewable. Third-instar larvae were reared in the laboratory and identified using taxonomic keys by Stojanovich et al. (1962) and Debang and Greenberg (1989). Adults were identified using the same keys just noted as well as those in *A Manual of Nearctic Diptera* (McAlpine 1993).

Samples of the larvae and adults of other arthropods present on the carcasses each day were collected and pinned or preserved in 80% ethanol. These samples were identified to family and genus using *An Introduction to the Study of Insects* (Borror et al. 1992), *How to Know the Immature Insects* (Chu and Cutkomp 1992), and *A Manual of Acarology* (Krantz 1978).

Succession patterns of the arthropods present for each carcass study are based on written observations, sticky board collections, collections of larvae and eggs, sweep net collections, pitfall collections, yellow pan collections, and the rearing of eggs, larvae and pupae to adulthood. The patterns were based on the presence or absence of arthropods on each day of observation. Collections were not made on days when the temperature was too cold to allow arthropods to be present and on days of heavy rainfall.

RESULTS AND DISCUSSION

Occurrence of Calliphoridae in Brazos and Burleson Counties: Table 1 shows the species of blow flies collected at each study site, and takes into account both larval and adult stages of each species. Most of the Calliphoridae species collected were present in both settings. *Phaenicia cuprina* Wiedemann was not collected at the rural study site. This species has been documented to prefer carrion located near human dwellings (Byrd and Castner 2001). Table 2 shows the blow fly species collected by season.

TABLE 1. List of Calliphoridae Taxa Collected from Swine Carcasses from October 1999-October 2000 at Two Study Sites in Brazos and Burleson Counties of Central Texas.

		Occurrence at study sites	
Genus	Species	Urban	Rural
Calliphora	*livida*	X	X
	vicina	X	X
Cynomyopsis (=Cynomya)	*cadaverina*	X	X
Phaenicia	*cuprina*	X	
	eximia	X	X
	coeruleiviridis	X	X
Cochliomyia	*macellaria*	X	X
Chrysomya	*rufifacies*	X	X
	megacephala	X	
Phormia	*regina*	X	X

The following distinguishing characteristics were noted from the different species of blow flies occurring in Brazos and Burleson Counties based primarily on observations of wild populations.

Cochliomyia macellaria (Fabricius). Representatives of this species typically displayed a dark green to blue metallic abdomen and thorax with three longitudinal stripes on the dorsum of thorax. In Brazos and Burleson Counties, this species occurred in late spring, throughout the summer, and into late fall (Table 2). Members of this species are

usually the first to oviposit on a carcass, and the larvae form large feeding masses. During the summer months, larvae of this species are often driven out from the carcass prematurely by those of *C. rufifacies*.

Phormia regina (Meigen). Adults of this species collected in Brazos and Burleson Counties typically displayed metallic dark olive green to metallic dark blue coloration of the thorax and abdomen with the anterior spiracle appearing dark orange or red, and a row of bristles present on the radial stem of wing. During the course of this study, this species occurred in late fall throughout late spring (Table 2). This species is well established in the area and occurred in great numbers in both rural and urban settings. Adults cluster together to lay large numbers of eggs and create huge masses of larvae.

Chrysomya rufifacies (Macquart). This species occurred during late spring, early summer, and into the fall months of the year (Table 2). Adults of this species are characterized by a row of bristles present on the radial stem of the wing, a bright white anterior spiracle, white buccal area, white, pubescent squamal lobes, and bright metallic green abdomen and thorax. The anterior dorsal portion of the thorax has three small black lines that do not stretch past the middle of the thorax and are often difficult to see without close inspection. This species will arrive first at a carcass, but does not tend to oviposit first. Females tend to lay eggs in masses on the underside of carcass (under jaw, under ribs, and any other part touching the ground). Larvae then migrate upwards into the carcass and eventually drive out populations of other species as noted by other authors (Tantawi and Greenberg 1993). This species is well established in Central Texas where this study was performed, is highly competitive, and the larvae form rolling masses. This species competes with *Phaenicia coeruleiviridis* (Macquart), *C. macellaria*, and *P. regina*.

Chrysomya megacephala (Fabricius). During the course of this study, only three specimens of this species were collected. This species does not have well-established populations in either of the two collection sites. The collections made of this species were at first accidental and afterwards only made by close observation. Random sightings of this species have been made in rural areas of College Station, Texas (Highway 39, personal observation). This species was collected in November (Table 2), and adults are characterized by a dark brown to black anterior spiracle and a row of bristles present on the radial stem of the wing. Males of this species have large eyes which take up most of the head area, while females tend to appear more like *C. rufifacies* with the exception of a yellow buccal area, and dark brown to black anterior spiracle.

Phaenicia coeruleiviridis (Macquart). Members of this species occur in the late spring and early months of the summer (Table 2). Adults form swarms above a carcass and lay large numbers of eggs. The larvae of this species form huge masses and are very similar in appearance to *P. cuprina* and *P. eximia* (Robineau-Desvoidy). Adults of this species have a very bright metallic green thorax and abdomen and sometimes appear bright metallic blue. Males of this species can be distinguished from *P. eximia* by the presence of one pair of reclinate frontoorbital bristles.

Phaenicia eximia (Robineau-Desvoidy). This species has often been misidentified because of its similarity to *P. coeruleiviridis* (Byrd and Castner 2001). In Brazos and Burleson Counties, it replaces *P. coeruleiviridis* during the late summer and early fall (Table 2). The larvae of this species are almost identical to *P. coeruleiviridis*. References to *P. eximia* in any of the larval keys that were used in this study were not found. This species occurred in small numbers in both settings. The best character for identification found during this study was that males of *P. eximia* do not have a pair of frontoorbital reclinate bristles. Females of this species are difficult to identify. The best character for identification of this species, at the moment, is the season when it occurs.

TABLE 2. Occurrence of Blow Flies by Season at Swine Carcasses Located in Brazos and Burleson Counties of Central Texas from October 1999-October 2000.

Genus	Species	Seasons of occurrence			
		Winter (Dec.-Feb.)	Spring (Mar.-May)	Summer (June-Aug.)	Fall (Sept.-Nov.)
Calliphora	*livida*	XXXXXXXXXXXXXXXXXX			
	vicina	XXXXXXXXXXXXXXXXXX			XXXXXXXXX
Cynomyopsis (=Cynomya)	*cadaverina*	XXXXXXXXXXXXXXXXXX			XXXXXXXXX
Phaenicia	*cuprina*		XXXXXXXXXXXXXXXXXXXXXXXXXXXX		
	eximia				XXXXXXXXX
	coeruleiviridis		XXXXXXXX		
Cochliomyia	*macellaria*	XXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXX			
Chrysomya	*rufifacies*		XXXXXXXXXXXXXXXXXXXXXXXXXXXX		
	megacephala				XXXXXXXXX
Phormia	*regina*	XXXXXXXXXXXXXXXXX			XXXXXXXXX

Phaenicia cuprina (Wiedemann). This species typically displays a bronze to metallic light green abdomen and thorax, with stout bristles over the entire body. This species tends to lay small numbers of eggs, and the larvae are often not collected. Larvae of this species are similar to those of *Phaenicia* spp. but differ in the number of branches on the anterior thoracic spiracles. This species occurs in late spring into the summer, and early fall in Central Texas (Table 2).

Cynomyopsis (=Cynomya) cadaverina Townsend. This species of bluebottle fly occurred from mid-winter to early spring during the course of this study (Table 2). Adults of this species are very similar in appearance to *C. vicina* and *C. livida*, having a dull gray to black thorax and a metallic blue or green and occasionally pollinose abdomen. Members of this genus are distinguishable from the *Calliphora* genus by the occurrence of one pair of preintra-alar bristles on the thorax. Females of this species tended to lay eggs in small clusters or scattered singly. Adults are strong flyers emitting a loud buzzing sound.

Calliphora vicina (Robineau-Desvoidy). This species of blow fly is highly endophilic. Many specimens of this species were caught inside the TAMU Mosquito Research Laboratory. Adults of this species are robust and strong fliers. The greatest numbers of individuals were caught in early to mid-spring.

Calliphora livida Hall. The morphology of adults of this species is very similar to *C. vicina*. Specimens collected in Brazos and Burleson Counties were smaller than *C. vicina* and *C. cadaverina*. The abdomen of this species has a more violet coloration than that of the other bluebottle species. Larvae of this species tended to be found in mixed samples of larvae belonging to other species. While not as abundant as *C. cadaverina* or *C. vicina*, this species is well established in the area. This species occurs in the central Texas area (Brazos and Burleson Counties) during the late winter and early spring.

Fig. 1 represents a diagram that was created to simplify the differences between *C. cadaverina*, *C. livida* and *C. vicina*. The morphological characteristics provided in Fig. 1 were compiled from previously mentioned sources (Shewell 1993, Byrd and Castner 2001).

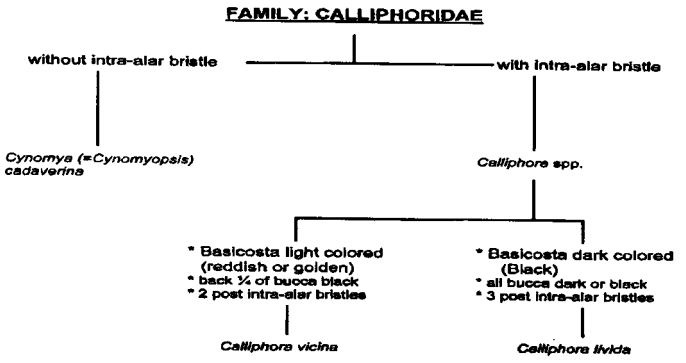

FIG. 1 Species tree diagram for *C. cadaverina*, *C. livida*, and *C. vicina*.

Other Arthropods Collected: Table 3 is a list of all other taxa collected at each of the study sites. Twenty-four families were identified, representing six orders of arthropods. The species of arthropods representing predators were more abundant in the rural setting, but the species of necrophagous arthropods present at the two sites did not differ. The species present seemed to vary more with season and temperature than with location.

The main predators present were fire ants (*Hymenoptera:Formicidae*), with wasps (*Hymenoptera:Vespidae*), robber flies (*Diptera:Asilidae*), and assassin bugs (*Hemiptera:Reduviidae*) also playing roles as predators in the rural setting. In the case of 11 of the 12 carcasses set out during this study, fire ants were present before the second day. It is possible that the presence of these arthropods retarded the oviposition patterns of some of the Diptera present, but not the presence of the adults. Fire ants carried away eggs and larvae whenever these were present during the same day.

Of the Diptera collected, flesh flies (*Diptera:Sarcophagidae*), and blow flies (*Diptera:Calliphoridae*) were the earliest arrivers, and the adults continued to be present into the later stages of decomposition. Larger flesh fly larvae were often found mixed in the large masses of blow fly larvae. Adults and larvae of *C. rufifacies*, *P. regina*, and *C. macellaria* were the blow fly species most commonly collected, and their larvae often made up the largest part of the larval masses. During the course of the study *P. coeruleiviridis* seemed to be an important species, but the discovery of *P. eximia* and the similarities between the larvae and female adults of these species point to a need for further studies of their interactions and times of occurrence. *Phaenicia cuprina* larvae and *C. megacephala* adults and larvae occurred less often than any of the other species of blow flies. *Calliphora livida* and *C. vicina*, were more rare than *C. cadaverina* which was the dominant early arriver during the winter months. Often *C. cadaverina* was the first to arrive, usually on the first day, and the females immediately began to lay eggs.

TABLE 3. List of All Other Taxa Collected at Both Carrion Study Sites in Central Texas October 1999-October 2000.

Class	Order	Family	Genus and Species[a]	Collection site Rural	Urban
Hexapoda	*Diptera*	*Sarcophagidae*		X	X
		Muscidae	*Musca domestica* (lLinnaeus)	X	X
			Fannia	X	X
			Hydrotaea/Ophyra	X	X
		Piophilidae	*Piophila casei* (Linneaus)	X	X
		Sepsidae		X	X
		Phoridae		X	X
		Stratiomyidae	*Hermetia illucens* (Linneaus)	X	X
		Asilidae		X	
	Coleoptera	*Silphidae*		X	X
		Staphylinidae	*Creophilus maxillosis* (Linneaus)	X	X
			sp. 1	X	X
			sp. 2	X	X
		Histeridae	*Saprinus pennsylvanicus* Paykull	X	X
			sp. 1	X	X
		Scarabeidae		X	X
		Trogidae		X	X
		Geotrupidae		X	
		Dermestidae	*Dermestes caninus* German	X	X
		Nitidulidae	*Omosita colon* (Linneaus)	X	X
		Cleridae	*Necrobia rufipes* (DeGeer)	X	X
		Tenebrionidae		X	
	Hymenoptera	*Vespidae*		X	
		Formicidae		X	X
		Chalcididae			X
	Hemiptera	*Reduviidae*		X	
Arachnida	*Acari*				
	Mesostigmata	*Macrochelidae*		X	X
	Astigmata	*Acaridae*		X	
Crustacea	*Isopoda*			X	

[a] Many of these arthropod specimens were only identified to family

Muscoid flies of the genera *Ophyra/Hydrotaea*, *Fannia*, and *Musca* did not occur until after the blow flies were well established, and the larvae of these genera were not found until final collections were made for each carcass. Adult cheese skippers (*Diptera:Piophilidae*) were present in large numbers during the fall months of this study very early in the decomposition process, but their larvae were only found during final carcass collections. Soldier flies (*Diptera:Stratiomyidae*) showed the same behavior as the cheese skippers, but their larvae, when present, were more numerous. Flies of the family *Sepsidae* were collected from a carcass at each study site, but only adults were collected and never in high numbers. These flies are scavengers and are very common on dung (Byrd and Castner 2001). In these cases, they may have been attracted to the carrion, but did not seem to find it a suitable food source.

The species of Coleoptera associating with each carcass were only present after the larvae of the blow flies and flesh flies became well established. *Creophilus maxillosis*

(Linneaus) *(Coleoptera:Staphylinidae)* was the dominant species of rove beetle present at both locations during the summer months, with other species of smaller rove beetles.taking over when the temperatures became cooler in the fall and winter. In each case, carrion beetles (*Coleoptera:Silphidae*), and clown beetles (*Coleoptera:Histeridae*) were present before the skin (*Coleoptera:Dermestidae*), checkered (*Coleoptera:Cleridae*), sap (*Coleoptera:Nitidulidae*), and hide (*Coleopera:Trogidae*) beetles. The only species of checkered beetles present at both locations was *Necrobia rufipes* (DeGeer), and *Omosita colon* (Linneaus) was the only species of sap beetles collected. All the adult beetles were conspicuous and easy to collect. They often wandered on the carcass and surrounding vegetation during the daylight hours, especially when the temperatures were warm. Hide beetles, and other beetles of the family Scarabeidae were rarely collected. When collected, they were found in and under the carcass during late decomposition and skeletonization. Beetle larvae were not present until after skeletonization except for one carcass (Rural Setting: 1/18/00-2/22/00) that was covered with carrion beetle larvae on Day 20. When the larvae of beetles were present, rove beetle larvae were the most abundant. They were often found inside the carcass and in surrounding vegetation.

As for other arthropods collected during the study, predatory mites (*Acari: Macrochelidae*) were more prevalent in the rural setting, and were often seen attached to the skin beetles. They were conspicuous and easily collected but did not arrive until the later stages of decomposition (i.e., putrefaction and skeletonization). A family of stored product mites (*Acari:Acaridae*) was also collected in huge numbers on several of the carcasses during the same stages of decomposition and were collected as adults and hypopial stages.

In summary, this study has helped to show that the species of carrion-associating insects in Central Texas vary with season and locale. Some of the blow fly species collected occurred only at the rural site of collection, while others were only collected at the urban collection site. This indicates that some blow fly species may prefer one environment to the other as previously noted in other studies (Wells and Greenberg 1994, Smith 1986). In addition, some of the species of blow flies collected have not previously been recorded as occurring in Central Texas. For example, *C. megacephala* is a new arrival to Texas. The collections of two males and one female during the course of this study are new records for the area.

As previously noted by Wells and Greenberg (1994), fire ants are an important factor in the calculation of a post-mortem interval. Whenever fire ants were present near the study sites, their foraging area included the carcasses put out for this study. They may delay blow fly oviposition as much as two days when they are present, further supporting the observations by other authors (Wells and Greenberg 1994).

Insect succession in the area that was studied followed a set pattern. The flies were always the first wave, followed by waves of beetles, more flies, and then more beetles. The blow flies were not always the first insects to colonize the carcass. Often, flesh flies arrived first, especially during the fall months of the year.

Insect activity did not cease at any point during the course of this study. The diversity of species was greatest during the warm months, but even on the coldest days of the year, the carcasses did not fail to be infested with some types of insects. Decomposition was retarded by the cold weather, but flies and some beetles were present. *P. casei* was one of the dominant flies during the fall and winter, when the weather was the coldest.

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COMPARISON OF SUSCEPTIBILITY OF *GEOCORIS PUNCTIPES*[1] AND *LYGUS LINEOLARIS*[2] TO INSECTICIDES FOR CONTROL OF THE TARNISHED PLANT BUG

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ABSTRACT

Comparison of the susceptibility of *Geocoris punctipes* (Say) and the tarnished plant bug (TPB), *Lygus lineolaris* (Palisot de Beauvois) to selected insecticides was determined in topical, tarsal contact, and field studies. In both topical and tarsal contact studies, *L. lineolaris* was more susceptible to imidacloprid and oxamyl residues than *G. punctipes*. However, oxamyl was more toxic to the pest than imidacloprid. Both insect species responded very similarly to fipronil, acephate, dicrotophos, and lambda-cyhalothrin, all of which were very toxic to these insects. In our field study, lambda-cyhalothrin had an equally negative impact on populations of TPB and *G. punctipes* concurring with previously published field studies. Results from our laboratory and other field studies indicate that oxamyl and imidacloprid would be effective against TPB while conserving populations of *G. punctipes* for biological control of lepidopteran larvae in cotton.

INTRODUCTION

The tarnished plant bug (TPB) *Lygus lineolaris* (Palisot de Beauvois) (Hemiptera: Miridae) is often a serious tissue-sucking pest of cotton, *Gossypium hirsutum* L. causing abnormal plant growth, fruit damage, delay in fruiting, and delayed boll maturity (Hanny et al. 1977). The big-eyed bug, *Geocoris punctipes* (Say) (Hemiptera: Lygaeidae), is a predator of many pest species including *H virescens* and *Helicoverpa zea* (Boddie) eggs and small larvae (Lingren et al. 1968). *Geocoris punctipes* also feeds on plants, increasing the likelihood of their survival during the absence of invertebrate hosts (Eubanks and Denno 1999).

Since the TPB and *G. punctipes* can occur in cotton fields concurrently, selectivity of insecticides with respect to these two insect species is an important issue in an integrated pest management program because insecticides recommended for *L. lineolaris* control may be harmful to *G. punctipes*. TPB and *G. punctipes* can be affected by insecticides through various routes of administration: topical contact on their bodies, tarsal contact with residues of insecticides, and feeding on insecticide treated plants and prey (for predator). The organophosphate, acephate, recommended for use in cotton for TPB control in 1977, provides effective TPB suppression (Bannister et al. 1995, Reed et al.1997, Robbins et al. 1998). The pyrethroid insecticides were first registered for use in cotton in 1978, and lambda-cyhalothrin was used very effectively against TPB (Graham and Gaylor 1988, Leonard et al. 1987). Another organophosphate insecticide, dicrotophos (Burris et al. 1986, Graham and Gaylor 1988, Leonard

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[5]Mention of a proprietary product does not constitute an endorsement or recommendation for its use by USDA.

et al. 1987, Langston and Schuster 1989), and the carbamate, oxamyl (Micinski 1983) also were used successfully for TPB control during this time. Pyrethroid resistance was first detected in 1993 in a field population of TPB in the Mississippi Delta, and resistant insects also were found to have multiple resistance to some organophosphate and carbamate insecticides (Snodgrass and Elzen 1995). Level of control with organophosphate, carbamate, and pyrethroid insecticides has decreased over the past several years in some areas of cotton production where resistance to all these insecticides has been reported. However, dicrotophos, oxamyl, and lambda-cyhalothrin, are still used effectively against susceptible TPB populations (Bannister et al. 1995, Pankey et al. 1996, Reed et al. 1997, Robbins et al. 1998, Russell et al. 1998). The new insecticides imidacloprid, an imidazolidinimine, and fipronil, a phenylpyrazole, have shown excellent activity on TPB even against TPB populations that are highly resistant to other classes of insecticides (Burris et al. 1994, Bannister et al. 1995, Scott and Snodgrass 1996, Shaw and Yang 1996, Teague and Tugwell 1996).

Most of the reported insecticide research on *G. punctipes* concentrates on residual toxicity of insecticides which has been low for some insecticides such as spinosad and oxamyl and high for other insecticides such as malathion and fipronil (Boyd and Boethel 1998, Elzen et al. 1998, Elzen and Elzen 1999, Tillman and Mulrooney 2001). The few topical toxicity studies conducted have demonstrated that malathion and other organophosphates, fipronil, and cyfluthrin are highly toxic to *G. punctipes* (Lingren and Ridgway 1967, Tillman and Mulrooney 2001). The single study reported on effect of feeding on dried residues of an insecticide on cotton leaves demonstrated that both species were susceptible to feeding on indoxacarb-treated plants (Tillman et al. 2001). Few studies have investigated the effect of insecticides on feeding by *G. punctipes*. Elzen (2001) reported that consumption of *H. zea* eggs by this predator was lower in malathion, profenofos, endosulfan, fipronil, azinphos-methyl, and imidacloprid treatments compared with the untreated control. Tillman et al. (2001) reported that indoxacarb-treated eggs were highly toxic to females feeding on these eggs.

Few studies have compared the effect of insecticides on both TPB and *G. punctipes*. In one field study, survival of *G. punctipes* was high for carbaryl and spinosad, moderately high for indoxacarb, and low for methyl parathion, lambda-cyhalothrin, and imidacloprid+cyfluthrin (Leverage)(Muegge and Payne 2001). Our research, conducted to compare susceptibility of *L. lineolaris* and *G. punctipes* to selected insecticides with current or potential usage in TPB control, involved bioassaying both insects via topical and tarsal contact routes of administration and evaluating the effect of lambda-cyhalothrin on field populations of these insect species.

MATERIALS AND METHODS

G. punctipes were collected from untreated cotton in the hill section of Mississippi and kept in plastic food containers with *H. virescens* eggs for food. *L. lineolaris* were collected from wild host plants in the Mississippi Delta (Washington Co.), kept in cardboard ice-cream cartons, and fed fresh green beans. Immature stages were monitored biweekly in the field so that adults could be collected when they were no older than 1-1.5 wk old.

Topical Toxicity Bioassay. This test included the following six treatments and rates: (1) acephate (Orthene 75 wettable soluable powder [0.56 kg (AI)/ha], Valent USA Corporation, Walnut Creek, CA), (2) lambda-cyhalothrin (Karate 1 emulsifiable concentrate [0.028 kg (AI)/ha], Zeneca, Wilmington, DE), (3) dicrotophos (Bidrin 8 emulsifiable concentrate [0.56 kg (AI)/ha], Novartis Crop Protection, Greensboro, NC), (4) fipronil (Regent 2.5 emulsifiable concentrate [0.056 kg (AI)/ha], Rhone-Poulenc Agric. Co., Research Triangle Park, NC), (5) imidacloprid (Provado 1.6 flowable [0.053 kg (AI)/ha], Bayer, Inc., Kansas City, MO), and (6) oxamyl (Vydate 2.76 concentrated low volume [0.28 kg (AI)/ha], Dupont Agricultural Products, Wilmington, DE). The recommended rate for *L. lineolaris* control in Mississippi was used for

all insecticides. A laboratory spray chamber was used to treat adult insects topically. The spray chamber used to apply the treatments was equipped with a conventional spraying system that was calibrated to deliver 93.5 liters/ha, using a single TX-8 nozzle (Spraying Systems, Wheaton, IL), while maintaining 138 kPa pressure. The height and speed of the nozzle above the spray surface were 35.6 cm and 6.4 km/h, respectively. A water control was included in the test.

Insects were aspirated into a new plastic petri dish (100 x 15 mm), anesthetized lightly (until slight knockdown or approximately 3-5 sec) with CO_2 and then placed uncovered in the spray chamber for treatment. Control insects were treated in the same manner to eliminate CO_2 knockdown as a source of mortality. Before the test, a hole (55 mm in diameter) was cut in the top of the petri dish and covered with organdy mesh to increase movement of the CO_2 into the dish from a CO_2 cylinder. A treatment replicate consisted of ten insects per species. Each treatment was replicated six times for a total of 60 insects per treatment for each species. Only adult females were sprayed. After spraying, the insects were transferred to a clean petri dish. Sprayed insects were provided food (green beans for *L. lineolaris* and *H. virescens* eggs for *G. punctipes*) and placed in an environmental chamber maintained at 25 ± 2°C, 50 ± 5% RH, and a photoperiod of 14:10 (L:D) h. All insects were checked for mortality 48 h after treatment.

Residual Toxicity Bioassay. Bollgard cotton (Monsanto, St. Louis, MO) was planted in plots 4 (1.02 m/row) rows x 61 m and were replicated four times. All insecticides were applied with a spray system pressurized by compressed air mounted on a John Deere 600 high clearance sprayer. The application parameters were: speed - 4.8 kph; pressure - 358 kPa; volume - 93.4 L/ha: and nozzles - TX-12 (Spraying systems, Wheaton, IL). In 1996, a test was repeated on 24, 25, and 28 June and included the following six treatments with the same rates as in the previous test: (1) acephate (2) dicrotophos, (3) fipronil, (4) imidacloprid, (5) oxamyl, and (6) untreated control. A randomized complete block design with four replications was used. On 27 June 1997 a second test was done in conjunction with the field study below and included the insecticide lambda-cyhalothrin at the rate in the previous test and an untreated control. For both tests, ten cotton leaves from the fourth node down from the terminal were collected from each treatment replicate for bioassay immediately after the insecticide dried (approximately 1 h after treatment). Leaves were placed in plastic bags, transported to the laboratory on ice, and placed in 15 x 100 mm petri dishes. One *G. punctipes* and one *L. lineolaris* were placed in each of the ten plastic petri dishes containing a treated cotton leaf. All insects were checked for mortality after 48 h.

Field Study. Bollgard cotton (Monsanto Company, St. Louis, MO) was planted in large plots, 40 rows (1.02 m/row) wide by 39.6 m long (0.162 ha), to minimize insect migration. A John Deere 600 high-clearance sprayer equipped with a conventional spraying system was calibrated to deliver 46.8 liters/ha using TX-8 nozzles (Spraying Systems, Wheaton, IL) and 275 kPa pressure. The test began 26 June 1997 and included two treatments: 1) lambda cyhalothrin at 0.0128 kg (AI)/ha, and 2) untreated control. A randomized complete block design with four replications was used. Sampling was done immediately before each application. Post application samples were made at 1, 3, and 5 days after application. Samples (four rows) were taken using a KISS sampler (Beerwinkle et al. 1997). This sampling method was used in preference to other sampling techniques to obtain sufficient insects to make comparisons between treatments.

Percentage mortality for topical and 1996 residual data were converted by arcsine transformation and then analyzed using PROC MIXED followed by a least significant difference test (LSD) (SAS Institute 2001) where appropriate. In the 1997 residual and field experiments, *t*-tests were used for comparisons of means between treatments and species.

RESULTS AND DISCUSSION

Topical Toxicity Study. A statistically significant difference was detected between species ($F = 4.32$; $df = 1, 65$; $P = 0.042$) and insecticide treatments ($F = 117.7$; $df = 6, 65$; $P =$

0.0001) when insects were exposed to topical applications of selected insecticides (Table 1).

TABLE 1. Topical Toxicity of Selected Insecticides to *G. punctipes* and *L. lineolaris* Adults in a Spray Chamber Bioassay.

		% Mortality[a]	
Treatment	Kg (AI)/ha	*G. punctipes*	*L. lineolaris*
Lambda-cyhalothrin	0.028	100.0 ± 0.0 a, 1	100.0 ± 0.0 a, 1
Dicrotophos	0.56	100.0 ± 0.0 a, 1	100.0 ± 0.0 a, 1
Acephate	0.56	96.5 ± 0.0 a, 1	94.7 ± 0.0 a, 1
Fipronil	0.056	93.3 ± 2.5 a, 1	93.3 ± 4.1 a, 1
Oxamyl	0.28	83.3 ± 7.1 b, 2	96.7 ± 4.0 a, 1
Imidacloprid	0.053	53.3 ± 15.8 c, 2	66.7 ± 10.6 b, 1
Water		0 d, 1	10.0 c, 1

[a]Means were assessed 48 h after treatment. Means within a column and row followed by the same letter and number, respectively, are not significantly different ($P > 0.05$; LSD).

Both oxamyl and imidacloprid were less toxic to *G. punctipes* than to *L. lineolaris* even though oxamyl was more toxic than imidacloprid to the former species. Imidacloprid was the least effective insecticide against *L. lineolaris* in this topical contact test. Lambda-cyhalothrin, dicrotophos, acephate, and fipronil were very toxic to both insect species. Other researchers have also reported that topical applications of organophosphates, synthetic pyrethroid, and fipronil are toxic to *G. punctipes* (Lingren and Ridgway 1967, Tillman and Mulrooney 2001).

Residual Toxicity Study. A statistically significant difference was detected between species ($F = 19.29$; $df = 1, 12$; $P = 0.0009$) and insecticide treatments ($F = 20.11$; $df = 5, 11.9$; $P = 0.0001$) when insects were exposed to residues of the selected insecticides (Table 2). *L.*

TABLE 2. Residual Toxicity of Selected Insecticides to *G. punctipes* and *L. lineolaris* in a Field-treated Cotton Leaf Bioassay.

			% Mortality[a]	
Treatment	Year	Kg (AI)/ha	*G. punctipes*	*L. lineolaris*
Dicrotophos	1996	0.56	89.2 ± 2.9 a, 1	98.3 ± 1.3 a, 1
Fipronil		0.056	88.3 ± 4.2 a, 1	93.3 ± 3.8 a, b, 1
Acephate		0.56	85.8 ± 5.6 a, 1	83.2 ± 5.0 b, 1
Oxamyl		0.28	70.8 ± 7.0 b 2	91.7 ± 3.5 a, b, 1
Imidacloprid		0.053	45.0 ± 8.8 c, 2	65.0 ± 7.3 c, 1
Control			0 d, 1	4.2 d, 1
Lambda-cyhalothrin	1997	0.028	87.5 ± 6.3 a, 1	87.0 ± 5.4 a, 1
Control			0 b, 1	0 b, 1

[a] Means were assessed 48 h after exposure to residues on leaves collected 1 h after insecticide application. For 1996, means within a column and a row followed by the same letter and number, respectively, are not significantly different ($P > 0.05$; LSD). For 1997, means within row and column followed by the same number are not significantly different ($P > 0.05$; *t*-test).

lineolaris was more susceptible to imidacloprid and oxamyl residues than *G. punctipes*. However, oxamyl was more toxic to the pest than imidacloprid. Both insect species responded very similarly to fipronil, acephate, dicrotophos, and lambda-cyhalothrin, all of which were very toxic to these insects.

For *G. punctipes,* residual toxicity was highest for dicrotophos, fipronil, lambda-cyhalothrin, and acephate. Toxicity of oxamyl to *G. punctipes* was lower than that of the four former insecticides, but oxamyl was still intermediate in toxicity to this predator. For *L. lineolaris*, residual toxicity was high for all insecticides except imidacloprid.

The pattern of the response between insecticides and species to prolonged tarsal contact with dried insecticide residues and topical contact with sprayed insecticides was similar for both insect species. Even though toxicity of insecticides administered topically appears to be higher than that of toxicity from prolonged contact to dried insecticide residues, the general pattern of toxicity between the insecticides remains similar for both methods of application. Since the entry of these insecticides likely would be through the exoskeleton for both types of administration, the pattern of toxicity between these insecticides should be similar for both application methods. Topical applications of insecticides may have resulted in higher toxicity than exposure to insecticide residues because more of the active ingredient could enter the insect with wet versus dry insecticides. Unfortunately, quantifying amounts of insecticides in treated insects was beyond the scope of this particular study.

Residual toxicity of imidacloprid to *G. punctipes* in this study was the same as that reported by Boyd and Boethel (1998), a little higher than that reported by Elzen et al. (1998), and a little lower than that reported by Mizell and Sconyers (1992). These variances in toxicity between tests are probably due to differences in amount of exposure of the insects to the insecticide (entire surface treated versus top of leaf treated) and level of coverage on substrates (sprayed versus dipped insecticide). Each study, nevertheless, has shown that imidacloprid is less toxic than the organophosphates used for TPB control. Toxicity of oxamyl residues reported by Elzen et al. (1998) was much lower than the toxicity we obtained for *G. punctipes*. The reason for our differences is not clear, but may be due to differences in plant feeding during the test. Nevertheless, we both found that oxamyl was less toxic to *G. punctipes* than organophosphates. Elzen et al. (1998) not only determined, as we did, that residues of fipronil were very toxic to *G. punctipes*, but also that they acted quickly (30 min.). McCutcheon and DuRant (1999) also showed that residues of acephate on cotton plants in field cages, in comparison to residues on leaves in a petri dish, were very toxic to *G. punctipes*. Except for Boyd and Boethel (1998), all the residual toxicity tests, including ours, were conducted by exposing the insects to residues of the insecticide on leaves of a plant. A problem with this protocol is that the residual test may be compounded by feeding on treated leaves, and feeding on insecticide treated leaves can have a detrimental effect on plant-feeding insects. *Geocoris punctipes* females were susceptible to feeding through dried residues of Steward after sprayed on young cotton plants (Tillman et al. 2001). To eliminate insect feeding, insects can be exposed to residues on the inside surface of a holding container. Walking on treated leaves, though, more closely imitates field conditions. A possible solution to obtain the best of both experimental protocols would be to prevent insects from feeding on treated leaves. Thoughtful consideration of feeding behavior of pests and predators should be taken into account when conducting tests to determine the effect of tarsal contact to residues of insecticides.

Field Study. The day before insecticide application, the number of insects was the same for treated plots and untreated controls for both species (Fig. 1). However, numbers of insects were much lower in the treated plots compared to the untreated controls 1, 3, and 5 days after insecticide application for both species. In comparison to the untreated control, a 100% reduction in TPB numbers occurred 1 day after lambda-cyhalothrin application while a 84% reduction occurred for the same treatment and time after application for *G. punctipes*. However,

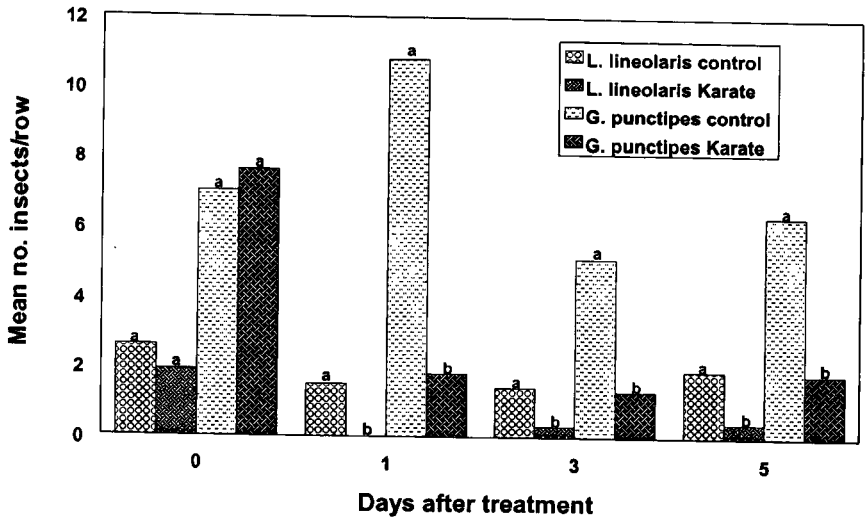

FIG. 1. Mean numbers of *G. punctipes* and *L. lineolaris* per row after application of cyhalothrin (0.028 kg/ha) on day 0. Means between treatments (untreated controls and Karate treated plots) within the same treatment day and insect species followed by the same letter are not significantly different ($P > 0.05$; *t*-test).

percentage of reduction in number for the treated plots in comparison to untreated controls was equal (approximately 80%) for each species day 3 and 5 after application. Thus, we concluded that lambda-cyhalothrin had an equally negative impact on populations of TPB and *G. punctipes* in the field. Other studies also have shown that although lambda-cyhalothrin was very effective against TPB in field plots, it reduced populations of cotton predators, including *G. punctipes* (Graham and Gaylor 1988, Studebaker 1997, Muegge and Payne 2001).

Acephate and dicrotophos, too, have been reported to be equally detrimental to TPB and cotton predators, including *G. punctipes*, in field plots 1, 3, and 7 days after treatment (DAT) (Burris et al. 1986, Graham and Gaylor 1988, Studebaker 1997). Logically lambda-cyhalothrin, acephate, and dicrotophos should be very toxic to TPB and cotton predators in the field since these insecticides are highly toxic to both insect species through both topical and residual routes of administration. However, fipronil which was equally toxic to both insect species in the lab, was shown to be effective against TPB, but not detrimental to cotton predators, in field plots 7 DAT (Studebaker 1997). Fipronil has feeding activity, since mortality of *G. punctipes* females was very high when they fed on fipronil-treated prey eggs (Elzen 2001). Thus, the reason for the differences in susceptibility of TPB and cotton predators to fipronil in these field tests is not apparent. It is possible that other species grouped into "cotton predators" are less susceptible to fipronil than *G. punctipes*, or a reduction in the predator population occurred before the 7 DAT sampling time. Muegge and Payne (2001) reported that imidacloprid was effective against TPB 3 and 7 DAT, but uneffective at 14 DAT at which time the population of *G. punctipes* dropped below that of the control. Since *G. punctipes* was less susceptible to imidacloprid than TPB in the lab, the differences in susceptibility between the two insect species in the field was not surprising. *G. punctipes* consumes fewer imidacloprid-treated prey than untreated prey (Elzen 2001). So the drop in population of *G. punctipes* at 14 DAT may be due to lower egg production, thus fewer nymphs would develop in the field or more simply adults died from lack

of suitable nutrition. Our topical and residual studies indicate that imidacloprid would be ineffective against TPB in the field, and yet it was reported to be very effective in field plot tests (Studebaker 1997, Muegge and Payne 2001). The insecticide may have feeding activity against TPB increasing effectiveness in the field. Oxamyl was very effective against TPB without reducing cotton predators in field plots (Studebaker 1997). Oxamyl has shown very little to no feeding activity against brown and green stinkbugs (Tillman, unpublished data), and thus, may have no feeding activity against cotton predators. A possible explanation for the observed difference in susceptibility of field populations of cotton predators and TPB could be that other predators are less susceptible than TPB to tarsal contact with residues of oxamyl as shown for *G. punctipes,* and they are unaffected when feeding on cotton with residues of oxamyl. Further studies on feeding activity of these two insecticides along with fipronil and the effect of these insecticides on *G. punctipes* in field plots clearly need to be done to fully understand the impact these insecticides would have on *G. punctipes* populations in the field. Nevertheless, results from our laboratory and Studebaker's (1997) and Muegge and Payne's (2001) field studies indicate that oxamyl and imidacloprid would be effective against TPB while conserving populations of *G. punctipes* for biological control of lepidopteran larvae in cotton fields.

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INSECTS ASSOCIATED WITH BLACK STAIN ROOT DISEASE CENTERS IN PINYON PINE STANDS

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ABSTRACT

Black stain root disease centers in pinyon-juniper woodlands near Cortez, Colorado were monitored in 1998 and 1999 for potential insect vectors of the fungus that induces black stain root disease (*Leptographium wageneri* var. *wageneri*). Baited Lindgren funnel and pitfall traps were placed in root disease centers from mid May to December in 1998 and mid May until early August in 1999. Insects representing four orders and 28 families were captured including13 bark beetle species (Scolytidae) and 13 weevil species (Curculionidae). More than 50% of the scolytid and curculionid species were root and stump breeding insects. These insects could be vectors of the pathogen because the fungus resides in roots and lower stem of pinyon pine trees. Most scolytids were trapped in May and June; whereas, the majority of curculionids were trapped in July and August. Three *Hylastes* species were the most commonly and consistently recovered beetles or weevils. Further studies are needed to determine if any of the insects are vectors of the black stain pathogen.

INTRODUCTION

Pinyon pine (*Pinus edulis* Engelm.), occupying more than 22.5 million hectares in the western United States, plays an important role in southwestern ecosystems (Mitchell and Roberts 1999). Pinyon pine occurs with *Juniperus* spp. in mixed woodlands on foothills, low mountains, mesas, and plateaus from 1,200 to 2,400 m (Buckman and Wolters 1987). Pinyon pines have died in unprecedented numbers in southwestern Colorado over the last decade (Eager 1999). Sustained mortality of pinyon pine has negatively affected recreational sites such as campgrounds and picnic grounds and the value of residential housing. The large numbers of dead and dying trees has increased fuels for wild fires (Eager 1999).

The two primary mortality agents in the region are black stain root disease caused by the fungal pathogen, *Leptographium wageneri* (Kendr.) Wingf. var. *wageneri* and *Ips* bark beetle, *Ips confusus* (LeConte). Black stain root disease is a vascular wilt disease (Landis and Helburg 1976) and was first recognized and described from observations at Mesa Verde National Park in 1942. When a pinyon pine is killed by black stain root disease, the fungus is found in the outer annual rings of roots and lower stem. Local spread of the pathogen occurs through root contacts and grafts between infected and healthy trees (Wagener and Mielke 1961). Ips bark beetles often attack pinyon pines affected by black stain root disease. *Ips* bark beetles can also kill pinyon pine without the involvement of black stain root disease (Kearns 2001). In ponderosa pine (*Pinus ponderosa* Laws.) and Douglas fir (*Pseudotsuga menziezii* (Mirb.) Franco.) ecosystems

the black stain pathogen is vectored by root feeding and root breeding beetles (*Hylastes* spp). and weevils including *Pissodes* and *Steremnius* spp. (Goheen and Cobb 1978, Harrington et al. 1985, Witcosky and Hansen 1985,Witcosky et al. 1986). Insects are suspected to vector the pathogen in pinyon pine (Landis and Helburg 1976), although none have been identified. We suspect insect transmission is not common because during the survey work of Kearns (2001) few root feeding or root breeding insect galleries were seen in black stain affected trees. Most newly infected trees occur near recently killed trees so it is assumed root contact is the prevalent infection method. However, new black stain root disease centers have been noted in southwest Colorado, so long distance transport of the pathogen does occur.

We report on the first steps taken to determine insect vector relationships. Our objective was to determine which insects may be involved as vectors of the black stain root disease pathogen. A survey of insects that visited black stain root disease centers in pinyon pine stands was conducted in the summers of 1998 and 1999 in Montezuma County, Colorado. The survey was conducted in locations with known black stain root disease, with declining and dying pinyon pine. Future studies are needed to isolate the pathogen from the suspected insects to determine which insect species actually vector the pathogen.

METHODS

Trap sites were established in 1998 and 1999 near McPhee Reservoir in the San Juan National Forest, 10 km north of Cortez, Colorado, at about 2,150 m. Insects were collected from a total of four sites in 1998 with two sets of traps per site. Trap sets were separated by 50-100 m at each site within a black stain root disease center and traps within a set were placed one meter from each other. Sites 1 and 2 were located on the west side of the reservoir and Sites 3 and 4 were located 3.2- 6.0 km away on the east side of the reservoir. An additional site, located in a housing development 1 km south of McPhee Reservoir, was added in 1999 for a total of five trap sites. Trapping ended in early August of 1999 because field crews were no longer available.

Each trap set used in 1998 included one pitfall trap sunk into the ground under the canopy of an affected tree and a flight trap suspended 3 m above ground from the same tree. Pitfall traps were Bio-Control Services® Multipher® traps baited with 10% (vol./vol.) pinyon pine resin in 95% ethanol and buried to ground level. Pitfall traps contained plastic cups with liquid propylene glycol to kill trapped insects. Flight traps were Lindgren funnels with 12 tiers to intercept and force insects into a collecting cup. Fight traps used alpha-pinene as an attractant. Small pieces (2.5 cm square) of dichlorvos (18%) impregnated plastic were placed in collecting cups to kill collected insects. Flight traps were not used in 1999 because the pitfall traps collected similar numbers and species as the flight traps (Fig. 1). Further, it could not be determined whether the dichlorvos insecticide in the Lindgren funnel traps was effective in retaining all insects.

Trap contents were removed and attractant material replaced at approximately 2-week intervals. Trap contents were transferred to glass jars with propylene glycol for short-term storage and then transferred to 95% ethanol for longer-term preservation. All insects captured were separated and identified to the family level at Colorado State University. Only curculionids and scolytids were further identified since insects in these groups vector the pathogen in other tree species. Identifications of the curculionids were provided by Charles W. O'Brien, Florida A&M University. Stephen Wood, Brigham Young University, provided identification of the scolytids respectively. We suspect that these two insect families were the most likely candidates of vectors due to their members feeding and/or breeding habits on roots or lower stems of pinyon pine. Other insects that were collected do not feed or breed in the xylem/phloem area where the pathogenic fungus resides. Other taxa collected were curated and identified on an anecdotal basis.

All specimens are on deposit in the C.P. Gillette Museum of Arthropod Biodiversity at Colorado State University.

RESULTS AND DISCUSSION

We identified insects representing four orders and 28 families (Table 1) including 13 bark beetle species (Scolytidae) and 13 weevil species (Curculionidae) (Table 2). More than 50 % of the trapped bark beetle and weevil species are known to feed or breed in roots or stumps (Arnett. 1971, Furniss and Carolin 1977, O'Brien and Wibmer 1982, Stephen Wood, Brigham Young University, Provo Utah, personal communication). Thus, 12 or more insects we found could be potential vectors.

TABLE 1. Insect Families Trapped within Black Stain Root Disease Centers in Pinyon Pine Stands near McPhee Reservoir in the San Juan National Forest in 1998 and 1999.

Hemiptera	Coleoptera		Diptera	Hymenoptera	
Reduviidae	Carabidae	Bostrichidae	Meloidae	Asilidae	Ichneumonidae
Lygaeidae	Staphylinidae	Anobiidae	Cerambycidae	Calliphoridae	Pompilidae
Coreidae	Histeridae	Trogossitidae	Chrysomelidae	Sarcophagidae	Vespidae
Pentatomidae	Scarabaeidae	Cleridae	Curculionidae	Tachinidae	
	Buprestidae	Ciidae	Scolytidae		
	Elateridae	Tenebrionidae			

Potential Scolytidae vectors include; *Dendroctonus valens* (LeConte), *Hylastes fulgidus* (Blackman), *Hylastes gracilis* (Leconte), *Hylastes macer* (LeConte), *Gnathotrichus denticulatus* (Blackman), *Hylurgops porosus* (LeConte), *Hylurgops reticulatus* (Wood), *Orthotomicus caelatus* (Eichhoff), *Orthotomicus latidens* (LeConte), and *Xyleborus intrusus* (Blandford). Only two Curculionidae, *Otiorhynchus ovatus* (L.) and *Otiorhynchus rugosostriatus* (Goeze) represent potential vectors. The most commonly trapped beetle, *Ips confusus,* was not assumed to be a vector because it is rarely seen breeding within a meter of the ground or in roots of pinyon trees. *Ips confusus* were active in the region during the collection period, which explains the high trap catches. *Hylastes* spp. and *Hylurgops porosus* were the most abundant of the potential vectors. More potential vectors may occur among the species we trapped but the life histories of many of these insects are not known. In fact, the life histories of most of the species listed as potential vectors are not well known.

Potential vectors were recovered from both pitfall and flight traps. Both scolytids and curculionids were trapped from May to November in 1998 and from May to August in 1999. With the exception of *I. confusus*, the majority of scolytids were trapped in May while the most curculionids were trapped in July and August (Fig. 1). The *Hylastes* spp. were collected in both trap types but predominantly in the pitfall traps.

Despite the fact that an insect has yet to be implicated as a vector of the pathogen of black stain root disease of pinyon pine, some evidence suggests a role for insect vectors. Landis and Helburg (1976) reported insect galleries, presumably made by bark beetles, adjacent to stain margins of *Leptographium wageneri* var. *wageneri* in diseased pinyon pine roots. Research and observations on other hosts of the black stain pathogen suggests a vector relationship in pinyon pine. Goheen and Cobb (1978) reported *Leptographium wageneri* var. *ponderosum* (Harrington and Cobb) conidiophores and perithecia only in insect galleries of diseased ponderosa pine roots. As insects are well-known vectors of various *Leptographium* and *Ophiostoma* pathogens, Goheen and Cobb (1978) suggested that insects might introduce *Leptographium wageneri* to ponderosa pine roots. Harrington et al. (1985) and Witcosky et al. (1986) demonstrated the ability of the

TABLE 2. Numbers of Adult Scolytids and Curculionids Trapped Near McPhee Reservoir in the San Juan National Forest in 1998 and 1999.

Taxa	Collection Date													
	1998						1999							
	5/20	6/19	7/10	7/26	8/12	8/29	11/7	12/15	5/25	6/10	7/12	7/26	8/9	[b]Total
							Scolytidae							
[a]*Dendroctonus valens LeConte*	-	5	7	-	5	-	-	1	10	14	2	7	5	56
[a]*Gnathotrichus denticulatus Blackman*	-	-	-	-	-	1	-	-	-	-	-	-	-	1
[ac]*Hylastes sp.*	62	3	1	2	6	17	50	10	147	27	8	19	5	357
[a]*Hylurgops porosus LeConte*	100	3	-	-	-	1	-	-	-	-	-	-	-	104
[a]*Hylurgops reticulatus Wood*	2	-	-	-	-	-	-	-	-	-	-	-	-	2
Ips confusus LeConte	-	-	-	14	554	2	-	18	-	-	-	2	2	592
[a]*Orthotomicus caelatus (Eichhoff)*	1	-	-	-	-	-	-	-	-	-	1	-	-	2
[a]*Orthotomicus latidens (LeConte)*	-	3	1	-		-	-	-	-	-	1	-	-	5
Pityophthorus confertus Swaine	-	81	-	-	34	-	-	-	-	-	-	-	-	115
Scolytus sp.	-	-	-	-	2	-	-	-	-	-	-	-	-	2
[a]*Xyleborus intrusus Blandford*	-	-	1	-	10	5	-	-	-	-	-	-	-	16
							Curculionidae							
Cimbocera conspersa Fall	-	1	-	-	-	-	-	-	-	-	-	1	-	2
Cossonus crenatus Horn	-	-	-	13	6	1	-	-	-	-	-	-	-	20
Gerstaeckeria basalis LeConte	-	-	-	-	-	-	-	-	-	-	1	-	-	1
Myrmek estriatus Casey	-	-	-	-	1	-	-	-	-	-	-	-	-	1
Orimodema protracta Horn	-	-	-	-	-	-	-	-	-	1	1	-	-	2
[a]*Otiorhynchus ovatus (L.)*	-	5	2	-	-	5	-	-	2	1	1	-	-	16
[a]*Otiorhynchus rugosostriatus Goeze*	-	2	1	1	1	5	-	-	-	-	-	-	2	12
Rhinocyllus conicus (Frolich)	-	1	8		3	3	-	-	-	-	-	5	4	24
Rhyncolus oregonensis Horn	-	-	-	2	-	-	-	-	-	-	-	-	-	2
Thinoxenus sp.	-	-	-	-	-	-	2	-	-	-	-	-	-	2
Thricolepis inornata Horn	-	1	7	1	-	-	-	-	-	-	-	-	-	9
Zascelis irrorata LeConte	-	-	-	1	1	-	-	-	-	-	1	-	-	3

a Insects known to feed and/or breed on roots /stumps. Insects without + do not feed/breed on roots or lower stems or hosts are unknown. bCombined totals of Lindgren and pitfall traps. cTaxa include *H. fulgidus* Blackman, *H. gracilis* LeConte, and *H. macer* LeConte.

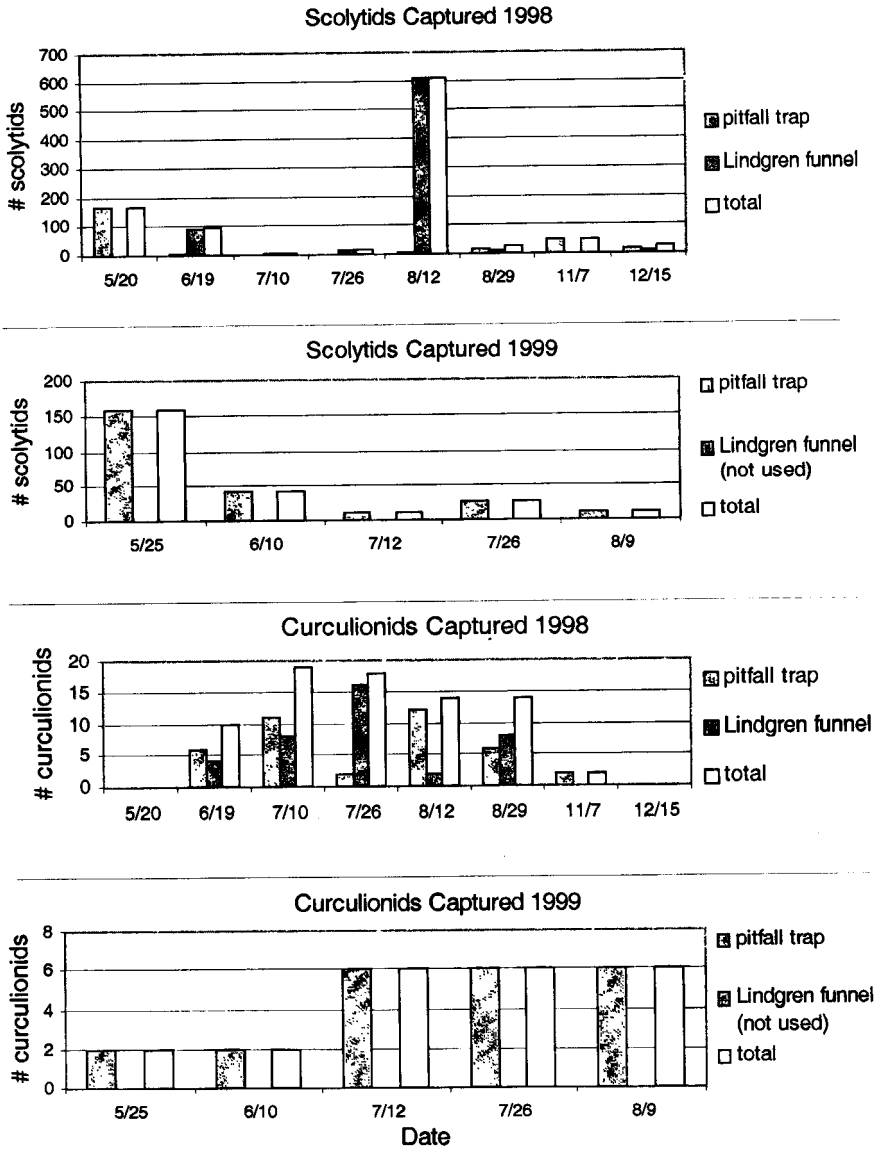

FIG. 1. Numbers of adult Scolytids and Curculionids by date that were trapped near McPhee Reservoir in the San Juan National Forest in 1998 and 1999.

scolytid *Hylastes nigrinus* (Mannerheim), and curculionid *Pissodes fasciatus* (LeConte) and *Steremnius carinatus* (Mannerheim) to vector *L. wageneri var. pseudotsuage* (Harrington and Cobb) in Douglas-fir. Adult beetles made feeding wounds and artificially contaminated adults successfully transferred the pathogen to feeding wounds. Additionally, at least two insects of these genera consistently appear in diseased Douglas-fir and ponderosa pine stands. Either recovered directly from affected trees or traps; *Hylastes* and *Pissodes* are reported to constitute upwards of 90% of insects recovered in these areas (Witcosky and Hansen 1985, Jacobi 1992).

We have identified a group of bark beetles and weevils that might be vectors of the black stain pathogen. The next step will need to focus on determining if any of the potential vectors identified in this study are vectoring the black stain pathogen. The biology of these insects in regards to pinyon pine and black stain root disease also will need further elucidation. A careful collection and rearing of insects found in roots of declining and recently dead pinyon pine affected by black stain root disease would also help explain the long distance transmission of the black stain pathogen.

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ARTIFICIAL FEEDING SYSTEM FOR THE SQUASH BUG, *ANASA TRISTIS* (DE GEER) (HETEROPTERA: COREIDAE)

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ABSTRACT

Squash bugs, *Anasa tristis* (De Geer) (Heteroptera: Coreidae), did not feed on liquid diet-filled feeding sachets used traditionally for feeding homopterans, or on pouch-like artificial feeding source (AFS) containing a meridic diet developed for rearing the western tarnished plant bug, *Lygus hesperus* Knight, another heteropteran. However, excised cubes of squash fruit, vacuum infiltrated with a suspension of the desired diet and offered above a screen barrier, were accepted. During a 48-hr testing period, all cube-fed adult insects survived, while 35% of those fed on the meridic diet offered in parafilm pouches and 40% of those fed on 5% sucrose offered in sachets died. After blue food coloring was added to the diets as a marker, the excretory fluids of 75% of insects fed on infiltrated cubes was blue in color indicating that feeding had occurred, while no blue defecation occurred when dye-amended diets were offered in the other two systems. The *A. tristis* AFS provides a convenient system for studying the feeding behavior of squash bugs and provides an alternative to whole plants for studying the etiology of cucurbit yellow vine disease.

INTRODUCTION

The squash bug, *Anasa tristis* (De Geer) (Heteroptera: Coreidae), occurs throughout North America and is considered a major pest of cucurbits. *A. tristis* feeding involves piercing of the plant's epidermis by the stylets and intracellular penetration to the mesophyll or vascular tissues (Beard 1940, Bonjour 1991). This 'lacerate and flush' method of feeding interrupts xylem transport of water due to vascular damage and blockage that results in collapse of plant structures distal to feeding sites (Neal 1993).

Cucurbit yellow vine disease (CYVD) is characterized by rapid and general yellowing of leaves appearing over a 3-4 day period, followed by gradual or rapid decline and death of the vine in several cucurbit crops (Bruton et al. 1998). *A. tristis* was reported (Pair et al. 2000) and confirmed (Bextine et al., unpublished data) to be a vector of *Serratia marcescens*, the causal agent of CYVD (Bruton et al., in press). This bacterium has been cultured from diseased field-grown watermelon, zucchini, pumpkin, and cantaloupe.

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Although artificial feeding systems (AFS) have been developed for many homopteran insects including leafhoppers, aphids, and whiteflies, few have been developed for heteropterans (Cohen 2000b). AFSs, along with artificial diets, have been developed for the western tarnished plant bug, *Lygus hesperus* Knight (Cohen 2000a; Debolt 1982; Debolt and Patana 1985), the reduviid bug, *Triatoma infestans* Klug (Schaub 1991), and the southern green stink bug, *Nezara viridula* (L.) (Ragsdale et al. 1979). In the latter case, the AFS was instrumental in associating insect feeding with microorganism transmission.

The relationship between the vector *A. tristis* and the pathogen *S. marcescens* is an important element of the etiology of CYVD, but investigations of pathogen-vector interactions were hampered by the lack of a convenient AFS by which pathogen acquisition by the squash bug could be assured and controlled. Preliminary attempts to feed *A. tristis* on known homopteran or heteropteran artificial diets were unsuccessful. The goal of this study was to develop an AFS and a suitable diet on which to maintain squash bugs during research studies and to provide a convenient acquisition source for the microorganism. Feeding acceptance and survivorship of *A. tristis* on traditional AFSs were compared with those on a new AFS composed of diet-infiltrated squash cubes.

MATERIALS AND METHODS

Colonies of *A. tristis*, initiated with adults collected from a field near Lane, OK, were maintained in screened cages (50 x 25 x 45 cm) in a growth room at 27°C, 12L:12D, and 45-50% RH. Colony insects were reared on pumpkin plants (*Cucurbita pepo* L. var. *pepo* "Connecticut Field") that were replaced at weekly intervals and supplemented with washed zucchini squash fruit (*C. pepo* L. var. *melopepo*).

Several AFSs and artificial diets used for sustaining other hemipteran insects were offered to *A. tristis* and observations of feeding were made. Feeding sachets, or stretched-parafilm membrane feeding systems, commonly used for studying leafhopper, aphid, and whitefly feeding (Mitsuhashi and Koyama 1971), hereafter referred to as the homopteran feeding system, consisted of 29.5ml flexi-cup medicine cups (Baxter Heathcare Corp., Deerfield, IL) covered by one layer of parafilm (American National Can, Greenwich, CT) stretched to 4X its original size over the opening of the medicine cup. A volume of 0.5ml of 5% sucrose (pH 7.0) was placed on the outer surface of the stretched parafilm and covered with another layer of stretched parafilm to enclose the solution (Mitsuhashi and Koyama 1971). Twenty insects, not separated by sex, were placed into the AFS, one per sachet, and the sachets were maintained at 27°C, 12L:12D, and 45-50% RH. Another five insects were individually offered a modification of this membrane feeding system in which squash slurry, made by liquefying approximately 10g squash fruit and 20ml sterile tap water in a blender, was brushed onto the insect-facing surface of the membrane (1ml squash slurry per 16cm^2 membrane surface) as a possible gustatory cue.

A non-membrane system was also tested by offering semi-solid preparations of 5% agarose or 3% gelatin. Ten ml of solution was poured into the bottom of each of five, 60 x 15 mm petri dishes (Ragesdale et al. 1979). A 70x70 mm, fiberglass screen barrier with 1-mm grid squares was placed so that it rested on the rim of the petri dish. Five insects (sex not determined) were placed on top of the screen in each dish, and the dish lids were positioned on top, their rims resting on the screen. The dishes were maintained for 48 hr at 27°C, constant light, and 45-50% RH.

Intact squash fruit were washed with hand soap and reverse osmosis (RO) water, and subsequently was cut with a sterile razor blade. The epidermis was removed and 6-mm^3 cubes were excised from the cortex. Squash cubes were vacuum infiltrated with blue dye so evidence of feeding could be determined by evidence of blue dye in squash

bug excretory fluid. For infiltration, squash cubes were submerged in RO water containing blue food coloring (McCormick and Co., Inc, Hunt Valley, MD) (0.1ml per 10ml H_2O) at a rate of 1 cube per ml in a 500-ml Erlenmeyer vacuum flask. A vacuum force was applied for 5 sec., during which negative pressure forced air from the intercellular spaces of the tissue, and as the vacuum was quickly released the blue-colored water entered the intercellular spaces.

Adult squash bugs were confined individually with a squash cube using an apparatus similar to that described by Ragsdale et al. (1979). The insect was placed in the bottom half of a 60 x 15 mm petri dish. A 70 x 70 mm square of sterile 1-mm fiberglass screen was placed over the dish, the squash cube was positioned in the center of the screen, and the petri dish lid was positioned on top. The squash cube was offered above the insect, rather than below, to avoid excretory fluid contamination of the cube. The feeding apparatuses were incubated in a humidity chamber (placed on a stand in a covered transparent plastic shoebox containing water at a depth of approximately 1cm) to minimize cube desiccation and were held under constant light at 27°C.

Three of the AFSs (the homopteran membrane feeding system, the tarnished plant bug feeding system, and the newly developed infiltrated squash cube AFS described above) amended with identical ratios of blue food coloring were tested for *A. tristis* acceptance over a 48 hr period at 27°C, constant light, and 45-50% RH.

In the homopteran membrane feeding system, 20 adult *A. tristis* were placed individually into the apparatuses and given access to 0.5ml of 5% sucrose (pH 7.0) containing blue food coloring (0.1ml per 10ml sucrose) (Mitsuhashi and Koyama 1971).

The tarnished plant bug feeding system (Cohen 2000a) consisted of meridic diet, a lima bean meal and wheat germ based formulation containing additional nutrient supplements and containing blue food coloring (0.1ml per 10ml diet) enclosed in 100mm x 70mm unstretched parafilm pouches as described by Debolt and Patana (1985). This diet was offered individually to 20 single insects (not separated by sex), with the parafilm pouches supported on mesh screening in six-well tissue culture plates with 35-mm wells.

The *A. tristis* feeding system was also offered individually to 20 *A. tristis* (not separated by sex). For all three feeding systems filter paper (Whatman no. 4), cut to fit the bottom of the apparatus, allowed collection of the droplets of squash bug excretory fluid. If this fluid was blue, squash bugs were assumed to have fed on the offered diet. Insect mortality over the 48-hr period was recorded.

For both detection of blue excretory fluid and insect mortality, chi square analysis was used to assess differences (critical P-value=0.05) between treatments (SAS 1996).

RESULTS AND DISCUSSION

In our preliminary experiments, when squash bugs were placed in several AFSs and offered artificial diets known to be acceptable to other homopteran and heteropteran insects, neither stylet insertion nor test probing was observed. These insects did not feed through any of the tested membrane based feeding systems, nor would they feed on the semi-solid agarose or gelatin formulations.

Squash bugs fed on blue, water-infiltrated squash cubes within one hour of introduction, with 15 of 20 test insects excreting droplets of blue fluid onto the filter paper after 48 hr (Table 1), whereas none of the insects offered the other two systems/diets excreted any fluid (chi-square=40, df=2, $P<0.001$). None of the twenty insects offered blue water-infiltrated cubes in our AFS died during the 48-hr period, compared to 7/20 of those offered the meridic diet in unstretched parafilm bags and 8/20 of those offered 5% sucrose in feeding sachets. These percentages were significantly different among treatments (chi-square=10.13, df=2, $P<0.01$).

It is unclear why squash bugs did not feed in the known homopteran or heteropteran AFSs tested. It is possible that physical, tactile, or gustatory cues present on or in plants are not present on these membranes, but coating the stretched membrane with

TABLE 1. Preference and Suitability of Three Different Artificial Feeding Systems by the Squash Bug, *Anasa tristis*.

Treatment[c]	Blue Excetory Fluid[a]		48 hr Mortality[b]	
	Blue	Not Blue	Survived	Died
A. tristis feeding system with squash cube diet[d]	15	5	20	0
Lygus system with Meridic artificial diet[e]	0	20	8	12
Feeding sachets with 5% sucrose diet[f]	0	20	7	13

[a]Excretory fluids: excretion of blue fluid was accepted as a sign that *A. tristis* had fed on blue dye-amended diet in the artificial system tested (chi-square=40, df=2, $p<0.001$).
[b]Mortality: proportion of insects that died within the 48 hr experimental period (chi-square=10.13, df=2, $p<0.01$).
[c]Diet was offered for 48 hr at 27°C, constant light, and 45-50% RH.
[d]*A. tristis* were offered 50 mm^3 squash cubes as a feeding source.
[e]*A. tristis* were offered unstretched parafilm bags containing the meridic diet.
[f]*A. tristis* were offered parafilm membrane feeding sachets containing 5% sucrose.

squash fruit slurry did not induce squash bug feeding behavior. Our *A. tristis* AFS was developed in response to observation of the insects feeding readily on squash fruit provided in colony cages.

That squash bugs fed readily from infiltrated squash cubes was demonstrated by the fact that 75% of the insects tested were determined to have fed within a 48-hr period. These data indicated that this system might be suitable for the oral delivery of bacteria to *A. tristis* in acquisition experiments. *A. tristis* acquisition of the CYVD bacterium, *S. marcescens*, in this artificial system has since been demonstrated by transmission experiments in which squash cubes were vacuum-infiltrated with a bacterial suspension (Bextine 2001). Un-infiltrated squash cubes were also useful as inoculation targets for bacteria-exposed squash bugs. Subsequent detection of the pathogen by polymerase chain reaction was facilitated because the cube size was convenient for DNA extraction.

The *A. tristis* AFS is actually only partially artificial because the primary nutrient source is the squash fruit, a natural squash bug food that is chemically undefined. Development of an artificial diet to be used in the AFS would offer better control of the feeding medium; however, the cube system is well-suited for the purpose of acquisition and inoculation of a bacterial phytopathogen in insect transmission studies.

Because *A. tristis* is a vector of *S. marcescens*, the causal agent of CYVD, it is important to better understand the relationship between the insect and the pathogen. Bacterial cultivation from infected plants ensured a reliable source of the pathogen, but a system of microorganism delivery to the insect was needed. The *A. tristis* AFS serves as a convenient method to study pathogen transmission by the squash bug. Using fruit cubes rather than infected plants as inoculum sources for *A. tristis* acquisition has several advantages; the fruit is readily available commercially, eliminating the need for plant care, and the amount and titer of the bacterial inoculum can be controlled and standardized in the vacuum infiltration step. Environmental conditions are also easier to control in the AFS than in a plant system. The cube system allows the interaction between the pathogen and the insect to be studied without the added dimension of the plant. However, eliminating the plant from the system does remove an important aspect of pathogen transmission. The pathogen titer in the cubes is probably higher than would

be expected in the plant. Because the CYVD pathogen is phloem-associated, testing *S. marcescens* transmission by *A. tristis* in the artificially inoculated squash cube AFS may not exactly mimic acquisition or inoculation of the pathogen in a naturally infected plant system. The vacuum infiltration of a squash cube saturates all intercellular spaces, which would not occur in plant infection, and does not assure bacterial entry into phloem sieve tubes. However, the AFS developed for *A. tristis* provides a tool that will facilitate the study of CYVD.

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LONGEVITY OF ULTRA-LOW-VOLUME SPRAYS OF FIPRONIL AND MALATHION ON COTTON IN MEXICO

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ABSTRACT

In 1996, fipronil and malathion residues were evaluated after four ultra-low-volume (ULV) spray applications in northeastern Tamaulipas, Mexico. Sprays were applied at 0.88 L/ha. Fipronil was applied at 28 and 56 g A.I./ha and malathion at 840 g A.I./ha. Four applications were made beginning 23 May at four, five and six day intervals. Leaf surface residues of malathion accumulated with each application. Leaf surface residues of fipronil applied at both rates dissipated >90% after 2 to 4 d after all applications.

INTRODUCTION

Probably the most extensive use of ultra-low-volume (ULV) insecticides in crop production has been in the Boll Weevil Eradication Program which began in 1978. From the beginning, ULV application of insecticides has been used to great effectiveness. The labor and money saved by using ULV application has helped to keep the cost of the program at an affordable level. Vast acreage can be most expeditiously treated with ultra-low-volumes of insecticides because aircraft are able to spend more time spraying and less time filling and ferrying to and from the airstrip.

Technical malathion is the insecticide of choice for area-wide eradication programs against the boll weevil, *Anthonomus grandis grandis* Boheman, in the United States. It is effective against this insect as an ultra-low volume (ULV) spray (Jones et al. 1996, 1997).

Fipronil (as Regent® from Aventis Environmental Science, Research Triangle, NC) is a phenyl pyrazole (Colliet et al. 1992) that has been demonstrated to be effective as a high volume spray at 0.056 kg A.I./ha against the boll weevil in field plots (Burris et al. 1994). Four applications of ULV sprays of malathion and fipronil in cottonseed oil at 0.88 L/ha were effective against the boll weevil in a field test (Reed et al. 1998).

How long an insecticide remains effective on the plant surface is an important factor in determining the optimum spray interval for controlling boll weevils. Costly over-treating can be avoided if the longevity of the insecticide being used to control a pest is known.

The objective of this test was to compare the longevity of ULV sprays of fipronil and malathion on cotton leaves after multiple applications.

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MATERIALS AND METHODS

The test was conducted in a 30-ha field of cotton planted on 18 February 1996 near Nuevo Progreso, in northeastern Tamaulipas, Mexico. The field was irrigated prior to planting and twice during the test. No rain fell during the test period.

Fipronil, as a 300 g/liter or 2.5 lbs/gallon emulsifiable concentrate, was tested at 28 or 56 g A.I./ha. Crude cottonseed oil (Olete de Monterrey, Nuevo Leon, Mexico) was used as a diluent of fipronil. Technical malathion (95%) (Chemica Lucava, Monterrey, N.L., Mexico) was tested at 840 g A.I./ha.

Applications of each treatment were made at 202 km/hr with a single-winged Ag-Cat airplane fitted with an eight-meter boom calibrated to deliver 0.88 L/ha through nine 8002 flat fan TeeJet nozzles (Spraying Systems, Wheaton, IL.). Applications were made on 23, 27 May and 1 and 7 June 1996. Boom pressure of all sprays was 1.75 kg/cm^2. The airplane made applications 1.0 m above the plant canopy and along the north to south rows. Applications were directly into or with a prevailing south to southeast wind at about 07:00 hours. Wind gusts did not exceed 24.1 km/hr during the applications.

The test field consisted of 383 rows on 1.0-m centers that averaged 750 m in length. Treated plots were replicated four times and were 28 rows wide with one swath/replicate. Treatments were randomly assigned to the plots.

Leaves were collected for residue analysis from the second or third nodes below the first fully expanded terminal leaf on each of 16 plants (four/plot) in row 15 of each plot. Leaves were sampled 0, 1, and 2 d after the first application and 0, 1, and 4 d after the second and fourth application, respectively. Leaves were not collected after the third application because the field was furrow irrigated.

Sixteen leaves (four leaves/plot) collected on each date were placed in 946-ml plastic bags, sealed, and placed in a cold box. The bags were placed in a freezer at 0 °C within 2 h after collection and held until residues were extracted. Within 3 to 7 d, residues of fipronil and malathion were washed from the upper and lower surface of each leaf using dual-side leaf washers (Carlton 1992) with 95% ethanol as a solvent.

Samples of both insecticides were transported in refrigerated containers to the USDA Laboratory in Stoneville, Mississippi, for analysis. Samples of fipronil were evaporated to dryness under a slow stream of ultra high purity (UHP) grade nitrogen. The dried samples were then reconstituted in 1 ml acetonitrile-methanol for analysis.

Residues of fipronil were determined by high pressure liquid chromatography with ultraviolet detector at 280nM (Mulrooney et al. 1998). Analytical fipronil was provided by Rhone-Poulenc Ag Company, Research Triangle Park, NC. All solvents were HPLC grade. Retention time of fipronil was 7.50 min. Mulrooney and Goli (1999) recovered 92% of fipronil from the cotton leaf using ethanol. Minimum detection limit of fipronil was <50 ppb.

The concentration of malathion in washes of upper and lower leaf surfaces was determined by gas chromatography using a flame photometric detector in phosphorus mode (Mulrooney et al. 1997). Retention time of malathion was 5.44 min. Minimum level of detection was 0.12 ppm.

Analysis of variance ($P<0.05$) using PROC MIXED, SAS (1997) and subsequent mean separation (PDIFF option) were applied to residues of malathion and both rates of fipronil on upper and lower surfaces of leaves after each application. Residues of both insecticides were collected after the first, second, and fourth applications.

Curves of residues of malathion and fipronil were best fitted by $Y = AX^B$, or using linear regression, log (residue + 1) = intercept + slope (log time + 1). Analysis was made using PROC MIXED of SAS (1997). Contrasts of trends (intercept and slope) and slopes of the low and high rates of fipronil were made.

RESULTS AND DISCUSSION

Analysis of variance showed significant differences in residues of malathion and high and low rates of fipronil between days ($F=5.0;df=3,3;P<0.02$) and applications ($F=10.53;df=2,3;P<0.0001$).

Malathion residues on the upper surface of the leaf ranged from 398 to 4,391 ng/cm^2 and from 61 to 1367 ng/cm^2 on the lower surface (Table 1). After the first application, residues on upper or lower surfaces of leaves did not significantly decrease until two days after application. After applications two and four, there were no significant differences in residues on either the upper or lower leaf surfaces between day zero and day two sample dates. Malathion residues on the lower surface significantly decreased from day two to day four after applications two and four.

A ULV spray of technical malathion at 2.8 kg A.I./ha near Brownsville, Texas (Wolfenbarger and McGarr 1971) deposited 6,490 ng malathion/cm^2 on leaves. In our study, the first and fourth application of 0.84 kg A.I./ha deposited 2,105 and 3,381 ng malathion/cm^2 on leaves (Table 1). The 2.8 g A.I/ha of malathion applied in Brownsville, Texas, resulted in 2- to 3- fold greater deposition than the 0.84 g A.I./ha rate used in our study. Residues of malathion on upper leaf surfaces sampled on day zero after one (1,621 ng/cm^2), two (2,168 ng/cm^2) and four (2,576 ng/cm^2) applications show increasing residues after multiple applications. Residues collected immediately after applications two and four increased 35 to 60% from application one. Residues at 2 d after applications two and four were about 10 fold greater than application one. There was no significant difference in residues on the upper leaf surface between 0 and 4 d after application four.

Malathion residues on the upper and lower surfaces of the cotton leaves increased after the first and second application (Table 2). Intercepts of regressions of residues after the second and fourth application were equal.

The slope of regression of malathion residue on the upper leaf surface was negative after applications one and two and positive after application four (Table 2). This result seems to indicate a decrease in the rate of degradation of malathion on the leaf surface as the season progressed.

In contrast to malathion, residues of fipronil on the upper surface of the leaf on day zero did not accumulate after each of the three applications (Table 1). Fipronil is a short-lived insecticide compared to malathion. At both rates, residues of fipronil decreased an average of 95% 2 and 4 d after the three applications. Residues of the low rate of fipronil on the upper leaf surface decreased significantly on each of the days after application.

Residues of fipronil on the lower leaf surface decreased 98% 2 d after the first application at the high rate and 94% and 93% 4 d after the second and fourth application, respectively. Residues of fipronil were not significantly different on days 0 and 1 after the first application. The residues found on the lower leaf surface at the high rate after applications two and four were significantly different from each other on each of the sample days.

Fipronil residue declined on both surfaces of the leaf after all applications (Table 3). The loss of fipronil was always greater on the upper surface of the leaf than on the lower surface. The intercept, which is an estimate of the initial deposit on the leaf, of the high rate of fipronil was always greater than that for the low rate. The intercept decreased from application one to application four for the low rate of fipronil, especially on the upper surface of the leaf. At the high rate, the intercept was variable on both leaf surfaces. The intercept of fipronil at the high rate far exceeded the low rate after applications two and four.

TABLE 1. Insecticide Residues (ng/cm^2) Recovered from the Upper and Lower Surfaces of Cotton Leaves.

	Application 1			Application 2			Application 4		
	Day after application			Day after application			Day after application		
Treatment	0	1	2	0	2	4	0	2	4
				Upper Surface					
Malathion	1621[a]a	1897a	398b	2168a	4053a	792b	2576a	4391a	3139a
Fipronil 28.0 g/ha	62.5a	7.8b	1.6c	30.6a	5.9b	0.9c	16.6a	0.9b	0.5c
Fipronil 56.0 g/ha	57.8a	8.0b	1.0c	78.9a	5.8b	1.4c	43.1a	2.4b	0.8b
				Lower Surface					
Malathion	484a	252a	61b	876a	1367a	389b	805a	935a	259b
Fipronil 28.0 g/ha	15.6a	3.4b	0.8c	14.4a	3.3b	1.3b	9.7a	5.6b	1.4b
Fipronil 56.0 g/ha	22.2a	14.7a	0.5b	39.2a	15.6b	2.3c	39.6a	8.8b	2.7c

[a]Means for each treatment over days within an application not followed by the same letter are significantly different ($P \leq 0.05$) as determined by PDIFF (SAS Institute, 1990).

TABLE 2. Equation (Residue= Intercept×Day^{Rate}) of Malathion, Applied at 0.88 l AI/ha, Residues as ng/cm^2 from Upper and Lower Surfaces of Cotton Leaves as a Function of Day (Application on Day 0).

Application	Intercept	Slope
	Upper Surface	
1	2080	-1.12
2	2893	-0.45
4	2864	0.12
	Lower Surface	
1	567	-1.77
2	1075	-0.36
4	1022	-0.61

TABLE 3. Equations (Residue = Intercept × Day^{Rate}) of Fipronil Residues from Upper and Lower Surfaces of Cotton Leaves as a Function of Day (Application on Day 0).

Rate (g A.I./ha)	Application	Intercept	Slope
		Upper Surface	
28.0	1	67	-3.51
56.1	1	68	-3.79
28.0	2	30	-2.13
56.1	2	80	-2.55
28.0	4	13	-2.24
56.1	4	41	-2.56
		Lower Surface	
28.0	1	16	-2.85
56.1	1	36	-3.23
28.0	2	15	-1.60
56.1	2	49	-1.71
28.0	4	9	-1.12
56.1	4	41	-1.71

Comparisons of trends, which includes both intercept and slope, of the low and high rates of fipronil indicated that greater degradation of fipronil on the upper leaf surface occurred after the second (F=4.85;df=2,75;P>F=0.0333) and fourth (F=7.56;df=2,75;P>F=0.0073) application of the high rate (Table 4). However, when only the slopes of each rate were compared, no significant differences were observed.

Comparisons of trends of the degradation of fipronil rates on the lower surface of leaves were significantly different for each of the three applications, with the greatest difference (F=15.57;df=2,80;P>F=0.0001) after the second application (Table 4). As with residues on the upper surface, comparisons of slopes showed no difference in degradation for any of the applications. Comparisons of trends, which include both the intercept (initial deposit) and the slope (rate of degradation), give a more complete picture of fipronil on the leaf surface.

TABLE 4. Contrasts of Trends and Slopes for Low and High Rates of Fipronil on Upper and Lower Surfaces of Cotton Leaves.

	Trend ($\beta_0 + \beta_1$)[a] Contrast		Slope (β_1) Contrast	
Application	F value	P>F	F value	P>F
		Upper Surface		
1	0.65	0.5225	0.55	0.4614
2	4.82	0.0333	1.86	0.169
4	7.56	0.0073	1.22	0.2722
		Lower Surface		
1	3.83	0.0256	0.68	0.4106
2	15.57	0.0001	0.13	0.7218
4	3.32	0.0574	2.69	0.1049

[a] β_0 = intercept, β_1 = slope.

Malathion has been effectively used in the eradication of the boll weevil. Its effectiveness along with its low cost will likely ensure its continued use in eradication. However, the EPA is carefully scrutinizing all organophosphorus insecticides for harmful effects on humans and the environment and their continued use could be questionable. If malathion was removed from the U.S. market, the eradication of the boll weevil could be accomplished with fipronil provided that it was labeled for use on cotton in the U.S. Substituting fipronil for malathion in an area-wide application program would have some consequences. Fipronil is highly toxic to aquatic organisms (Harmon et al. 1996) and being a relatively new insecticide, it would likely cost more than malathion.

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INJURY AND DISTRIBUTION OF ONION THRIPS (THYSANOPTERA: THRIPIDAE) IN RED CABBAGE HEADS

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Onion thrips, *Thrips tabaci* Lindeman, has been a serious pest of onions in south Texas for decades (Royer et al. 1986, Sparks et al. 1998, Liu and Chu, unpublished data). *T. tabaci* can cause significant damage to crops adjacent to onion fields shortly before, during or shortly after onion harvest between March and May because of the dispersal and migration of high thrips populations from the onion fields. Direct feeding by larvae and adults of *T. tabaci* not only causes direct cosmetic damage of the cabbage head with rough brown blisters on green or white cabbages, but also significantly reduces storage duration and market value (Fox and Delbridge 1977, Stoner and Shelton 1988, Shelton et al. 1998).

In the spring 2000, we observed that a severely infested red cabbage field adjacent to an onion field near Mission, Texas. The cabbages were planted in mid-December 1999. On 10 March 2000, we collected 50 red cabbage plants from this field. We carefully cut the plants with all leaves at the ground level and placed them in plastic bag for transport to the laboratory for examination. Before assessing injury and thrips on the cabbage heads, we removed all old wrapper leaves. We then peeled off individual leaf layers from each cabbage head, numbering respectively from the outmost toward the center of the head. We visually evaluated the injury level of each leaf based on the following four categories: 0 = no injury; 1 = 1-5 small injured spots or blisters; 2 = >5 small injured spots but still somewhat isolated; and 3 = general injury across much of the area covered by the leaf. At the same time, we counted all thrips (larvae and adults) on both surfaces of each peeled leaf from each cabbage head. We stopped peeling off leaves when no injury or thrips were found from five consecutive leaves of the cabbage head. We collected the adult thrips and identified them under a microscope. We found that all thrips collected were *T. tabaci*. We did not identify larvae, but assumed that all of them belonged to the same species. Voucher specimens were deposited in the Insect Collection of Texas A&M University Agricultural Research and Extension Center at Weslaco. The correlation between leaf injury and number of thrips found on that leaf was analyzed using PROC CORR (SAS Institute 2001).

On green or white cabbage, the feeding damage symptom caused by *T. tabaci* adults and larvae appeared as bronze discolored blisters or spots on the leaf (Fox and Delbridge 1977, Stoner and Shelton 1988). However, the blister-like spots were even more distinct on the red leaves than on green or white cabbage leaves. Therefore, the cosmetic damage and market value were even more significant for red cabbage than for green or white cabbage. The whole field sampled was destroyed without harvesting.

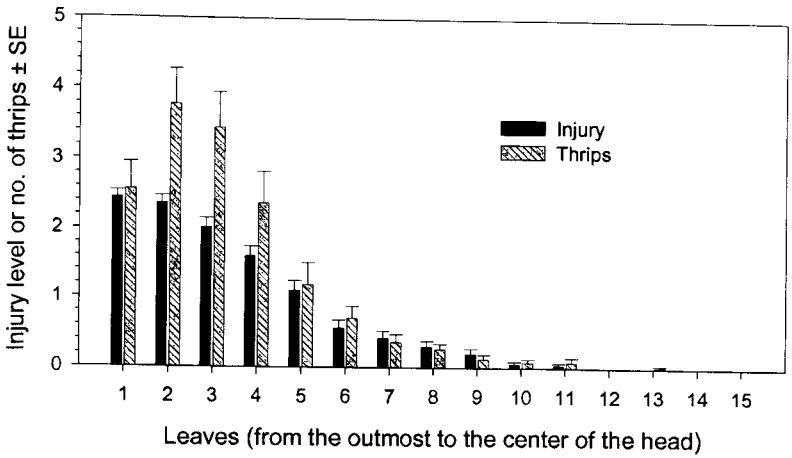

FIG. 1. Injury and number of *T. tabaci* (larvae and adults) on leaves of red cabbage head (n = 50).

The injury levels from the outmost older leaf of the head, with wrapper leaves previously removed, to the younger leaves toward the center of the head are shown in Fig. 1. Most damage occurred on the outer older leaves. The injury level in each of the first three layers of leaves was >2, considered as severe damage. The fourth and fifth leaves had moderate damage with an injury level of 1.1 and 1.6, respectively, and the next six inner leaves had little damage. No injury was found from the twelfth and younger leaves of the head. Most heads examined would have required removal of the wrapper leaves and at least five outer leaves from the head in order to reduce damage to an acceptable level, which would have reduced head size and marketability to an unacceptable level. Consequently, the level of damage in this field and similarly infested fields generally results in complete loss of the crop.

Similar to the injury level of the leaves, more thrips were found on the outer leaves of the heads than on the inner or younger leaves (Fig. 1). The outer four leaves had 81.3% of the total thrips, and the outer eight leaves 98.0% of the total thrips. An average of <1 thrips was found on the ninth to eleventh leaves. One thrips was found on the thirteenth leaf in one of the 50 cabbage heads.

As many as 49 thrips were found on one head, and the third and fourth leaves had 13 and 17 thrips, respectively. As shown in Fig. 1, the outmost leaf had the highest injury level among all injured leaves of the head, but it had fewer thrips than the second and third leaves. The possible reason was that the outer surface of the leaf was uncovered.

Numbers of thrips and injury levels on the leaves of the cabbage head were relatively well correlated ($r = 0.68$, $P = 0.01$). This result is similar to that found by North and Shelton (1986), that thrips (primarily *T. tabaci*) can be found as deep as 11 layers (22 leaves) into the green cabbage head.

In South Texas, the primary time of thrips movement into cabbage and other crop fields coincided with movement of thrips populations from onions. If thrips populations on onions were high, they moved to the adjacent crop fields as the onions matured, dried, and were harvested. This occurred during the period of onion harvest between March and May in South Texas. This problem generally was worse with later maturing onions, as onions growing later in the spring generally have higher thrips populations. The numbers of airborne immigrant thrips from onion fields can spread rapidly to cover extensive areas on a diversity of vegetables and field crops. Therefore, we recommend that cabbage and other vegetables that will not be harvested prior to onions should be planted as far away from onion fields as possible, or should be planted upwind to the onion fields in spring.

We thank C. Medelez for technical assistance. Publication of this manuscript has been approved by the Director of Texas Agricultural Experiment Station at Weslaco, and the Head of the Department of Entomology, Texas A&M University, College Station, TX.

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RANGE EXTENSION, NEW HOSTS, NEW MORPHS, AND A SYNONYM OF THE SEDGE APHID[1] *RHOPALOSIPHUM MUSAE* (SCHOUTEDEN)

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During a study of the aphid genus *Rhopalosiphum* Koch which culminated in a phylogeny for this economically important group of insects (Taber 1994), a population of one species was discovered where it might never have been suspected. The species was *Rhopalosiphum musae* (Schouteden), and the population was found in western Maryland at Orchard Pond, Allegany County, Greenridge State Forest on 23 November 1991. This aphid previously was known in the United States only from the western part of the country and from no closer to the Mid-Atlantic States than the Rocky Mountains, a distance of roughly 1,500 miles. The species has also been reported in the far west of Canada, in British Columbia (Forbes and Chan 1983). The Maryland host plant was a sedge or bulrush of the genus *Scirpus* (Cyperaceae). This genus is the only known summer host of a seasonally migrating aphid that uses cherry, almond, and other *Prunus* species as winter hosts (Gillette and Palmer 1932a,b; Patch 1938; Palmer 1952; Richards 1960; Smith and Parron 1978). Winged adults, wingless adults (Fig. 1), nymphs, and parasitized mummies were clustered in large numbers on bulrushes near the waterline. With dropping autumn temperatures the plants were dying back rapidly and the insects were destined to follow suit.

Microscope slides borrowed from museum collections yielded three previously unreported life cycle stages and two previously unreported host plants. The stages or morphs are the fundatrix and spring migrant (from unidentified host, Ash Creek, Utah, 1-V-1934, coll. G. F. Knowlton, British Museum slide "BM 1984-340 *Rhopalosiphum scirpifolii* = *musae*", det. D. H. Ris Lambers) and the fundatrigenia (from *Prunus fasciculata*, San Bernardino Co., Cal., 10 May 1968, coll. R. C. Dickson). Photographs of all three morphs are available but not published here due to space limitations.

One previously unknown host plant is the native desert almond *Prunus fasciculata* upon which the fundatrigenia morph was found, and the other is the exotic bird-of-paradise or crane flower *Strelitzia reginae* ("Hawaii via California via Florida: West Palm Beach"; Florida State Collection of Arthropods). The ornamental bird-of-paradise is a close relative of banana for which the aphid was named when it was first described from Belgian greenhouse specimens on leaves of *Musa ensete* (Schouteden 1906).

Occurrence on banana was reported again decades later (Hughes and Eastop 1991). A study of this material corroborated the opinion of David Hille Ris Lambers that *Rhopalosiphum scirpifolii* Gillette and Palmer is a junior synonym of *R. musae*. Voucher specimens will be sent to the United States National Museum in Washington, D.C.

[1]Hemiptera: Aphididae

FIG. 1. *Rhopalosiphum musae;* apterous alienicola on sedge.

I thank Mr. Wright Welton of Kiefer, Maryland, for showing me the secluded habitat which I would otherwise never have seen and Green Ridge State Forest Manager Mr. Francis Zumbrun for permission to collect voucher specimens. Slide material was loaned by Mr. Harold A. Denmark of The Florida Collection of Arthropods, Mr. Saul I. Frommer of the University of California at Riverside, and Mr. Paul A. Brown of the Natural History Museum (London, England).

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RANGE EXTENSION, HABITAT, AND REVIEW OF THE RARE ROBBER FLY[1] *ORTHOGONIS STYGIA* (BROMLEY)

Stephen W. Taber[2] and Scott B. Fleenor[3]

While conducting a biodiversity survey in the Ottine Swamps of south central Texas (Gonzales County), we captured a remarkable inch-long robber fly that was unfamiliar to us despite years of field work in the wetlands and forests of this area. It was identified as *Orthogonis stygia* (Bromley), the only Nearctic species of a mainly Old World genus (Hull 1962, Martin and Wilcox 1965, Joseph and Parui 1981, Poole 1996), with the aid of the standard key to Nearctic Asilidae (Wood 1981). This is the first published report from the southwestern United States. Our single specimen is a female; the male sex remains unknown to science. Furthermore, the biology of the species, even as regards such basic information as habitat, is unknown. Our purpose here is to provide basic biology, to alert others to search for the missing male, and to provide the first illustration of *O. stygia* to aid those wishing to continue our work (Fig. 1). An intriguing line drawing of the wing may be seen on page 558 (Fig. 14) in Wood (1981). It bears the male symbol and if that accurately described the specimen at hand, then paradoxically the known female has never been illustrated in any way though the unknown male has.

O. stygia has been reported previously only from the southeastern United States. The roughly half dozen reported specimens are from Florida (Gainesville), North Carolina (Stovall), and Mississippi (Ovett) (Bromley 1931, 1950). Thus, more than half a century has elapsed since the last published encounter with this striking predator that mimics a spider wasp with its black and metallic blue coloration. The Texas A&M University Insect Collection in College Station has two additional females. Both were collected by Bromley himself in 1934 in Liberty, Texas, and both are shown here beneath our own specimen. Bromley's site is 160 miles east and slightly north of our collection locality.

We found the asilid on a trail in an ash swamp characterized by dwarf palmetto, *Sabal minor* (Jacquin) Persoon, and green ash, *Fraxinus pennsylvanica* Marshall. These plants are more typical of the southeastern U.S., where the fly has been previously reported on two occasions, than of central Texas or the southwest, and the palmetto does reach its western limit nearby as does the loblolly pine and presumably *O. stygia* (Maxwell 1970, Schultz 1997). Our specimen and all those reported in the literature except one were captured in June. The single exception is one of Bromley's Texas flies captured on 2 July 1934. Prey, oviposition site, and larval habit remain to be discovered. We confirm the statement made in the original description of the species, that the species is "undoubtedly very rare" (p. 434, Bromley 1931). Yet we modify the statement that *O. stygia* is confined to the southeastern United States. Its absence from a report on the Asilidae of Texas (Bromley 1934) is explained by the discovery of Texas specimens in the same year

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FIG. 1. *Orthogonis stygia*, females; wingspan of upper specimen = 1.70 inches.

as the publication and at a time when the manuscript was already published or in press. We thank Assistant Curator Ed Riley for loaning material from the Insect Collection at Texas A&M University and David Riskind of the Texas Parks and Wildlife Department for providing Scientific Study Permit No. 21-01.

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BIOLOGY OF *COLEOTHORPA DOMINICANA FRANCISCANA* (LECONTE)[1]

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ABSTRACT

The life stages of *Coleothorpa dominicana franciscana* (LeConte), a case-bearing leaf beetle, are discussed, and photographs of each stage are presented. Eggs are oviposited in clusters near the tip of spines on the pricklypear cactus, *Opuntia engelmannii* Salm-Dyck, and the female beetle constructs a unique, previously undocumented, funnel structure on the spine to protect the eggs. Larvae are active for 5 - 8 months, and the overwintering stage lasts about 6 months. Development time from egg to adult was one year in the laboratory. This clytrine is associated with the ant *Formica neoclara* Emery, but there was no evidence that the ant was necessary for larval development.

INTRODUCTION

The biology of *Coleothorpa dominicana franciscana* (LeConte) is poorly understood, even though information on the general biology of this case-bearing leaf beetle was reported as early as 1874 (Riley 1874). Eggs are covered with platelets of fecal material that are molded around the egg, as described by Riley (1874), Erber (1988), and Stiefel and Margolies (1998). Rather than being dropped to the ground as occurs with many clytrines, each egg of *C. dominicana* is attached to one end of a short stalk that is fixed at the other end to the oviposition substrate, an arrangement similar to lacewing eggs (Neuroptera: Chrysopidae). In his review of the biology of case-bearing leaf beetles (Camptosomata), Erber (1988) reported that many species in the subfamily Clytrinae were totally or partly myrmecophilous, and Riley (1891) indicated that *C. dominicana* (F.) was associated with the ants *Formica obscuripes* Forel, *Formica* sp., and *Camponotus* sp. Eggs of several clytrines are carried by ants into their nests (Erber 1988, Stiefel and Margolies 1998) where the eggs hatch. The larvae remain in the ant nest and develop into adults. After hatching, the larvae live within the egg case for the remainder of the larval and pupal stages. As the larva grows, it uses fecal material, fine sand and small pieces of vegetative material to enlarge the case. Clytrinae adults are polyphagous and feed on leaves from trees and bushes, frequently without a definite preference (Erber 1988).

The reared beetles in this study were identified using the current taxonomy of Moldenke (1970), a work that treats the North-American Chrysomelidae of the subfamily Clytrinae. The present generic placement of this beetle (in *Coleothorpa* vs. *Coscinoptera*) follows Moldenke (1981). Most specimens of *Coleothorpa dominicana franciscana* (LeConte) from the southwestern United States differ in their outward appearance from those originating from the

[1] Coleoptera: Chrysomelidae: Clytrinae

more eastern portion of the subspecies' range. The southwestern specimens, including the adults reared in this study, closely match the type specimen of *Coscinoptera dorsalis* LeConte (images of LeConte's type specimen can be viewed in the Museum of Comparative Zoology type specimens database, at http://mcz-28168.oeb.harvard.edu/mcztypedb.htm). *Coscinoptera dorsalis* is presently considered a junior synonym of *Coleothorpa dominicana franciscana*.

Coleothorpa dominicana franciscana occurs in Texas Rolling Plains rangelands, but its biology from this region has not been documented. This article reports general life history of this species and provides photographs of each life stage.

MATERIALS AND METHODS

The study area was located 4.0 km north of Knox City, Knox Co., Tex., on the east side of Highway 6 at the junction with a paved county road. During the years 1990 to 1995, the site was consistently searched for eggs and adults weekly from early April to mid-June. Sporadic observations were made in 1989 and in 1996-1998.

When eggs were found, they were taken to the laboratory at Vernon, Texas. Attempts were made to rear the larvae from the eggs every year. However, only two were reared successfully to adult in 1995-96. Larvae were maintained in a 85-mm diameter petri dish; the lid had a 15-mm diameter hole in the middle that was coved with fine-mesh screen to allow ventilation. The filter paper in the dish was moistened daily with a drop of water. The rearing arena was cleaned every-other-day, and small pieces of freshly diced cactus pads, small pieces of moldy bread, several smears of pinto bean diet (Shorey and Hale 1965), and two, 1-cm lengths of dental wick soaked in 10% honey water were placed on the filter paper as food sources. Also, small amounts of fine sand and dried plant material were added to aid the larvae in enlarging their cases. Rearing was conducted in the laboratory under room conditions [14:10 (L:D) photoperiod, temperature 24-27°C].

The two larval cases with the last instar larvae were transferred from laboratory room conditions into an environmental control chamber on 29 November 1995. Photoperiod in the chamber was 11:13 (L:D), and temperature was 18.3°:15.6°C (L:D). On 4 April 1996, the larval/pupal cases were returned to the laboratory room, and on 22 April they were transferred to an environmental control chamber maintained at 26.7°C with a 14:10 (L:D) photoperiod.

DEVELOPMENTAL STAGES AND BIOLOGY

Eggs. Eggs are oviposited in clusters near the tip of a spine on pricklypear cactus, *Opuntia engelmannii* Salm-Dyck. Each egg is attached to the cactus spine by a short, thin filament. The length of the egg stalk was not measured, but Gilbert (1981) reported the length of the stalk was about 5 mm in *Coscinoptera panochensis* Gilbert. The ridges and depressions of the molded fecal material covering the egg give the impression of a small pine cone. Egg clusters are preceded on the spine by a previously undocumented, funnel-shaped structure which completely encircles the spine (Figs. 1a, 1b). The funnel appears to be composed of the same fecal platelet material that covers the individual eggs. The pointed end of the funnel faces the egg cluster while the open end of the funnel faces the cactus cladophyll (pad). The funnel is reminiscent of the rat barrier shields utilized on a ship's mooring lines and may function to protect the eggs from predators. The funnels were present with six of eight egg clusters; two of the six funnels were collapsed around the spine, perhaps due to faulty construction or to damage by beating rains.

The earliest oviposition was recorded on 8 May and the latest on 2 June. The dates that egg clusters were found and numbers of eggs per cluster (in parenthesis) were: 8 May 1989 (17), 20 May 1991 (21), 12 May 1993 (12), 24 May 1995 (19), 2 June 1995 (14), and three clusters

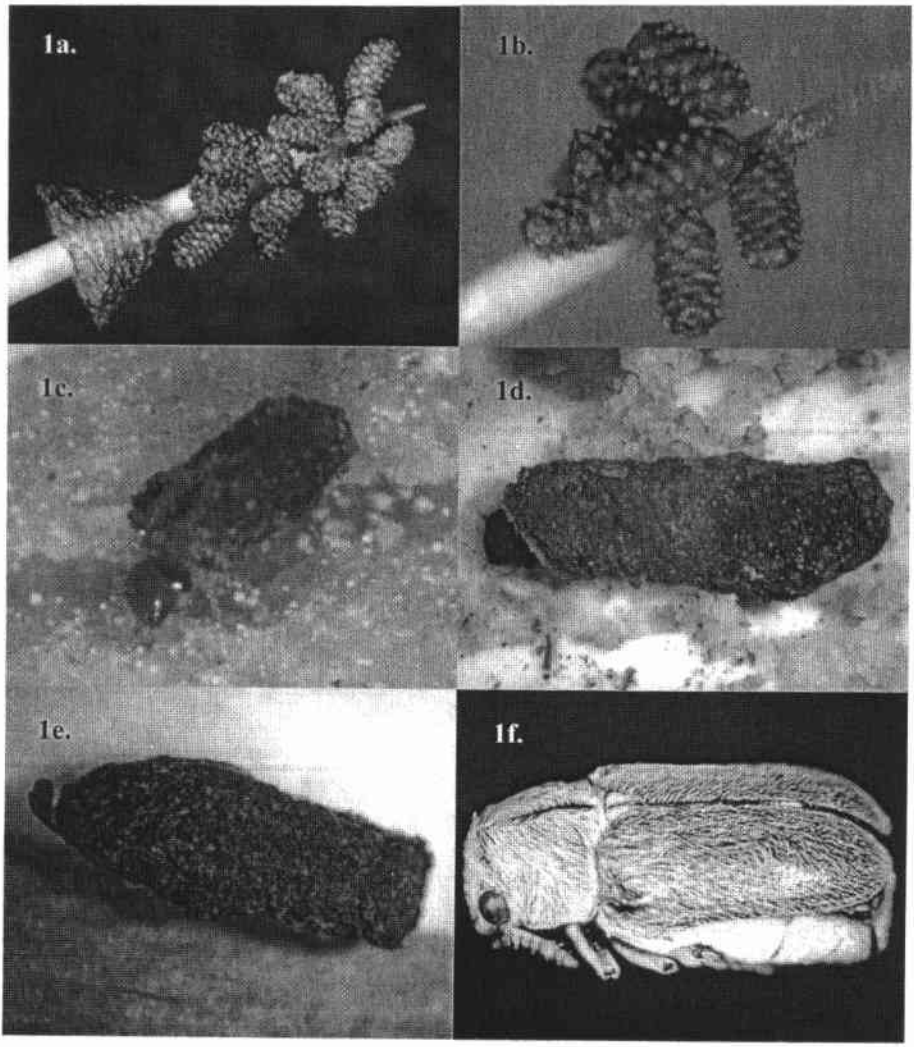

FIG. 1. Life stages of *Coleothorpa dominicana franciscana* (LeConte):1a. egg cluster with funnel; 1b. eggs with filament attachment to spine; 1c. young larva feeding on moldy bread; 1d. mature larva (1cm long); 1e. overwintering case with sealed end; 1f. adult.

on 8 May 1997 (18, 11, and 18). Gilbert reported up to 13 eggs for *C. panochensis*. Egg clusters held in the laboratory at room temperature hatched in 5-9 days. Ten of the 33 eggs (9 of the 19 egg-group, and 1 of the 14 egg-group) did not hatch in 1995; otherwise, all eggs hatched in the other years.

Larvae. If the arena became too wet, a larva would exit its case, or it might chew and destroy the case, and eventually die. In some years, smashed ants or other insects were added to the petri dish. However, the only time that larvae fed readily on insect remains was in 1993 when ground-up pieces of *Formica neolcara* Emery were provided, but this behavior was not consistent. Mesquite leaves cut into small pieces were frequently provided, but the larvae did not appear to feed on this fresh material. Larvae were observed to feed consistently on mold growing on both the bread (Fig. 1c) and cactus pieces, and larvae were frequently found under or on top of the honey-soaked cotton wicks. The larvae did not feed on pinto bean diet in 1997. Molds appeared to be a nutritional requirement, but the larvae frequently became entrapped in the mold and died. Riley (1874, 1882) successfully reared *C. dominicana* larvae on dead and decaying leaves.

In 1993 one larva remained active through late January 1994, and two larvae remained active until mid-December in 1997 before dying. In 1995, the two larvae that survived to become adults sealed their larval cases several times during the summer and fall, apparently to molt. The cases were sealed for the final time during October. The active larval stage lasted 5-8 months. A case containing a mature larva was ca. 1 cm long (Fig. 1d).

Overwintering Stage. The overwintering stage (note the sealed flat end of the case in Fig. 1e) lasted about six months. The timing of occurrence of the pupal stage is not known because the larval/pupal cases were not disturbed. Erber (1988) indicated the pupal period lasted 12-28 days.

Adults. One adult (Fig. 1f) emerged on 3 May and the other on 10 May 1996. Adult longevity was approximately 56 days. Adult emergence in the laboratory coincided with the timing of oviposition in the field, but adults were never observed at the collecting site. Under laboratory conditions, the complete life cycle from egg to adult was one year. However, and it may have been coincidence, eggs were only found every-other-year from 1989 to 1997, which implies a two-year life cycle.

Pinned specimens identified as *Coscinoptera dominicana franciscana* LeConte, in the collection at Texas Tech University, Lubbock, TX, were collected 4 June 1970 (Big Bend National Park, Brewster Co., Texas, Chisos Basin) and 3 April 1969 (San Angelo, Texas, Irion Co.). Smith and Ueckert (1974) collected *Coscinoptera* sp. from mesquite flowers and pods in Dickens, Lubbock, and Lynn Counties in west Texas. Specimens of *Coscinoptera aeneipennis* LeConte and *C. axillaris* LeConte, in the Texas Tech University museum, were also collected from mesquite in west Texas.

Range Site. Available evidence indicates *C. dominicana franciscana* is associated with rangeland habitats dominated by honey mesquite, *Prosopis glandulosa* Torr., in north and west Texas. The oviposition site on pricklypear spines and construction of the protective funnel suggests a preference for slender objects as spines, thorns, or small, bare stems on plants. Spines and thorns are present on mesquite, hackberry trees, *Celtis* sp., skunkbush, *Rhus* sp., pencil cholla (tasajillo), *Opuntia leptocaulis* DC var. *leptocaulis,* and silverleaf nightshade, *Solanum elaeagnifolium* Cav., in the area, but eggs have been located only on pricklypear spines.

The ant *F. neoclara* is common in the study area, and many individuals were frequently seen climbing on and under the pricklypear cactus. One large colony was located under a fallen mesquite limb near the pricklypear, but no *C. dominicana franciscana* larvae were found in the ant colony. Erber (1988) indicated that the myrmecophilous Clytrinae are carnivorous, but in our rearing attempts, insect remains were rarely fed upon. Insect remnants, either fresh or dried, were not part of the diet in 1995 when the two larvae were reared to adult. Erber (1988) reported

that *C. dominicana* may not require an association with ants, and while *Anomoea flavokansiensis* Moldenke is associated with ants, it is not known if the ants are necessary for survival of the larvae (LeSage and Stiefel 1996).

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HEAT SHOCK PROTEIN 70 DURING DEVELOPMENT OF THE LEAFCUTTING BEE, *MEGACHILE ROTUNDATA* (HYMENOPTERA: MEGACHILIDAE)

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ABSTRACT

Expression of a 70,000 M_r heat shock protein (HSP70) in *Megachile rotundata* (Fabr.) was studied from diapause to the pupal stage to determine whether HSP70 levels change during pupal development. We examined levels of HSP70 in the head capsules of prepupae that were sampled from three temperature regimes over 10 days: refrigerator (RF) at 0-7°C, room temperature (RT) at 21-24°C, and incubator (IN) at 30°C. Western blots using a monoclonal antibody against HSP70 detected a single band at 70 kDa in diapausing prepupae and during pupal development. Enzyme-linked immunoabsorbent assays (ELISA) revealed only minor fluctuations in mean levels of HSP70 during diapause and pupal development. Overall, the percentage of HSP70 in head capsules of *M. rotundata* ranged from 0.8-1.7%. Although large variation in HSP70 levels among individual larvae was observed, no significant changes in HSP70 levels occurred during post-diapause development to pupal stages.

INTRODUCTION

The alfalfa leafcutting bee, *Megachile rotundata* (Fabr.), is an increasingly important pollinator of seed alfalfa in the USA (Torchio 1990, Peterson et al. 1992). It overwinters as a diapausing prepupa inside brood cells constructed within preexisting cavities. Unlike its native megachilid counterparts in the northwestern USA, *M. rotundata* will nest in open grassland and, despite exceedingly high temperatures found there, construct nests in exposed habitats that can exceed 45°C (Barthell 1992, Barthell et al. 1998). Because of the unusual temperature extremes encountered by this species and the numerous proteins differentially expressed during development (Rank et al. 1982, Rank et al. 1989), this bee provides an opportunity to study variation in the levels of HSPs during its life cycle. Among the 11 proteins with altered expression during pupal development of *M. rotundata*, the expression of one 70 kDa protein notably declines in developing pupae and teneral adults (Rank et al. 1982). The pattern of expression of the 70 kDa protein in *M. rotundata* appears similar to post-diapause patterns of expression for the HSP70 family HSPs in *Sarcophaga crassipalpis* (Joplin and Denlinger 1990) and *Lymantria dispar* (Yocum et al. 1991).

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The purpose of this study was to determine whether *M. rotundata* possesses a 70 kDa HSP that undergoes altered expression during pupal development. We specifically chose to study HSP70 expression in the head capsule of *M. rotundata* because expression of HSP70 in the insect brain protects nervous system function during development (Krebs and Feder 1997) and because different tissues may express different isoforms in the HSP70 family of HSPs (e.g., Joplin and Denlinger 1990). We used SDS-PAGE and Western blotting to determine whether the 70 kDa band in *M. rotundata* was possibly a HSP70 family HSP, and employed an enzyme-linked immunoabsorbent assay (ELISA) to quantify HSP levels in head capsules.

MATERIALS AND METHODS

Diapausing *M. rotundata* prepupae (in cocoons) were obtained from International Pollination Systems (Manitoba, Canada). These were kept in a refrigerator at 0-7°C to maintain diapause, a dormant state with respect to developmental morphogenesis, prior to our experiment. We measured the amounts of soluble protein and HSP70 family proteins in the head capsule of diapausing and developing prepupae over a period of 10 days at three temperature regimes: refrigerator (RF) at 0-7°C, room temperature (RT) at 21-24°C, and incubator (IN) at 30°C. These conditions approximate those used during storage and incubation of *M. rotundata* for commercial pollination (see review in Peterson et al. 1992). Maximum and minimum temperatures were recorded for each of the temperature regimes, except the 30°C (constant) regime, using a maximum-minimum thermometer (Taylor Instruments, Fletcher, NC). Because pronounced expression of HSP70 is well-documented in larval brain tissue of at least one insect, *Drosophila melanogaster* (Meigen), we chose to focus on changes in the head capsule of the prepupae (Krebs and Feder 1997). Cocoons were collected from each of the temperature regimes on Days 0, 2, 4, 6, 8 and 10 of the experiment and immediately frozen and stored at -80°C for subsequent analyses. Teneral adults were collected from the IN temperature regime on Day 26. We identified the molecular weight of HSP70 family HSPs in prepupae by SDS-PAGE and Western blotting, and determined soluble protein and heat shock protein concentrations by spectrophotometry.

Homogenates were prepared using one (teneral adult) to five (prepupae) head capsules of *M. rotundata* homogenized in cold 10 mM phosphate buffered saline pH 7.6 (PBS), with 0.2% sodium azide and 2 mM tosyl arginine methyl ester (TAME). Homogenates were centrifuged at 16,000 x g for 20 minutes to remove debris. Supernatant soluble protein was measured by the Bradford assay (Bradford 1976) and was the protein source for SDS-PAGE and the ELISA. For Western blots, we separated 80 μg of soluble protein using one-dimensional SDS-PAGE as described by Laemmli (1970). The gel consisted of a 7% acrylamide resolving gel (pH 8.6) and stacking gel (pH 6.8). Aliquots of homogenates were combined (1 vol/2 vol) with sample reducing buffer containing bromophenol blue (0.1%) and denatured for 4 minutes at 95°C. Proteins were separated by electrophoresis at 20 V for 8 hours on a minigel (Bio-Rad Laboratories, Hercules, CA) and electrophoretically transferred to nitrocellulose membranes overnight at 90 mAmps in tris-glycine-methanol pH 8.6 buffer using a miniblot apparatus (Bio-Rad Laboratories, Hercules, CA). General proteins were detected using Ponceau-S stain (Sambrook et al. 1989), after which membranes were washed 10 minutes with distilled water before immunodetection of HSPs. HSP70 family HSPs were immunologically detected using a monoclonal antibody (1:1000 dilution) for bovine HSP70 (Sigma-Aldrich, St. Louis, MO) and an alkaline phosphatase-conjugated secondary antibody (Bio-Rad Laboratories, Hercules, CA) at a 1:3000 dilution. Three replicate gels were run to evaluate the consistency of the gel results.

To quantify HSP70 proteins recovered from head capsules of *M. rotundata*, we modified the monoclonal-antibody ELISA described by Yu et al. (1994). We coated Costar microplates (Bio-Rad Laboratories, Hercules, CA) with 80 μg of soluble protein per well and allowed binding to occur overnight in coating buffer: 0.01 M sodium carbonate and bicarbonate buffer (pH 9.6). We then washed the plate with PBS-Tween (PBST, 10 mM PBS, 0.05% Tween 20) once and blocked wells for one hour at 37°C with PBST-BSA (1% BSA) to reduce nonspecific binding of the antibodies. The plate was washed once with PBST and monoclonal antibodies for bovine HSP70 (Sigma-Aldrich, St. Louis, MO) were added at a 1:1000 dilution. After a one hour incubation at 37°C, the plates were washed four times with PBST and the alkaline phosphatase-conjugated secondary antibodies were added at a 1:3000 dilution. Following a one-hour incubation at 37°C, plates were washed six times with PBST and 200 μL of the colorimetric detection buffer were added to each well. After a 90 minute incubation at room temperature, the $O.D._{405}$ for each well was determined by using a Dynatech MR5000 EIA reader (Dynex Technology, Chantilly, VA). Each microplate contained HSP70 standards consisting of different dilutions of bovine HSP70 (Sigma-Aldrich, St. Louis, MO). Three replicates, each loaded in triplicate on microplates, were analyzed for each temperature regime. We tested for differences in soluble protein and HSP70 concentrations among treatments on each sampling day using the one-way ANOVA (Days 0-8) and t-test (Day 10) of SYSTAT 8.0. The experimentwise error rate for each variable was adjusted for the number of statistical tests using the Bonferroni method.

RESULTS

Prepupae maintained in the RF and RT temperature regimes showed no obvious morphological change for the 10 days of the experiment. In the IN temperature regime, prepupae terminated diapause and experienced marked morphological development by Day 8 when cocoons contained pupae. Immunodetection of HSP70 on Western blots of all *M. rotundata* in diapause or post-diapause development revealed one band at 70 kDa (Fig. 1).

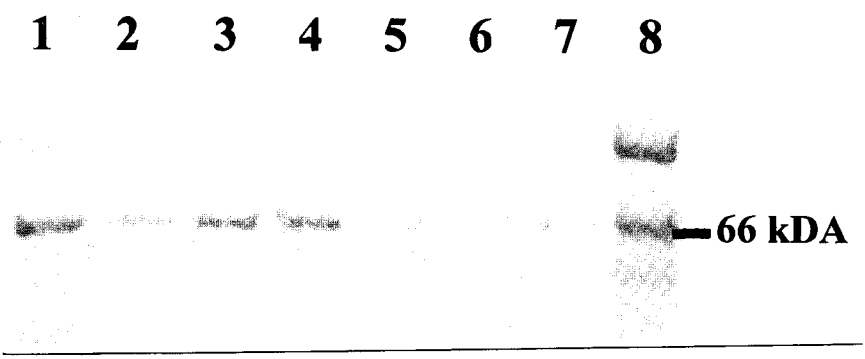

FIG. 1. Western blot of proteins with mouse anti-bovine HSP70 antibodies. Each lane contained 80 μg of soluble protein from head capsule homogenates of prepupae (5 per homogenate) or teneral adults (1 per homogenate). Lanes 1-3: prepupae from the RF, RT, and IN temperature regimes (left to right) on Day 0; Lanes 4-6: prepupae from the RF, RT, and IN temperature regimes (left to right) on Day 8; Lane 7: teneral adult on Day 26; Lane 8: molecular weight markers.

At Day 0, samples of diapausing prepupae that were placed in each temperature regime showed nearly equal levels of expression of this 70 kDa heat shock protein band. Although equal amounts of soluble protein were supplied in each lane, amounts of HSP70 on Day 8 appeared lower in RT and IN prepupae than RF prepupae. Low amounts of HSP70, comparable to HSP70 levels in RT and IN prepupae, were also observed in the teneral adults.

No change in soluble protein concentration of head capsules occurred among the prepupae from the three temperature regimes (Table 1). At the onset of the experiment, means for soluble protein concentration in the RT and IN prepupae were 13.4% and16.5% higher than RF prepupae, respectively. By Day 4, the soluble protein content of head capsules of RT and IN prepupae declined to levels that were 8.5% and 20.5% lower than the RF temperature prepupae. Soluble protein concentration in developing prepupae from the IN temperature regime declined through Day 8 such that head capsules of IN prepupae contained levels 27.9 and 39.5% less soluble protein than their RT and RF counterparts. By Day 10, soluble protein concentration of head capsules of diapausing prepupae in the RF and RT regimes increased from the levels detected at the onset of the experiment. Pupal development in the IN temperature regime was accelerated and most of the cocoons contained pupae on Day 10, with too few prepupae to study HSP70 expression in that temperature regime.

TABLE 1. Soluble Protein and HSP70 Concentrations in Prepupae During Diapause and Post-Diapause Development.

	Soluble Protein (μg/mL)				HSP70 Concentration (μg/mL)			
Day	RF[a]	RT[a]	IN[a]	Prob[b]	RF[a]	RT[a]	IN[a]	Prob[b]
0	368.41	417.70	429.02	NS	26.71	24.96	24.15	NS
	(9.16)	(14.23)	(51.29)		(3.45)	(1.51)	(0.51)	
2	416.50	414.48	455.20	NS	22.81	22.72	21.29	NS
	(22.85)	(36.72)	(30.41)		(1.73)	(2.84)	(1.30)	
4	562.51	514.62	447.34	NS	23.64	22.84	20.44	NS
	(54.19)	(110.23)	(22.75)		(3.88)	(3.29)	(0.57)	
6	568.74	462.11	357.34	NS	23.62	23.06	22.16	NS
	(94.87)	(8.96)	(18.33)		(1.80)	(2.38)	(1.38)	
8	518.32	434.62	313.57	NS	24.22	21.77	19.67	NS
	(46.21)	(15.29)	(27.34)		(1.93)	(2.51)	(1.08)	
10	528.34	445.38	NA[c]	NS	22.57	21.39	NA[c]	NS
	(44.41)	(38.80)			(3.72)	(2.99)		

[a]Means (standard errors in parentheses) are for three replicates per sample day in each temperature regime (RF = refrigerator, RT = room temperature, IN = incubator).
[b]Prob = probability in a one-way ANOVA, NS = not significant at $\alpha = 0.05$.
[c]NA = too few prepupae for analysis.

Although the highest levels of HSP70 occurred on Day 0, the mean concentration of HSP70 never differed among treatments. After Day 0, mean HSP70 levels declined slightly, with small fluctuations through the remainder of the experiment (Table 1). The same rankings occurred among temperature regimes on all sampling days, with diapausing prepupae in the RF temperature regime possessing the highest mean HSP70 concentrations and the IN prepupae containing the lowest mean HSP70 concentrations. The variation in HSP70 concentrations, measured as the coefficient of variation (CV) in HSP70 concentration, was greater in *M. rotundata* from the RF (CV = 13.1% - 28.6%) and RT (CV

= 10.5% - 24.9%) temperature regimes than in the IN (CV = 3.6% - 10.8%) temperature regime. The percentage of HSP70 in soluble protein of homogenates ranged from 0.8% to 1.7% among all samples.

DISCUSSION

We report a 70 kDa HSP in head capsules of *M. rotundata* that is immunologically related to the HSP70 family HSPs. This HSP70 family protein possesses the same molecular weight as a 70 kDa protein described by Rank et al. (1982). Although HSP70 concentrations were highly variable among replicates, HSP70 levels accumulated to about 0.8-1.7% of the soluble protein in the head capsules of *M. rotundata* at the temperatures used to maintain diapause and promote pupal development. These percentages are comparable to the percentage HSP70 reported in other ectotherms (e.g., Feder et al. 1997, Yu et al. 1994).

Variation in levels of HSP70, which was two to three times higher in the diapausing prepupae than in developing prepupae, may have resulted from either ontogenetic expression or individual variation in stress-induced expression of HSP70 in prepupae. An ontogenetic explanation for the variation HSP70 in *M. rotundata* is supported by other observations showing that expression of HSP70-family HSPs during insect larval development causes dramatic changes in the amount and number of isoforms present in tissues of different developmental stages of insects (Joplin and Denlinger 1990, Krebs and Feder 1997). Similarly, a stress-induced explanation is plausible because numerous studies show that stress protein expression is sensitive to both the magnitude and duration of a stressor (Feder and Hoffman 1999), a phenomenon illustrated by studies reporting cold-shock induced expression of HSPs in insects. A variety of HSPs in insects, including those in the HSP70 family, are commonly induced by cold-shock. Severe, acute cold-shock (-10 to -20°C for 1-8 h) induces HSP70 family HSPs in *Sarcophaga crassipalpis* (Joplin et al. 1990, Joplin and Denlinger 1990) and *Lymantria dispar* (Yocum et al. 1991). Intensified severity, either temperature or duration, of the cold stress increases the duration of HSP expression (Joplin et al. 1990, Yocum et al. 1991). Even though lower variation in HSP70 levels corresponded with rapid development at 30°C, suggesting developmental canalization of HSP70 expression after diapause, we can not differentiate stress-induced expression of HSPs among individual prepupae from developmentally controlled patterns of expression during diapause and pupal development.

While HSP70 expression has been characterized in some species of Hymenoptera, earlier studies show that HSP expression in Hymenoptera may be induced by two different stressors, heat shock and parasitic infections (Severson et al. 1990, Gehring and Wehner 1995, Gregorc and Bowen 1999). Although the functional significance of HSPs during diapause and pupal development of *M. rotundata* remains to be elucidated, others have described how high or low temperature extremes can delay emergence of *M. rotundata* from the pupal stage (Underraga and Stephen 1980a, 1980b). Small differences in temperature (≤ 5°C) can also produce significant differences in survival among pre-adult developmental stages as well as in the size and survivorship of adult bees (Tepedino and Parker 1986, Richards et al. 1987, Whitfield and Richards 1992). Because heat-shock proteins can serve as useful biomarkers for environmentally-induced physiological stress (dePomerei 1996, Feder and Hofmann 1999), HSP70 levels may serve as a useful bio-indicator of stress in developmental stages of *M. rotundata.* If HSP70 expression in *M. rotundata* is highly sensitive to environmental stressors (e.g., toxicants, pesticides, stresses during storage or incubation of developmental stages), then it could be used together with traditional indices, such as emergence success rates and body size, to study storage and rearing conditions for *M. rotundata.*

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ATTRACTION OF DIAMONDBACK MOTH[1] TO THREE COMMERCIAL SEX PHEROMONE LURES UNDER LABORATORY AND FIELD CONDITIONS

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ABSTRACT

Several commercial pheromone lures for diamondback moth, *Plutella xylostella* (L.) were used for monitoring male moths in the Lower Rio Grande Valley, Texas, in the 1990's; however, none were successful in capturing male moths. In this study, three commercial sex pheromone lures (Pherocon™, Scenturion 1st Watch™, and IPM™) for *P. xylostella* were tested in a cabbage field and walk-in field cages using the Scentry and TRÉCÉ traps. All three pheromone lures were effective for capturing adult males of *P. xylostella* under field conditions. Among the three lures, Pherocon™ and IPM™ lures were significantly more effective in general than the Scenturion 1st Watch™. The effectiveness of the three lures lasted longer than seven weeks. There was no significant difference among the three lures in a no-choice test in the field cages. Mating disruption of the lures on male *P. xylostella* was observed in the two-lure-choice tests in the walk-in field cages. The ratio of the three components, (Z)-11-hexadecenal, (Z)-11-hexadecenyl acetate, and (Z)-11-hexedecenol was found to be 3:1:2 from 1- to 6-d old *P. xylostella* female abdominal tips using GC-MS analysis. The level of pheromones produced by females reached a maximum from 3-4 d old females. The ratios of (Z)-11-hexadecenal, (Z)-11-hexadecenyl acetate, and (Z)-11-hexedecenol were detected as 1.15:1.00:0.04, 1.60:1.00:0.23 and 0.52:1.00:0.03 from the commercial pheromone lures of Pherocon™, Scenturion 1st Watch™ and IPM™, respectively.

INTRODUCTION

The diamondback moth, *Plutella xylostella* (L.), is one of the most destructive worldwide pests of crucifers such as cabbage, cauliflower, broccoli, watercress, mustard, and rape (Talekar and Shelton 1993). Texas is one of the largest U. S. producers of fresh market cabbage with a yearly value of $30-60 million depending on market prices (Anonymous 2000). Cabbage production represents a $19,224,000 industry in south Texas alone (Anonymous 2000). Average insecticide expenditure to

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manage lepidopterous and aphid pests in south Texas is over $1 million per year with no guarantee of adequate control due to the current threat of insecticide resistance, especially with *P. xylostella* and cabbage looper, *Trichoplusia ni* (Hübner). After the mid-1980's, the diamondback moth became a key pest in south Texas coincidental with the increase in use of pyrethroid insecticides and subsequent diamondback moth resistance to them (Magaro and Edelson 1990).

The sex pheromones of *P. xylostella* consist of three compounds: (Z)-11-hexadecenal, (Z)-11-hexadecenyl acetate, and (Z)-11-hexadecenol. Lin et al. (1982, 1984) and Koshihara et al. (1980) reported that the mixture of Z11-16:Al, Z11-16:Ac, and Z11-16:OH in the ratio of 5:5:0.1 or 3:7:0.1 were quite attractive to males of *P. xylostella* in cabbage. Little information is available on the use of pheromone lures of *P. xylostella* in the Rio Grande Valley, Texas. The objective of this study was to compare the effectiveness of three commercial sex pheromone lures (Pherocon™, Scenturion 1st Watch™, and IPM™) to attract male *P. xylostella*, to determine their longevity, and to compare the chemical ratios of commercial pheromone lures with that produced by sex glands of females from the Lower Rio Grande Valley, Texas.

MATERIAL AND METHODS

Plutella xylostella larvae were reared at 25 ± 2°C with a photoperiod of 14:10 (L:D). Larvae were fed artificial diet (Shelton et al. 1991), and adults were fed a 5% sugar solution. Male adults from 1 through 5-d old were bioassayed simultaneously to study insect response to pheromone. For pheromone extraction, female adults aged 1 to 6-d were used.

All synthetic standard compounds, (Z)-11-hexadecenal (Z11-16:Al), (Z)-11-hexadecenyl acetate (Z11-16:Ac), and (Z)-11-hexedecenol (Z11-16:OH) were purchased from Bedoukian Research, Inc. for GC-MS analysis. The standards were used to compare with the commercial lure products. The pheromone lures used in these experiments were obtained from TRÉCÉ Pherocon, Inc. (Grey septa, lot # 33600781 and lot # 3360331; Salinas, CA), IPM Technologies, Inc. (Red septa, lot # 06239913; Portland, OR) and Scenturion 1st Watch, Inc. (Grey septa, lot # 19711661; Clinton, WA). The pheromone traps were the Wing Kit K902 (Scentry, Inc., Billings, MT) and the 1C trap (TRÉCÉ Pherocon). Both traps are the same type used commercially for *P. xylostella*. Each pheromone-impregnated rubber septum was suspended with an insect pin in the interior center of the trap.

Pheromone components were quantified by gas chromatograph-mass spectrometry (GC-MS) using a Hewlett Packard 6890 GC coupled to a HP 5973 Network Mass Selective Detector with a Solgel-Wax capillary column (SGE: 60 m x 0.25 mm ID x 0.25 μm film thickness). Carrier helium gas flow was 1 ml/min. Oven temperature was programmed from 60° to 250°C at 20°C/min. Maximum temperature was maintained for 10 minutes. The system was controlled by a HPMS Chemstation. Initial analysis was performed in the electron impact (EI) mode (70 eV) with the mass spectra range scanned from m/z 40 to 550. Injection was performed in the splitless mode and the injector was purged after 1 minute. Injector and MS detector were maintained at 250° and 280°C, respectively. Three injections of each sample were made. The volatile emissions from three different lures were analyzed by the methods of Mayer and Mitchell (1998) and replicated three times. One hundred μg of n-tetradecane was used as an internal standard. We recovered 98.5% of the extraction standard.

Pheromone glands of 1 to 6-d old females of *P. xylostella* were excised in the morning. The eighth and ninth abdominal segments of *P. xylostella* females were extended by placing pressure on the abdomen using forceps, then excised, extracted in methylene chloride (500 µl) for 10-20 minutes, and ground and centrifuged at 11,600 rpm for 10 minutes at 4°C. Generally, 20-40 females were analyzed in each age group. GC-MS methods were used to quantify the three components from female sex glands. An internal standard was used for the quantification of the compound of 100 ng of n-dodecane. We recovered 98.0 % of the extraction standard.

Walk-in Field Cage Trial. Three polypropylene screened (5 threads per cm) cages (6.5 m long x 1.8 m wide x 2.2 m high) supported by a frame were placed in an alfalfa field. Cages were placed 16m apart. Traps were hung 0.3m above the ground on a wooden pole within the cages. Two tests, a no-choice lure test and a two-choice lure test, were conducted in the cages. In the no-choice lure test, one trap with one of the three lures was placed in the center of each of the three cages. In each cage, 100 male *P. xylostella* adults were released in late afternoon on the day of lure placement. The number of male *P. xylostella* caught in each trap was counted at 24, 48 and 72-h after release. There were three replicates per lure. In the two-choice lure test, two traps with different lures were randomly placed in each cage about 6.2m apart, with moths released at a point midway between the two traps. In each cage, 100 male *P. xylostella* were released at 09:30. Captured male *P. xylostella* were recorded at 1, 2, 4, 8, 12, and 24-h after release. The two-choice lure tests were repeated three times. The cages were randomly chosen for the lure combinations. These tests were conducted in November and December 2001 at the USDA-ARS, Vegetable IPM Laboratory, Weslaco, Texas.

Cabbage Field Trial. This trial was conducted for 8 weeks from 25 September to 20 November 2001 at the Texas A&M Agricultural Research and Extension Center, Weslaco, Texas. Cabbage was planted on 4 September 2001 on a 1-m bed with 30-cm spacing. The field size was 92.1m long by 3.1m wide. Pheromone traps were hung in the cabbage field on wooden stakes 10.2m apart from 0.2 to 0.3m above the ground. One of each lure type was replicated three times for a total of nine traps in the test plus three controls without lures. Each trap was moved to the next trap's position to avoid location bias after each weekly examination. During the test period, temperature ranged from 11° to 36°C and the relative humidity from 11 to 100%. Naturally occurring male diamondback moths were present and these were relied on for evaluating the lures.

The total number of *P. xylostella* captured per week per lure was used for statistical analysis for the cabbage field data. The capture rates were calculated for no- and two-choice pheromone lure tests. The capture rate (%) is the percentage of captured moths over total released moths. For the cabbage field test data, the analysis included lure and week as main effects and the interaction between them. The no-choice-lure test data was analyzed using a one-way classified model. The cage and lure nested within the cage were factors used for the two-choice lure test. All tests were performed using SAS Packages (SAS Institute 2000).

RESULTS AND DISCUSSION

Walk-in Field Cage Trial. In the no-choice lure tests, numbers of male *P. xylostella* captured decreased with increasing time following male release (Fig. 1). The majority of *P. xylostella* were caught in the first 24-h. The capture rates of male *P. xylostella* on Day 1 were 35%, 43%, and 59% from the 1st Watch™, Pherocon™, and IPM™ lures, respectively. On Days 2 and 3, the capture rate of male *P. xylostella* caught was less than 4%. Capture rates were not significantly different among the three

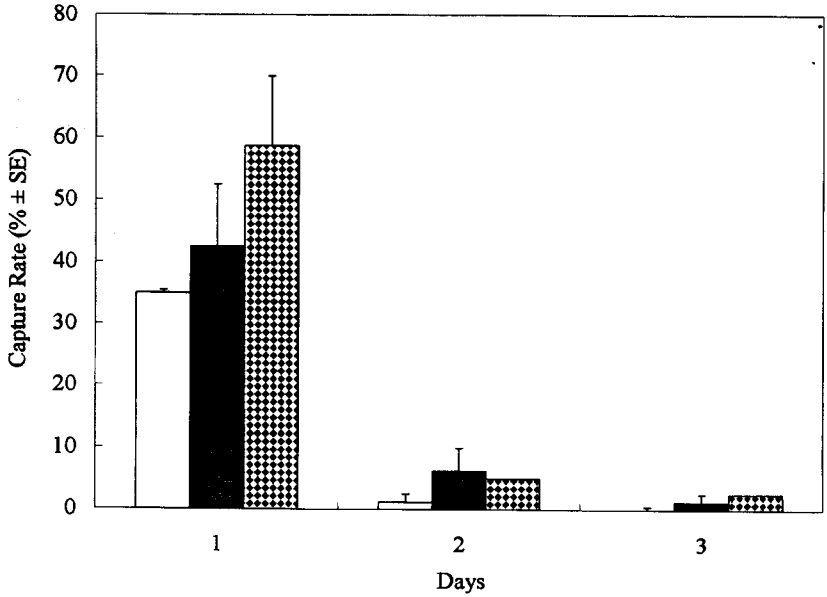

FIG. 1. Capture rates of male *P. xylostella* captured in the no-choice lures test in walk-in field cages (white bar: 1st Watch™, black bar: Pherocon™, diamond bar: IPM™).

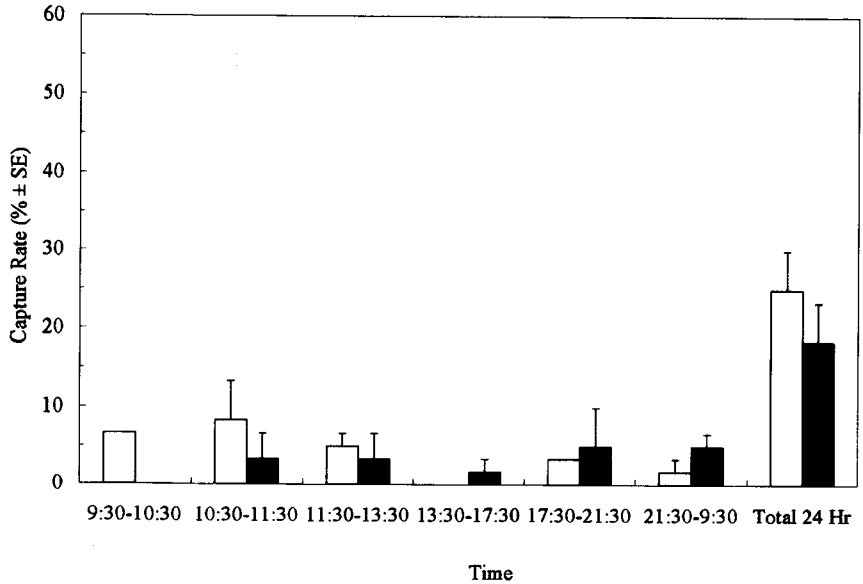

FIG. 2a. Capture rates of male *P. xylostella* captured in the two-lure-choice tests: IMP™ (white bar) versus Pherocon™ (black bar) in walk-in field cages.

lures on Days 1, 2, and 3 or over the 3-d period of observation.

In the two-choice lure tests, capture rates of *P. xylostella* were less than 3% on the second day for all lures. The majority of male adults were also captured within the first day. The Pherocon™ and IPM™ lures attracted 33% ($P = 0.0006$) and 23% ($P = 0.0038$), respectively, more male *P. xylostella* (Figs. 2b and 2c) than 1st Watch™ lures. These results showed a similar pattern to that of the no-choice lure tests (Fig. 1). When Pherocon™ and IPM™ lures were placed in the same cage, they had a lower total capture rate (Fig. 2a) than the other combinations of the lures in other cages (Figs. 2b and 2c). The difference in total capture rates between Pherocon™ and IPM™ lures was about 7% ($P = 0.2381$). Reduction of the capture rate with these two lures may have been caused by the amount of the pheromones released, affecting the ability of males to follow an odor plume to its source. This is known as the mating disruption effect (Chow 1990) and has been used as a control technique to prevent males from locating individual females for mating. The capture rate also could be affected by temperature, wind direction, and wind speed in the field. Reddy and Urs (1996) reported that the peak period of attraction for male diamondback moth was from sunset to midnight; however, there was no obvious peak for the capture rates within the first 24-h period in this study. This observation was similar to that of Furlong et al. (1995) who reported that male *P. xylostella* were attracted to synthetic lure throughout the initial 24-h period.

Cabbage Field Trial. Numbers of male *P. xylostella* attracted varied greatly among the three commercial lures (Fig. 3), whereas the no-lure control traps caught no male *P. xylostella*. Both week and lure effects on the number of male *P. xylostella* captured were significantly different ($P = 0.001$), but the interaction between these two main effects was not significant ($P = 0.9363$) (Table 1). Attraction was highest (28-55 moths) during Week 4 for all lures. There were no significant differences ($P = 0.1641$) between attractiveness of IPM™ and Pherocon™ lures, whereas the capture numbers of the 1st Watch™ lure were significantly lower than IPM™ ($P = 0.0147$) and Pherocon™ lures ($P = 0.0002$) (Table 2). The rubber septa lures effectively captured male moths for more than 7 weeks, similar to the findings of Reddy and Urs (1996). Under the field conditions in the Rio Grande Valley, Texas, we recommend that lures be replaced every 7 weeks for maximum efficiency in attraction of male *P. xylostella*.

TABLE 1. Analysis of Variance Parameters for Attraction of *P. xylostella* Males in the Cabbage Field Trials

Source of Variance	df	F-value	P-value
Model	23		
Week	7	3.83	0.0013
Lure	2	7.94	0.0009
Week x Lure	14	0.50	0.9363
Error	48		
Corrected Total	71		

TABLE 2. Contrast of Number of *P. xylostella* Males Captured among Different Lures for the Cabbage Field Trials

Contrast	Difference	t-value	P-value
IPM™ vs Pherocon™	-4.8	-1.41	0.1641
IPM™ vs 1st Watch™	8.7	2.52	0.0147
Pherocon™ vs 1st Watch™	13.6	3.93	0.0002

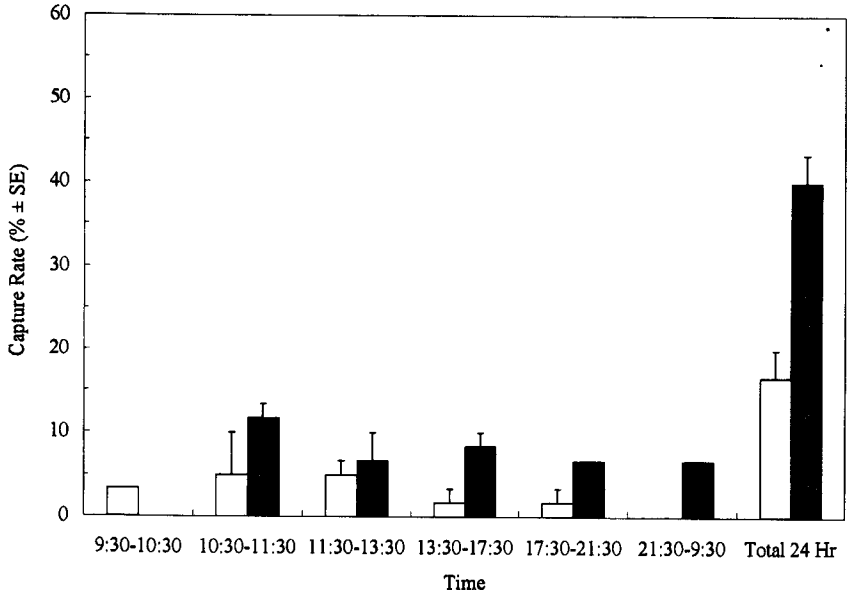

FIG. 2b. Capture rates of male *P. xylostella* captured in the two-lure-choice tests: IPM™ (black bar) versus 1st Watch™ (white bar) in walk-in field cages.

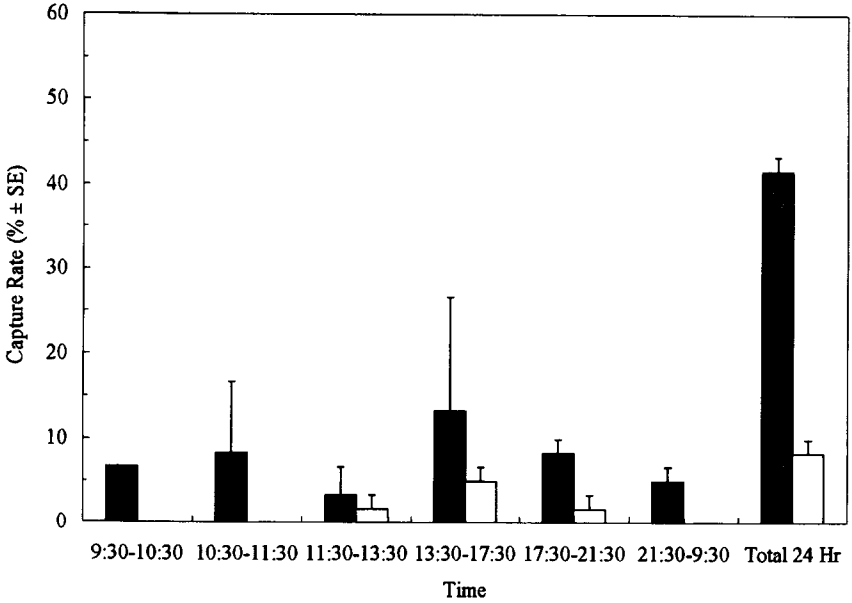

Fig. 2c. Capture rates of male *P. xylostella* captured in the two-lure-choice tests: Pherocon™ (black bar) versus 1st Watch™ (white bar) in walk-in field cages.

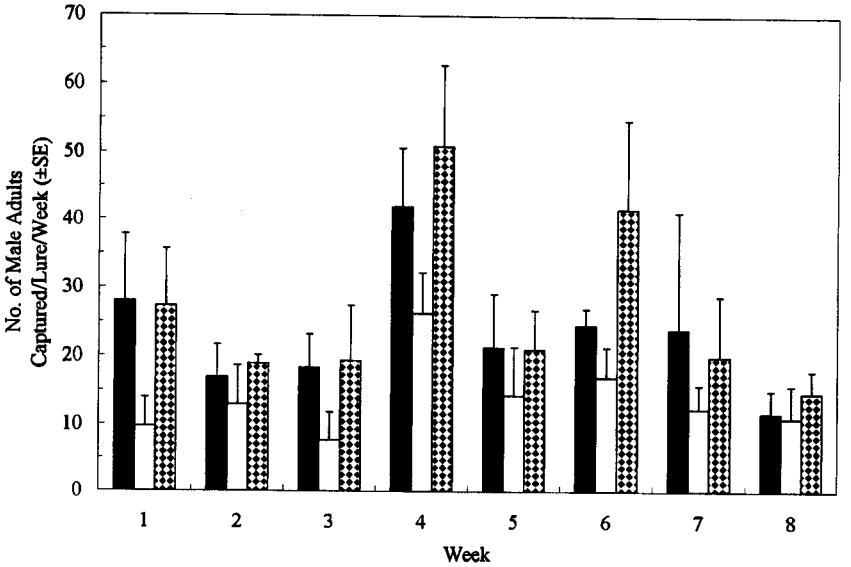

FIG. 3. Numbers of male *P. xylostella* captured using three commercial pheromone lures (black bar: IPM™, white bar: 1st Watch™, diamond bar: Pherocon™) in cabbage field (Weslaco, TX).

Chemical differences among the commercial pheromone lures were analyzed by GC-MS. The ratios of pheromone components varied among the three commercial lures (Table 3). The ratio of the 1st Watch™ lure was different from those of Pherocon™ and IPM™ lures. The ratios of Z11-16:Al, Z11-16:Ac, and Z11-16:OH are important to the capture rate of *P. xylostella* (Schroeder et al. 2000). The emitted ratios of Z11-16:Al to Z11-16:Ac were 0.52-1.60:1.0 from three different lures, which was within the range of 0.6- 4.0:1.0 (Mayer and Mitchell 1999) using septa. At Week 8 of the test, the lures from Pherocon™ and IPM™ still attracted male adults although the Z11-16:OH was not detectable, indicating that the Z11-16:Al and Z11-16:Ac are major attractive components to male adults. However, these pheromones are not very stable because of the Z-double bond isomers. We also detected other isomers from the lures, such as E11-16:OH, and E11-16:Ac. These findings emphasize the need for rigorous quality control in the preparation and testing of pheromone formulations. The inefficiency of the lures could cause decrease in mating disruption and reduction in capture of the male moths (Roelofs et al. 1979).

Female *P. xylostella* produce three pheromone components: Z11-16:Al, Z11-16:Ac, and Z11-16:OH from sex glands (Tamaxi et al. 1977, Koshihara and Yamada 1981). The amount of pheromones was the highest for the 3 to 4-d old female adults (Fig. 4). The ratio of Z11-16:Al, Z11-16:Ac, and Z11-16:OH was found to be 3:1:2 , which was in agreement with the results of Chow et al. (1978). They reported attraction of virgin females increased with age, reaching a maximum attraction on days 4 and 5. However, Reddy and Urs (1996) reported that five-day-old virgin female *P. xylostella* did not attract male moths because they lacked sex pheromone.

TABLE 3. Ratios of (Z)-11-Hexadecenal, (Z)-11-Hexadecenyl Acetate and (Z)-11-Hexedecenol from Three Commercial *P. xylostella* Sex Pheromone Lures before and after Eight Weeks of the Cabbage Field Trials Using GC-MS Analysis

Name	Z11-16:Al	Z11-16:Ac	Z11-16:OH
	Before		
1st Watch™	1.60	1.00	0.23
Pherocon™	1.15	1.00	0.04
IPM™	0.52	1.00	0.03
	After		
1st Watch™	0.68	1.00	0.06
Pherocon™	0.36	1.00	ND
IPM™	0.23	1.00	ND

ND: Not Detectable

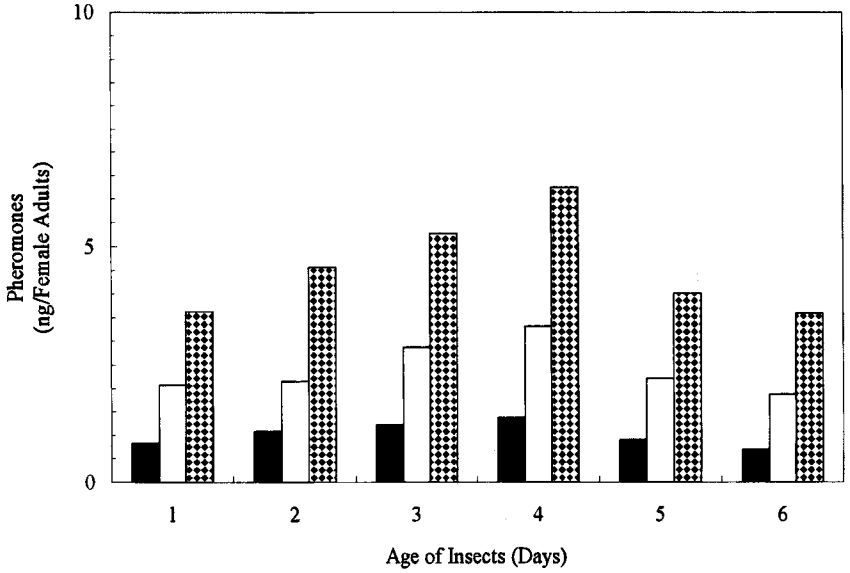

FIG. 4. Effects of age of female *P. xylostella* on Pheromones (black bar: Z11-16:Ac, white bar: Z11-16:OH, diamond bar: Z11-16:Al) in CH_2Cl_2 extracts of abdominal tips using GC-MS analysis.

Our results showed that all three commercial pheromone lures placed in Scentry or TRÉCÉ traps effectively attracted male *P. xylostella*. Sex pheromone baited trap techniques can be useful in an integrated pest management system for insect detection and surveillance, as well as for the timing, evaluation, and control of population levels of diamondback moth in the Rio Grande Valley, Texas. This technique can be used to monitor *P. xylostella* population, which will aid in the decision making process for management of this important pest.

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PHEROMONE PRODUCTION IN CORN EARWORM[1] : EFFECT OF TEMPERATURE AND HUMIDITY

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ABSTRACT

Females of the corn earworm, *Helicoverpa zea* (Boddie), produce a sex pheromone to attract conspecific males for mating. Laboratory experiments were conducted to study the effect of three temperatures and three levels of relative humidity (RH) on pheromone titer in the females measured as the quantity of Z11-hexadecenal, the major component of corn earworm sex pheromone. Under normal temperature and RH condition, females produced an average of 36.1 ± 3.9 ng. Lowest pheromone titer (2.8 ± 1.1 ng/female) was observed in females kept at cool (15°C) temperature and normal (50%) RH. Warm temperature (35°C) with any of the RH combinations (10, 50, or 90%) resulted in low pheromone production. Pheromone titer could be increased significantly by injecting pheromone biosynthesis-activating neuropeptide (PBAN) into females maintained at cool temperature; this treatment had no effect at the warm temperature. Thus, low availability of PBAN seems to be at least one of the factors contributing to the decreased pheromone titers at the cool temperature.

INTRODUCTION

Female-produced sex pheromones are commonly utilized by males for mate finding among lepidopteran species. Sex pheromone of the corn earworm, *Helicoverpa zea* (Boddie) is a blend of four components, of which *Z*11-hexadecenal accounts for approximately 92% (Klun et al. 1980). Earlier studies referred to relative percentages rather than absolute amounts of pheromonal components. The first quantitative determination of pheromone titers from single corn earworm females was reported by Raina and Klun (1984).

Pheromone production is regulated by endogenous and exogenous factors (Cardé and Webster 1980). McNeil (1991) reviewed the role of factors affecting emission and reception of pheromones in moths. Among the exogenous factors, the roles of photoperiod and temperature were studied extensively. Almost all the studies involving these factors measured the incidence of calling without actual determination of pheromone titers. Thus calling, a behavior associated with release of pheromone, was reduced significantly in the codling moth, *Laspeyresia pomonella* L., when temperature decreased from 23° to 16°C (Castrovillo and Cardé 1979). Calling was eliminated completely in the Oriental fruit moth, *Grapholita molesta* (Busck) at temperatures warmer than 32° and cooler than 15°C (Baker and Cardé 1979). However, very little information is published on the effect of relative humidity (RH) on

[1] *Helicoverpa zea* (Boddie) (Lepidoptera: Noctuidae)

pheromone production/calling (McNeil 1991). Baker and Cardé (1979) reported that RH ranging from 25 to 100% did not affect calling in the Oriental fruit moth. Studies of the European corn borer, *Ostrinia nubilalis* (Hübner), revealed that the onset of calling was earlier and the proportion of females calling greater at higher humidities (Webster and Cardé 1982, Royer and McNeil 1991).

The pheromone biosynthesis-activating neuropeptide (PBAN) regulates pheromone production in several species of moths (Raina 1993). Raina et al. (1991) suggested that low pheromone production in corn earworm females at 14°C might be due to inhibition of PBAN release and lower pheromone biosynthesis.

This study was conducted to determine the effect of temperature and RH on pheromone titer in corn earworm females and to examine the role of PBAN in regulating pheromone production under experimental conditions.

MATERIALS AND METHODS

Weekly shipments of eggs of corn earworm were received from the Insect Biology and Population Management Research Laboratory, Tifton, GA. In Beltsville, MD, the eggs were allowed to hatch and the larvae placed on artificial diet (Southland Products, Lake Village, AR) in environmental chambers maintained at 26:21°C, 60 ± 5% RH, and a 16:8 (L:D) photoperiod with lights off at 0800 and on at 1600 hours EDT. After pupating, males and females were kept in separate environmental chambers. Adults were provided 10% sucrose as food.

Pheromone titers (as quantity of Z11-hexadecenal) were determined from females at 3-4 hours into the third scotophase and quantified by gas chromatography using the internal standard method as described by Raina and Kempe (1992). Combinations of three temperatures and three relative humidities were used to determine the effect on pheromone production. For temperatures, 15°, 25°, and 35°C were considered as cool, normal, and warm, respectively. Similarly, 50% RH was considered as normal and 10 and 90% RH as low and high. High humidity (90%) was obtained by placing a cage with adults inside a larger cage, and then spraying water into the outer cage which subsequently was covered with a polyethylene bag. A hygro-thermograph with a remote sensor placed inside the cage with the adults was used to continuously monitor temperature and RH. Six newly emerged females were placed under each test condition, and the pheromone titer was determined after 51 hours (three hours into the third scotophase).

To determine whether the low pheromone observed under some of the experimental conditions was caused by lack of PBAN production/release, females under each experimental condition were divided into two groups, each consisting of ten individuals. One group was injected with 20 µl saline and the second group with 5 pmol PBAN (Peninsula, Belmont, CA) in 20 µl saline at one hour into the third scotophase. Pheromone was extracted from excised ovipositors three hours after injection of the peptide and quantified using GC analysis.

For the experiments with temperature and RH, PROC MIXED (SAS Institute 1996) with temperature and RH as the effects was used to analyze the pheromone data as a two-factor general linear model. Data on the effect of PBAN were analyzed as a three- factor general linear model, with temperature, RH, and treatment (check or PBAN) as the effects. To correct for variance heterogeneity, treatments were grouped into similar variance groups for analysis. Means were compared using pair-wise contrasts.

RESULTS AND DISCUSSION

Pheromone titers under various combinations of temperature and RH are shown in Fig. 1. Pheromone production was generally low (36.1 ± 3.9 ng/female) even at the optimal

temperature and RH conditions. Least pheromone production (2.8 ± 1.1 ng/female) was observed at cool temperature and normal RH. Comparing the effects of temperature and RH on pheromone production, statistical analysis of the data indicated that the effect of temperature and the interaction between temperature and RH were highly significant ($F = 36.4$, df = 2, $P \leq 0.0001$ and $F = 54.7$, df = 4, $P \leq 0.0001$, respectively). At cool temperatures, changing the RH to either low or high resulted in a significant increase in pheromone production ($P \leq 0.05$). At high temperature and any combination of RH, little pheromone was produced. At normal temperature, both low and high RH caused a significant reduction in pheromone titer ($P \leq 0.05$).

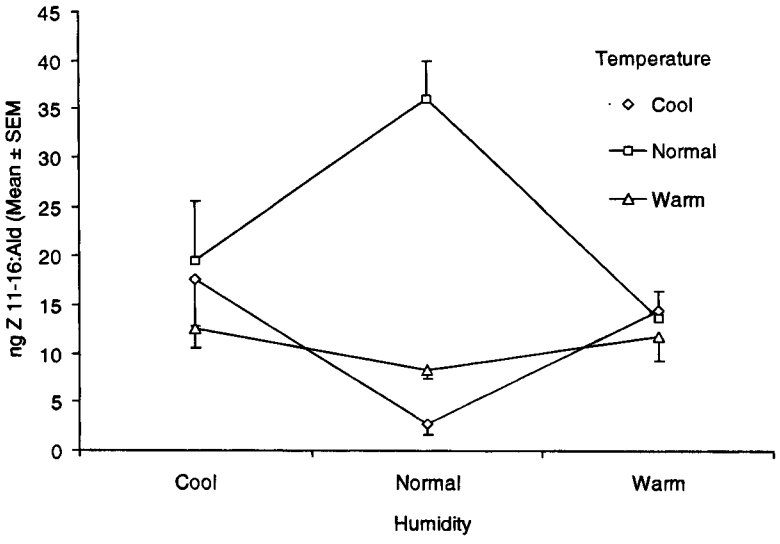

FIG. 1. Sex pheromone titers of laboratory-colony corn earworm females kept at combinations of three temperatures (cool = 15°, normal = 25°, and warm = 35°C) and three RHs (low = 10, normal = 50 and high = 90%). Pheromone was extracted 3-4 hours into the third scotophase. Average ± SEM, N = 6

Injection of PBAN had no significant effect ($P \leq 0.05$) on pheromone titer at high temperature in combination with low, normal, or high RH (Fig. 2). The maximal effect of PBAN injection was evident at low temperature in combination with normal and high RH ($P \leq 0.001$). At normal temperature, PBAN injection resulted in a significant increase of pheromone titer only at normal and high RH.

In nature, pheromones play a key role in mate location among nocturnal species of moths. Pheromone production and release are separate events and, whereas, we know much about how pheromone production is regulated in many species of moths, not much is known about the intrinsic factors that regulate the release of pheromone. Among the extrinsic factors, the role of light, particularly moonlight, on the performance of light traps has been studied extensively (Hardwick 1972, Mizutani 1984, Dent and Pawar 1988, Yela and Holyoak 1997). Similarly, the effects of temperature and other factors have been studied and reviewed thoroughly by McNeil (1991). In almost all of these studies, calling was used as a measure of pheromone release. Pheromone titer as a measure of pheromone production has rarely been used. Exposure

of corn earworm females to the warmer temperature (35°C) in the present study resulted in significantly low pheromone titers. Varying the humidity from 10 to 90% at this temperature had no effect on pheromone titer. Giebultowicz et al. (1992) reported that temperatures of 33° and 35°C caused pheromone titer to decrease to almost 0 in laboratory-reared and wild gypsy moth, *Lymantria dispar* (L.) females, respectively. Ono (1994) reported that females of the potato tuber moth produced very low amounts of pheromone at 15°C for the first 2 days of adult life. In the true armyworm, *Pseudaletia unipuncta* (Haw.), Delisle and McNeil (1987) reported that lowering the temperature from 25° to 10°C caused a shift in calling but no decrease in pheromone titer.

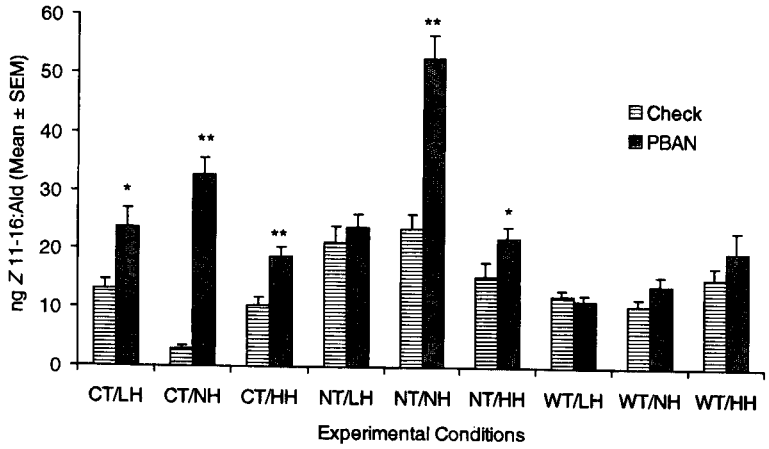

FIG. 2. Pheromone titer of laboratory-colony females kept under nine combinations of temperatures and RH. Check females were injected with 20 µl saline and test females with 5 pmol PBAN in 20 µl saline. Asterisks indicate significant difference (* $P \leq .005$, ** $P \leq 0.001$) between check and test under each experimental condition. N = 10.

The least pheromone titer was found in females maintained at 50% RH and cool temperature. Exposure to high or low RH at the cool temperatures resulted in a significant increase in pheromone titer. Apparently, the two extreme humidities partially suppressed the effect of cool temperature. In all of these cases, the pheromone titer was about 10 ng or greater than that which may be the low threshold for calling. Raina at el. (1991) observed a relationship between initiation of calling and pheromone titer in corn earworm. Pheromone titer in females at the time of initiation of calling during the scotophase was10 ng or greater.

In Beltsville during the corn-growing season (June - September) of 1997, the average weekly low temperature consistently was about 12°C except during 2 weeks when it was >15°C. Similarly, the average daytime high for the same period did not exceed 35°C, except during this same 2-week interval (MD State Climatologist Office). Temperatures were coolest late at night or in early morning; whereas, most of the sexual activity by corn earworm adults is confined to dusk and the early part of the night (Agee 1969). Insects probably will not consistently encounter temperatures of 35°C or warmer during evening/early night. During a period of cool temperatures combined with high humidity after an evening shower in summer, females should be able to produce sufficient pheromone (Fig. 1) to effectively attract males. Agee (1969) stated that ideal conditions for reproductive activity included RH between 50 and 100% and temperatures from 21° to 28°C.

PBAN had no significant effect in elevating pheromone levels at warm temperature, indicating that low pheromone titer may have been due to low pheromone biosynthesis and not lack of PBAN. Low pheromone titer at warm temperature also may have resulted from a high turnover (high synthesis combined with high release/degradation). However, at cool temperature, PBAN had a significant effect, leading to increased pheromone production, suggesting that release of PBAN was the limiting factor at cooler temperatures. Insects generally are inactive at cooler temperatures. Therefore, even if females were to produce pheromone, the males might not be able to fly to the calling female. Elsey (1982) reported that mating in the pickleworm, *Diaphania nitidalis* (Stoll), occurred when temperature was warmer than 16°C. Significant increase in pheromone production caused by injection of PBAN into females kept at normal temperature and normal humidity was expected.

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EFFECTS OF APHIDS, BARLEY YELLOW DWARF, AND GRASSY WEEDS ON GRAZED WINTER WHEAT

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ABSTRACT

The effects of grazing winter wheat, *Triticum aestivum* L., on abundance of aphids, barley yellow dwarf virus (BYDV), and grassy weeds (primarily cheat, *Bromus secalinus* L.), and on grain yield and yield components were determined during crop years 1999-2000 and 2000-2001. Cheat and aphids were suppressed or enhanced to acquire a range of aphid and grassy weed infestations in each field. During the 1999-2000 season, grazing reduced aphid abundance (as much as 87%) and BYDV levels (as much as 70%), but often promoted greater abundance of cheat. Stepwise regression analyses of 1999-2000 growing season data indicated that grazing had little to no effect on yields or yield components when aphids were part of the wheat system. In contrast, grazing was significantly correlated with reduced yields when aphids were not present during the 2000-2001 season. These findings may suggest that the negative effects of grazing are not detectable when aphid abundance is reduced as cattle feed on winter wheat.

INTRODUCTION

Approximately 50% of the wheat planted in Oklahoma is utilized as forage and for grain production (dual-purpose). Greenbug, *Schizaphis graminum* (Rondani), and birdcherry-oat aphid, *Rhopalosiphum padi* (L.), are two of the most important insect pests of winter wheat in the Southern Great Plains. These aphids often reduce yields of small grains, particularly when infestations occur during early plant growth stages (Burton et al. 1985, Kieckhefer and Kantack 1988, Kieckhefer et al. 1995, Riedell et al. 1999, Kindler et al. 2002). Additionally, both greenbug and birdcherry-oat aphid transmit barley yellow dwarf virus (BYDV) which can further reduce yields (Burton et al. 1985, Massey 1993, Hunger et al. 1996, Hoffmann and Kolb 1998). Riedell et al. (1999) reported 21% grain loss caused by birdcherry-oat aphid, but 58% reduction by birdcherry-oat aphid + BYDV.

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Dual-purpose winter wheat is often planted in early September, and grazing is terminated in late winter (Winter and Musick 1991, Redmon et al. 1996). Planting wheat early often leads to increased greenbug and birdcherry-oat aphid intensities (number per tiller), and subsequent infection by BYDV (Dedryver and Tanguy 1984). Insecticide applications that effectively reduce aphid intensity may have little effect on BYDV levels because most viral transmission is thought to occur before aphid intensities reach economic thresholds (Hunger et al. 2001). Seed treatments such as Gaucho™ (imidacloprid) can inhibit aphid abundance and subsequent BYDV levels, but are relatively expensive (Gourmet et al. 1996, Hunger et al. 1996). Planting wheat early may promote infestations by grassy weeds. Massee (1976) reported that downy brome is often more abundant in early planted wheat fields because final tillage to prepare the seedbed, which dramatically reduces establishment of weeds, occurs before downy brome seedlings emerge.

Grazing winter wheat benefits cattle production in dual-purpose systems but seems to increase the abundance of grassy weeds and reduce wheat yields. Dockage, which is primarily influenced by amounts of cheat, *Bromus* secalinus L., was 9% greater in grazed versus nongrazed wheat (Koscelny and Peeper 1990 a, b). In contrast to the negative effects of grazing winter wheat, Arnold (1981) demonstrated that grazing reduces abundance of aphids. Grazing could be potentially utilized to manage aphids and BYDV, which would reduce insecticide use and lower inputs. However, for dual-purpose wheat, the effect of reducing aphid abundance (via grazing) on grain yield has not been quantified. The trade-off between aphid and BYDV suppression and the negative impact of increased cheat in grazed wheat are unknown.

The objective of this research was to investigate the potential of cattle grazing as a biologically based aphid/disease management approach over a broad range of dual-purpose winter wheat systems. The effects of grazing winter wheat on aphid intensity, BYDV levels, infestations by grassy weeds, and the subsequent impact of these factors on grain yield and yield components were evaluated in several dual-purpose wheat fields (with variable grazing intensities) in Oklahoma and Texas from 1999–2001. To evaluate the applicability of this aphid suppression approach and avoid purely experimental effects, care was taken to select a diverse set of dual-purpose systems representative of the region.

One important question relative to this research was whether aphid suppression, attributable to cattle grazing in wheat, resulted in yield protection. Yield protection via aphid suppression may be evident when aphids are reduced in grazed wheat, but yields are similar between grazed versus nongrazed wheat; i.e., yield reductions caused by aphids in nongrazed wheat are equivalent to yields in grazed wheat that are a function of the positive effect of removing aphids and the negative effect of grazing. This idea would be further supported if aphids were absent and yields were less in grazed versus nongrazed wheat.

MATERIALS AND METHODS

Wheat Field Locations. Experiments were conducted during the 1999-2000 growing season at three locations (near Perkins and Stillwater, OK, and Sunray, TX), and at four locations during the 2000-2001 growing season (Perkins and Stillwater, OK, and Bushland and Hartley, TX). Wheat fields near Perkins, Stillwater and Bushland were at Oklahoma State University and Texas Agricultural Experiment Station research farms, while the Sunray and Hartley fields were on private farms (Table 1). Soil was analyzed for macro elements (N-P-K) at each location to ensure that adequate amounts were added before seeding for a 3,360 kg/ha targeted yield goal in a dual-purpose system.

Adapted hard red winter wheat cultivars were drilled at 100 to 135 kg/ha into 20-cm rows (Table 1). Planting dates at each location were optimal for dual-purpose production (Krenzer 2000). With the exception of Perkins and Stillwater during the first growing season, all fields were irrigated after planting to ensure seedling emergence.

Experimental Approach. The goal at each location was to establish experimental units with quantitative measures of grazing (average leaf area), cumulative aphid intensities over time (aphid-days), BYDV index (incidence × severity), and infestations by grassy weeds (percentage weeds), and subsequently examine the influence of these measures on wheat yields and selected yield components. Limited land availability and number of cattle at each location, along with logistic difficulties associated with excluding cattle from a large number of independent plots, dictated that we arrange experimental units within grazed and nongrazed whole-plots (split-plot design). At each location, eight 23 × 10.2-m whole-plots were established within each field. Single-strand electric wire fences excluded cattle from the four randomly selected nongrazed whole-plots. Four 10 × 3.6-m sub-plots were established within each whole-plot. Each sub-plot had a 1-m border separating it from other sub-plots and the electric fence. Cattle often herd and excessively trample wheat near electric fences. To prevent excessive damage to grazed wheat, nongrazed and grazed whole-plots were 10 m apart. For each location, sub-plots were defined as experimental units for stepwise regression analyses (see below) examining the influence of measured variables on wheat yields and selected yield components (M. E. Payton, personnel communication). This was justified based on (1) the observation that cattle did not uniformly graze sub-plots within designated grazed whole-plots, (2) the observed range of quantitative measurements (non-categorical) for all variables among sub-plots, and (3) measured variables were assessed identically in all sub-plots throughout the study.

Manipulation of sub-plots within each nongrazed or grazed whole-plot allowed establishment of a quantitatively measurable range of aphid-days, BYDV index, and percentage infestation by grassy weeds. Within each whole-plot, cheat seeds were broadcast at 30 kg/ha in two randomly assigned sub-plots (Koscelny and Peeper 1990b) after wheat emergence in 1999 and before planting in 2000. Natural infestations by cheat were suppressed with Maverick™ herbicide (MON 37500) in sub-plots not receiving cheat seed. At Stillwater, hand hoeing among rows was used to remove all other grasses not controlled by Maverick (mostly rye grass, *Lolium* species). During the 1999-2000 season, natural abundance of aphids was partially suppressed with insecticides in two randomly assigned sub-plots per whole-plot (one with cheat seeding and one without). Malathion was applied before grazing initiation (October-November) and Lorsban 4E® (chlorpyrifos, DowElanco) after grazing was terminated (March); both were applied at recommended rates. During the 2000-2001 season, aphids were nearly absent; thus no insecticides were used. Artificial establishment of aphids (greenbug and birdcherry-oat aphid) was attempted in designated sub-plots, but was not successful. Grazing was initiated in November or December and was terminated in late February or early March (Table 1) before the advancement of the spike above the soil surface (at first hollow stem), where it would be removed by grazing (Redmon et al. 1996).

Measurements. Throughout the growing season, the following measurements were made in each sub-plot every 4-14 days as weather permitted: tillers in two random 10-cm rows, aphid intensity (number per tiller) including greenbug, birdcherry-oat aphid, English grain aphid, *Sitobion avenae* F., and corn leaf aphid, *Rhopalosiphum maidis* Fitch, on 10 randomly selected tillers carefully clipped at ground level, and leaf area of the same 10 tillers (measured by a belt-fed leaf area meter). Cumulative aphid-days (an aphid-day was defined as one aphid feeding on one tiller for 24 hours), were estimated under field conditions (Gerloff and Ortman 1971, Pike and Schaffner 1985). Before

TABLE 1. Management Practices for Grazing Experiments During 1999-2000 and 2000-2001 Seasons.

Management	1999-2000			2000-2001			
	Perkins	Stillwater	Sunray	Perkins	Stillwater	Bushland	Hartley
Cultivar	2137	2174	TAM 202	2137	2174	Ogallala	2173+L-horn
Planting date	15 Sept.	9 Sept.	15 Sept.	25 Sept.	29 Sept.	12 Sept.	20 Sept.
Seeding rate (kg/ha)	135	135	100	135	124	100	135
Cheat seeding date	21 Sept.	4 Nov.	22 Nov.	Before planting	Before planting	17 Oct.	10 Oct.+14 Nov.
Grazing initiated	8 Nov.	14 Dec.	28 Nov.	21 Nov.	29 Nov.	6 Dec.	10 Jan.
Grazing terminated	21 Feb.	1 Mar.	27 Feb.	5 Mar.	2 Mar.	20 Mar.	15 Mar.
Stocking rate (cattle/ha)	8.6	1.7	1.0	8.6	2.5	2.5	0.7
kg/cattle head[a]	290	318	364	290	318	180	295

[a] Average weight per animal

harvest, percentage of infestation by grassy weeds (% weeds relative to wheat) was assessed in each sub-plot during tiller sampling (per 10-cm row). Incidence of BYDV disease was determined using visual ratings as a percentage of plants that showed symptoms, whereas severity of symptoms was evaluated on a scale of 0 to 9 (Qualset 1984), where 0 = disease free, and 9 = marked dwarfing, complete yellowing, few or no spikes with considerable sterility, forced maturity or dying of plants. An overall assessment of the level of BYDV disease was estimated by multiplying incidence by severity (all estimates were greater than 0) and calculating a BYDV disease index. During the 1999-2000 season, correlations between calculated disease index and both incidence and severity were significant ($r > 0.8$, $P < 0.001$). Thus, this disease index seems to standardize the variability between incidence and severity measurements and might be the most appropriate way to represent the overall effect of BYDV on wheat. During the 1999-2000 season, ELISA was used to confirm the presence of the virus and identify BYDV strains (PAV, RPV and RMV) in three symptomatic tillers from each sub-plot at each location (Rochow 1979). During 2000-2001, BYDV was not detected.

At maturity, height of three or four randomly selected wheat tillers was measured in each sub-plot. Before harvest, 20 spikes were randomly collected from each sub-plot to determine weight of kernels per spike and 100 kernel weight. A small combine that retained both cheat and wheat seeds was used to harvest plants in a 13.4-m^2 area in each sub-plot. Harvested kernels were cleaned with a small seed cleaner to remove cheat seeds so wheat yields and dockage could be determined. Yields of wheat were adjusted to 13.5% moisture.

Statistical Analyses. Data from each location for each year were analyzed separately because (1) stocking rate varied among locations, (2) wheat cultivars and the predominant aphid and weed species varied among locations, and (3) preliminary regression analysis revealed significant effects of location on yield ($P < 0.001$). Sub-plots at each location provided a broad range of quantitative measurements of grazing (average leaf area per tiller), aphid abundance (cumulative aphid-days), BYDV (disease index) and grassy weed infestation (percentage weeds). Average leaf area, cumulative aphid-days, disease index, and percentage weeds from each sub-plot were used as independent variables in a backward elimination stepwise regression model (PROC REG, SAS Institute 1999). Yield (kg/ha), plant height, tiller number, weight of kernels per spike and 100-kernel weight were included as separate dependent variables. Because combinations of measures (variables) may best describe dependent variables, the stepwise regression model included simple measures and all possible combinations. The significance level chosen for stepwise regression analyses was $P = 0.05$.

RESULTS AND DISCUSSION

Trends for Independent Variables in Regression Models. As expected, and similar to observations by Koscelny and Peeper (1990b), average leaf area was reduced at each location (as much as 46%) in grazed sub-plots. During the 1999-2000 season, birdcherry-oat aphid and greenbug were the predominant aphid species in Oklahoma and Texas, respectively. Aphid intensities (number per tiller) at the Oklahoma locations were relatively low (<15), whereas high levels (<45) were common in wheat fields in Texas. Following introduction of cattle to experimental locations, aphid-days were noticeably reduced (as much as 87%) in grazed compared to nongrazed sub-plots (within sub-plots with no insecticides and where cheat was suppressed) at all locations. Similar results were observed by Arnold (1981), who recommended grazing as a means of greenbug suppression in wheat. During the 2000-2001 season, aphids were nearly absent in the fall and spring and thus, it could not be determined whether their abundance was affected by

grazing. This low abundance of aphids may be attributed to the late emergence of wheat caused by severe fall drought throughout the Southern Great Plains.

Evaluations by use of ELISA (1999-2000 season) detected the presence of three BYDV strains: PAV, RPV and RMV, with PAV the most commonly observed. Rochow (1979) observed a similar prevalence of PAV in field–collected samples from multiple states during a 20-year period. Strain PAV was detected in all sub-plots at Perkins and many sub-plots at Stillwater and Sunray. The RPV strain was found only in a few sub-plots at Perkins, while the RMV strain was sparsely detected at all locations. Calculated BYDV index (incidence × severity) was markedly reduced (as much as 70%) in grazed sub-plots (within sub-plots with no insecticides and where cheat was suppressed) at Stillwater and Sunray during the 1999-2000 season. At Perkins, calculated BYDV index measures were similar among all sub-plots. During the 2000-2001 season, no symptoms of BYDV were observed.

With the exception of Stillwater during both growing seasons, grazed sub-plots (with cheat seeding) at each location had more grassy weeds (mainly cheat). At Perkins, grazed sub-plots (with cheat seeding) had as much as 15.4% more weeds than nongrazed sub-plots during both growing seasons; very small increases in percentages of weeds were observed at all other locations. At Stillwater, rye grass was prevalent in nongrazed and grazed sub-plots during both growing seasons, but the prevalence was decreased by grazing. Using relatively high stocking rates, Koscelny and Peeper (1990 a, b) reported an increase in cheat biomass caused by grazing. Grazing tends to remove the wheat canopy that shades slower developing cheat seedlings, thus allowing greater light penetration and shifting the competitive advantage to cheat. More weeds in grazed sub-plots at Perkins may have been the result of the high grazing intensity (8.6 heads/ha) (Table 1). As observed during this study, the high stocking rate at Perkins was sufficient to remove the wheat canopy. All other locations were moderately grazed which may have resulted in less light penetration among rows and consequently less growth of weeds.

Step-Wise Regression: Yield Components. At all locations, grazing (decreased average leaf area) was the most significant and often the only factor influencing (negatively) tiller numbers ($F > 15.6$, $P < 0.001$, Partial $R^2 > 0.26$). Reductions in tiller numbers caused by grazing have been observed by several researchers (Sharrow and Motazedian 1987, Winter and Thompson 1987 and 1990, Christiansen et al. 1989).

Similar to previous findings (Pumphrey 1970, Koscelny and Peeper 1990b, Winter et al. 1990,), grazing (decreasing leaf area) resulted in shorter plants at maturity ($F > 11.2$, $P < 0.001$, Partial $R^2 > 0.24$) for all locations except Sunray (1999-2000) and Perkins (2000-2001). At these locations, grazing combined with aphid-days and weeds negatively influenced plant height at maturity ($F > 7.5$, $P < 0.010$, Partial $R^2 > 0.26$).

Koscelny and Peeper (1990b) found that wheat kernels weighed less in grazed wheat at only one of three locations. In our study, the effects of grazing on weight of kernels per spike and 100-kernel weight were variable. During the 1999-2000 season at Stillwater and Sunray, weight of kernels per spike was not significantly influenced by any combination of variables ($P > 0.05$). At Perkins (1999-2000), grazing, aphid-days, percentage of weeds, and BYDV index combined had a small but significant negative influence on weight of kernels per spike ($F = 6.4$, $P = 0.017$, Partial $R^2 = 0.18$). During the 2000-2001 season at Stillwater, Bushland and Hartley, weight of kernels per spike was negatively influenced by grazing or percentage of weeds ($F > 8.9$, $P < 0.006$, Partial $R^2 > 0.22$). At Perkins during the 2000-2001 season, weight of kernels per spike was not significantly influenced by any combination of variables ($P > 0.05$). During the 1999-2000 season, 100-kernel weight was not significantly influenced by any combination of variables ($P > 0.05$). However, during the 2000-2001 season, 100-kernel weight was

negatively influenced by grazing and/or percentage of weeds at all locations ($F > 4.7$, $P < 0.038$, Partial $R^2 > 0.13$).

Step-Wise Regression: Yields. During the 1999-2000 season at Perkins, aphid-days and percentage of weeds combined were the only variables correlated with reduced yield (Table 2). At Stillwater and Sunray, grazing (leaf area), aphid-days, percentage

TABLE 2. Statistical Output for Stepwise Regression Analyses (Backward Elimination) for Simple and Combined Variables Related to Yield (kg/ ha) at Each Location During 1999-2000 and 2000-2001 Seasons.

Location	Variable	Parameter estimate	F	P	Partial R^2
		1999-2000			
Perkins, OK	Intercept	2209.7866	482.59	<0.001	
	AD-PW	-2.4323	20.36	<0.001	0.38
	LA-AD-PW-BYDV	-0.0001	9.99	0.004	0.19
	AD-PW-BYDV	0.0041	10.44	0.003	0.19
	LA-AD-PW	0.0523	20.09	<0.001	0.37
	LA-PW	0.9357	8.22	0.008	0.15
Stillwater, OK	Intercept	3182.3667	174.55	<0.001	
	LA-PW	-0.5610	21.83	<0.001	0.40
	AD-BYDV	-0.2082	9.11	0.005	0.17
	AD-PW-BYDV	0.0044	7.20	0.012	0.13
Sunray, TX	Intercept	4552.9479	36.67	<0.001	
	LA-PW-BYDV	-0.0164	10.40	0.003	0.26
		2000-2001			
Perkins, OK	Intercept	4812.9336	1210.10	<0.001	
	PW	-43.3103	82.12	<0.001	0.30
	LA-PW	0.5691	10.00	0.004	0.04
Stillwater, OK	Intercept	6785.6026	1224.60	<0.001	
	PW	-70.4425	46.67	<0.001	0.28
	LA-PW	0.9866	9.52	0.004	0.06
Bushland, TX	Intercept	3720.1574	101.38	<0.001	
	LA	52.3345	8.92	0.006	0.23
Hartley, TX	Intercept	1317.1948	0.37	0.550	
	LA	241.9042	8.54	0.007	0.22

LA = Average leaf area per tiller (cm^2) representing grazing.
AD = Aphid days.
PW = Percentage weeds/ sub-plot.
BYDV = Barley yellow dwarf virus disease index (incidence × severity).

weeds and BYDV disease only slightly affected yield. Many studies have demonstrated that aphid-days can be a useful predictor of grain yield loss at harvest (Kieckhefer et al. 1995, Kindler et al. 2002). In our study, however, aphid-days represented a minor component of the yield model at Perkins during 1999-2000. Interestingly, based on parameter estimates (Table 2), measured variables had little meaningful effect on yield at any of the locations during 1999-2000.

During the 2000-2001 season, aphid-days and BYDV disease were not detectable; thus, fewer variables were included in the stepwise regression model. For both Perkins and Stillwater, the only variable greatly affecting (negatively) yield was percentage of weeds (Table 2). Based on previous work in winter wheat (Koscelny and Peeper 1990a), when other factors such as aphids were not present, negative correlations between yield and abundance of grassy weeds are to be expected.

Redmon et al. (1996) demonstrated that increasing grazing intensity reduced wheat yields. In our study, the only strong negative correlations between yield and grazing (average leaf area) were detected at Bushland and Hartley (Table 2). At both Bushland and Hartley, cheat was not well established and therefore did not cause noticeably reduced yield. Based on these results, it seems that in such wheat systems as at Bushland and Hartley, when weeds and aphids are absent or scarce, negative effects of grazing are clearly demonstrated.

Probably the most important question relative to this study was whether suppression of aphids in grazed wheat resulted in yield protection. Yield protection via aphid suppression may be evident (1) when aphids are reduced in grazed wheat, but yields are similar among grazed versus nongrazed sub-plots, and (2) when aphids are not present and yields are less in grazed versus nongrazed sub-plots. Both of these ideas are supported by trends for yields observed during this study. At each location, aphids were clearly reduced in grazed sub-plots during the 1999-2000 growing season; during 2000-2001, aphids were absent. Based on stepwise regression analyses, grazing (leaf area) had little to no effect on yields during the 1999-2000 growing season when aphids were part of the wheat system. In contrast, during the 2000-2001 season, grazing was significantly correlated with reduced yields when aphids were not present. These findings may suggest that the negative effects of grazing are not detectable when aphid abundance is reduced as cattle feed on winter wheat.

When greenbugs and birdcherry-oat aphids are common in the late fall or early spring, grazing will reduce their abundance. Based on our observations, when cattle are removed in the spring, aphid abundance is unlikely to reach a damaging threshold as wheat plants grow to maturity. Additionally, reducing aphid abundance seems to decrease the potential for development of BYDV. Therefore, grazing has the potential to be a biologically based insect/disease management tactic within the integrated crop and livestock wheat systems of Oklahoma and Texas. However, over-grazing seems to promote competition from grassy weeds. Further study is needed to determine if moderate grazing in wheat systems throughout the Southwestern U.S. holds promise for reducing pesticide inputs commonly used in wheat fields.

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THE NEGATIVE BINOMIAL DISTRIBUTION AS A MODEL FOR DESCRIBING COUNTS OF GREENBUGS, *SCHIZAPHIS GRAMINUM*,[1] ON WHEAT[2]

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ABSTRACT

One-hundred-thirty-two samples in which one or more tiller was infested with greenbugs, *Schizaphis graminum* (Rondani), were taken during the fall growing season, and 232 samples with one or more greenbugs were taken during spring. The frequency distribution of greenbug counts differed significantly from that expected for a negative binomial distribution for six of 132 samples from fall (4.5%) and for six of 222 samples from spring (2.7%). Estimates of k were highly variable and changed systematically as $\bar{x}$ increased. The parameters of a quadratic regression model fitted to samples with $\bar{x} > 0.25$ greenbugs per tiller did not differ significantly between samples from fall and spring. The expected value of k from the quadratic regression model varied from approximately 0.3 to 0.7 as $\bar{x}$ varied from 2 to 12 greenbugs per tiller. Variation in estimates of k about the regression curve provided an indication of the extent to which k varied independent of greenbug population intensity. The mean square error of the regression was equal to 0.043 and was relatively large compared to k. This indicates that a sequential sampling scheme based on the negative binomial distribution should be evaluated by simulation and/or direct validation to verify the utility of a sampling scheme before operational use in a pest management program.

INTRODUCTION

Each year, more than two million ha of winter wheat, *Triticum aestivum* L., are planted in Oklahoma. The greenbug, *Schizaphis graminum* (Rondani), commonly infests winter wheat in Oklahoma and causes severe damage, with estimates of economic losses exceeding $100 million in some years (Webster 1995). A sequential sampling scheme based on binomial count (presence-absence) sampling of greenbugs on individual wheat stems (tillers) was recently developed for the greenbug in winter wheat (Giles et al. 2000). This sampling scheme was derived from a commonly used empirical equation relating the

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[1]Homoptera: Aphididae

[2]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.

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mean number of insects per sample unit to the proportion of infested sample units (Kono and Sugino 1958, Gerrard and Chiang 1970). While this empirical equation can be used to develop sequential sampling schemes, those based on Wald's (1947) sequential probability ratio test (SPRT) are sometimes more precise. This is because it is not necessary with SPRT to incorporate variance of the regression relationship of the proportion of sample units infested with the particular pest to the mean number of pests per sample unit (Nyrop and Binns 1991). To develop a sampling scheme using the SPRT, it must be known that the distribution of counts of insects in samples fits a particular probability distribution.

For insects such as the greenbug, which have aggregated spatial distributions, the negative binomial distribution sometimes provides an acceptable fit to sample data (Southwood 1978). The negative binomial distribution is characterized by two parameters, the mean and an exponent denoted by k (Southwood 1978). When using SPRT to derive a sequential sampling scheme based on the negative binomial using SPRT, k is assumed to be constant. Failure of k to remain constant complicates development of a sampling scheme because the probabilities of rejecting the hypotheses that greenbug population intensity is less than or greater than an action threshold differ from the nominal values established in developing the scheme (Nyrop and Binns 1991). The robustness of the sequential sampling plan may depend on how much k varies at densities near the economic threshold for the insect. The two objectives of this study were to determine whether: 1) the negative binomial distribution provided a good fit to data collected for greenbugs from winter wheat fields in Oklahoma, and 2) k varied over the range of values of the sample mean ($\bar{x}$) typically observed in greenbug-infested fields, and if it differed, to describe mathematically the form of the relationship between k and $\bar{x}$.

MATERIALS AND METHODS

From September 1997 through May 2001, the number of greenbugs per tiller was estimated in fields of hard red winter wheat located throughout central and western Oklahoma, including the Panhandle. Fields sampled from September through December were considered to have been sampled during the fall growing season, and those sampled from January through May were considered to be fields sampled during spring. Tillers were sampled from a field by walking a u-shaped transect through a field and cutting at ground level an individual tiller approximately every 10 m. The number of greenbugs on each of 80 to 200 tillers was counted in the field and recorded. The number of tillers inspected in a field depended on greenbug population intensity, with more tillers being inspected when greenbugs were scarce than when they were abundant. In total, 132 samples in which one or more tiller was infested with greenbugs were taken during the fall growing season, and 232 samples with one or more greenbugs were taken during spring.

The data acquired by sampling were subjected to statistical analyses. The parameter k of the negative binomial distribution was estimated by using the maximum likelihood method described by Bliss and Fisher (1953) for each sample. The goodness of fit to the negative binomial distribution was determined for each sample by using a chi-square test (Bliss and Fisher 1953). The probability of rejecting the hypothesis that the observed data fit the negative binomial was set at $\alpha = 0.05$. Analyses were accomplished with a computer program written in Compaq Visual Fortran 6.6©.

The relationship between k and the mean number of greenbugs per tiller ($\bar{x}$) in the samples was investigated by regression of k on $\bar{x}$. A previous study indicated that parameters of a statistical model relating the population intensity of greenbugs to the proportion of tillers infested with greenbugs, which implicitly depends on the negative binomial distribution, differed for wheat fields sampled during fall compared with fields

sampled in spring (Giles et al. 2000). Therefore, we used heterogeneity of slopes regression to test for a difference in the relationship of k to $\bar{x}$ between fall and spring. Regression analyses were accomplished using the REG procedure of the Statistical Analysis System (SAS Institute 1990). A quadratic regression model with a single linear and quadratic parameter for data from both seasons was also fitted.

RESULTS AND DISCUSSION

Maximum likelihood estimates of the parameter k varied from 0.002 and 145.1 for fields sampled in fall, and from 0.001 to 259.2 for fields sampled in spring. Estimates of k were highly variable when the mean number of greenbugs per tiller ($\bar{x}$) was less than approximately 0.25 for fields in the fall and spring (Fig. 1). The value of k increased slightly as $\bar{x}$ increased for greenbugs in fields sampled in fall and spring and then decreased as $\bar{x}$ increased to more than 15 greenbugs per tiller.

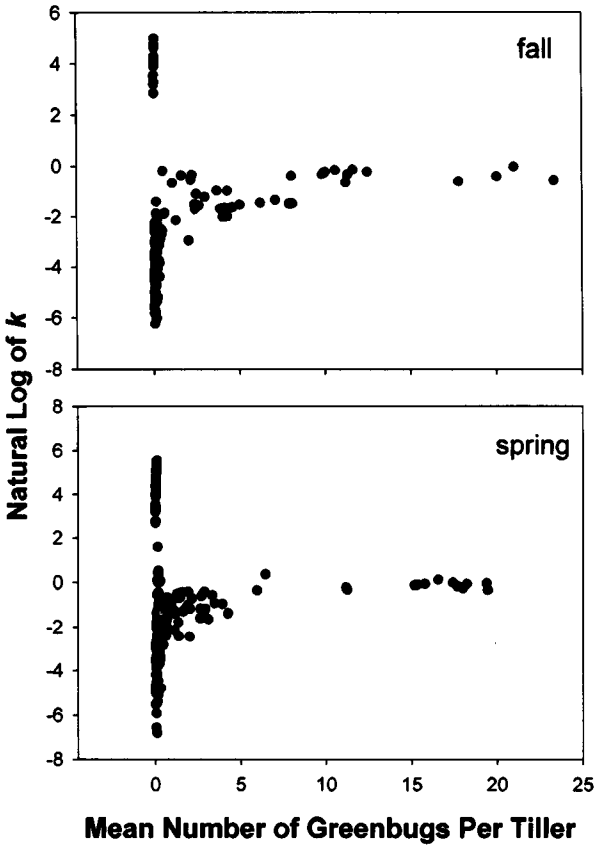

FIG. 1. Natural log of maximum likelihood estimates of the parameter k versus the mean number of greenbugs per wheat tiller for fields sampled in fall and spring.

The frequency distribution of counts of greenbugs per tiller differed significantly from that expected for a negative binomial probability distribution for six of 132 (4.5%) of the fields sampled in fall and for six of 222 (2.7%) of the fields sampled in spring. These percentages were only slightly less than the percentage of samples expected to differ significantly by chance given that α was set equal to 0.05 for each chi-square test. The general agreement of observed to expected frequencies indicates that the negative binomial is an acceptable probability model for describing the frequency distribution of counts of greenbugs on wheat tillers for fields sampled in the fall or spring.

Because of the apparent non-linearity in the relationship of k to $\bar{x}$, a quadratic regression model was used to fit the relationship. Samples with $\bar{x} < 0.25$ were excluded for the purpose of model development because of the great variability of the estimates of k. Linear and quadratic terms were significant for fields sampled in fall and spring (Table 1). All regression parameters were significantly different from zero, but a regression of heterogeneity of slopes indicated there were no significant differences for any of the parameters between fall and spring. Even though parameters of the quadratic regressions differed for fall and spring sampled fields, the differences were not significant. Therefore, fall and spring data were combined to construct a single regression model. Parameters for linear and quadratic terms were significant for the model using the combined data. The model explained 48% of the variability in k.

TABLE 1. Parameters of Quadratic Regression Models Relating the Parameter k of the Negative Binomial Distribution to the Mean Number of Greenbugs per Tiller ($\bar{x}$) for Fall and Spring Sampled Winter Wheat Fields, and for All Sampled Fields.

Season	n	Intercept	Slope	Slope^2	r^2
Fall	50	0.12 (0.047)[a]	0.060 (0.014)	-0.0014 (0.0007)	0.54
Spring	95	0.20 (0.099)	0.088 (0.014)	-0.0015 (0.0007)	--
Both Seasons	145	0.20 (0.025)	0.062 (0.011)	-0.0015 (0.0006)	0.48

[a]Standard errors of estimates are in parentheses.

Agreement of frequency distributions of greenbug counts on wheat tillers with expectations of the negative binomial distribution does not assure that the negative binomial probability distribution correctly describes the observed probabilities (Hurlbert 1990). However, from a practical perspective, the small number of significant deviations indicates that at the very least, the negative binomial distribution provides a good empirical probability model to describe the distribution of counts. Under this circumstance, the negative binomial is acceptable for developing sequential sampling schemes by using the SPRT (Binns 1994).

The systematic variation of k with $\bar{x}$ observed will complicate development of sequential sampling schemes for greenbugs. The extent of this problem will depend on the amount of variation in k over the range of mean population intensity for which control decisions are typically made (Nyrop and Binns 1991). The value of k seems to vary from about 0.3 to 0.7 for $\bar{x}$ varying from approximately two to 12 greenbugs per tiller (Fig. 2), the range of population intensities for which control decisions typically are made in winter wheat in Oklahoma (Royer et al. 1998). Thus, variation in the value of k with respect to greenbug density is not extreme over the range of $\bar{x}$ for which control decisions are made.

Variation around the regression curve (Fig. 2) indicates the extent to which estimates of k can vary independent of greenbug population intensity. The mean square error of the regression is a good estimate of the potential maximum of this variability (Nyrop and

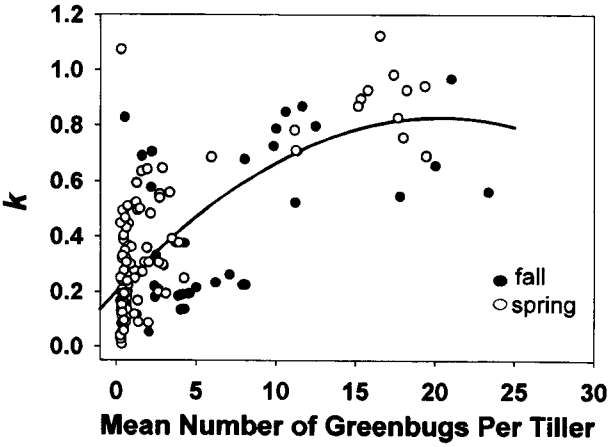

FIG. 2. Linear regression equation for k versus $\bar{x}$ for wheat fields sampled in fall and spring. Samples with $\bar{x} < 0.25$ were excluded from the regression analysis.

Binns 1991). The mean square error for the regession (MSE = 0.043) was large as a proportion of k, ranging from 0.14 for k = 0.3 to 0.06 for k = 0.7. An unknown fraction of the variation in k is caused by sampling error in the estimate, and will have no effect on the performance of a sequential sampling scheme apart from its influence on the precision of the estimate of k used in developing the plan. However, true variation in k independent of $\bar{x}$ is probably also present, which will affect the performance of the sampling scheme. The large variation in k indicates that a sampling scheme based on the negative binomial distribution should be evaluated using Monte Carlo simulation (Nyrop and Binns 1991) and/or direct validation using independent sampling data to accurately assess the utility of the sampling scheme before using it to make greenbug control decisions.

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PROTECTION OF THE SPORE-TOXIN COMPLEX OF *BACILLUS THURINGIENSIS* SEROVAR *ISRAELENSIS* FROM ULTRAVIOLET IRRADIATION WITH ALUMINUM–CMC ENCAPSULATION AND PHOTOPROTECTORS

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ABSTRACT

A *Bacillus thuringiensis* serovar *israelensis* (*B.t.i*) spore-toxin complex was encapsulated within aluminum carboxymethylcellulose (CMC) using green malachite, ponceau red, and congo red photoprotective agents against ultraviolet light. Two percent CMC was found to be a stable encapsulated spore toxin-complex. The LC_{50} and LC_{90} values of the spore-toxin complex were increased 13.9 and 29.1%, respectively, and slope decreased 13.6% through the microencapsulation process. When malachite green, ponceau red, and congo red were added, the LC_{50} changed from 0.6155 mg/l of spore-toxin complex encapsulated to 0.6127, 0.6207 and 0.6738 mg/l, respectively. Twenty-four hour exposure of the free spore-toxin to ultraviolet light reduced stability to zero as indicated by tests against third-instar *Aedes aegypti* (L.) larvae. When the spore-toxin complex was microencapsulated within aluminum carboximethylcellulose alone and with ponceau red, malachite green, or congo red as photoprotector agents and then exposed to UV light for 24 hrs, percentage mortality against third-instar *Ae. aegypti* larvae was 23, 50, 60 and 88%, respectively. The aluminum-CMC encapsulated form of the spore toxin complex of *B.t.i.* with photoprotectors avoided the limitation of this agent in controlling mosquito larvae caused by ultraviolet light.

INTRODUCTION

Bacillus thuringiensis serovar *israelensis* (*B.t.i*), a gram-positive bacterium, is highly toxic to dipteran larvae such as mosquitoes and blackflies which are vectors of several tropical diseases as malaria and dengue (de Barjac 1978). The entomocidal activity of *B.t.i.* is due to crystalline inclusions produced during sporulation. Inclusions are ingested by susceptible larvae, solubilized by the high pH of the larval midgut, and the protoxin is activated by proteases. The gut cells swell and lyse, and the insect stops feeding and eventually dies (Ellar 1994). The use of *B.t.i.* is limited by the low efficacy of current preparations under field conditions (Tyanyun and Mulla 1999). Major reasons for the low efficacy of these preparations include particle sedimentation (Rushed and Mulla 1989), protein adsorption onto silt particles and organic matter (Ohana et al. 1987), consumption by other organisms to which the toxin is not lethal (Blaustein and Margalit 1991), dissolved tannins (Lord and Undeen 1990), and inactivation by sunlight (Pusztai et al. 1991). Photoinactivation seems to be one of the major environmental factors affecting the stability of *B.t.i.* delta endotoxin (Leong et al. 1980). In other subspecies of *B. thuringiensis* active against lepidopterans, polymeric formulations and photoprotective agents have been used to encapsulate the spore-toxin complex to protect the δ-endotoxin from environmental factors (e.g., Tamez-Guerra et al. 1999, McGuirre et al. 1996, Behle et al. 1996, Ridgway et al.

1996, Castro-Franco et al. 1998). However, these formulations cannot be used against dipteran larvae because their behavior, and habitat needs are different from those of lepidopteran larvae. In the subspecies *israelensis,* some studies have been reported on the protection of the delta endotoxin against environmental conditions (e.g., Kase and Branton 1986, Yu-Tien et al. 1993). In this paper, we present the results of a study on the photoprotection of mosquitocidal activity of a spore-toxin complex with carboximethylcellulose and aluminum sulfate using malachite green, congo red, and ponceau red as photoprotective agents.

MATERIALS AND METHODS

B.t.i. was obtained from the Pasteur Institute, Paris, France. An active culture was maintained by streak-inoculating nutrient agar-slant tubes, incubated at 30°C for 2 days, and stored at 4°C. The culture was transferred to new agar slants at approximately two-month intervals. Two milliliters of LB medium (1.0% tryptone, 0.5% yeast extract and 1.0% NaCl) were inoculated with 50µl of suspension of grown agar cells ($O.D._{620}$= 2.0 approximately). The culture was incubated overnight at 30°C and then transferred to 50ml of medium in a 250-ml flask. The composition of the medium (expressed as g/l in distillated water) was as follows: tryptone, 15; Na_2HPO_4, 7.1; $MgSO_47H_2O$, 0.02; $FeSO_47H_2O$, 0.001; $ZnSO_47H2O$, 0.005; and $CuSO_45H_2O$, 0.005 with pH=7.0 ± 0.2 (Pearson and Ward 1988). The culture was incubated in a shaker at 300 rpm and 30°C and harvested at 48 hrs when lysis reached at least 90%. The fermented broth was centrifuged at 5,000 rpm for 10 min, washed three times with 0.1 M NaCl, and then with distilled water. The spore-toxin complex was dried at 60°C for 48 hrs. Solutions of a 1.0% (w/v) spore-toxin complex were made up in sterile water containing 0.5-3.0% (w/v) sodium CMC (Sigma) to which 2.0% (w/v) of the respective photo protective agent was added (i.e., malachite green, congo red, or ponceau red). The solutions were magnetically stirred overnight at 4°C and then transferred into 0.05 M $Al_2 (SO_4)_3$ (pH=3.4) as described by Elcin et al. (1995) using a hypodermic needle at a drop rate of 60 drops per minute. The mixture was stirred at 4°C until aluminum CMC microcapsules were formed. Microcapsules were filtered through a sterile filter (MF millipore 1.2 µm), washed several times with sterile 0.05M $Al_2(SO_4)_3$ (pH=3.4), and then lyophilized. Protein content of the microencapsulated samples were assayed by the Lowry et al. (1951) method. Bioassays were carried out according to the test procedures obtained from McLaughlin et al. (1984). Larvae of *Aedes aegypti* (L.) were obtained from colonies maintained at the insectary of Ministry of Health of Veracruz, Mexico. Fifty milligrams of the spore-toxin complex or microencapsulated powder were placed in 5ml of deionized water. Stock solutions were prepared in test tubes by serial dilutions of the homogenates in deionized water, and appropriate dilutions were tested against third-instar *Aedes aegypti* (L.) larvae. Five different concentrations of each powder were prepared in duplicate for each assay. In each assay, 20 mosquito larvae were added to a final test volume of 100ml. Each test was repeated three times on different dates. Duplicate cups with 20 mosquito larvae in 100ml of deionized water without test material served as a control. Mortality was determined at 24 hrs, based on the number of dead larvae. Mortality was subjected to probit analysis (Finney 1977) on a computer program and the LC_{50}, LC_{90} and curve slope were calculated for the respective spore-toxin complex and microencapsulated powder. Efficacy of formulations, LC_{50}, LC_{90}, and slopes values were compared by analysis of variance ($p \leq 0.05$) using a Minitab® 10.2 statistic program. Stability of free and encapsulated spore-toxin complex mixtures against ultraviolet light was tested using an UV germicidal lamp (257 nm). Samples were suspended in 100ml of deionized water and exposed to UV irradiation at a distance of 60cm. Duplicate cups were collected at 6, 12, 18 and 24 hrs, and

Table 1 shows the probit analysis of the concentration-mortality response of third-instar *Ae. aegypti* larvae to the encapsulated products. These results indicated that LC_{50} values for spore-toxin complex were 15.9% lower than that for the encapsulated product without the photoprotector, and 15.4, 16.9 and 26.9% lower for the encapsulated products with malachite green, ponceau red, and congo red, respectively. However LC_{50} values were not significantly different for the unencapsulated toxin and the encapsulated toxin with and without a photoprotector. The highest LC_{90} value for the encapsulated products was for the congo red formulation (1.6218 mg/l), and the lowest was for the formulation without a photoprotector (1.3872 mg/l). All encapsulated products showed an increase of 29 to 52% in the LC_{90} value with respect to the free spore-toxin complex formulation. The LC_{90} value was significantly different for encapsulation with ponceau and congo red with respect to the free toxin. The slopes ranged from 13 to 28% less in value for the encapsulated formulations compared to that for the unencapsulated spore-toxin complex; here we also observed values significantly different for ponceau and congo red.

TABLE 1. Toxicity (LC_{50} and LC_{90}, mg/l) of Microencapsulated Formulations of the *B.t.i.* Spore-Toxin Complex to Third Instar *Aedes aegypti* Larvae Over 24 Hours.

PRODUCT	LC_{50} +/- s.d.	LC_{90} +/- s.d.	SLOPE+/- s.d.
Spore-toxin complex	0.5310^{a} +/-0.0266	1.0740^{a} +/-0.0835	4.2728^{a} +/-0.5840
Encapsulation without photoprotector	0.6155^{b} +/-0-0473	1.3872^{ab} +/-0.1388	3.6916^{ab} +/-0.2637
Encapsulation with malachite green	0.6127^{ab} +/-0.0597	1.4488^{ab} +/-0.3796	3.6278^{ac} +/-0.6715
Encapsulation with ponceau red	0.6207^{ab} +/-0.0330	1.6342^{b} +/-0.2648	3.0416^{bc} +/-0.5049
Encapsulation with congo red	0.6738^{ab} +/-0.0788	1.6218^{b} +/-0.1655	3.4002^{bc} +/-0.4183

[a] Different letters on a given determination indicate significant differences among formulations by one factor analysis of variance ($p \leq 0.05$).

Fig. 3 shows the effects of ultraviolet irradiation on the stability of the encapsulated and normal spore-toxin product. These results indicate that UV irradiation affected the activity of the unencapsulated product to a point where, after 24-hrs irradiation, less than 5% mortality was realized when the irradiated formulation was tested against third-instar *Ae. aegypti* larvae. The aluminum-CMC encapsulated spore-toxin complex without a photoprotector was also affected by ultraviolet irradiation, resulting in less than 25% larval mortality after irradiation for 24 hrs. Results of the larvicidal activity test involving the aluminum-CMC encapsulated formulations containing the photoprotectors malachite green or ponceau red demonstrated that major protection of the spore toxin against ultraviolet light was realized, with these particular formulations still causing 55% or more larval mortality after 24 hrs of irradiation. In the case of the congo red encapsulated formulation, almost the same larval mortality rates (88%) resulted after irradiation as before (Fig 3).

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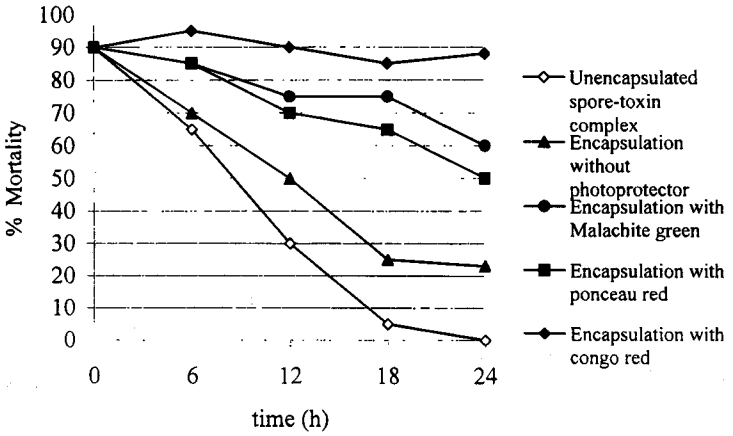

FIG.3. - Efficacies of *B.t.i.* spore-toxin complex microencapsulation formulations after 24 hours ultraviolet irradiation (257 nm) under laboratory conditions.

DISCUSSION

One of the major drawback in the use of *B.t.i* is its rapid inactivation mainly due to sunlight (Priest 1992, Morris 1983, Dunkle and Shasha 1989, McGuirre et al. 1996). The irradiation of UV light at wavelengths ranging from 250-380 nm has a detrimental effect on the viability and toxicity of *B.t.* (Pusztai et al. 1991). Yu-Tien et al. (1993) reported that *B. thuringiensis. israelensis* completely lost its toxicity to mosquito larvae when exposed to 1.34 x 105 J/m2 of irradiation at 253 nm. In this regard, our data demonstrated that UV irradiation, at least under the conditions that were used, adversely affected the larvicidal activity of the aluminum CMC-encapsulated formulation of the spore-toxin complex of *B.t.i.* containing congo red as a photoprotective agent (Fig 3). Elcin et al. (1995) showed that the aluminum-CMC encapsulation of *Bacillus sphaericus* did not protect larvicidal activity of this agent from the detrimental effect of UV irradiation. In this study, a rapid loss of toxicity of the unencapsulated formulation of the *B.t.i.* spore–toxin for *Ae. aegypti* larvae was observed when exposed to UV irradiation for up to 24 hrs. Also, the encapsulation of this particular spore-toxin complex without a photoprotector did little to increase the toxin's stability and toxicity for mosquito larvae. These results are consistent with those of other authors. Poszgay et al. (1987) showed that exposure of *B. thuringiensis* to 40 hrs of UV irradiation resulted in lost activity. Since protein photoinactivation produced by UV light seems to be the primary factor affecting the stability of the toxin, attempts were made by others to achieve photoprotection using exogenous chromophores to absorb light and protect the delta-endotoxin of this agent. Yu-Tien et al. (1995) achieved photoprotection of the spore-toxin complex by addition of melanin. Castro-Franco et al. (1998) tried malachite green, congo red, and charcoal as UV protectors in formulations of *B. thuringiensis aizawai.* The authors used the formulations against *S. frugiperda* and obtained 78, 70 and 45% UV protection, respectively. Ignoffo et al. (1991) showed that activated carbon and polyflavonoids can also be used effectively to protect entomophatic viruses from the adverse effects of UV irradiation. In this work, the most effective chromogen protector at least up to 24 hrs of UV exposure was congo red followed by malachite green and ponceau red. However, this photoprotector significantly increased the LC_{90} value from 1.074 to 1.6218 (51%) and the slope decreased from 4.2728 to 3.4002 (20%). Castro-Franco et al. (1998)

considered malachite green a better photoprotector than congo red. This difference is probably due to the composition of these author's formulations compared to ours. As for encapsulations itself, results indicated that 2% CMC forms a stable formulation of the *B.t.i.* spore-toxin complex. It was observed that percentage mosquito larval mortality for the 2% CMC encapsulated formulation without photoprotector compared to those encapsulated formulations with ponceau red, malachite green, or congo red was not statistically different. The encapsulation using 2% aluminum CMC had high capacity to produce consistent capsules. As observed in Fig. 1, the decrease in the mosquito mortality after encapsulation was probably due to the free spore-toxin being eliminated during filtering and/or washing. In the case of their effect on *Ae. aegypti* larva mortality, there was no statistical preference of which formulation performed best.

Results indicate that the encapsulation material and the photo protectors used are good prospects as formulating agents for *B.t.i.* endotoxin to be applied against mosquito larvae. The use of phagostimulants might serve to increase the preferences or feeding rates of mosquito larvae for these formulations (Farrar and Ridgway 1994) and, thereby, make these formulations even more effective.

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GENETIC VARIABILITY AMONG POPULATIONS OF *TRIATOMA LONGIPENNIS*[1], VECTOR OF CHAGAS DISEASE IN WESTERN MEXICO

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ABSTRACT

Enzyme polymorphism in *Triatoma longipennis* Usinger, one of the most important vectors of Chagas disease in Mexico, was analized using starch gel electrophoresis. Seven geographic locations were sampled in order to determine gene flow among populations and to characterize intraspecific differences. Of 18 enzymes assayed, three (ES, MDH, and ME) were successfully resolved and then used to score genetic variation. ES was used to differentiate between populations. Both polymorphism and heterozygosity indicated genetic variability in the populations studied. Gene flow between some populations was found to be high. This finding and the low genetic distance between populations indicate similarity among most of the nearby localities, suggesting an important epidemiological threat.

RESUMEN

Se realizó un estudio para estimar el polimorfismo enzimático entre poblaciones de *Triatoma longipennis* Usinger, uno de los principales vectores de la enfermedad de Chagas en México, mediante análisis electroforéticos en geles de almidón. Se colectaron ejemplares en siete localidades con la finalidad de detectar flujo interpoblacional de genes y para caracterizar diferencias intraespecíficas. De las 18 enzimas probadas, sólo tres (ES, MDH y ME) mostraron suficiente resolución. El polimorfismo y la heterocigocidad indicaron variabilidad genética dentro y entre poblaciones. El flujo de genes entre poblaciones resultó alto. Lo anterior, aunado a la baja distancia genética entre la mayoría de las poblaciones cercanas, podría indicar un riesgo epidemiológicamente importante.

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INTRODUCTION

Chagas disease is a public health threat throughout Latin America where it has been estimated that 16-18 million people are infected (TDR 2000). In Mexico, the blood-sucking bug *Triatoma longipennis* Usinger (Hemiptera:Reduviidae) is one of the most important vectors of *Trypanosoma cruzi* Chagas, the causative agent of Chagas disease (Velasco-Castrejón 1991, Velasco-Castrejón et al. 1994, Velasco-Castrejón and Salazar-Schettino 1996, Magallón-Gastélum et al. 1998, Vidal-Acosta et al. 2000, Martínez-Ibarra et al. 2001). The state of Jalisco, located in Western Mexico, is considered one of the Mexican states with high risk of transmission of *T. cruzi* to human populations by triatomines, specially *T. longipennis* (Magallón-Gastélum et al. 1998, Martínez-Ibarra et al. 2001). This state also had the highest number of asymptomatic acute cases detected by parasitoscopic tests (Velasco-Castrejón et al. 1994).

One of the main problems in controling *T. cruzi* vectors is the recolonization of domiciliary habitats through migration of triatomine bugs from sylvatic to domestic foci (Wisnivesky-Colli 1994, López and Moreno 1995, Noireau et al. 1995, Gajate et al. 1996) and the migration between neighboring localities (Dujardin et al. 1996). To determine the mobility between sylvatic and domiciliary habitats, it is useful to analyze the genetic structure of representative populations and to estimate gene flow.

Enzyme systems provide useful genetic markers for population studies, allowing genetic structures to be elucidated on the basis of polymorphic loci. Isoenzyme polymorphism has been used to characterize populations of *T. infestans*, the most important vector of *T. cruzi* in many South American countries. Research in Bolivia concluded that the triatomines found inside some homes after a insecticide treatment were survivors and not reinfestanting individuals (Dujardin et al. 1996). Also in Bolivia, Breniere et al. (1998) investigated the population genetic structure of this species, concluding that the panmitic unit was smaller than previously estimated. In Brazil, Costa et al. (1997) confirmed by isoenzyme polymorphism the existence of four different chromatic populations of *Triatoma brasiliensis* Neiva. In México, Flores et al. (2000) detected differences between Mexican triatomine species, but failed to find species-specific loci to distinguish among three species of the *T. phyllosoma* complex [*T. pallidipennis* (Stal), *T. longipennis* Usinger and *T. picturata* Usinger]. The present study addresses genetic variability of three isoenzymes in sylvatic, domiciliary and peridomiciliary populations of *T. longipennis*, and the rate of gene flow between sylvatic and domiciliary habitats.

MATERIALS AND METHODS

Seven locations were selected for this study. Each location was about 30 km apart from each other, between Tepic, Nayarit (21° 30' N, 104° 54' W) and Guadalajara, Jalisco (20° 40' N, 103° 20' W) and between Guadalajara and Autlán, Jalisco (19° 46' N, 104° 22' W). The presence of triatomines in different habitats in each locality provided appropriate conditions to study gene flow and made feasible the characterization of genetic variability between populations of *T. longipennis*

Two hundred and thirty-eight domiciliary triatomines were collected from the seven localities in the study area, whereas only 11 sylvatic specimens were collected from a site 10 km North of Jala, Nayarit (104° 26' N., 21° 03' W). A total of eight populations were assayed (Table 1).

A quarter of the thorax from each 40-day starved adult was macerated separately in 250 µl of an enzyme stabilizer solution (tris-HCl buffer, sacarose, ascorbic acid,

polivinilpirrolidona, sodium matasulfate and mercaptoetanol) (Acquaah 1992). The extracts were frozen at –30°C until they could be analyzed by electrophoresis.

Standard horizontal starch gel electrophoresis procedures and enzyme development conditions described by Acquaah (1992) were employed. The following 18 enzymes were assayed: aconitase (ACO, EC 4.2.1.3); α-glutamate dehydrogenase (α-GDH, EC 1.4.1.2); aminopeptidase (PEP, EC 3.4.13.9); catalase (CAT, EC 1.11.1.6); 6-diaphorase (DIA, EC 1.6.2.2); fumarase (FUM, EC 4.2.1.2); glucosephosphate isomerase (GPI, EC 5.3.1.9); glutamate oxalacetate transaminase (GOT, EC 2.6.1.1); hexokinase (HK, EC 2.7.1.1); isocitrate dehydrogenase (IDH, EC 1.1.1.42); leucine aminopeptidase (LAP, EC 3.4.11.1); malate dehydrogenase (MDH, EC 1.1.1.37); malic enzyme (ME, EC 1.1.1.40); mitocondrial isocitrate-NADP (ICDH, EC 1.1.1.42); phosphoglucomutase (PGM, EC 2.7.5.1); phosphogluconate dehydrogenase (6PGDH, EC 1.1.1.43); soluble esterase (ES, EC 3.1.1.1); and superoxide dismutase (SOD, EC 1.15.1.1). The electrophoretic patterns were recorded photographically.

Gene flow among populations was estimated with the equation $F_{ST} = 1/(1 + 4N_em)$ (Wright 1931), where F_{ST} is a measure of gene frequency variance among populations and was calculated according to Nei (1975); N_e represents the effective population size and m, the migration rate.

Genetic similarity and distance among populations were estimated with the Identity Index (I) and the Genetic Distance Index (D) of Nei (1975) and the Similary Index (S) of Rogers (1972). Comparisons of allele frequencies between samples was performed with the independency G test of Sokal and Rohlf (1979). Values of mean heterozygosities were compared with the Wilcoxon, Mann and Whitney U-test (Infante-Gil and Zárate de Lara 1997).

RESULTS AND DISCUSSION

Three out of the 18 systems tested gave readable results for the seven populations of *T. longipennis*. Enzymes ES, ME and MDH were resolved and showed only one locus each. FUM, GPI, HK, LAP, and PEP showed no activity, ACO, α-GDH, CAT, DIA, GOT, ICDH, IDH, 6PGDH, PGM and SOD produced only weak bands and were discarded from the analysis.

Polymorphism was observed only in ES; ME and MDH were monomorphic. A locus was considered polymorphic when the frequency of the most common allele was not higher than 99% in at least one of the samples. The polymorphic isozyme profiles are shown in Table 1.

The allele frequencies were different among some populations; Sylvatic Jala, San Martín Hidalgo and Autlán populations were the most different. Alleles for the ES locus in Sylvatic Jala were significantly different ($P < 0.05$) as were alleles for the ES locus in San Martín Hidalgo and Autlán ($P < 0.05$) (Table 1).

All F_{ST} values were zero and N_em was 0.25. Nei's genetic distance (D) ranged between 0.001 and 0.038. Gene flow was 0.25, indicating low migration between populations. Nei's genetic distance (D) between populations was low, except between the Sylvatic Jala population and the other four populations from Nayarit (even the nearest Jala population, 10 km distant) and Cárdenas population. In agreement with the low genetic distance, similarity (S) was substantial in most populations and low between the Sylvatic Jala population and the other four populations from the Nayarit state, and between San Martín Hidalgo and the nearest population, Cárdenas (Table 2). Heterozygosity was similar (zero) in all populations since heterozygotes were not detected.

TABLE 1. Allele Frequencies of Polymorphic Locus (ES) in Populations of *T. longipennis* from Western Mexico.

Population	n	Allele		
		110	100	90
Puga[a]	41	0	0.93	0.07
Xalisco[a]	34	0	0.94	0.06
Compostela[a]	43	0.02	0.91	0.07
Sylvatic Jala[b]	10	0.30	0.70	0
Jala[a]	11	0	1.0	0
San Martín Hidalgo[c]	63	0.11	0.81	0.08
Cárdenas[a]	33	0	1.0	0
Autlán[c]	12	0.17	0.83	0

[a,b,c] Groups of locations according to their allele frequencies

TABLE 2. Nei's Genetic Distances (D) (Above Diagonal) and Roger's Similarity (S) Below Diagonal) between Populations of *T. longipennis*.

Population	Puga	Xalisco	Comp.	S-Jala	Jala	Sn. Martín Hidalgo	Cárdenas	Autlán
Puga	____	0.000	0.000	0.019	0.001	0.002	0.001	0.000
Xalisco	0.833	____	0.000	0.023	0.000	0.002	0.000	0.001
Compostela	0.325	0.430	____	0.027	0.001	0.001	0.001	0.002
S-Jala	0.084	0.110	0.093	____	0.038	0.008	0.038	0.001
Jala	0.382	0.369	0.733	0.080	____	0.005	0.000	0.005
Sn. Martín Hidalgo	0.154	0.138	0.224	0.075	0.219	____	0.005	0.001
Cárdenas	0.282	0.157	0.296	0.056	0.609	0.071	____	0.005
Autlán	0.405	0.051	0.325	0.830	0.209	0.167	0.063	____

Differences in allelic frequencies between populations allowed populations to be divided into three groups: (a) Sylvatic Jala, (b) San Martín Hidalgo and Autlán and (c) the remaining four localities. Since Sylvatic Jala was the only sylvatic population collected, it was the most different population, as expected. San Martín Hidalgo and Autlán populations were similar to each other, but different from the other populations, perhaps because they were the two only locations where most specimens were collected in or immediately outside of sleeping rooms, which could be associated with a higher rate of preference for people as a blood meal source. On the other hand, the population of Cárdenas was different from that of San Martín Hidalgo in spite of the fact that they are located no more than 10 km from each other. The difference in this case may be due to the habitats (sleeping rooms and chicken roosts in San Martin Hidalgo and backyards in Cárdenas) where triatomines were collected. Since most houses in the latter are surrounded by brushwood, an active colonization by triatomines from the brushwood to the close backyards could be inferred, different from the more stable populations in San Martín Hidalgo. Unfortunately, no sylvatic specimens could be collected from the brushwood to corroborate such speculation.

Heterozygosity was zero in all populations, since only homozygotes were detected. According to Pasteur et al. (1987), the lack of heterozygotes may de explained by undetected null alleles. This explanation was discarded because an absence of enzymatic activity (homozygotes for the null allele) was not observed in any individual. Two other explanations include unknown locally acting selective factors and biological factors impeding random mating. Selection against heterozygotes is uncommon, especially for enzyme loci, and non-random matings produce local departures from panmixis. However, in the absence of fixed diagnostic alleles allowing the recognition of two sets of different genotypes, this hypothesis is difficult to refute. Genetic similarity among distant localities does not favor this hypothesis.

The subdivision of one locality into smaller panmictic subunits (i.e., the Walhund effect) appears to be the most likely explanation for the overall results. If the subunits differed in allele frequency at a locus with two alleles, and a sample set is collected that includes some members from each unit, then a deficiency in the number of heterozygotes will be observed relative to predictions under Hardy-Weinberg equilibrium conditions (Wahlund 1928). The reasons why these subunits differed in gene frequencies could be nil or low levels of genetic exchange, allowing genetic drift, and probable founding of populations by very few females. In low or recent house infestations by triatomines (low endemicity), it is possible that a population at a single site may arise from a single female, as suggested for *T. infestans* in Bolivia (Breniere et al. 1998).

According to our results, the seven domiciliary populations were very similar and could be considered part of a larger population in the entire study area. It could be inferred that the observed genetic uniformity may be the result of a recent and rapid dispersal of the species in Western Mexico. Further investigations involving more field populations are necessary to validate this hypothesis.

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OCCURENCE OF THE MAIZE BILLBUG, *SPHENOPHORUS MAIDIS*[1] IN EASTERN GAMAGRASS[2]

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Eastern gamagrass, *Tripsacum dactyloides* (L.) L., has a potential to produce 21,300 kg/ha (19,000 lbs/acre) per year of cured hay (Barnett 1988) or an estimated 78-112 kg/ha (70-100 lbs/acre) of pure live seed (Flaim 2001). Maize billbug, *Sphenophorus maidis* (Chittenden), is a well documented pest of corn, *Zea mays* L., as well as sorghum, *Sorghum* spp., cattails, *Typha* spp., and many species of wild grasses, reeds, rushes and sedges (Hayes 1920, Fenton 1940, Vaurie 1951, Manley 1999). Satterthwait (1919) reported the maize billbug utilized corn and eastern gamagrass as food sources, but no observations were made of damage to eastern gamagrass stands.

Damage to the previous year's reproductive and vegetative culms of eastern gamagrass was noted in April 2002 in plantings at Woodward, Oklahoma, in Woodward County and near Fort Supply, Oklahoma, in Harper County. Underground shoots were hollowed out during larval feeding (Fig. 1) and then utilized for pupation.

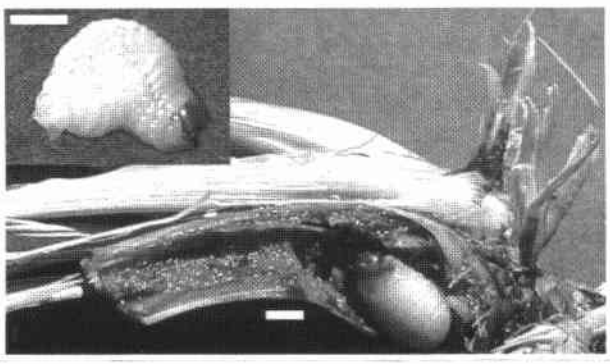

FIG. 1. Larval stage of maize billbug, *Sphenophorus maidis* (Chittenden), consuming the underground stalk of eastern gamagrass, *Tripsacum dactyloides* (L.) L. Inset is larvae removed from stalk. Scale: bar equals 5mm.

[1]Coleoptera: Curculionidae

[2]Contribution of the USDA-ARS Southern Plains Range Res. Stn., Woodward, Oklahoma and the Oklahoma Agric. Exp. Stn., Stillwater, Oklahoma. All programs and services of the USDA are offered on a nondiscriminatory basis without regard to race, color, national origin, religion, sex, age, marital status, or handicap.

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Larval feeding also damaged nearby shoots either by direct damage or by allowing access to opportunistic organisms. Injury to shoot bases by newly hatched larvae often resulted in partial dieback of one side of the leaf blade from the midrib outwards. Characteristic adult billbug feeding injury in corn (Satterthwait 1919), consisting of transverse rows of holes across the blade, was observed in eastern gamagrass plants.

Fifty plants randomly selected from 800 plants in a six-year-old eastern gamagrass germplasm nursery at Woodward, Oklahoma, were found to be 100 % infested. Similar infestations were found in a six-year-old seed production block of 'FGT-1' eastern gamagrass germplasm (Dewald and Kindiger 1996) at Woodward and a 12-year-old grazed 'Pete' eastern gamagrass (Fine et al. 1990) pasture near Fort Supply. Adult, larval and pupal stages of the maize billbug were collected from infested plants. Taxonomic identification of adult maize billbugs was confirmed by Don Arnold, Entomologist, Oklahoma State University, Stillwater, Oklahoma. Voucher specimens were deposited at the K. C. Emerson Entomological Museum, Oklahoma State University, Stillwater, Oklahoma.

Damage inflicted during the life cycle of the pest will have a negative economic impact on seed production from the loss of reproductive tillers. Maize billbug damage may also contribute to the rate of center 'die out' of the plant crown of eastern gamagrass. Although eastern gamagrass is a perennial relative of corn, the control measures used in corn production may not be effective for gamagrass. Research is needed to characterize the life cycle of maize billbug in eastern gamagrass and to determine effective methods of control.

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PRESENT STATUS OF *TRICHOBARIS CHAMPIONI* BARBER (COLEOPTERA:CURCULIONIDAE) IN MEXICO

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Approximately 45,043 hectares of husk tomato, *Physalis ixocarpa* Brot., resulting in the production of >580,000 tons with an estimated value of ca. US $176 million, are cultivated annually in Mexico. The main producer-states are Sinaloa, Jalisco, Michoacán, Puebla, and the state of Mexico (SAGARPA 2002). The most important insect pests attacking husk tomato are *Trialeuurodes vaporariorum* Westwood, *Lema trilineata daturaphila* Kogan and Goeden, *Epitrix* sp., *Macrodactylus mexicanus* Burm., *Trichobaris mucorea* (LeConte), *Trichobaris* sp., *Heliothis subflexa* (Guenée), *Myzus persicae* (Sulzer), *Diabrotica undecimpunctata* Harold, *Euschistus* sp., *Murgantia histrionica* (Hahn), *Lygus* sp., *Draeculacephala* sp., *Paratrioza cockerelli* (Sulc), and *Meromyza* sp. (Jiménez et al. 1992, González 1994).

Collections of insects on husk tomato crops, conducted in early 2000, in the states of Mexico, Morelos, and Puebla indicate an altitudinal distribution of at least two pests that attack husk tomato: *Heliothis subflexa* Guenée and *Trichobaris championi* Barber. *H. subflexa* is found in denser populations at altitudes equal to or higher than 1,800 m above sea level, where it is the major pest of the crop; the presence of *T championi* is negligible at this altitude. However, between 1,800 and 2,200 m above sea level, *T. championi* predominates as the principal pest of husk tomato, and the incidence of *H subflexa* is less than 5%.

Trichobaris championi (Coleoptera:Curculionidae), commonly known as "borreguillo" (larva) or "capichi" (adult), has recently become the major pest attacking husk tomato in the vegetable producing region of the Altiplano Poblano, particularly in the municipalities of Acatzingo, Atoyatempan, Cuapiaxtla de Madero, Felipe Angeles, Huixcolotla, Los Reyes de Jáurez, Pálmar de Bravo, Quecholac, and Tochtepec. This insect was initially observed as a pest in the mid 1990's; today, damage is considered severe. This insect has reduced production from twelve harvestings of ten years ago to three at present, equivalent to a loss of approximately 75% (personal communications with farmers and technical personnel of the State Committee of Plant Health of the State of Puebla).

From early April to September 2002, twelve samples were taken every 15 days in five different husk tomato plots in the Puebla high plateau. Sampling consisted of selecting five sites inside the plot, in which five husk tomato plants were removed and dissected to count the insects present in the stalks and branches. Additionally, adult insects present on the plants also were counted. On the basis of these samples, it was determined that during the vegetative growth stage, from late April to early June, the incidence of plants infested by larvae bored into stems and branches varied between 68.9 and 87.3%. At the end of the crop cycle (second week of June), 77.4% of the plants were found with late-instar larvae, pupae, and adults inside the stems distributed as follows: eggs, 0.0%; larvae boring in stems, 1.3%; pupae in cocoons, 51.6%; pharate adults inside cocoons, 26.6%; and, adults inside stems, 20.5%.

Eight samples taken every 15 days in five different plots in the Puebla high plateau from January to April 2002 indicate that early infestations by adults in seedlings

occur in the months of March and April on land bordering empty lots 5 to 7 days after planting, with a percentage of infestation that varies between 15.3 and 56.5% (average 26.8%). One to three adults were found on the seedlings; typically there was one, to a lesser degree two, and rarely three. Based on these samples, it appeared that the percentages of seedlings infested with *T. championi* eggs fluctuated between 37.6 and 65.2%. The adults arrived on the crop, fed on the foliage and stems for three to five days, mated, and began to oviposit one day later on stems in the middle region of the plant where the first ramification begins.

To oviposit, females made several oval holes on the stems with their rostrum. Holes were approximately 3 mm long and 1-2 mm deep; the length of the hole was aligned along the longitudinal axis of the plant stem. Some of these holes were selected by females for oviposition. Females deposited eggs on the upper edge, almost on the surface, in the direction of the length of the oval, inserting only one egg per hole. Holes used for oviposition were different from the others in that they had a small "tongue" of stem tissue covering the egg which was wrapped inside a bag. Eggs were kidney-shaped, yellow, and measured 0.5-0.7 mm. During the crop's seedling stage, adults fed on foliage, apparently without causing death of the leaf or seedling. Later, damaged seedlings tended to recover so that this damage was of no economic importance.

Originally, *T. championi* was collected from wild plants belonging to the genera *Datura, Solanum* and *Physalis* at sites in the states of Aguascalientes, Colima, Chihuahua, State of Mexico, Morelos, Oaxaca, and Veracruz, and in the Federal District (Mexico City) (Barber 1935). At that time, the insect was reported as a new species, with no mention of it as a pest of husk tomato. Thus, this is the first report documenting the presence of *T. championi* in the Puebla high plateau and its pest status on husk tomato in this region.

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SCIENTIFIC NOTE

PHYLLOPHAGA CRINITA (COLEOPTERA: SCARABAEIDAE): A NEW PEST IN THE HUASTECAS AREA OF MEXICO

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The white grub *Phyllophaga crinita* (Burmeister) is an important soil insect pest of corn, sorghum, wheat, sugarcane, grassland, and turfgrass in Texas and northern Tamaulipas, Mexico (Reinhard 1940, Teetes 1973, Fuchs et al. 1974, Merchant and Crocker 1995, Rodríguez-del-Bosque et al. 1995). Occasional damage has also been reported in parsley, cabbage, field beans, spinach, and sugar beet (Reinhard 1940, Plapp and Frankie 1976, Rodríguez-del-Bosque 1988). Adults have been observed feeding on the foliage of pecans, citrus, *Tamarix* sp., and sunflowers (Reinhard 1940, Rodríguez-del-Bosque 1988), although the host plants range should be numerous during the flight periods. *P. crinita* has a one-year life cycle in northeastern Mexico and southern Texas, although a proportion of the population may have a two-year life cycle in northern Texas (Reinhard 1940, Teetes et al. 1976, Huffman and Harding 1980, Rodríguez-del-Bosque et al. 1995). Reproductive flights of *P. crinita* in northern Tamaulipas peak from late April to early June (Rodríguez-del-Bosque et al. 1995); whereas, most larval feeding activity occurs from July to September, decreases gradually from October to December, and ceases in January-February (Rodríguez-del-Bosque 1996a).

P. crinita is particularly abundant in northern Tamaulipas, where monoculture of grain sorghum for the last five decades might have favored building up its populations in this region (Rodríguez-del-Bosque 1984, Rodríguez-del-Bosque et al. 1995). For instance, *P. crinita* adults captured on standard light traps in northern Tamaulipas (Rodríguez-del-Bosque et al. 1995) are much higher than those captured in several locations of Texas (Reinhard 1940, Gaylor and Frankie 1979, Stone 1986).

The most southern known distribution of *P. crinita* was San Fernando, Tamaulipas (Rodríguez-del-Bosque et al. 1995), 137 km south of the Mexico-USA border. During mid-September 2000, soil sampling in several grain sorghum fields near Tampico, southern Tamaulipas (292 km south of San Fernando), showed high populations of white grubs. Two-hundred larvae were collected from those fields, and reared under laboratory conditions following procedures by Rodríguez-del-Bosque (1996b). All emerging adults (71) were identified as *P. crinita* by Edward J. Riley (Texas A&M University, College Station, TX.). During May-June 2001 and 2002, adults of *P. crinita* were commonly captured in light traps close to the fields sampled in 2000, which confirmed the presence of this species in southern Tamaulipas. In addition, during June 2002, more than 500 adults of *P. crinita* were captured in light traps in Ebano, eastern San Luis Potosí, 55 km west of Tampico, Tamaulipas. In southern Tamaulipas and eastern San Luis Potosí, known as the

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"Huastecas" area of Mexico, nearly 500,000 hectares are planted with different field and horticultural crops. During this period, there have been increasing reports about crops losses by white grubs in this region, including corn, grain sorghum, soybeans, and onions. To minimize damage by white grubs, chemical control is being considered by growers in this area, where application of soil insecticides was not a common practice in the past. These findings indicate that *P. crinita* has become a new potential insect pest in the Huastecas area of Mexico.

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MINUTES OF THE 2003 ANNUAL MEETING OF THE EXECUTIVE COMMITTEE OF THE SOCIETY OF SOUTHWESTERN ENTOMOLOGISTS.

The Executive Committee met at 3:30 p.m., February 24, 2003, at the Renaissance Hotel in Oklahoma City during the Annual Meeting of the Southwestern Branch of the Entomological Society of America. Present were President John Jackman, Darrell Bay, Editor, and Secretary-Treasurer Allen Knutson. The Editor's and Secretary-Treasurer's reports were reviewed and approved. The proposed name change of the Society and progress to compile all back issues of the Southwestern Entomologist onto a CD were discussed. The Committee approved of the contract with Bill Sames for scanning the back issues of the journal and supplement and authorized payment of $500.00 to Bill Sames for expenses. The ballots for President-elect were counted, and there being no further business, the meeting was adjourned at 4:00 p.m.

MINUTES OF THE 2003 ANNUAL MEETING OF THE SOCIETY OF SOUTHWESTERN ENTOMOLOGISTS.

The Annual Meeting of the Society was called to order by President John Jackman at 4:00 p.m., February 24, 2003, at the Renaissance Hotel, in Oklahoma City, OK, during the Annual Meeting of the Southwestern Branch of the Entomological Society of America. The minutes of the 2002 annual meeting as published in the June issue of the Southwestern Entomologist were distributed and approved as printed. The Secretary-Treasurer's report and Editor's report were distributed, reviewed and approved. Editor Bay noted that only the Supplement was mailed in October and that the September and December issues were combined to meet the US Post Office requirement that four issues be mailed each calendar year ending December 31. Appreciation was extended to Carlos Blanco for assisting several members in Mexico with making payment for their membership.

President Jackman, reporting for the Electronic Publication Committee, stated that the Texas A&M web site hosts some information about the Society, but a stand alone web site dedicated to the Society is needed. The cost to maintain a web site would be about $100-200 per month. President Jackman also reported that Bill Sames had scanned all of the back issues of the Southwestern Entomologist and Supplements and that these would be made available as a for-sale CD. The need to update this CD each year and the sale to libraries was discussed. Of a survey of the membership conducted during the annual membership renewal mailing, 65% of the 153 members responding said they would be interested in purchasing this CD at an estimated cost of $25.00. Scott Russell and Bart Drees reported on progress to update the Society's membership brochure and distributed a draft copy. President Jackman recognized Pat Morrison and Frank Gilstrap for their service on the Nominating Committee and reported that Jonathan Edelson had been elected President-elect by the majority of the 154 members who returned ballots. Also, 72% of the members approved of the proposal to change the name of the Society to the Society of Southwestern Entomologists. President-elect Drees offered to assist the Society in making this transition. President Jackman also reminded the members that the Society would share the cost with the Branch and provide a one year's membership to the winners in the Student Poster and Student Paper Competition. President Jackman then thanked the officers of the Society and

its members for their support and passed the gravel to incoming President Bart Drees. Editor Bay awarded John Jackman with a plaque in recognition of his service to the Society. There being no other business a motion to adjourn was made and approved.

Respectfully submitted,
Allen Knutson, Secretary-Treasurer

SECRETARY-TREASURER'S REPORT
for fiscal year February 1, 2002-January 31, 2003

Balance on hand February 1, 2002		$6,190.21
Income:		
Memberships	$ 5,660.00	
Subscriptions	650.00	
Page Charges	27,320.00	
Back Issues	247.65	
Royalties	65.54	
Total Income		$33,943.19
Expenses:		
Journal:		
Editor's Fee	$ 2,000.00	
Printing	25,016.22	
Secretary Fee	2,000.00	
AdMail Mailing Service	1,317.86	
Postage	1,500.00	
Supplies	200.00	
Society:		
Secretary Fee	$ 1,000.00	
Supplies	137.64	
Postage	576.98	
President Plaque	120.97	
Secretary-Treasurer Fee	1,500.00	
Student Paper Competition Award	600.00	
Total Expenses		$35,969.67
Balance on hand January 31, 2003		$4,163.73

As of January 31, 2003, there were 341 members paid for 2002 and 96 institutional subscribers in the Southwestern Entomological Society. There were 10 unpaid page charges totaling $ 2,723.00

Respectfully submitted,
Allen Knutson, Secretary-Treasurer

EDITOR'S REPORT

There were 37 manuscripts, totaling 314 pages, published in the four regular issues of Volume 27 of the Southwestern Entomologist during 2002 compared to 42 manuscripts and 386 pages in Volume 26 for 2001. Volumes 3 and 4 were combined into a single issue in 2002 due to U.S. Postal Service mailing constraints. Additionally, Supplement No. 25, consisting of 137 pages, also was published during the year.

A total of 69 manuscripts were received for consideration for publication during 2002, compared to 55 in 2001. A number of these are still in the review process; however, seven have been rejected as of this date. The total of seven manuscripts rejected is a decrease of five from the twelve manuscripts rejected during 2001, and represents a rejection rate of approximately 10%.

Editor's Financial Report

Date	Description	Receipts/Expenditures	Balance
01/01/02	Balance Forward		$14.94
01/12/02	Postage	2.63	12.31
02/09/02	Postage	8.81	3.50
03/08/02	Postage	9.73	-6.23
04/13/02	Postage	9.74	-15.97
04/27/02	Postage	11.68	-27.65
05/13/02	From Treasurer	100.00	72.35
06/01/02	Postage	5.60	66.75
06/15/02	Postage	15.70	51.05
07/13/02	Postage	12.42	38.63
08/17/02	Postage	41.25	-2.62
09/07/02	Postage	7.88	-10.50
09/18/02	From Treasurer	100.00	89.50
11/23/02	Postage	8.43	81.07
12/27/02	Postage	15.38	65.69
12/30/02	Postage	18.12	47.57

Cash Summary

Balance Forward 01/01/02	$ 14.94
Receipts	200.00
Expenditures	167.37
	$ 47.57 Ending Balance

Respectfully submitted,
Darrell E. Bay, Editor

AUDIT COMMITTEE REPORT

I have examined the financial records of the Society and the Secretary-Treasurer's report for February 1, 2002 though January 31, 2003, and they were found to be in order.

Respectfully submitted
Bart Drees

ECONOMIC INJURY LEVEL FOR THE GREENBUG[1], *SCHIZAPHIS GRAMINUM*, IN OKLAHOMA WINTER WHEAT

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ABSTRACT

The effect of greenbug, *Schizaphis graminum* (Rondani), on the yield of lines of winter wheat *Tricicum aestivum* L., was studied during four years in central Oklahoma. Each year, 0.4-ha of 'Karl' (1993), 'Karl-92' (1995, 1996, and 1997), or '2163' (1997) winter wheat was planted between 1 and 15 October. One-meter square plots were established and infested during the three- to four-leaf growth stage (fall infestations) or during the tillering growth stage in late winter (spring infestations) with varying numbers of biotype-E greenbugs. Infestations by greenbugs achieved by artificial infestation of plots were great enough to affect the wheat yield from infested plots, but the intensity of infestations varied among years and growing seasons. A regression model was constructed to estimate yield loss for the wheat lines tested as a function of the maximum number of greenbugs occurring per tiller in the plot. The regression model predicted a 14.5 kg/ha (0.22 bu/ac) loss of yield for each greenbug per tiller during years with near average precipitation levels, and a loss of 34.3 kg/ha (0.51 bu/ac) under severe drought conditions.

INTRODUCTION

We previously reported on a four-year study of the effect of greenbugs, *Schizaphis graminum* (Rondani), on yield of winter wheat in the field (Kindler et al. 2002). In that report, a model was presented that expressed yield loss of winter wheat as a function of the greenbug-days accumulating per tiller during the growing season. Greenbug-days is an intuitively appealing concept because it relates yield to the intensity of an infestation by greenbugs by integrating greenbug abundance (number of greenbugs per tiller) during the duration of the infestation, and exhibits a strong relationship to yield of winter wheat infested with greenbugs (Kieckhefer et al. 1994, Kindler et al. 2002). However, the greenbug-day concept is not useful as a quantitative measure to determine economic injury levels in practical integrated pest management programs because winter wheat fields are rarely sampled for greenbugs more than one or two times per growing season, thus making it impossible to estimate adequately the number of greenbug-days that accumulate for a field during the growing season.

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[1]Homoptera: Aphididae

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The maximum number of greenbugs that occur per tiller in a winter wheat field is also correlated with yield of the wheat. Although the maximum number of greenbugs per tiller does not predict yield of winter wheat as precisely as greenbug-days does (Kindler et al. 2002), it has the advantage of practical utility. Typically, winter wheat fields in Oklahoma are sampled for greenbugs only if a pest manager has reason to believe that an infestation by greenbugs is building in the field. In this case, the pest manager can conclude that the maximum number of greenbugs that will occur per tiller in the field is the number observed during sampling because the field will be treated with insecticide if this number exceeds an economic threshold. Here we construct a linear regression model to relate yield loss caused by greenbugs to the maximum number of greenbugs that occurs per tiller in a field of winter wheat. In addition, we create a table that lists economic injury levels based on the maximum number of greenbugs per tiller in relation to the cost of insecticide treatment and the value of the winter wheat grain.

MATERIALS AND METHODS

A detailed description of field experimental methods is presented in Kindler et al. (2002). Therefore, only a brief description is presented here. The study was conducted during four wheat growing seasons in a field 7.5 km west of Stillwater, Oklahoma. 'Karl' (1993), 'Karl-92' (1995, 1996, and 1997), or '2163' (1997) winter wheat was planted in 0.4-ha plots between 1 and 15 October each year. One-meter-square plots were established and infested during the three- to four-leaf growth stage (fall infestations) or during the tillering growth stage in late winter (spring infestations) with varying numbers of biotype-E greenbugs. Aphids were excluded from some plots for the duration of the study by periodic treatment with malathion® insecticide. Fall infestations were allowed to persist until heavy freeze each (approximately 20 December), after which plants were kept free of greenbugs by periodic insecticide treatment. Plots infested during spring were kept aphid free during autumn and were allowed to persist until wheat plants headed. Greenbugs were sampled by cutting 10 tillers from random locations in each plot. In the laboratory, the number of greenbugs per tiller was determined for each sample. Samples were taken approximately every two weeks starting 7 days after initial infestation in fall. Sampling was discontinued during the winter period, when no aphids were present in any of the plots. When the wheat seed ripened, five 0.3-m samples of row were harvested from each plot for yield determination.

Heterogeneity-of-slopes linear regression (Neter and Wasserman 1977) was used to relate yield for each plot to the maximum number of greenbugs per tiller measured during the growing season in the plot. Regression analyses were accomplished using the PROC REG procedure of the Statistical Analysis System (SAS Institute 1990).

RESULTS AND DISCUSSION

Our previous analysis revealed that slopes of regression lines relating yield to greenbug-days did not differ significantly for any of the years or infestation periods (fall or spring) during which wheat was infested, with the exception of the 1995-1996 growing season during which severe drought occurred in central Oklahoma (Kindler et al. 2002). As would be expected, regression of yield against the maximum number of greenbugs per tiller was similar to the previously reported model in that slope parameters did not differ significantly among years or infestation periods except for the 1995-1996 wheat growing season. Here we report two regression models, one that includes unique intercepts for each year and wheat line and unique slopes for 1995-96 and all other years combined, and a

second model in which a single slope parameter is used to describe the relationship between yield and the maximum number of greenbugs per tiller (Table 1). For the regression model with different slope parameters for the drought (1995-1996) and growing seasons with approximately average or above average precipitation, the coefficient of determination was $r^2 = 0.78$. The estimate of the slope was equal to -34.3 ($SE = 5.9$) for the growing season with severe drought. The slope estimate for the growing season with severe drought was over twice in magnitude of the estimate of –14.5 ($SE = 1.8$) obtained for the other three growing seasons. The intercepts for various growing seasons differed among wheat growing seasons for Karl-92 (the only wheat line tested in more than one year) from 2,667 kg/ha for 1995-1996 to 4,613 kg/ha in 1997-1998 (Table 1). As was expected, the slopes relating yield to the maximum number of greenbugs per tiller were negative for all growing seasons. For the regression model with all four growing seasons combined the coefficient of determination was $r^2 = 0.74$. The estimate of the slope parameter was -17.7 ($SE = 1.9$).

TABLE 1. Parameters for Linear Regression Models Relating kg/ha Wheat Yield (bu/ac in Parentheses) to the Maximum Number of Greenbugs per Wheat Tiller.

Growing Season/ Wheat Line	Multiple Slopes[a]		Single Slope[b]	
	Intercept	Slope	Intercept	Slope
1993-1994 / Karl	3,155 (46.9)	-14.5 (-0.22)	3,170 (47.1)	-17.7 (-0.26)
1995-1996 / Karl-92	2,667 (39.7)	-34.3 (-0.51)	2,268 (33.7)	-17.7 (-0.26)
1996-1997 / Karl-92	2,880 (42.8)	-14.5 (-0.22)	2,899 (43.1)	-17.7 (-0.26)
1997-1998 / Karl-92	4,613 (68.6)	-14.5 (-0.22)	4,687 (69.7)	-17.7 (-0.26)
1997-1998 / 2163	5,494 (81.7)	-14.5 (-0.22)	5,573 (82.9)	-17.7 (-0.26)

[a]The linear regression model has two slopes, one for growing seasons with approximately average or above average precipitation, and one for drought conditions.
[b]The linear regression model has a single slope parameter for all growing seasons.

Our results distinguish an increase in the loss in yield of winter wheat caused by greenbug infestations under conditions of severe drought from that under normal or above normal precipitation. However, our data are insufficient to provide guidelines on the nature of the relationship between greenbug infestation levels and drought. Therefore, it seems prudent at this time to use the regression model with a single slope parameter for all growing seasons in estimating yield loss of winter wheat caused by greenbugs. Economic injury levels based on the yield loss model for all years are listed in Table 2 in terms of grain value per bushel in relation to per-acre control costs. Economic injury levels range between two and 18 greenbugs per tiller depending on grain value and control cost. The wide range in economic injury levels illustrates the importance of estimating the number of greenbugs per tiller in the field before making control decisions, or using sequential sampling plans with appropriately chosen stop-lines (e.g., Giles et al. 2000) when making control decisions for greenbugs in winter wheat in Oklahoma.

TABLE 2. Economic Injury Levels for Greenbugs (Number /Tiller) in Relation to Control Cost and Grain Value.

Grain Value ($ / bu)	Control Cost ($ / ac)								
	4.00	5.00	6.00	7.00	8.00	9.00	10.00	11.00	12.00
2.50	6	8	9	11	12	14	15	17	18
3.00	5	6	8	9	10	12	13	14	15
3.50	4	5	7	8	9	10	11	12	13
4.00	4	5	6	7	8	9	10	11	12
4.50	3	4	5	6	7	8	9	9	10
5.00	3	4	5	5	6	7	8	8	9
5.50	3	3	4	5	6	6	7	8	8
6.00	3	3	4	4	5	6	6	7	8
6.50	2	3	4	4	5	5	6	7	7
7.00	2	3	3	4	4	5	5	6	7

ACKNOWLEDGMENT

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OVIPOSITION BY *LYGUS HESPERUS*[1] AND ITS EGG PARASITOID, *ANAPHES IOLE*[2], IN COTTON, ALFALFA, AND A WILD MUSTARD

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ABSTRACT

Oviposition patterns of *Lygus hesperus* Knight and its parasitoid *Anaphes iole* Girault were observed in three host plants. The majority of the *L. hesperus* eggs were deposited in the upper third of cotton, *Gossypium hirsutum* L., Var. Deltapine 16, and mixed cultivars of alfalfa, *Medicago sativa* L., and in the upper 2/3 of wild mustard, London rocket, *Sisymbrium irio L.*, plants in cages. In choice tests, *L. hesperus* deposited significantly more eggs (78%) in alfalfa than in cotton but did not show a significant preference between alfalfa and London rocket. *Anaphes iole* readily parasitized eggs of *L. hesperus* in all three plant types, but appeared to search and parasitize eggs on all sections of the plants rather than restrict searching to the areas where most of the host eggs were located. Significantly, more *L. hesperus* eggs were parasitized in alfalfa than in cotton, but a greater percentage of eggs were parasitized in cotton. More eggs were parasitized in London rocket than in alfalfa, but the percentages parasitized were not significantly different.

INTRODUCTION

Lygus hesperus Knight is the predominate species of three *Lygus* spp. that feed on agricultural crops in the southwestern U.S.A. (Graham et al. 1982). *L. hesperus* feeds on a wide variety of host plants (Scott 1977) and migrates from one host to another as they become available during the year (Graham et al. 1986). Alfalfa, *Medicago sativa* L., and a wild mustard, London rocket, *Sisymbrium irio* L., are common host plants of *Lygus* species in southern Arizona crop areas (Stitt 1949; Fye 1972, 1975; Graham et al. 1986). Cotton, *Gossypium hirsutum* L., is not a preferred host of *L. hesperus* (Sevacherian and Stern 1974), but cotton may suffer damage when large numbers of *L. hesperus* move from recently cut alfalfa to feed. Strip-cutting alfalfa and interplanting alfalfa with cotton were proposed as methods to prevent this movement (Stern et al. 1967, Stern 1969). In addition, populations of *L. hesperus* may develop on London rocket and then move to alfalfa when the weeds mature (Graham et al. 1986). Damage to alfalfa grown for hay is not considered serious; however, damage can be economically severe when alfalfa is grown for seed. London rocket germinates in late fall and flowers from December to April (Graham et al. 1986), but the growth period may be extended or contracted by the timing and amount of rainfall (Parker 1972). Stands are usually restricted to patches along roadsides, ditch banks, and ends of fields but may cover large areas of fallow

[1] Heteroptera: Miridae
[2] Hymenoptera: Mymaridae

fields. London rocket also grows as a weed in alfalfa during winter and early spring, when alfalfa is still growing slowly so that the two plant types may be found growing in the same agricultural area or interspersed in the same field.

The mymarid wasp, *Anaphes iole* Girault, is a parasitoid of *Lygus* spp. eggs and the most common parasitoid of *L. hesperus* (Graham and Jackson 1982, Jackson and Graham 1983, Graham et al. 1986). *A. iole* has been reared from alfalfa and London rocket stems and from cotton leaves collected from the field (Graham and Jackson 1982, Graham et al. 1986). *Lygus* spp. deposit large numbers of eggs in alfalfa and London rocket in southern Arizona and large percentages of these eggs may be parasitized by *A. iole* so that both plant types are key hosts for survival and population increase of both pest and parasitoid. The relationship between *Lygus* spp. and its hosts, alfalfa and London rocket, and the effect of this relationship on parasitism of *Lygus* spp. are of obvious importance. An understanding of this relationship is necessary before a decision to control London rocket in alfalfa fields and in the surrounding areas is made.

I did a series of tests to determine ovipositional preference by *L. hesperus* and *A. iole* for plant type (cotton vs alfalfa and alfalfa vs London rocket) and to further determine egg distribution on each of these plant types.

MATERIALS AND METHODS

Cotton (DPL 16), alfalfa (mixed cultivars), and London rocket plants were used for experiments on ovipositional behavior of *A. iole* and *L. hesperus*. The stages of the plants that are most susceptible to damage or those on which the largest populations of *L. hesperus* occur were used. For alfalfa, these were plants with buds or blooms and for cotton, these were plants with small buds (squares) or blooms. Plant stage was less critical for London rocket because blooms are present during much of its growth. For each set of tests, plants of about the same height were used. All plants were grown in the greenhouse to prevent oviposition by wild *L. hesperus*, with the exception of some London rocket plants which were transplanted from the field. These were held in a greenhouse until any *Lygus* spp. eggs had hatched and parasitoids had emerged.

The *L. hesperus* were collected in the field from alfalfa or London rocket with the exception of four cages of one test, when they were reared in the laboratory on green beans. The use of field-collected *L. hesperus* probably added to the variability of the data because age and ovipositional status could not be controlled. However, Zaugg and Nielsen (1974) found that laboratory-reared *L. hesperus* responded differently to odors from alfalfa than did field-collected ones. The *A. iole* were collected from alfalfa near Tucson, Arizona, and maintained as a laboratory culture that was supplemented with field collections each year. One-day-old females, which had been caged with males since emergence, were exposed to *L. hesperus* eggs in beans four to six hours before use in the tests.

Two types of cages were used in these tests. Cage Type-1, used to study preference by *L. hesperus* and *A. iole* for plant species and preference by *L. hesperus* for plant height, consisted of a wooden frame, 51cm on each side and 62cm in height, that was screened with nylon organdy. Four slots, 2x14cm, were cut into the bottom so that the stems of plants in pots fit into the slots and the cage bottom rested on top of the pots. Type-2 cages, used to study preference by *A. iole* for cotton plant height, were made of cylindrical sections designed to cover a 15-cm vertical portion of the cotton plant. The 29-cm diameter cylinders were made of 5x7 per cm^2 mesh aluminum screening that prevented movement of *L. hesperus* adults between sections. Two or three of the individual sections were used for each plant, depending on the plant height.

The data were analyzed by one-way analysis of variance or by Kruskal-Wallace one-way ANOVA on ranks when the data were not normally distributed (SigmaStat

1995). The numbers of *L. hesperus* eggs and parasitoids reared from the stems were transformed by square root and percentages were transformed by arcsin square root. Numbers are given as the mean and standard error of the mean.

Oviposition by L. hesperus on Cotton and Parasitism by A. iole. Eight Type-1 cages were used to determine *L. hesperus* ovipositional patterns on cotton and parasitism of *L. hesperus* eggs by *A. iole*. Potted cotton plants were placed in the cages, two plants per pot and four pots per cage. Twenty *L. hesperus* females were put into each cage for 48 h, then removed and replaced with eight one-day-old *A. iole* females for 24 h. After the unparasitized *L. hesperus* eggs on the plants hatched (8-10 days), the plants were cut into 15-cm sections from the apex and the numbers of hatched and unhatched eggs in each section counted to determine the number of eggs at each height. All sections of the two plants in each pot were held together to obtain oviposition data on *A. iole* so that no information on height preference was collected for the parasitoid. Ten additional days were allowed for parasitoid emergence, after which the stems were examined for eggs with the caps (opercula) still intact. These were dissected for evidence of development, but unemerged parasitoids were added to the number emerged to obtain a total count of parasitoids.

Parasitism of L. hesperus Eggs at Different Heights on Cotton Plants by A. iole. Individual cotton plants were enclosed with sectional cages (Type-2) to determine if *A. iole* preferred a particular height on cotton plants to parasitize *L. hesperus* eggs. The number of leaves in each 15-cm section was counted before the cages were put into place. One *L. hesperus* female per large leaf was confined to each section for 48 h, after which the sectional cages were removed, the plants placed individually in Type-1 cages, and the whole plant exposed to female parasitoids for 24 h. One female parasitoid for each five leaves (rounded to the nearest five) was released into each of nine cages with plants with two sections as was one female for each 2.5 leaves in six cages with plants with three sections.

One week after exposure to the parasitoids, the plant stems were divided into the marked 15-cm sections, the numbers of eggs were counted, and each section was held separately until parasitoid emergence. The plants sections were re-examined 8-10 days later for eggs containing unemerged parasitoids, and the total number of emerged and unemerged parasitoids was recorded.

Oviposition by L. hesperus and Parasitism by A. iole on Cotton vs. Alfalfa. Five Type-1 cages, each containing two pots with two cotton plants each and two pots with one alfalfa plant each, were set up to test preference between these two plant types. All but six of the longer stems were clipped from each alfalfa plant, so that there were 12 alfalfa stems and four cotton plants in each cage. The pots with the two plant types were placed in the four corners alternately around the cage and the positions were rotated in the next cage. Alfalfa plants with buds, but not blooms, and cotton plants with flower buds were used. The plants in each cage were exposed to 20 female *L. hesperus* for 48 h and then to 16 *A. iole* females (one per cotton plant and alfalfa stem) for 24 h.

Oviposition by L. hesperus and Parasitism by A. iole on Alfalfa vs. London Rocket. Type-1 cages were used to determine the preference by *L. hesperus* to oviposit in alfalfa or London rocket and the preference by *A. iole* to parasitize *L. hesperus* eggs in these two plant species. The number of eggs laid by *L. hesperus* in the two plant types was tested in 19 replicate cages. *L. hesperus* (20 per cage) were enclosed with two alfalfa plants and two London rocket plants in each cage for three days, after which they were removed and replaced with eight *A. iole* for three days, so that both pests and parasitoids were given a choice of plants. Plants were selected for similar heights.

The number and percentage of *L. hesperus* eggs parasitized by *A. iole* were determined in the same 19 cages plus eight additional cages (27 replicates total). For the eight additional cages, sixteen potted alfalfa plants and 16 London rocket plants were

enclosed individually (no choice of plant species) with ten *L. hesperus* females for three days. The *L. hesperus* were removed and the plants were combined in the eight cages. Each cage contained two pots with one alfalfa plant in each pot and two pots with one London rocket plant in each. Eight *A. iole* were released in each cage for three days. Therefore, only the parasitoids were given a choice of the two plant types in these eight cages.

The plants from nine cages were divided into 15-cm sections after exposure to *L. hesperus* and *A. iole* females to determine the distribution of *L. hesperus* eggs and from four cages to determine the distribution of parasitized eggs. After the eggs hatched, the numbers of *L. hesperus* eggs deposited in each section were counted. The plant sections were held an additional ten days or until the adult *A. iole* emerged and were counted. The eggs with the caps still intact were examined for unemerged parasitoids. These were counted and added to the number of emerged parasitoids for a total count.

RESULTS AND DISCUSSION

Oviposition by L. hesperus on Cotton and Parasitism by A. iole. The average height of the portions of cotton plants exposed to the insects in the eight cages was 46.5 cm. There were 105 flower buds (squares) on the plants. *L. hesperus* deposited 54.5% of 2,040 eggs in the upper 15cm of the plants, 32.8% in the section 15-30cm below the top of the plant, and 12.7% in the bottom section. This trend for more eggs located in the upper part of plants is supported by the work of Benedict et al. (1981) who showed that 66% of *L. hesperus* eggs were deposited in the top one-third (approximately 24cm) of glanded cotton (Acala 4-42-77); 21% were found in the middle third and 13% in the bottom portion.

L. hesperus deposited 81.4% of eggs in the leaves (Table 1). Most eggs were deposited in the petioles within 2cm of the enlarged portion at the base of the leaf blade. These results agree with those of Benedict et al. (1981) who found that 69-87% of the *L. hesperus* eggs were deposited in leaves of glanded Acala 4-42-77; 64-78% of these were found in the leaf petioles in the upper half of the plants.

The eight *A. iole* in each of the eight test cages parasitized 42.6 ± 6.91 (17.4 %) *L. hesperus* eggs within a 24-h period. The number of *A. iole* reared from *L. hesperus* eggs did not increase significantly with the number of host eggs in a plant pair ($r = 0.2588$, $P = 0.1526$, $df = 1, 30$). The plant sections and plant parts were not held separately, so that no data were obtained on parasitism of eggs in the separate sections.

Tingey and Leigh (1974) were concerned that differences in plant height might affect *L. hesperus* egg deposition in cotton varieties of different heights in cages. They found that *L. hesperus* oviposited significantly more in the taller plants (varieties) in cages when given a choice. The plants in this test were of similar height to eliminate a choice of taller plants.

Parasitism of L. hesperus Eggs at Different Heights in Cotton Plants by A. iole. For this test the *L. hesperus* were confined to distinct 15-cm sections of the plant for oviposition. The parasitoids were given access to the entire plant to determine if there was a preference to parasitize *L. hesperus* eggs in a particular plant section. Even though the *L. hesperus* were not given a choice of plant height, 72.2% of the eggs were deposited in the top 15cm of the plants with two sections (Table 2). In taller plants with three sections, similar numbers were deposited in the 0-15 (39%) and the 15-30-cm (41.7%) sections. The percentages of eggs parasitized in the different plant sections were not significantly different ($P > 0.05$). Thus, the data show that *A. iole* did not show a preference for searching a particular height, but searched all sections of the plants used in this test (Table 2). Variability in the percentage of eggs parasitized are partially due to the low numbers of parasitoids released in the cages. Averages of 2.4 and 4.8 female

parasitoids were released in cages with plants with two and three sections, respectively.

TABLE 1. Location of *L. hesperus* Eggs in Various Parts of Cotton Plants Held in Greenhouse Cages.

Location	Number of eggs in indicated section[a]				% of total eggs
	0 - 15	15 - 30	30 - 45	> 45	
Main stem	87	15	10	0	5.5
Leaf petiole	671	401	153	0	60.0
Leaf base	123	126	52	0	14.8
Leaf vein	55	28	5	0	4.3
Leaf scar	0	6	10	0	0.8
Terminal[b]	64	--	--	--	3.1
Axillary branch					
Stem	61	17	1	0	3.9
Square	14	2	0	0	0.8
Stem of Square	7	3	0	0	0.5
Bud[c]	15	39	27	0	4.0
Leaf petiole	12	26	1	0	1.9
Leaf base	4	4	0	0	0.4
Leaf vein	0	1	0	0	0.05

[a] Stems were divided into 15-cm sections from the apex of the plants.
[b] For this test, "terminal" is all parts of the plant above the topmost expanded leaf, usually 2-4cm in length.
[c] "Bud" refers to the axillary meristem.

Oviposition by L. hesperus and Parasitism by A. iole on Cotton vs. Alfalfa. When offered a choice between alfalfa and cotton plants, *L. hesperus* preferred to oviposit in alfalfa (Table 3). Significantly more ($F = 58.7597$, df = 1,18, $P < 0.0001$) eggs were found in alfalfa than in cotton. Overall, 78.2% of 2,409 *L. hesperus* eggs were deposited in the alfalfa plants. Significantly more ($F = 18.2457$, df = 1,18, $P < 0.0004$) eggs were parasitized in alfalfa plants than in cotton plants. However, a larger percentage ($F = 5.7521$, df = 1,18, $P < 0.05$) of the eggs in cotton plants were parasitized than of those in alfalfa. These results show that *A. iole* searched both plant species. Considering that *A. iole* searched for only 24 h, the 22.3% parasitism of *L. hesperus* eggs on alfalfa and 36.5% on cotton was substantial.

An average of 53–63% of the total number of eggs in both cotton and alfalfa was deposited in the top 15cm and the number decreased with each successively lower section (Table 4). This trend was observed for the cotton plants in previous tests. Approximately one-half of the eggs in alfalfa plants were deposited in the main stem in the top 15cm. Graham and Jackson (1982) found a similar distribution of *Lygus* spp. eggs in field-collected alfalfa stems.

In the present study, the different plant sections were not put in separate cages for parasitoid emergence, so parasitism data was not obtained for the different plant sections of either plant species. However, in a field study by Graham and Jackson (1982), parasitism levels of 4, 17, 18, and 20% were found for eggs in successive 15-cm segments from the top to bottom of alfalfa plants. Lower parasitism levels of eggs in the

TABLE 2. Parasitism of *L. hesperus* Eggs by *A. iole* in Different 15-cm Sections of Cotton Plants[a].

No. plant sections	No. plants	0 – 15cm		15 – 30cm		30 – 45cm	
		Eggs per plant	% parasitism[b]	Eggs per plant	% parasitism[b]	Eggs per plant	% parasitism
2	9	106.5 ± 16.71	5.1 ± 1.83	40.9 ± 8.47	2.4 ± 1.34		
3[c]	6	68.2 ± 11.19	9.9 ± 3.65	72.8 ± 14.34	9.6 ± 4.80	33.5 ± 12.56	10.6 ± 3.99

[a] Data are presented as the mean ± SEM. Plants were measured from the top.

[b] Differences in percentage parasitism of eggs in the plant sections in each row were not significant ($P > 0.05$) as shown by ANOVA of arcsin square root transformed data ($F = 0.1459$, df = 1,16 for plants with two sections and $F = 0.1420$, df =2, 15 for plants with three sections).

[c] Twice as many *Anaphes iole* were released in the 3-section plants (one per 2.5 leaves) as in the 2-section plants (one per 5 leaves).

TABLE 3. *L. hesperus* Oviposition and Parasitism by *A. iole* in Choice Tests of Cotton and Alfalfa Plants Caged in a Greenhouse.

Plant type	No. eggs deposited[a] $\bar{x}$ ± SEM	No. eggs parasitized[a] $\bar{x}$ ± SEM	% eggs parasitized[a] $\bar{x}$ ± SEM
Alfalfa	188.3 ± 15.92	39.6 ± 4.51	22.3 ± 3.10
Cotton	52.6 ± 9.43	17.0 ± 2.83	36.5 ± 5.06
Significance level[b]	<0.0001	0.0004	0.0275

[a] Numbers of eggs deposited and parasitized per plant were transformed to the square root and the percentage of eggs parasitized by arcsin square root (N = 10).
[b] Significance levels were determined on plants (alfalfa) or pairs of plants (cotton) by ANOVA or by Kruskal-Wallace one-way ANOVA on ranks.

TABLE 4. Oviposition by *L. hesperus* in Sections of Cotton and Alfalfa Plants in Choice Tests in Cages in a Greenhouse.

Plant sections (cm)[a]	Number eggs ($\bar{x}$ ± SEM)[b]		Distribution (%) eggs	
	Cotton	Alfalfa	Cotton	Alfalfa
0 - 15	33.1 ± 5.46	98.9 ± 13.98	62.9	52.5
15 - 30	10.7 ± 2.04	53.1 ± 6.34	20.3	28.2
30 - 45	7.9 ± 2.89	21.1 ± 4.27	15.0	11.2
> 45	0.9 ± 0.38	15.2 ± 3.57	1.7	8.1

[a] Plant sections were measured from the apex; the > 45 cm section sometimes exceeded 15 cm in length.
[b] Means are based on two cotton plants and one alfalfa plant per pot and two pots per cage in five cages (N = 10).

tops of the plants in the field may have been due to rapid plant growth, so that eggs there would have been recently deposited and exposed to the parasitoids for a shorter period of time.

Oviposition by L. hesperus and Parasitism by A. iole on Alfalfa vs. London Rocket. The number of eggs deposited by *L. hesperus* in the two plant species was compared in 19 cages. There was no significant difference (F = 1.6376, df = 1,36, P = 0.2088) in the number of eggs deposited in alfalfa and London rocket plants. Parasitism of *L. hesperus* eggs in the two plant types was compared by the number of parasitized eggs and by the percentage of eggs parasitized per cage (27 cages) (Table 5). Significantly fewer parasitized eggs were found in alfalfa than in London rocket (F = 4.5378, df = 1,53, P = 0.0379), but the percentages of parasitized eggs were not significantly different (F = 1.6847, df =1,52, P = 0.2000) (Table 5). These data indicate that neither *L. hesperus* nor *A. iole* demonstrated a marked preference for either plant species under the test conditions, but that there was a tendency to choose London rocket over alfalfa. In this test, the plants were chosen for the growth stages on which the largest *Lygus* spp. populations were found, but differences in growth stage could possibly alter preference for a particular species.

Data for the distribution of the eggs of *L. hesperus* and those parasitized by *A. iole* in different sections of alfalfa and London rocket plants were collected from nine cages

TABLE 5. *Lygus hesperus* Oviposition and Parasitism by *A. iole* in Alfalfa and London Rocket Plants in Greenhouse Cages in Choice Tests.

Plant	No. cages	No. eggs Per cage [a]	N	No. eggs parasitized [a]	N	% parasitism [b]
Alfalfa	19	73.3 ± 7.41	27	4.8 ± 0.94	27	5.7 ± 1.17
London rocket	19	91.2 ± 8.47	27	9.8 ± 1.75	27	8.6 ± 1.66
Significance		$P = 0.2088$		$P = 0.0379$		$P = 0.2000$

[a] Number of eggs and numbers of eggs parasitized in the two plant types were analyzed by ANOVA of square root transformed counts. The average numbers per plant type per cage were used to get the $\bar{x}$ ± SEM.

[b] The percentages of eggs parasitized were transformed by arcsin square root before analysis by Kruskal -Wallace one-way ANOVA on ranks.

for the *L. hesperus* eggs and four cages for the parasitized eggs (Table 6). Most of the *L. hesperus* eggs were deposited in the upper 30cm in both alfalfa and London rocket, but there was a difference in the distribution on the plant species. They largest proportion of the eggs was found in the top 15cm in alfalfa (58.7%) and in the top 30cm in London rocket (69.7%). Graham and Jackson (1982) found similar trends for *Lygus* spp. eggs in alfalfa and London rocket for field-collected stems.

TABLE 6. Distribution of Parasitized and Unparasitized Eggs of *L. hesperus* in Alfalfa and London Rocket Plants in Cages in a Greenhouse.

Plant section	No. eggs deposited per plant[a]		% Eggs in plant sections[a]		%Eggs parasitized[a]	
	Alfalfa	London rocket	Alfalfa	rocket	Alfalfa	rocket
0 - 15	42.7 ± 4.35[b]	18.3 ± 4.63[b]	58.8	26.8	1.1	13.07
15 - 30	18.8 ± 4.2	29.2 ± 6.14	25.8	42.9	2.1	6.2
30 - 45	8.3 ± 2.26	9.4 ± 1.84	11.8	13.8	0.0	0.0
> 45	2.6 ± 0.86	11.9 ± 2.30	3.5	16.5	2.6	1.6

[a] The percentage of parasitized eggs was based on four cages (8 plants of each kind) and the number and distribution of *L. hesperus* eggs by section on nine cages (18 plants of each kind).

[b] Numbers are $\bar{x}$ ± SEM.

The data for the distribution of parasitized *L. hesperus* eggs are limited, especially for those in alfalfa stems, but a general trend for the parasitized eggs on London rocket is apparent from the data (Table 6). Very low levels of parasitism were found in the alfalfa stems and no conclusions are apparent. Graham and Jackson (1982) studied the distribution of parasitized eggs in field-collected alfalfa and London rocket stems over a two-year period. The total numbers of parasitized eggs in their study were also low, but trends were obtained. In alfalfa stems, more parasitized eggs were found in the 15-30-cm section, followed by the 0-15-cm section, but the 0-15-cm section had the lowest percentage of parasitism. Parasitism levels at 15-30, 30-45, and more than 45cm from the apices of the stems were all similar (17-20%). In field-collected London rocket stems, the distribution of the number and percentage of parasitized eggs was more even

among plant sections (Graham and Jackson 1982). Percentage parasitism ranged from 21% in the 15-30-cm section to 45% in the 0-15 and 30-45-cm sections.

In summary, *L. hesperus* showed a preference to oviposit in alfalfa over cotton in choice tests, but showed no significant preference between alfalfa and London rocket. *Anaphes iole* parasitized *L. hesperus* eggs on all three plant types. The number of eggs parasitized was greater in alfalfa, where the larger number of eggs were deposited, than in cotton, but the percentage of eggs parasitized was higher in cotton than alfalfa. There was no difference in the number of eggs deposited in alfalfa or London rocket, nor was there a difference in the percentage of eggs parasitized. However, a larger number of the eggs in London rocket were parasitized. *Lygus hesperus* tended to oviposit in the top one-third of cotton plants and the top two-thirds of alfalfa and London rocket. *Anaphes iole* searched and parasitized eggs in all parts of the plants.

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INCIDENCE OF THE BEET LEAFHOPPER, *CIRCULIFER TENELLUS* (HOMOPTERA:CICADELLIDAE) IN NEW MEXICO CHILE

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ABSTRACT

Chile fields were sampled for the beet leafhopper, *Circulifer tenellus* (Baker), vector of beet curly top virus (BCTV) during 2001 and 2002 in two New Mexico counties every two weeks using yellow sticky traps. Weed hosts of the beet leafhopper and BCTV were collected from the fields at the same times and tested for virus using PCR. Higher numbers of leafhoppers were collected and more virus detected in 2001 than in 2002. In both years, leafhopper flights into chile fields initiated in April to May, peaked in June-July, and dropped off by late October to mid-December. However, the timing of leafhopper flights differed between the two counties sampled. Virus incidence in the weeds was higher (1.3%) in 2001 than 2002 (0.06%), and no virus symptoms were observed in the weeds.

INTRODUCTION

Beet curly top virus (BCTV) epidemics have occurred sporadically in the southern chile pepper growing areas of New Mexico since the first report in the state in 1927 (Crawford 1927). In three of the last 10 years, New Mexico chile sustained substantial losses due to curly top. In 2001, a year with high curly top incidence in New Mexico, the chile yield per acre was 12% less than in 2000 or 2002, years with minimal curly top pressure (NASS-USDA 2003). Chile plants infected with curly top are often severely stunted, with chlorotic leaves and, if infected early, do not produce fruit. Chile fruit that is produced by plants infected later in the season is usually small and round. No effective control measures are known for curly top on chile.

BCTV is a monopartite geminivirus of the genus *Curtovirus*, which is characterized by a circular ssDNA genome within twin spherical particles. Molecular characterization of BCTV in sugarbeet has demonstrated that the virus primarily exists as three strains (CFH, Worland, and California/Logan), and variants of these strains (Stenger and McMahon 1997). BCTV infects a broad range of hosts that, in addition to chile, includes numerous crops and weeds in many plant families (Bennett 1971).

The virus is transmitted in a circulative manner by the beet leafhopper, *Circulifer tenellus* (Baker), a member of the subfamily Deltocephalinae of the homopteran family Cicadellidae. The beet leafhopper is endemic throughout the western and southwestern US, preferring arid and semi-arid conditions. The leafhopper vector feeds and breeds on an extensive range of plant families (Cook 1967). *C. tenellus* exists as three morphological types: a summer morph, a winter morph, and a migratory morph (Severin 1921). The summer morphs survive 3-4 months, while the winter morphs live longer and consist

primarily of mated overwintering females. Migratory morphs of the leafhopper are capable of flying several hundred miles (Dorst and Davis 1937).

The flight patterns of the leafhopper in California are well described, where *C. tenellus* overwinters in the foothills on weeds, with the nymphs presumably acquiring virus from these hosts (Cook 1967). As these weeds dry, the mature leafhoppers migrate into the interior valleys in the spring, feeding on and infecting crops and weeds. Spring movements of the leafhoppers in California have also been correlated with temperature accumulation (Cook 1945). The leafhoppers progress through several generations before moving back into the foothills in the fall.

The current migratory patterns of the beet leafhopper in New Mexico are not as well studied. The Rio Grande Valley was reported to be a spring and summer breeding area for the beet leafhopper in 1939, where they undergo three to five breeding cycles (Romney 1939). The leafhoppers were thought to spread in the spring from this area to areas north and east, including northern New Mexico, west Texas, and portions of Oklahoma, Kansas, and Colorado. However, the land use patterns in New Mexico have changed dramatically since the 1930's when sugarbeet was a primary crop.

The weed host range of the beet leafhopper is well-documented (Bennett 1971), although data on relative BCTV incidence in weeds is limited (Creamer et al. 1996). A perennial mustard was reported to be the most important overwintering host of the leafhopper in much of the breeding area of New Mexico in the 1930s (Romney 1939). The same weed is now scarce at best. Given the changes in crops and weeds in the past 60 years and the reoccurring losses to chile due to the virus, we felt an update of the status of the beet leafhopper in New Mexico was necessary. This paper reports the incidence of beet leafhoppers in chile fields in two years in New Mexico, one with high curly top disease pressure and one with low pressure. In addition, we report the disease incidence in weed hosts for the two years.

METHODS AND MATERIALS

Incidence of leafhoppers in chile was assayed by trapping insects from the margins of chile fields from February 2001 through December 2002. In 2001, nine fields were sampled, five in Luna County and four in Doña Ana County, New Mexico, while in 2002, ten fields (five from each county) were sampled. Since chile is not usually grown in the same field in consecutive years, chile fields located near those sampled in 2001 were chosen for testing in 2002. Four yellow sticky traps (20 x 25 cm) were placed approximately 61 cm from the ground at the margins of each test field. Traps were changed every two weeks and leafhoppers identified and counted. Meteorological data were gathered from weather stations located near the two groups of chile fields. Degree days were calculated using the single sine method using a 50°F (10°C) lower threshold.

Incidence of BCTV in chile fields was estimated by counting symptomatic plants in 100 randomly chosen plants in test fields and selected additional fields. This method underestimates the total amount of virus infection in a field in a season, since symptomatic plants are removed during crop thinning.

At two-week intervals, weeds were collected from the margin of each chile field. After the plants were identified to species, 0.5-g leaf samples were ground in liquid nitrogen, and total DNA extracted (Palmer et al. 1998). BCTV was amplified by PCR using a viral specific primer set 5'-GTGGATCAATTTCCAGACAATTATC-3' and 5'-CCCATAAGAGCCATATCAAACTTC-3', which amplifies a portion of the coat protein gene. Primers that specifically amplify the Worland strain (5'-CCAGGACTTAAGGGCTTCATTT-3' and 5'-GGAGGCCAGCAGACGGCTAA-3') and CFH strain (5'-TCTACGTCATCAATGACGTT-3' and 5'-AGCTCCTCGCTATAAATACA-3') were used to determine the strain of BCTV after

plants had been determined to contain the virus. PCR reactions were carried out in a total volume of 50 µl containing 20.5 µl H_2O, 5 µl 10X buffer (100 mM Tris-HCl, pH 8.3 500 mM KCl, 0.01% gelatin), 1 µl 10 mM dNTPs, 3 µl 25 mM $MgCl_2$, 5 µl each of 5mM primers, 4 U Taq DNA polymerase, and 10 µl of 1:10 dilution of purified DNA.

Amplification using primers to detect all BCTV strains and BCTV-Worland was carried out with the following parameters: 35 cycles consisting of 94°C for 30 sec, 59°C for 60 sec, and 72°C for 90 sec. A final extension of 72°C for 5 min followed. Amplification to detect BCTV-CFH was done similarly except that the annealing step was done at 53°C for 60 sec. Amplification products were separated by electrophoresis on a 2% agarose gel, and the bands visualized with ethidium bromide.

RESULTS AND DISCUSSION

The beet leafhopper vector of BCTV was trapped from all fields assessed during the nearly two-year period. *C. tenellus* appeared in chile fields in Doña Ana County during the end of March and beginning of April in 2001 and 2002, and in Luna County during early to middle May in 2001 and 2002 (Figs. 1, 2). Leafhopper numbers in 2002 peaked in June in Doña Ana County, while in Luna County, the leafhopper numbers peaked in August. In both areas surveyed, the number of leafhoppers trapped per field decreased below 20 by the end of October 2002, while in 2001, leafhopper numbers did not drop below 20 per field until mid-December. In both years, more leafhoppers were trapped from Doña Ana County fields than from Luna County.

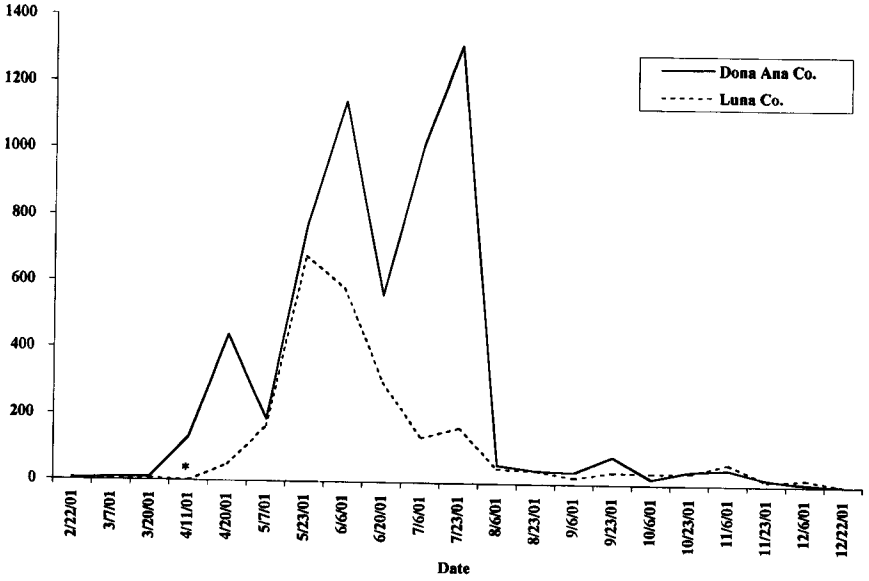

FIG. 1. Mean numbers of adult *Circulifer tenellus* caught on yellow sticky traps from the margin of chile fields in two New Mexico counties in 2001. Note maximum value on Y axis. Asterisk denotes date when no data was available for Luna County due to severe dust storms.

FIG. 2. Mean numbers of adult *Circulifer tenellus* caught on yellow sticky traps from the margin of chile fields in two New Mexico counties in 2002. Asterisks denote dates when no data was available for Luna County due to severe dust storms.

The maximum number of leafhoppers trapped in a field in 2001 (3,500/field, Fig. 3) was substantially more than in 2002 (145 leafhoppers/field). In 2001, one field located in Rincon, NM, trapped much higher leafhopper numbers than any other field from mid-April through July (Fig. 3), although disease incidence was not different from the other fields in Doña Ana County tested.

Although increased numbers of beet leafhoppers appeared in Doña Ana County chile fields at least a month earlier than Luna County fields, the 2001 and 2002 January to April temperatures (accumulated degree days) were similar between the two areas. Doña Ana County fields accumulate 411°F (210°C) degree days (DD) in 1 January-31 March 2001 and 398°F (203°C) DD for the interval during 2002, compared to 388°F (196°C) DD for Luna County for the same three months in 2001 and 442°F (226°C) DD in 2002. This also suggests that temperature was not the primary factor in determining the flight of the beet leafhopper in this study. This differs from Cook's (1945) results which suggest that beet leafhoppers leave their breeding grounds as soon as they achieve maturity.

During the January to April period for both years Luna County received more precipitation (1.36 in., 1 January-31 March 2001; 1.35 in., 1 January–31 March 2002) than Doña Ana County during the same interval (0.99 in., 2001; 0.93 in., 2002). The extra precipitation could have delayed the leafhoppers departure from overwintering weed hosts in Luna County compared to Doña Ana County, by delaying the drying of the weeds. Drying of the overwintering weed hosts is thought to be one of the primary factors that contribute to spring flights (Carter 1930, Severin 1933).

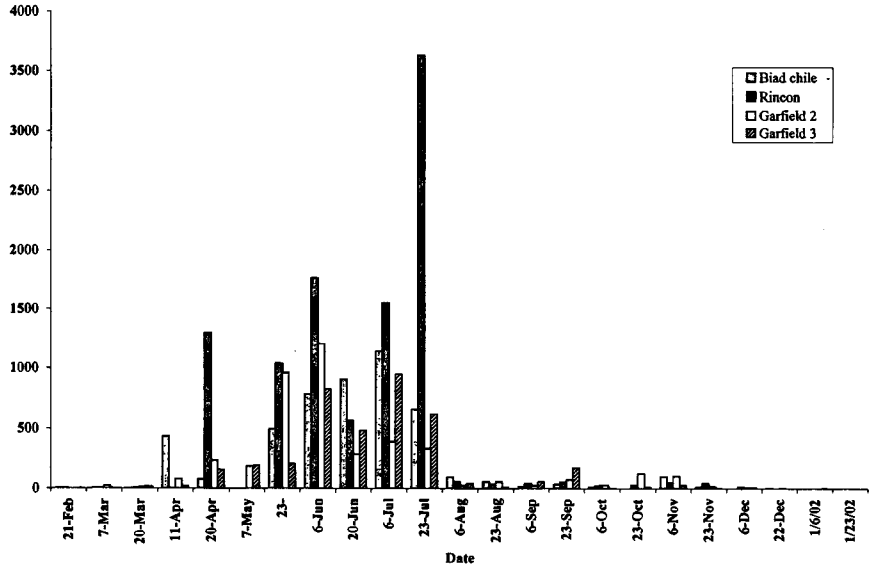

FIG. 3. Numbers of adult *Circulifer tenellus* caught on yellow sticky traps from the margin of chile fields in 4 fields within Doña Ana County in 2001. Note maximum value on Y axis.

The incidence of curly top in chile in 2002 (0.5-1%) was substantially less than in 2001 (30-50%). The BCTV incidence in weeds was correspondingly lower. The incidence in weeds is likely a more accurate estimate of the actual level of disease in the environment since infected weeds do not show disease symptoms, thus sampling is not biased. We found 1.3% incidence of BCTV in weeds in 2001 (9 infected / 686 plants tested) compared to 0.06% in 2002 (1 infected / 1,768 plants tested).

Four weed species were found to be infected with BCTV (Table 1). These have all been previously reported as hosts for the virus (Bennet 1971), and all have been reported as infected in field collections in California (Creamer et al. 1996). Other frequently tested plants that did not test positive for BCTV infection included bindweed (*Convolulus arvensis* L.), 119 plants tested; spurred anoda (*Anoda cristata* (L.) Schlecht), 114 plants tested; ground cherry (*Physalis wrightii* Gray), 197 plants tested; and Russian thistle (*Salsola iberica* Senne & Pau), 315 plants tested. The lack of infected Russian thistle in the field, even though collected for 10 months of the year, is similar to our findings in California where although the plant was frequently collected, it was never found to be infected with BCTV (Creamer et al. 1996).

TABLE 1. Beet Curly Top Virus-Infected Weeds Collected in 2001-2002.

Species	Number infected / number tested	
	2001	2002
Amaranthus sp. (pigweed)	2/237	0/174
Sisymbrium irio L. (London rocket)	2/115	0/252
Chenopodium sp. (lambsquarters)	2/57	0/125
Kochia scoparia (L.) (Schrader)	3/18	1/195

All the BCTV-infected plants were found to contain the Worland strain of the virus. In addition, one *Chenopodium* sp. was also found to be infected with the CFH strain. Infected plants were collected in June, July, August, and September of 2001 and in August of 2002. The 2002 virus-infected sample was collected from Doña Ana County, while in 2001, eight infected samples were collected from Doña Ana County and one from Luna County.

The collection of BCTV-infected weeds at the margins of chile fields through much of the 2001 growing season emphasizes the need for more stringent weed control. The four species of infected weeds are all reported to be hosts for the beet leafhopper (Cook 1967) as well as the virus, and thus, could serve as source of virus for infection into chile fields.

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BIOLOGY OF THE VINE MEALYBUG[1] IN VINEYARDS IN THE COACHELLA VALLEY, CALIFORNIA

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ABSTRACT

Vine mealybug, *Planococcus ficus* (Signoret) (Homoptera: Pseudococcidae), biology was studied in several vineyards in the Coachella Valley from 1994–1998 and in 2000 using a variety of sampling techniques. It was determined that all life stages of VMB could be found on all parts of the vine throughout the year, above and below ground. In spring, densities of VMB increased dramatically, peaking in mid-late spring. This increase in density was probably due to increased reproduction and movement of VMB throughout the vine. Densities of VMB declined dramatically in early summer and remained low throughout the summer, fall, and winter. Rates of parasitism on VMB were low in all of the years of sampling. Those mealybugs that were exposed (e.g., leaves and clusters) were more heavily parasitized than those on more protected parts of the vine (e.g., roots). The primary parasites, *Anagyrus pseudococci* (Girault), *Pseudaphycus* spp., and *Leptomastidea abnormis* (Girault) (Hymenoptera: Encyrtidae), were recovered. A secondary parasite, *Chartocerus* spp. (Hymenoptera: Signiphoridae), was also recovered.

INTRODUCTION

In the early 1990's, grape growers in the Coachella Valley of California began experiencing a reduction in the quality and yield of their table grapes due to a mealybug. It was assumed that the mealybug causing these problems was the grape mealybug, *Pseudococcus maritimus* (Ehrhorn) (Homoptera: Pseudococcidae), a pest of grapes in central and northern California. However, specimens sent to the California Department of Food and Agriculture-Plant Pest Diagnostic Laboratory and the US National Museum in 1994 were identified as the vine mealybug, *Planococcus ficus* (Signoret) (Homoptera: Pseudococcidae) (Gill 1994). Until this identification, the vine mealybug (VMB) was known as an economic pest of grapes only in the Mediterranean region of Europe, Africa and the Middle East, South Africa, Pakistan, and Argentina. In these areas of the world, VMB has also been reported to attack fig, avocado, mango, and pomegranate (Gill 1994).

Management of VMB is complicated by the cryptic nature of this mealybug, which spends much of its life cycle either beneath the bark of the vine or on roots at the soil-air

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interface (Duso 1989). Therefore, biological control was the management tactic targeted for development in a cooperative project initiated in 1994 among the California Department of Food and Agriculture–Biological Control Program, the Riverside County Department of Agriculture, and the University of California at Riverside. The objectives of this project were to identify the indigenous parasitoids attacking VMB, elucidate VMB biology on grapes in the Coachella Valley, and to conduct foreign exploration and introduce exotic parasitoids of VMB. Each agency had a specific role assigned: California Department of Food and Agriculture–Biological Control Program and Riverside County Department of Agriculture surveyed indigenous parasitoids and conducted studies on the biology of VMB, and the University of California conducted foreign exploration and introduction of exotic parasitoids. Results of the surveys for indigenous parasitoids and studies on the biology of VMB on table grapes in the Coachella Valley are reported.

MATERIALS AND METHODS

All studies were conducted in vineyards in the Coachella Valley from 1994 through 1998 and in 2000. The vineyards sampled were selected for having large densities of VMB. The number of vineyards surveyed and the methods used to sample VMB varied with the specific objectives of each study. Although information on parasitoids was recorded over the 5-year period, the survey for indigenous parasitoids was emphasized in 1994 and 1995. From 1996–1998 and in 2000, the studies conducted emphasized the biology of VMB.

Indigenous parasitoids of VMB were surveyed by collecting plant parts infested with VMB and holding the mealybugs in rearing containers for the emergence of any parasitoids. From 15 November 1994 through 28 May 1995, collections of leaves, fruit clusters, roots, and canes were made every other month in four vineyards. Beginning on 26 June 1995, samples were taken monthly until 4 October 1995, and then every other month from 27 November 1995 through 19 March 1996. In each vineyard, ten vines were selected to sample using systematic sampling with a random start. At each vine, the trunk, roots, leaves, clusters (when present), and "buried canes" (i.e., canes in contact with the soil), when present were sampled. For the trunk, the sampling consisted of searching for 3 minutes by scraping bark from the trunk and inspecting the scraped area for VMB. The roots of the vine were dug out, and then searched for 3 minutes. Sections of roots containing VMB were clipped and placed in vials. At each vine, six leaves were also collected, and if clusters were present, one cluster was collected. The soil surface near the sampled vine was also inspected for the presence of buried canes, which were collected when found. All plant material was returned to the laboratory and sorted. The number of VMB and VMB mummies (i.e., parasitized VMB) were recorded for each plant part and sample date. The VMB were placed in rearing containers with a sprouted potato to allow the development of any parasitoids that may have been present. The VMB mummies were placed in vials and held for emergence of the adult parasitoid.

The biology of VMB was investigated from 1996 through 1998 and in 2000 by assessing VMB density and activity on various plant parts using a variety of sampling methods. In 1996, 1998, and 2000, all studies were conducted in one vineyard, a certified organic block of Superior Seedless grapes. In 1997, studies were conducted in the certified organic vineyard and in a block of conventionally managed Thompson Seedless grapes. For all years, VMB mummies that were found were collected, returned to the laboratory, and held for the emergence of the adult parasitoid. The number and identity of the parasitoids were recorded.

The biology of VMB in 1996 was investigated using three sets of 12 vines that were sampled monthly from 14 May through 19 November and at bimonthly intervals

from 19 November through 21 January 1997. The first set of 12 vines was randomly selected and sampled repeatedly. On 19 March 1996, loose bark was removed from the trunk of each vine, a 250-cm^2 area marked on the exposed trunk, and then covered with a layer of burlap. In addition, a section of a small root (1-2cm diameter) was exposed, loosely wrapped in a sheet of plastic, and reburied. The intention of these manipulations was to delimit specific areas on the vine for repeated sampling beginning 14 May, while providing physical protection from predators of VMB. On each sample date, the coverings on the trunk and roots were removed, the number of VMB and mummies counted in situ, and the coverings replaced. In addition, eight leaves were collected from each vine and returned to the laboratory. The number of VMB and mummies found on each leaf were recorded.

The second set of 12 vines was immediately adjacent to the first set and was used to investigate the effects of artificially covering the exposed trunk. For these vines, the bark was removed from the trunk; a 250-cm^2 area was marked on the trunk, but no covering was placed over the exposed area. On each sample date, the number of VMB and mummies were counted in the delimited area on the trunk.

The third set of 12 vines was used in an attempt to track VMB movement throughout the vine. On each sample date, 12 vines were randomly selected and the bark removed from three areas of the cordon and green cane (i.e., current year's growth). These areas were proximal, medial, and distal to the trunk. In these areas, sticky traps consisting of two widths of double-sided sticky tape (width of tape=1.9cm) were placed encircling a cordon or cane. On each sample date, the traps were removed from the field. The number of each size class of VMB on each tape, and the length of each tape were recorded.

Studies on the biology of VMB in 1997 were conducted on 18 vines in the certified organic vineyard and six vines in the conventionally managed vineyard beginning 24 February 1997 with sampling at monthly intervals from 2 April through 24 November 1997. The vines were specifically selected for having observable VMB infestations in January. On each sampling date, one side of the trunk (from the soil line to about 30cm above the soil line) was examined, and the number of VMB and mummies found recorded. In addition, ten leaves, one bud, and one cluster, when present, were collected for each vine and returned to the laboratory. The numbers of VMB and mummies found on each plant part were recorded. In the organic vineyard, the roots on six vines, selected at random from the 18 sample vines, were exposed, and the number of VMB and mummies found recorded. No roots were examined in the conventionally managed vineyard.

To investigate VMB movement, sticky traps were placed around an arm, a cordon, a brown cane (i.e., previous year's growth), and a green cane on each vine. Each trap consisted of a single width (1.9cm) of double-sided tape. The tapes were replaced on each sample date. The number of each life stage of VMB and mummies on each tape and the length of the tape were recorded.

In 1998, all studies were conducted on 12 vines that were selected for having a readily observable mealybug infestation in March. Two types of sticky traps and refuge areas were placed on the vines on 22 April and monitored through 26 August 1998. On each vine, a single width (1.9cm) of double-sided tape was placed around an arm, cordon, and brown cane. In addition, a yellow sticky trap (7.6cm x 12.7cm) was hung under the canopy of each vine. The sticky tapes and yellow traps were replaced every 2 weeks. The number of each life stage of VMB and mummies on each tape, and the length of the tape were recorded. The number of male VMB and the number and species of parasitoids found on each yellow trap were recorded.

Refuge areas were also established on an arm, cordon, and brown cane on each vine opposite the sticky tapes. Each refuge consisted of a 5.08-cm wide strip of bubble wrap (~3.5 bubbles per cm^2) wrapped around the appropriate plant part and held in place with

duct tape. When initially establishing the refuge areas, a series of three wraps was placed on each plant part on each vine. Each wrap in a series was assigned at random to a 6-week, 12-week, or 18-week sampling interval. Those wraps assigned to the 6-week interval were replaced with new wraps when removed for the next 6-week sampling interval. The wraps that were removed were placed in a vial along with any mealybugs adhering to the vine underneath the wrap and returned to the laboratory. The numbers of VMB and mummies found under each wrap were recorded.

In 2000, VMB biology was investigated using sampling methods that had been the most successful in previous years. This included field counts, sticky tape traps, yellow sticky traps, and refuge areas. On 24 February, 270 vines with active VMB infestations were selected and randomly assigned to one of three groups: field counts (50 vines), trapping (20 vines), and refuge areas (200 vines). Sampling was conducted monthly from 24 February through 7 November 2000.

The field count method was used to investigate changes in the VMB population on different parts of the vine through time. At the beginning of the study, each vine within the 50-vine group was assigned at random to one of ten subgroups to be used on one sampling date. All vines in one subgroup were examined for VMB on the trunk, cordon, and roots. The VMB and mummies were collected, returned to the laboratory for counting, and held for parasitoid emergence and identification. The number and size class of VMB, the number of mummies, and the parasitoid species found on the various parts of the vine were recorded.

The trapping method was used to investigate VMB movement through space and time, and to determine the general activity of VMB males and parasitoids. The same 20 vines were sampled throughout the study. In February, the loose bark was removed from portions of the trunk and one cordon on each vine. A sticky tape trap was applied to the exposed areas. A yellow sticky trap was hung in the canopy of each vine. All traps were replaced monthly. The number of each size class and/or life stages of VMB and the number of each VMB parasitoid species were recorded for each trap.

The refuge areas were used to investigate parasitism of VMB. The 200 vines were assigned randomly to one of ten subgroups. Each subgroup was used on a different sampling date. On each sampling date, the loose bark was removed from an area of the trunk and cordon on each vine, and a refuge area (i.e., band of bubble wrap) was applied to the exposed area. After one month, the refuge areas were removed, returned to the laboratory, and the size class of VMB, their numbers, and the number of mummies recorded. The VMB and mummies were then held for parasitoid emergence and identification.

The counts of VMB on various plant parts were summarized as means and standard errors per plant part for each sample date and vineyard. The sticky trap data was summarized by calculating the means and standard errors for VMB per cm^2 of tape on each plant part for each sample date and vineyard. The yellow sticky trap and refuge data were summarized by calculating the total number of VMB, mummies, and parasitoids recovered for each sample date. The levels of parasitism for VMB from the field counts and refuge areas were calculated as the ratio of the number of mummies (intact and emerged) to the total number of VMB collected (expressed as percentage) for each sample type, sample date, and vineyard.

RESULTS AND DISCUSSION

The preliminary survey of indigenous parasitoids attacking VMB in the Coachella Valley revealed low levels of parasitism (Table 1). The primary parasitoid *Anagyrus pseudococci* (Girault) (Hymenoptera: Encyrtidae) was recovered on all sample dates for

which parasitism was detected. From the samples collected 6 September and 27 November 1995, another parasitoid belonging to the genus *Chartocerus* (Hymenoptera: Signiphoridae) and thought to be hyper-parasitic was recovered. On 6 September 1995, 70% of the parasitoids recovered were hyperparasitoids, and 14.6% were hyperparasitoids on 27 November 1995.

Although generally low, the amount of parasitism varied with the exposure of the mealybug. For VMB with few places to hide such as on leaves or clusters, parasitism rates were as high as 22% and 13%, respectively (Table 1). For those VMB that were hidden or protected, such as on roots or buried canes, parasitism rates rarely exceeded 1% (Table 1).

TABLE 1. Parasitism and Total Numbers of VMB on Different Parts of the Vine in the Coachella Valley in 1994 – 1996.

	Percent Parasitism (Total No. of VMB)			
Date	Root	Buried Cane	Cluster	Leaf
15 Nov 1994	0.1 (711)	0.4 (6,983)	NS[a]	0.7 (143)
16 Jan 1995	0 (276)	NS	NS	NS
28 Mar 1995	0 (1,379)	NS	0 (16)	0 (104)
23 May 1995	0 (314)	NS	0 (14)	0.3 (179)
26 Jun 1995	0 (36)	0.4 (510)	12.9 (74)	2.4 (40)
2 Aug 1995	0 (117)	0.2 (1,367)	9.8 (37)	22.0 (8)
6 Sept 1995	1.2 (81)	0.4 (4,548)	NS	0 (15)
4 Oct 1995	0 (59)	0.3 (3,060)	NS	5.3 (18)
27 Nov 1995	0 (458)	0.1 (1,224)	NS	0 (18)
23 Jan 1996	0 (14)	NS	NS	NS
19 Mar 1996	0 (112)	NS	NS	0 (4)

[a]NS = No sample taken

The patterns of VMB seasonal abundance were similar for all sampling methods over the four years of study. Field counts of VMB on various parts of the vine in the organic vineyard in 1996, 1997, and 2000 began to increase in late May through June, peaking in May or June (Figs. 1, 2a, 3). Densities of VMB generally declined during the summer with a slight increase in density in September and October (Figs. 1, 2a). There was a second peak in density in August in 2000 (Fig. 3). For this peak, three out of the five vines sampled had very large densities of VMB, and two had no VMB. This demonstrates the clumped nature of VMB distribution within the vineyard, and, consequently, the limitations of data from only a few samples.

The counts of VMB on various plant parts also revealed that there was a resident population of VMB on the trunk, and to a lesser degree, on the roots throughout the year (Figs. 1, 2a, 3). The extent of VMB habitation of roots is influenced by many factors such as ant activity, soil texture, temperature, and irrigation regimes. Even in January when vines were pruned, VMB could be found throughout the vine (e.g., trunk, roots, the base of buds, pruning scars, etc.). In addition, reproducing VMB females could be found throughout the year (J. Ball, unpublished data). As the vines developed canes, leaves, and clusters in the spring, VMB moved outward from the main stem or trunk colonizing the new plant parts (Figs. 1, 2a, 3).

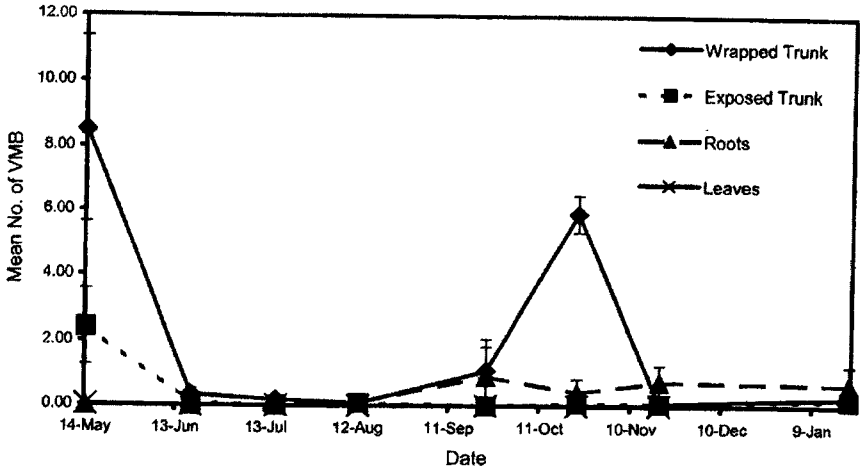

FIG. 1. The mean number of VMB found on the various parts of the vine in field counts in 1996. The bars represent the standard errors of the means.

The trend in VMB density in 1997 on vines in the conventionally managed vineyard was similar to that seen in the organic vineyard in (Figs. 1-3). The density of overwintering VMB in February was much lower in the conventionally managed vineyard (Fig. 2b). In addition, this vineyard was treated with methomyl in late March, further reducing VMB densities in April (Fig. 2b). From May through August, VMB densities resurged, followed by a decline in September through November (Fig. 2b). No VMB were found on leaves or clusters, possibly due to the lower density of VMB on the trunk early in the season.

In the field counts in 2000, parasite mummies were recovered from February through October. No mummies were recovered from the root samples. The parasite *A. pseudococci* was recovered a few months prior to the recovery of *Leptomastidea abnormis* [(Girault), Hymenoptera: Encyrtidae]. Both parasites had been released within the vineyard from 1998 through 2000, but outside the study plots.

The collections made on sticky tape traps in 1996–1998 and in 2000 demonstrated the same population trends as those found using the direct count method. In general, there was an increase in the number of VMB trapped throughout the spring with a decline occurring in mid-summer. There appeared to be a resident population of VMB on the trunk throughout most of the year with movement outward to other plant parts during the spring (Figs. 4–6). In the conventionally managed vineyard in 1997, the movement outward was delayed slightly due to the application of methomyl in late March (Fig. 5b).

The trap catches on the yellow sticky traps placed in the vineyard in 1998 and 2000 show patterns consistent with the field count and sticky tape trap data. VMB male flight peaked in mid-July in 1998, and in April in 2000 (Fig. 7). The peak in VMB male flights occurred about 1 month after the peak trap catch for the younger life stages (Fig. 6). The delay in development of VMB males in 1998 compared to 2000 was due to a much colder than normal spring in 1998, and a warmer than normal spring in 2000 (www.ncdc.noaa.gov).

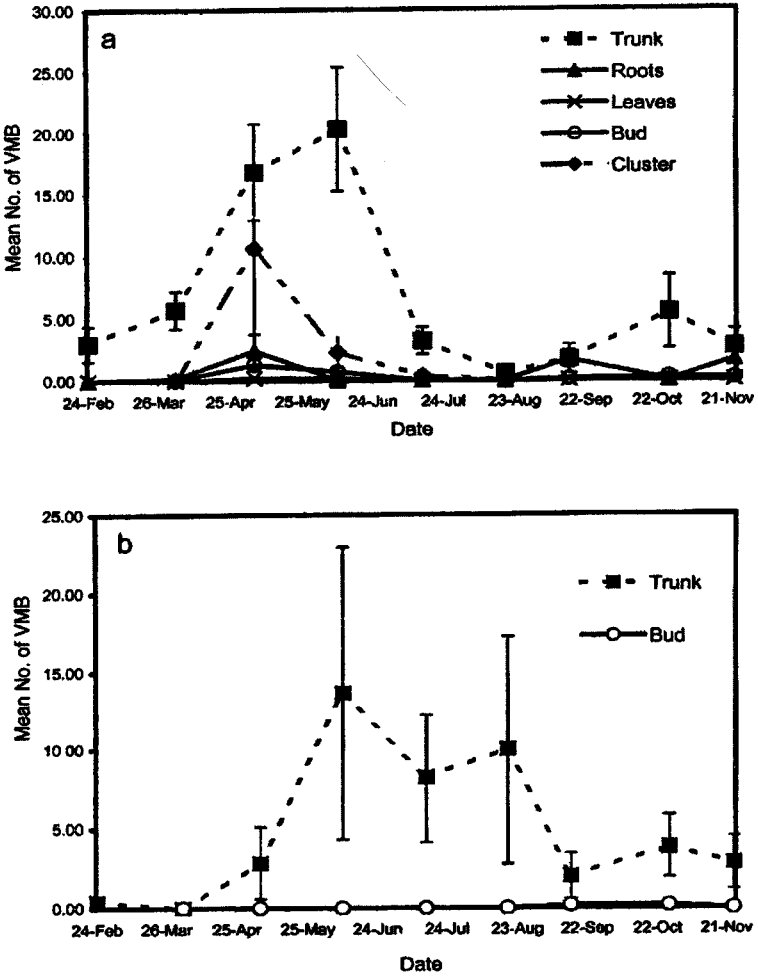

FIG. 2. (a) The mean number of VMB found on various parts of the vine in field counts in an organic vineyard in 1997. (b) The mean number of VMB found on various parts of the vine in field counts in a conventionally managed vineyard in 1997. Methomyl was applied to this vineyard in March. For both graphs, the bars represent the standard errors of the means.

The yellow sticky traps also trapped the adults of the two parasites that attack VMB. *Anagyrus pseudococci* was found on the traps in both years before *L. abnormis*. In 1998, peak trap catches for both parasites occurred in July, whereas, *A. pseudococci* peaked in May and *L. abnormis* peaked in June in 2000 (Fig. 7).

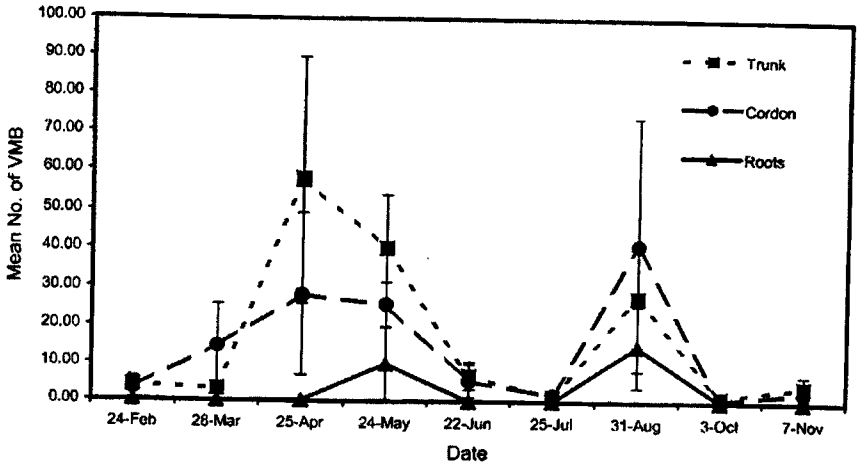

FIG. 3. The mean number of VMB found on various parts of the vine in field counts in 2000. The bars represent the standard errors of the means.

The refuge areas placed on the vines in 1998 and 2000 did harbor parasitized VMB (Table 2). Refuge areas left in the field over short periods of time allowed better detection of the parasites because the parasitized VMB and/or the evidence of parasitism was not destroyed with the passage of time (Table 2). The parasites from the refuge areas were found at approximately the same time as the adult parasites were found on the yellow sticky cards (Table 2 and Fig. 7).

Over all years of sampling, the rates of parasitism on VMB were relatively low. Those VMB that were more exposed (e.g., on leaves and clusters) were more heavily parasitized than those in protected locations (e.g., on roots and buried canes). Augmentation of the parasite populations did increase the amount of parasitism on VMB within the release vineyard.

Combining the data from all years and all sampling techniques, the biology and seasonal phenology of VMB were pieced together. The data were consistent with the idea that there was a resident population of VMB on all parts of the vine throughout the year. Even in winter when VMB was at its lowest density, it could be found high on the vine such as under bud scales (J. Ball, unpublished data), and some of the aerial population was probably removed during winter pruning. There was no winter aestivation or diapause because all life stages of VMB could be found throughout the year. In spring, densities of

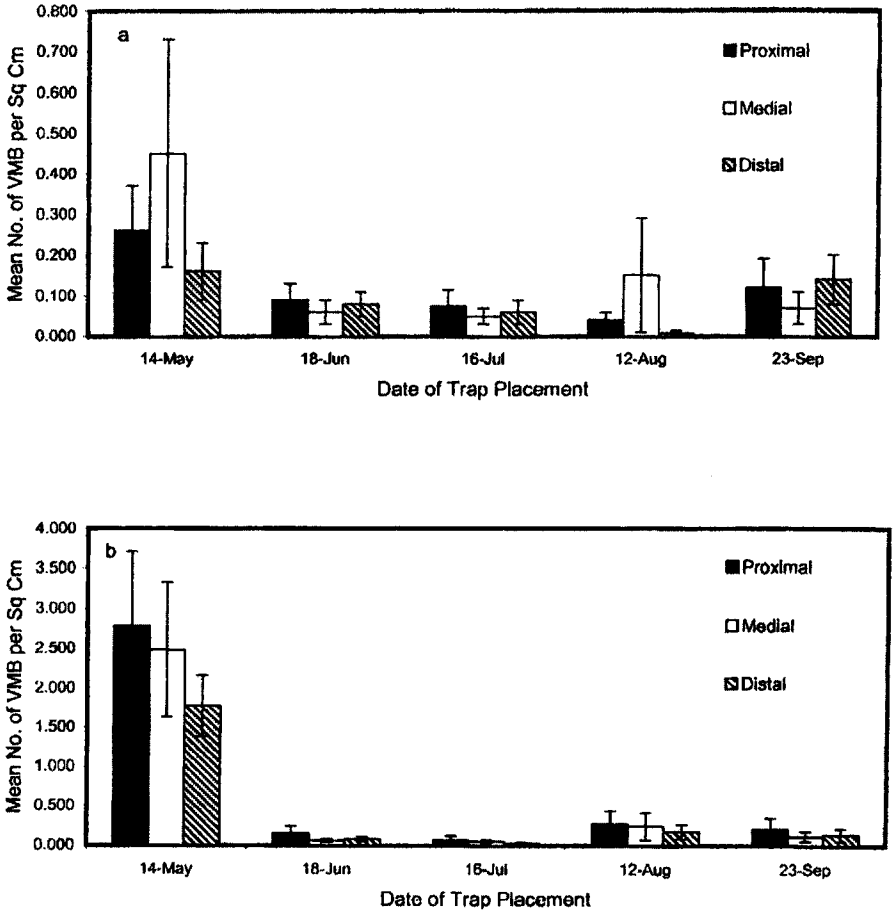

FIG. 4. (a) The mean number of VMB per sq cm of sticky tape trap placed on canes in a vineyard in 1996. (b) The mean number of VMB per sq cm of sticky tape trap placed on cordons in a vineyard in 1996. For both graphs, the bars represent the standard error of the means.

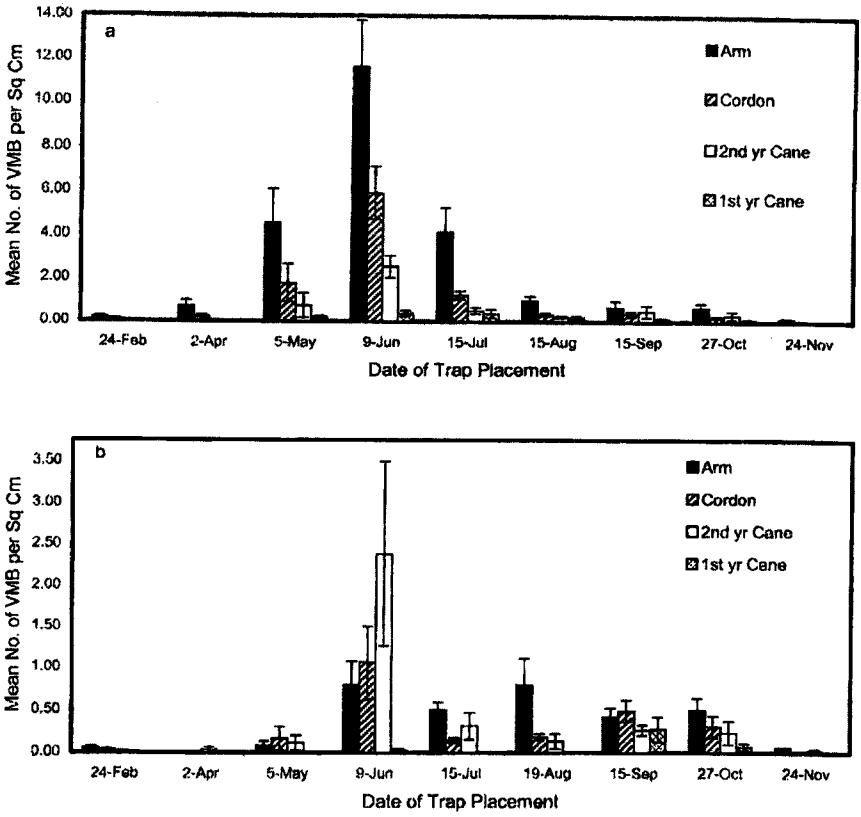

FIG. 5. (a) The mean number of VMB per sq cm of sticky tape trap placed on various parts of the vine in an organic vineyard in 1997. (b) The mean number of VMB per sq cm of sticky tape trap placed on various parts of the vine in a conventionally managed vineyard in 1997. For both graphs, the bars represent the standard errors of the means.

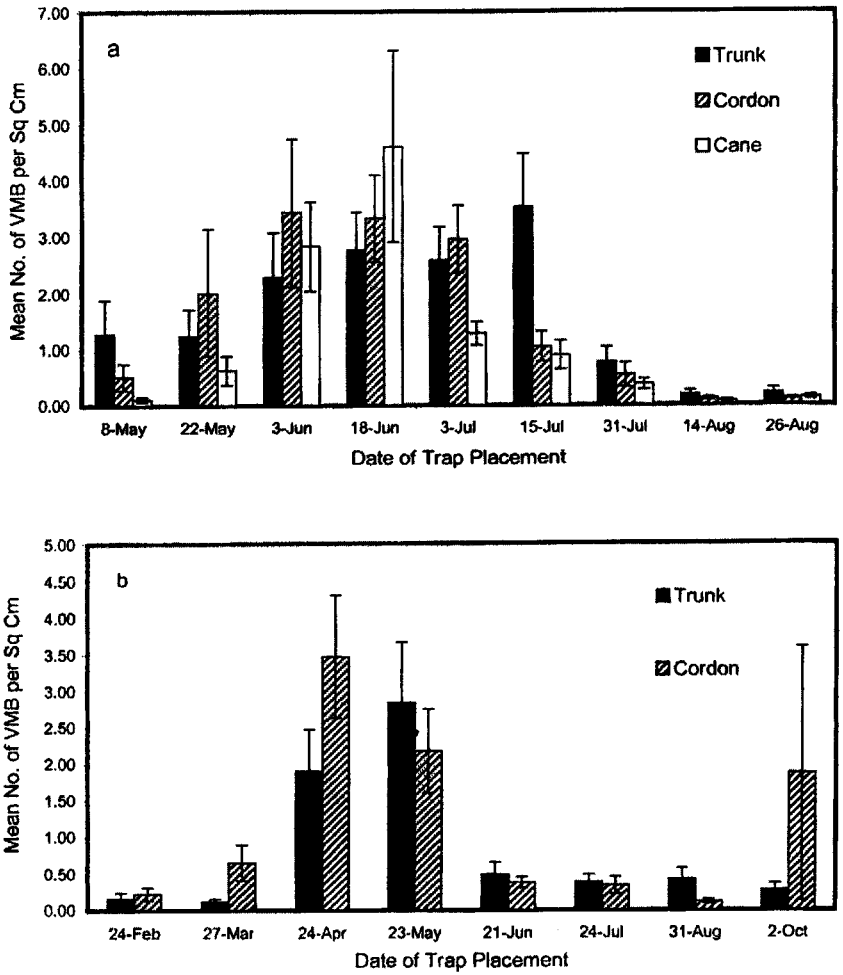

FIG. 6. (a) The mean number of VMB per sq cm of sticky tape trap placed on various parts of vines in 1998. (b) The mean number of VMB per sq cm of sticky tape trap placed on various parts of the vine in 2000. For both graphs, the bars represent the standard errors of the means.

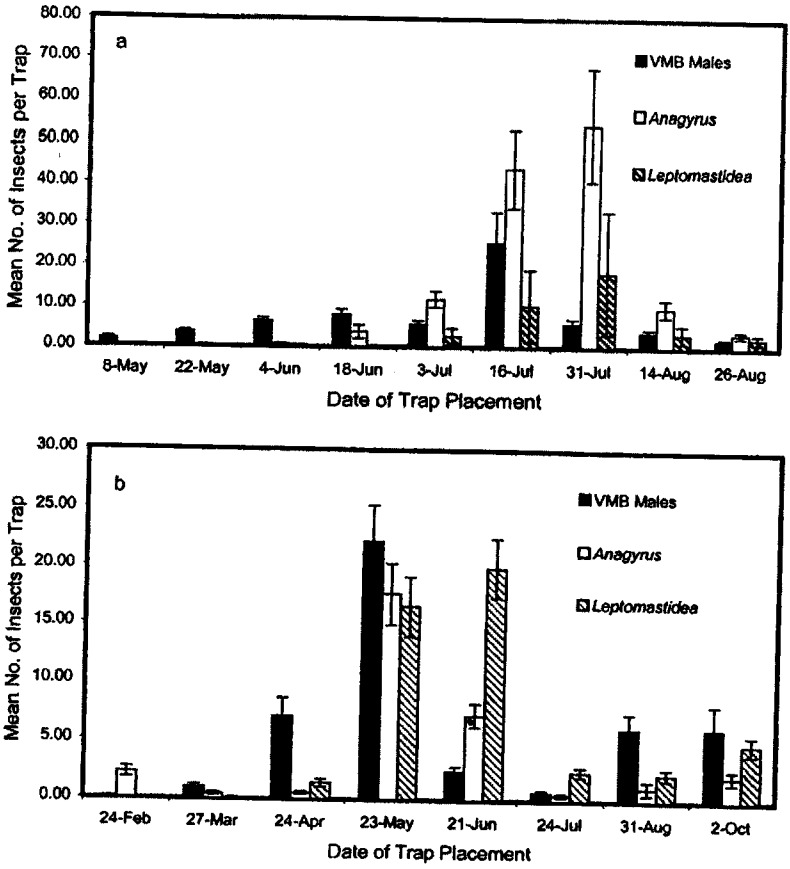

FIG. 7. (a) The mean number of adult insects per yellow sticky card placed in the vine canopy in 1998. (b) The mean number of adult insects per yellow sticky card placed in the vine canopy in 2000. For both graphs, the bars represent the standard errors of the means.

TABLE 2. Mean Numbers of VMB and Total Numbers of Parasites Recovered from Refuge Areas in 1998 and 2000.

Date Refuge in Field	Mean No. of VMB per sq cm (± S.E.)			Total No. of Parasites Recovered[a]		
	Trunk	Cordon	Cane	Trunk	Cordon	Cane
			1998			
Apr – Jun	0.15 (± 0.05)	0.14 (± 0.06)	0.67 (± 0.28)	1 Anag & 3 Pseud	0	0
Jun - Jul	1.30 (± 0.35)	0.35 (± 0.10)	0.29 (± 0.08)	3 Chart	2 Anag & 6 Chart	16 Anag & 4 Chart
Jul - Aug	0.002 (± 0.002)	0	0.003 (± 0.003)	3 Chart	0	0
Apr - Jul	0.004 (± 0.002)	0.003 (± 0.002)	0.02 (± 0.007)	1 Anag	0	5 Anag
Apr - Aug	0	0	0	0	0	0
			2000			
Feb - Mar	0.011 (± 0.007)	0.008 (± 0.007)		0	0	
Mar - Apr	0.02 (± 0.006)	0.04 (± 0.01)		0	1 Anag	
Apr - May	0.04 (± 0.01)	0.02 (± 0.006)		1 Lept & 4 Anag	1 Anag	
May - Jun	0.006 (± 0.002)	0.012 (± 0.008)		2 Anag & 1 Chart	1 Anag, 1 Lept & 3 Chart	
Jun - Jul	0.002 (± 0.001)	0.005 (± 0.0005)		0	0	
Jul - Aug	0.02 (± 0.02)	0.006 (± 0.003)		0	0	
Aug - Oct	0.0004 (± 0.0004)	0.0004 (± 0.002)		0	0	
Oct - Nov	0.0004 (± 0.0004)	0.003 (± 0.003)		0	0	

[a]Anag = *Anagyrus pseudococci* adults; Pseud = *Pseudaphycus* spp. adults; Lept = *Leptomastidea abnormis* adults; Chart = *Chartocerus* spp. adults

VMB increased dramatically, peaking in mid-late spring. This dramatic increase in density was probably due to increased reproduction and movement of VMB throughout the vine. In early to mid-summer, the densities of VMB declined dramatically and remained low throughout the fall and winter. The role of the roots on VMB seasonal dynamics requires further study because many factors may impact that role. A similar pattern in phenology was reported by Berlinger (1977) for VMB populations in southern Israel.

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LYGUS[1] SPECIES ASSOCIATED WITH TEXAS HIGH PLAINS COTTON, ALFALFA AND WEEDS

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ABSTRACT

A preliminary collection of *Lygus* species from cotton, *Gossypium hirsutum* L., on the Texas High Plains in 1999 indicated that *Lygus hesperus* Knight and *Lygus elisus* Van Duzee were the most common, economically damaging species, followed by very low densities of *Lygus lineolaris* (Palisot de Beauvois) and *Polymerus basalis* (Reuter). Further intensive surveys in 2000 and 2001 revealed that during June and July the probability of finding *L. hesperus* and *L. elisus* in production cotton or alfalfa *Medicago sativa* L. was essentially the same. Alternate weed hosts such as kochia, *Kochia scoparia* L., lambsquater, *Chenopodium album* (L.) Schrad., yellow sweet clover, *Melilotus officinalis*, L. and redroot pigweed, *Amaranthus retroflexus* L, were acceptable hosts for both *L. hesperus* and *L. elisus* and also *Polymerus basalis (Reuter)*, a related species that feeds mostly on weed florets and is not a damaging pest of cotton.

INTRODUCTION

Lygus is the genus name for a group of closely related plant feeding insects in the family Miridae that have a broad host range of weeds, legumes and cultivated crops such as cotton, *Gossypium hirsutum* L. This group of insects can be devastating to cotton yield because they feed specifically on the newly developing squares, anthers and small bolls. The cotton plant can compensate a portion of yield potential until it becomes too late in the season for squares and bolls to develop and mature. Cotton is most susceptible to *Lygus* feeding from the time it begins to develop squares to peak-bloom, although young bolls are susceptible late in the season. There are over thirty Nearctic species of *Lygus* in North America (Schwartz and Foottit 1998) but not all are damaging to cotton. Identifying *Lygus* to species is very difficult, and few entomologist can make positive identifications without the aid of a mirid taxonomist.

Within the cotton growing regions of the United States, the most universal species found in the southeastern regions is *Lygus lineolaris* (Palisot de Beauvois), also known as the tarnished plant bug; whereas, in the western cotton production regions the western tarnished plant bug, *Lygus hesperus* Knight, is cited as the most economically threatening species to field crops and specifically cotton (Layton 2000). Less frequently cited mirids of economic concern in the western production regions are the pale legume bug, *Lygus elisus* Van Duzee

1 Hemiptera: Miridae

(McGregor 1927, Fye 1975, Sevacherian and Stern 1972, Karner et al. 2000) and *Lygus desertus* Knight (Stitt 1949, Clancy 1968).

Lygus have become more of a significant pest of cotton on the Texas High Plains over the last four years along with *L. lineolaris* in the southeastern cotton production regions (Layton 2000). The Texas High Plains produces between 1.2 to 1.6 million ha of cotton a year, which accounts for about 60% of the total production for the state (Sansone et al. 2002), in a short production season. Many entomologists believe that plant bugs and stink bugs have become more important insect pests because of recent changes in cotton pest management strategies. These changes include the utilization of Bt (*Bacillus thurgiensis*) cotton varieties, the availability of target specific insecticides, and boll weevil eradication. Fewer insecticides are applied in early season, which allows for plant bug reproduction or continued movement into cotton and ultimately results in more damage. The mirid complex that is damaging to Texas High Plains cotton has not been identified. The purpose of this research was to identify the *Lygus* complex in cotton, alfalfa and associated weed hosts during the spring and summer of 1999 through 2001. The result of this survey should help identify *Lygus* species that damage cotton and differentiate from those species that may be related but do not cause damage. Some mirid species may be incidental in cotton but their identification will result in less confusion and possibly fewer insecticides applications.

METHODS AND MATERIALS

Lygus spp. were sampled from twelve production cotton fields that ranged from 23-60 ha in 1999 from Lubbock, Lamb, Castro and Swisher counties to determine if species other than *L. hesperus* infested Texas High Plains cotton (Fig 1). Fifty sweep-net samples with a standard 38-cm diameter sweep-net were taken along with sampling 15-m of linear row with a KISS sampler (Beerwinkle 1998) within each field. From the 1999 collections, it was determined that three species from the genus *Lygus* and one related species from the genus *Polymerus* were found in cotton and warranted a broader host survey of other crop species and weed hosts. *Lygus* were sampled from all counties in the Texas High Plains where cotton, alfalfa, peanuts, and sunflowers could be found during the months of May to August for 2000 and 2001 seasons (Fig. 1). Weed hosts were also sampled along crop borders and roadsides when they were present around cultivated species. Sampling was conducted with either a 38-cm sweep net for the broadleaf weeds or KISS sampler for crop species. A minimum of 50 sweep samples and a minimum of 15-m of linear row were sampled from crops. The contents of the sampler bag were transferred to plastic self-sealing bags and placed in a freezer until the adults could be pinned and identified. Nymphs of all species were collected and counted for all samples but not identified to species. The proportions of each species from the total collected on a given host plant were calculated. For cotton and alfalfa, the survey samples were consistent enough from each county that the distributions were plotted for the months of May, June, July and August. *Lygus* distributions from weed species were not plotted over this four month period because weeds were a viable host for only a few weeks.

RESULTS AND DISCUSSION

Four mirid species were collected in cotton in 1999: *Lygus hesperus, L. elisus, L. lineolaris,* and *Polymerus basalis* (Table 1). The proportions of adults was in favor of *L. hesperus*, followed by *L. elisus* and less than five specimens each of *L. lineolaris* and *P. basalis. Lygus hesperus* has been considered the main *Lygus* pest of Texas High Plains

cotton, however, little is know or mentioned about other species such as *L. elisus*. *Lygus lineolaris* is considered an eastern pest of cotton but does occur in the west (Anderson and Schuster 1983, Schwartz and Foottit 1998). *Polymerus basalis* has been recovered from the Mississipi Delta from weed hosts of the family Asteraceae but is not considered a cotton pest (Snodgrass et al.1984).

Alfalfa and canola were dominate hosts for *L. hesperus* in 2000 regardless of the sample method used, followed by only a few *L. elisus* and *L. Lineolaris* (Table 1.) Cotton sampling resulted in a high number of individuals with a greater proportion of *L. elisus* (0.58) compared to *L. hesperus* (0.40) during 2000 using the KISS sampler but sweep samples were all *hesperus* (Table1).

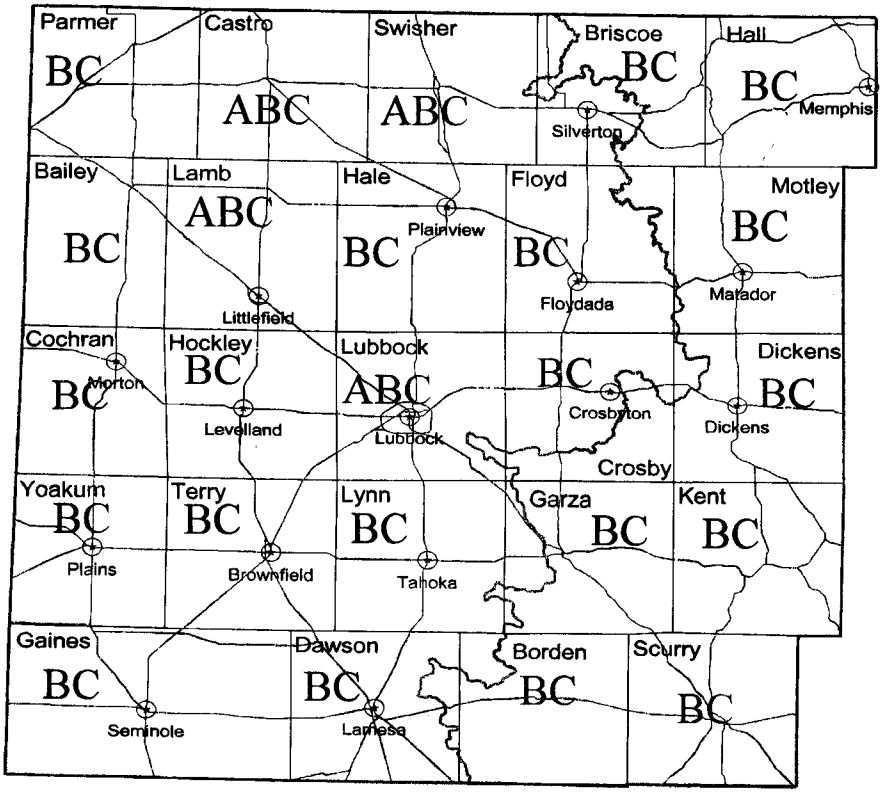

Fig. 1. Counties sampled for mirid species on the Texas High Plains. A's represent counties sampled for cotton in 1999, B's were counties where cotton, alfalfa and weed hosts were sampled in 2000, and C's are counties sampled for cotton alfalfa and weed hosts in 2001.

Corn or peanut do not appear to be an important host of *Lygus* species, although corn has been implicated as an important host for *L. lineolaris* southeast (Young 1986), and peanut has been suspected as being a good host from the southern high plains where peanut acreage has expanded significantly over the last ten years.

TABLE 1. Proportions of *Lygus hesperus* (Lh), *Lygus elisus* (Le), *Lygus lineolaris*, and *Polymerus basalis* (Pb) Collected From Sweep-Net (Number of Sweeps) or KISS (Number of Row Feet) Samples From Crop and Weed Hosts On the Texas High Plains, May-August, 1999-2001.

Plants Sampled	Sample Method	Number samples	Total adult bugs (nymphs)	Proportion of Adult Species			
				Lh	Le	Ll	Pb
Cultivated species			1999				
Cotton, *Gossypium hirsutum* L.	KISS	12 (183)	176 (22)	0.69	0.27	0.01	0.03
Cotton, *Gossypium hirsutum* L.	Sweep	12 (600)	78 (10)	0.82	0.14	0.02	0.02
Cultivated species			2000				
Alfalfa, *Medicago sativa* L.	KISS	6 (92)	127 (64)	0.94	0.04	0.02	0.00
Alfalfa, *Medicago sativa* L.	Sweep	11 (550)	166 (54)	0.94	0.05	0.00	0.01
Canola, *Brassica napus* L.	Sweep	4 (200)	358 (57)	1.00	0.00	0.00	0.00
Canola, *Brassica napus* L.	KISS	1 (15)	7 (5)	1.00	0.00	0.00	0.00
Corn, *Zea maize* L.	Sweep	16 (800)	0	-	-	-	-
Cotton, *Gossypium hirsutum* L.	KISS	23 (351)	836 (116)	0.40	0.58	0.02	0.00
Cotton, *Gossypium hirsutum* L.	Sweep	3 (150)	32 (95)	1.00	0.00	0.00	0.00
Peanut, *Arachis hypogaea* L.	Sweep	15 (750)	4 (0)	1.00	0.00	0.00	0.00
Weed species							
Lambs quarter, *Chenopodium album* L.	Sweep	3 (150)	21 (47)	0.95	0.05	0.00	0.00
Kochia, *Kochia scoparia* (L.) Schrad.	Sweep	2 (100)	21 (5)	0.95	0.05	0.00	0.00
Redroot Pigweed, *Amaranthus retroflexus* L.	Sweep	4 (200)	100 (23)	0.04	0.07	0.01	0.88
Ragweed, *Ambrosia artemisiifolia* L. &							
Russian thistle, *Salsola iberica* Sennen &							

TABLE 1. CONTINUED

Kochia, *Kochia scoparia* (L.) Schrad.	Sweep	1 (50)	75 (38)	0.03	0.16	0.00	0.81
Common sunflower, *Helianthus annus* L.	Sweep	1 (50)	1 (0)	1.00	0.00	0.00	0.00
Common sunflower, *Helianthus annus* L.	KISS	1 (30)	17 (0)	0.88	0.00	0.00	0.12
Yellow sweet clover, *Melilotus officinalis* L.	Sweep	5 (250)	71 (46)	0.58	0.28	0.14	0.00
			2001				
Cultivated species							
Alfalfa, *Medicago sativa* L.	Sweep	26 (1400)	724 (1,123)	0.43	0.57	0.00	0.00
Corn, *Zea maize* L.	Sweep	18 (900)	0	-	-	-	-
Canola, *Brassica napus* L.	Sweep	10 (500)	643 (97)	1.00	0.00	0.00	0.00
Cotton, *Gossypium hirsutum* L.	Sweep	42 (4,250)	148 (111)	0.52	0.48	0.00	0.00
Cotton, *Gossypium hirsutum* L.	KISS	1 (80)	7	0.57	0.43	0.00	0.00
Peanut, *Arachis hypogaea* L.	Sweep	16 (800)	2	1.00	0.00	0.00	0.00
Weed species							
Gumweed, *Grindelia squarrosa* (Rush) Dunal	Sweep	2 (100)	0	-	-	-	-
Common ragweed, *Ambrosia artemisiifolia* L.	Sweep	6 (300)	11	0.45	0.55	0.00	0.00
Kochia, *Kochia scoparia* (L.) Schrad	Sweep	8 (600)	78	0.40	0.60	0.00	0.00
Lambsquarter, *Chenopodium album* L	Sweep	2 (200)	45 (16)	0.64	0.36	0.00	0.00
Redroot pigweed, *Amaranthus retroflexus* L.	Sweep	16 (800)	38 (4)	0.39	0.61	0.00	0.00
Russian Thistle, *Salsola iberica* Sennen	Sweep	11 (550)	10	0.30	0.70	0.00	0.00
Yellow clover, *Melilotus officinalis*	Sweep	9 (450)	201 (84)	0.72	0.28	0.00	0.00
Flixweed, *Descurainia sophia* (L.) Webb.	Sweep	4 (200)	17	0.42	0.58	0.00	0.00
Common sunflower, *Helianthus annus* L.	Sweep	5 (250)	11	0.36	0.64	0.00	0.00

Weed species sampled in 2000 favored *L. hesperus* with the exception of redroot pigweed and a mixed sweep sample of ragweed, Russian thistle and kochia where *P. basalis* (Reuter) made up >80% of the adult collections (Table 1). Yellow clover served as the most diverse host of the weed species with a significant number of nymphs collected.

The 2001 samples from alfalfa were predominated by *L. elisus* compared to *L. hesperus,* which was a complete contrast to the 2000 samples where *L. hesperus* was the dominate species. Corn again did not prove to be a host of *Lygus*. The ratios of adult *L. elisus* and *L. hesperus* were almost identical when collected from cotton in 2001 (Table 1). Peanut has been implicated as a good host for *Lygus* and a possible reason for increased problems on the High Plains in cotton but from limited sampling for two years; only an occasional *L. hesperus* was collected. *Lygus elisus* was the most prevalent species on most weeds in 2001 with the exception of lambsquarter and yellow clover where *L. hesperus* was in higher proportions (Table 1). No other *Lygus* or related species were collected from weeds in 2001.

The seasonal distribution of *Lygus* collected from cotton and alfalfa show some similar trends (Fig. 2). May was dominated by higher proportions of *L. hesperus*; June

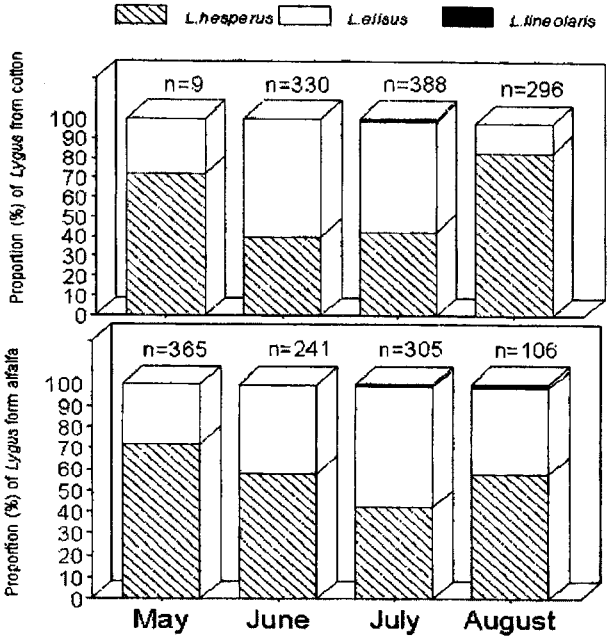

Fig. 2. Seasonal distributions of the percentage of *Lygus* species collected from cotton (top graph) and alfalfa (bottom graph) surveyed monthly from Texas High Plains counties for the 2000 and 2001 production seasons combined.

and July were usually in favor of *L. elisus*, and August was again dominated by *L. hesperus*. Alfalfa was responsible for generating high numbers of May; whereas, few *Lygus* of either species were collected in cotton. Cotton is planted from early to late May and is not producing fruiting forms or flowers to attract *Lygus*. *Lygus lineolaris* is found only occasionally in cotton or alfalfa.

The 1999 survey from cotton and the 2000-2001 survey of crop species and weeds clearly indicated that *L. hesperus* and *L. elisus* were the damaging species in field crops on the Texas High Plains. *L. lineolaris* was found only occasionally in cotton or alfalfa and usually in July or August. *Polymerus basalis* could be found in association with broadleaf weeds and was included in this survey because it can be misidentified as a damaging species.

The results of this *Lygus* survey were different from any other cotton production region in the United States in that *L.hesperus* or *L. elisus* could be found infesting cotton or alfalfa in similar proportions. *Lygus lineolaris* is reported as a threatening cotton pest in the Northern Blackland Prairies of Texas (Womack and Schuster 1987) while *L. hesperus* is recorded as the prevalent species threatening cotton in southwestern Oklahoma (Karner et al. 2000). This is the first report where *L. hesperus* and *L. elisus* are equal in terms of host plants and distribution. The proportions of these two are very similar when cotton was setting fruit in June and July. If insecticides are used as a control measure, it could be important to know which species is present because the efficacy of a given insecticide may not be the same for the two species. Insecticide efficacy and insecticide resistance are documented in *L. hesperus* (Grafton-Cardwell et al. 1997), but no information if any is available for *L. elisus*.

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ARTHROPODS PRESENT ON REMOVED FOLIAGE FROM AN APPLE PACKING LINE

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ABSTRACT

The effectiveness of packing house operations in removing arthropods on leaves was evaluated by surveying four apple cultivars, *Malus domestic* Borkh., from 17 grower lots over six months of inspection. Among the 6,400 leaves inspected, damage from the western tentiform leafminer, *Phyllonorycter elmaella* Doganlar & Mutuura (Lepidoptera: Gracillariidae) was seen in 63% of the leaves. Twospotted spider mites, *Tetranychus urticae* Koch (Acari: Tetranychidae), found on 62 leaves, and predatory mites, *Typhlodromus* spp. (Acari: Phytoseiidae), found on 34 leaves, were the most frequent live arthropods. Six other live arthropods (three parasitic wasps, a psocid, an aphid, and a spider) were also observed. Only one live arthropod, a predator mite, was found at the final pack sampling station. The numbers of arthropods decreased with fruit processing along the packing line. Data from this study indicate that foliage packed with fruits is not a likely pathway for spreading live arthropods.

INTRODUCTION

Expanded global trade has increased the opportunities of exotic pests to expand into new territories (LaGasa et al. 1997). The Pacific Northwest is a major exporter of domestic apples, *Malus domestic* Borkh. From the 2000 harvest, about 5×10^5 M.T. (6.5×10^5 tons) of apples from Washington State were exported (NHC 2002). Because of this large volume, there is concern that foliage included with the packed fruits may be a potential source for the introduction of new pests. Knowledge of the numbers of arthropods surviving the packing line is essential for establishing pest risk assessment (Griffin 2001). Currently, only Mexico limits the amount of foliage at two leaves per carton (Erikson et al. 1996), and only Japan requires apples to be fumigated as condition for entry (Hansen et al. 2000). However, future restrictions from other countries are possible because of expanding world trade.

Previous studies on the occurrence of arthropod pests in packed tree fruits have emphasized the fruits themselves rather than extraneous plant material such as leaves (Curtis et al. 1992, Johnson et al. 2000, Knight and Moffitt 1991, Moffitt 1990, Hansen and Schievelbein 2002). Yet, the risk of introducing exotic pests from leaves packed in fruit containers has not been evaluated. A thorough investigation would involve inspecting leaves

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removed at points throughout the conventional packing process, beginning with the arrival of freshly harvested fruits and examining the foliage for the presence of arthropods as evidence of phytosanitation. Thus, the objective of our study was to observe discarded leaf material along a typical packing line to determine where arthropods are eliminated.

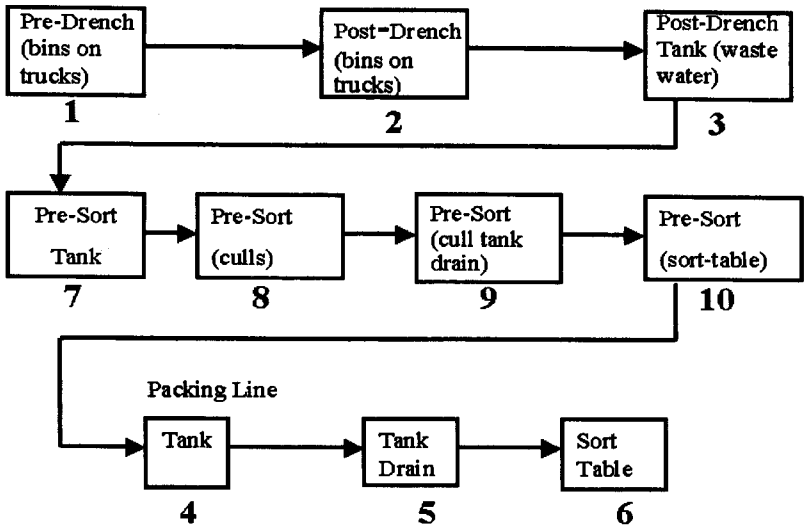

FIG. 1. The arrangement of sampling stations along the packing line, each with an identification code number.

MATERIALS AND METHODS

Apple leaves from four cultivars ('Gala,' 'Fuji,' 'Delicious,' and 'Golden Delicious') were obtained from 17 grower lots (fruit harvested on a particular date from one orchard) over six months. Foliage samples were collected from ten stations during packing (Fig. 1), with the majority of the samples consisting of 'Delicious' leaves. The amounts varied by grower lot, but a minimum of 20 leaves per lot per site were collected in most cases. Regardless, all leaf material was suppose to be removed before fruits were packed. In the pre-sort samples, foliage was obtained while fruits were still in bins and from drench tanks. Some of the fruit was packed immediately, whereas others were placed in conventional refrigerated storage or controlled atmosphere storage to be packed later. Samples were obtained between pre-sort and the sort table from fruits in rinse tanks, from leaf traps on tank drains, and from fruits along the packing line. Foliage samples were brought to the laboratory where they were inspected under a 30x binocular microscope. Arthropods were counted, removed, identified, and their viability determined.

Data were analyzed using SAS (SAS Institute 1989). PROC MEANS was used for univariate statistics. Because of heterogeneity in variances, nonparametric tests were used to determine significant differences by first arranging data by PROC RANK, then performing PROC GLM. This is the equivalent to a Wilcoxon rank sum test for two samples and the Kruskal-Wallis *k*-sample test for more than two samples.

RESULTS AND DISCUSSION

The total number of leaves examined was 6,408 among 336 samples. The quantity of leaves collected varied among the sampling sites because of differential removal. Damage by the western tentiform leafminer, *Phyllonorycter elmaella* Doganlar & Mutuura (Lepidoptera: Gracillariidae) was the most prevalent, occurring in about 63% of the leaves. However, because this insect is not a pest on fruits, it was excluded from any further analysis. Only ten leaves were collected at Station 3, and these data were also excluded.

The largest group of live arthropods observed on leaves was the twospotted spider mite, *Tetranychus urticae* Koch (Acari: Tetranychidae); there were 62 leaves with spider mites, which were generally collected at Stations 7, 8, and 10. The next most abundant group was the predatory mites, *Typhlodromus* spp. (Acari: Phytoseiidae), recovered from 34 leaves also at the same stations. The greatest number of live insects collected throughout the study were three parasitic wasps, although a live psocopteran, and an aphid were found at Station 8. Among all cultivars, the only live arthropod found at the final pack (Station 6) was a predacious mite.

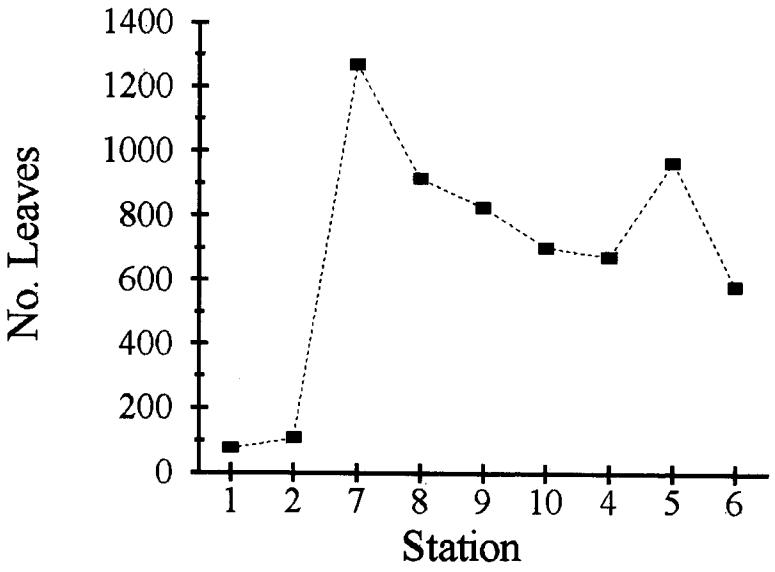

FIG. 2. Total number of 'Delicious' leaves collected at each of the sampling stations along the fruit packing line.

Among the cultivars examined, only 'Delicious' had leaf samples collected at each station (Fig. 2). The percentage of clean 'Delicious' leaves was significantly different among the sample stations ($F = 3.21$; $df = 8, 175$; $P < 0.01$). Significant difference in the percentage of clean leaves was also found for 'Golden Delicious' among Station 4 through 7 ($F = 3.94$; df

= 3, 55; P < 0.05). The cleanest station for both these cultivars was Station 6 ('Delicious': mean ± SEM = 95.5 ± 1.9%; 'Golden Delicious': mean ± SEM = 89.1 ± 3.5%). There were no significant differences among the sampling stations for the remaining cultivars. Station 7 was the only sampling station that had a significant difference (F= 9.72; df = 2,55; P < 0.01) in percentage of clean leaves per sample among cultivars ('Delicious': mean ± SEM = 70.6 ± 3.4%; 'Golden Delicious': mean ± SEM = 57.5 ± 7.8%; 'Fuji': mean ± SEM = 94.5 ± 2.1%.), perhaps due to the different growing conditions. Overall, 'Delicious' had the least percentage of clean leaves among sample stations (mean ± SEM = 80.4 ± 3.1%), but this may because the samples were collected from more stations than for any other cultivar.

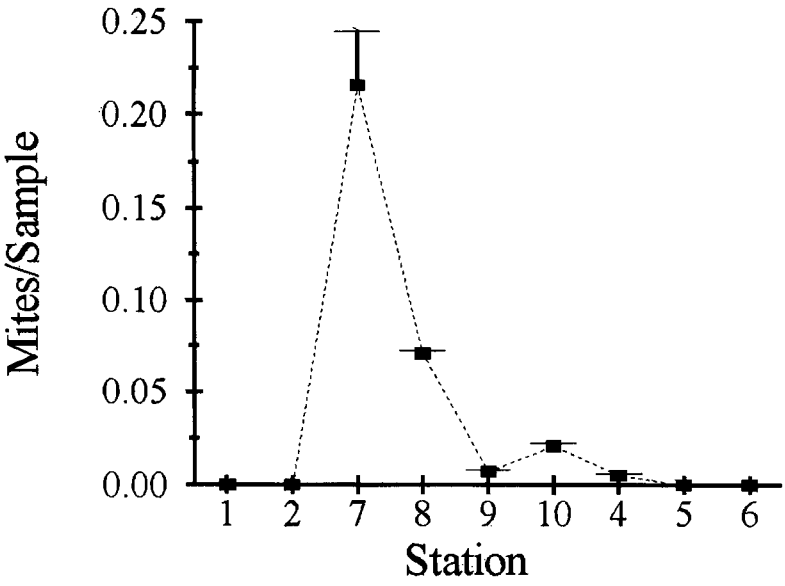

FIG. 3. Mean (± SEM) number of live spider mites per sampling station for 'Delicious' leaves.

If cleaning was effective along the packing line, then the number of living pests should be reduced as the fruits are processed. To test this, we examined the live infestation rate of the most populous pest, spider mites, on 'Delicious' among the sampling stations and found that the infestation rate decreased rapidly after Station 7 from a mean (± SEM) = 0.22 (± 0.18) mites/sample to a mean (± SEM) = 0.01 (± 0.01) mites/sample at Station 9 (Fig. 3). Samples from the sorting stations averaged less than 0.01 mites/sample. Hence, either live mites were eliminated early in the process or they died by the time they reached the packing table. The only mites, including the European red mite, *Panonychus ulmi* (Kock) (Acari: Tetranychidae), and predacious mites, collected at Station 6 were dead.

The findings of our study provides support for the systems approach (Jang and Moffitt 1994, Moffitt 1990, Moffitt 1997) to quarantine security. In the systems approach, production components, starting with field control and ending with fruit cleaning and sorting in the packing house, accumulatively assure a pest-free product. Besides being efficacious, other proven

advantages of the systems approach are maintenance of fruit quality without additional treatment facilities, reduced labor costs without compromising work safety, and reduced harmful effects to the environment through deceases in waste disposal. No further treatment may be needed to prevent potential spread of arthropod pests from foliage including those in the containers holding the packed fruits.

To assess pest risk, Griffin (2001) recommended determining initial pest infestation levels as the first step. In our study, very few live arthropods reach the packing house, regardless of the types of apples harvested. Those that went through the cleaning, sorting and packing operations did not survive. If this study is characteristic of other conventional packing houses, foliage material that may be inadvertently included with the packed fruits very likely does not harbor any live arthropods.

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EFFECT OF SPINOSAD ON HONEY BEES (HYMENOPTERA: APIDAE) IN GUATEMALA

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ABSTRACT

A field evaluation of the effects of aerially applied spinosad bait spray (Success 0.02 CB™), used by the area-wide Mediterranean fruit fly, *Ceratitis capitata* (Wiedemann) eradication program in Guatemala, on honey bees, *Aphis mellifera* Linnaeus, and hive activity was conducted during the 2000 spray season. The parameters monitored were adult bee mortality, brood size, pollen reserves, panels of honey, panels of adults, and hive weight. Statistical analysis of the data revealed no significant differences between parameters in the treated and control area, demonstrating that there were no deleterious effects on the bee population due to the spinosad applications.

RESUMEN

Se efectuó una evaluación en campo del efecto producido por el insecticida spinosad con cebo (Success 0.02 CB™), aplicado en aerosol, sobre abejas adultas y la actividad de sus colmenas durante la temporada de fumigación del año 2000. Este compuesto químico se utiliza en áreas tratadas por el programa de erradicación de mosca del mediterráneo. Los parámetros monitoreados fueron: mortalidad de adultos, cría operculada, reservas de polen, panales de miel, panales de adultos y peso de la colmena. El análisis estadístico de los datos no indica una diferencia significativa entre el área de tratamiento y el área de control, demostrando que no existe un efecto nocivo en la población de abejas debido a la aplicación de spinosad.

INTRODUCTION

The Mediterranean fruit fly, *Ceratitis capitata* (Wiedemann), commonly known as Medfly, is one of the most destructive phytophagous insect pests in the world, with larvae attacking more than 300 species of fruits and vegetables (Liquido et al. 1991). Because of its destructive nature, large-scale control programs have been implemented in various parts of the world including Mexico and Guatemala. Medfly was first detected in Guatemala in 1976 and in Mexico in 1977. These detections led to a major effort that resulted in its eradication from Mexico in 1982 and the establishment of a barrier zone in Guatemala.

The chemical component of the eradication program consisted of a mixture of malathion and hydrolyzed protein that was integrated with the release of sterile flies and the establishment of an effective quarantine program. The barrier prevented the northward movement of Medfly back into Mexico until 1999, at which time 274 outbreaks were

detected in southern Mexico. In 1999, emergency funds were appropriated to eradicate Medfly from Mexico and Guatemala, and plans were made to begin the aerial spraying phase of the program in February 2000. The chemical product selected for this phase of the Guatemala program was Success 0.02 CB™ produced by DowAgroSciences (Indianapolis, IN). This product contains spinosad, a mixture of spinosyn A and spinosyn D, derived from the fermentation of the actinomycete *Saccharopolyspora spinosa,* which has improved environmental properties over older insecticides (Cleaveland et al. 2001). Success 0.02 CB™ bait is reported to be an improved bait combining low use rates and a reduced risk toxicant to non-target insects (Anonymous 2003); however, its potential effect on honey bee, *Aphis mellifera* Linneaus, was an obvious concern since one of the components is sugar.

In 1999, prior to recommending the use of Success 0.02 CB™ as a component of the eradication program, Guatemala APHIS PPQ Methods Development conducted a series of field cage evaluations, as well as a large field evaluation in southwestern Guatemala, to determine the effects of Success 0.02 CB™ on honey bees. The conclusions from these evaluations were that Success 0.02 CB™ was not toxic to bees and that the product had no deleterious effects on normal hive activity when used according to label directions (Rendón et al., 2000). In February 2000, the Guatemalan Ministry of Agriculture, Ministerio de Agricultura, Ganadería y Alimentación (MAGA), appointed a commission of MAGA personnel to conduct another field evaluation on the effects of Success 0.02 CB™ on honey bees in collaboration with USDA-APHIS-IS and Moscamed (Spanish for Medfly) program personnel. This evaluation was done in conjunction with the Medfly area-wide spray program to evaluate the effects from actual field use of repeated applications across multiple geographies and climates.

MATERIALS AND METHODS

The evaluation was conducted from 16 February to 2 April 2000. Apiaries located inside the spray zone were near the villages of Somberito Bajo and Caserío Los Perez of the municipality Nuevo Progreso, San Marcos, Guatemala; apiaries outside the spray zone were between San Antonio Las Flores and Palín, also in Nuevo Progreso, San Marcos. The area is 400-600 m above sea level; coffee is the predominant agricultural crop. Sixteen bee hives from five apiaries were selected within the treatment area and 11 hives from four apiaries were selected outside the treatment area to serve as controls. Control apiaries were 1.5-5.5 km from the spray zone. Prior to the initiation of the evaluation, hives were examined to determine their general condition and absence of disease. Two aspects of honey bee activity were evaluated in order to determine the potential effect of Success 0.02 CB™; the first aspect considered adult mortality around the opening of the hive while the second considered the effect of the product upon hive vitality. In order to determine adult bee mortality, apron traps (Rhodes and Wilson 1978) were constructed immediately in front of the hives. The apron traps were constructed by placing a white cloth on the ground and surrounding it with concrete blocks 20 cm high, creating an area 1x1 m. Dead bees falling onto the cloth in the 1-m^2 area were counted and removed daily. The effect of Success 0.02 CB™ upon colony activities was determined by taking measurements of brood (square centimeters), pollen reserves (square centimeters), honey (number of panels), and overall hive weight. Initially, measurements were taken weekly, but the last two series of measurements were taken at two-week intervals.

The Success 0.02 CB™ concentrate was mixed with water (3 parts water:2 parts Success) to produce an 80 ppm active ingredient mixture which was aerially applied to the treatment area using LET-410 and Turbo Thrush fixed-wing aircraft at the rate of 0.38 g a.i./ha. Aerial applications were made at weekly intervals for a total of seven applications.

In order to compare the number of dead bees from hives inside and outside the spray zone, the Wilcoxon test for paired samples was used. The non-parametric Mann-Whitney test for two independent samples was used for comparing hive activity variables (Infante and Zarate de Lara 1996).

RESULTS AND DISCUSSION

Honey bee mortality is illustrated in Fig. 1. The daily means of bee mortality were 20 and 13 for the hives located in the control and treated areas, respectively. Statistical analysis revealed no significant difference between the two areas (Z= 1.8781; p-value= 0.0604).

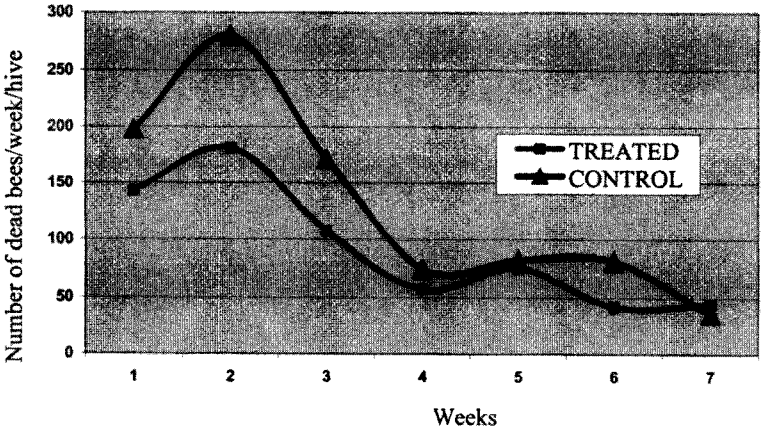

FIG 1. Honey bee mortality from area treated with Success 0.02CB™ vs non-treated area in Guatemala

The effects of Success 0.02 CB™ on hive activity is presented in Table 1. Only during the first week were pollen reserves and panels of adults significantly higher in the control hives than the hives in the treated area. The only other significant differences were near the end of the evaluation in which panels of adults, brood size, and pollen reserves were significantly higher in the treated area than the untreated area. This is counter to the hypothesis that the applications of Success 0.02 CB™ may have a deleterious effect on hive viability.

Results of this evaluation are consistent with the findings of the evaluation previously done by Rendón et al. (2000). In that study, there was no bee mortality caused by the aerial applications of Success 0.02 CB™; nor was there any negative impact upon normal hive activity as measured by brood size, pollen reserves, panels of honey, panels of adults, and hive weight. Burns et al. (2001) also reported no effects on hive conditions or brood from spinosad bait spray applied to commercial citrus to control Med fly and Caribbean fruit fly, *Anastrepha suspensa* (Loew) in Florida.

The susceptibility of bees to direct application of spinosad has been reported by various investigators. Mayes et al. (2003) have reviewed much of this work. The absence of acute adult mortality and lack of deleterious effects upon hive activity in this study is most likely the result of a combination of factors. One is the avoidance of the Success 0.02CB™ mixture by the bees due to the presence of 1% ammonium acetate. The

TABLE 1. Effect of Success 0.02 CB™ on Certain Parameters of Honey Bee Hive Activity.

Week	Mean Value of Measurement									
	Brood Size (cm^2)		Pollen Reserve (cm^2)		Honey Panels (Number)		Adult Panels (Number)		Hive Weight (kg)	
	Treated	Control	Treated	Control	Treated	Control	Treated	Control	Treated	Control
1	3467.41	3091.48	105.61[c]	200.58	1.72	1.86	12.50[a]	14.91	34.57	37.52
2	2653.22	3334.32	174.19	92.64	5.08	3.77	11.12	13.00	38.15	41.94
3	2814.12	3010.57	113.68	77.00	6.37	6.09	12.19	10.54	42.78	42.65
4/5	3061.28	2695.07	37.87	14.32	4.56	4.28	11.69[a]	9.36	39.32	35.62
6/7	2972.77[b]	1864.51	268.19[b]	117.29	4.46	1.91	11.07[a]	7.37	36.62	26.70

[a,b,c] Treated value is significantly different from control value at $P < 0.05$, $P < 0.01$, and $P < 0.001$, respectively.

repellency of this chemical compound to honey bees has been well documented by Tarshis Moreno (2001). Although the inclusion of ammonium acetate into the Success 0.02CB™ mixture attracts Medfly and *Anastrepha* spp. which readily feed upon the mixture to obtain the protein necessary for egg development, bees will avoid the mixture given the opportunity to do so, as in a field cage or field situation (Rendon et al. 2000). Edwards et al. (2003) reported significant honey bee mortality when exposed to GF-120 (Success 0.02 CB™) fruit fly bait in the laboratory. However in that exposure study, bees were confined to petri dishes where their normal avoidance behavior was negated and direct contact unavoidable because of the restricted space.

Other important factors leading to the absence of any effects were the low dosage rate and low volume of the aerial applications (droplet size of 3000-6000 microns in diameter dispersed at the rate of 60-80 droplets per m^2). The probability of a honey bee in flight receiving enough spinosad to cause death is very remote. Additionally, through a very active environmental monitoring program in Guatemala, chemical analyses of water and vegetation samples have verified that residues of spinosad in the field are broken down rapidly and do not accumulate (USDA-APHIS-IS 2000, USDA-APHIS-IS 2001). These findings are consistent with residue persistence studies done by Saunders and Bret (1997) who found that spinosad degraded rapidly in the field due to photodegradation by sunlight.

These investigators are in agreement with the position taken by Stark et al. (1995) that predictions regarding the safety of insecticides to beneficial invertebrates must be validated with field data. These data indicate that under the conditions that the Medfly area-wide eradication program currently operates, no detrimental effects to bees or honey production have occurred or are likely to occur from applications of Success 0.02 CB™.

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ARTIFICIAL SUBSTRATES FOR OVIPOSITION OF *NOTONECTA IRRORATA* UHLER (HEMIPTERA:NOTONECTIDAE).

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ABSTRACT

A substrate and depth preference study was carried out with the mosquito larval predator *Notonecta irrorata* Uhler in artificial pools from March to December 1996. Four artificial substrates (cotton thread, nylon thread, *Agave* sp. fibers and tree twigs) were tested at three depths (10, 20 and 30 cm) as oviposition site by notonectid female. Results showed two peaks in egg production and preference for *Agave* sp. fibers and the 30-cm depth level as ovipositional sites.

INTRODUCTION

Notonectis are voracious predators of mosquito larvae and are therefore considered promising group for biological control (García 1983, García and Des Rochers 1984). The predatory capacities of several species of backswimmers has been reported previously (Ellis & Borden 1970, Toth and Chew 1972, Gittelman 1974, Fox and Murdoch 1978, Chesson 1984). Miura and Takahashi (personal communication) released *Notonecta unifasciata* Guerin eggs to control *Culex tarsalis* Coquillet in rice field with excellent results. Neri-Barbosa et al. (1977) released adults of *Notonecta irrorata* Uhler in artificial containers to control larvae of *Aedes aegypti* (L.) and *Culex pipiens* L.; Their results showed low mosquito larval densities and notonectid numbers after ten months. However, cost of colonization and mass production of these notonectid species, coupled with the logistics of their distribution, handling and timing of release at the appropriate breeding site have impeded their use as mosquito control agents (Legner 1995). Miura (1986), and Legner (1995) have suggested inoculating mosquito breeding areas with backswimmer eggs collected on artificial oviposition material. The objective of this study, therefore, was to determine the preference of *Notonecta irrorata* to different artificial substrates and substrate depth levels as oviposition sites. This information may useful as a support tool for integrated management of mosquitoes in environments inherent to Mexico.

MATERIAL AND METHODS

The study was carried out from March to December of 1996 at the Agricultural Field Station of Instituto Tecnológico y de Estudios Superiores de Monterrey (ITESM) in the municipality of Apodaca, Nuevo León. Two concrete pools, each 8x8x1m, with a continuous

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water supply were used in the study. Submerged vegetation, with *Chara* sp. being the dominant plant, was present in the bottom of these pools at all times. The water temperature in the pools ranged between 20 to 39° C and pH varied from 7.5 to 8.1. The pools were continuously exposed to solar radiation.

The artificial substrates examined as oviposition sites for *Notonecta irrorata* were 30-cm strips of cotton thread, nylon threads, *Agave* sp. fibers, and tree twigs. Substrates were suspended from the surface water by red thread attached to 5x2.54-cm polystyrene squares at three 10-cm. depth levels denoted as level 1, 2 and 3.respectively. A plummet was attached to each strip in order to keep it vertical. There were four replicates for each substrate. Substrates were changed every 15 days, and the numbers of notonectids eggs on each substrate at each depth level were recorded. Analysis of variance was used to determine significance in oviposition preference for substrates and depth level according to Zar (1974).

RESULTS AND DISCUSSION

Notonecta irrorata eggs were first observed on the artificial substrates approximately 6 months into the 8 month study (i.e., 22 May 1996) (Fig. 1). The highest number of eggs recorder occurred in late spring (22 May) with 117 eggs and early summer (26 June) with 125 eggs as shown in Fig. 1. Number of eggs decreased on the remaining sample dates. This pattern of oviposition and the relative low number of eggs suggest that, during these months, the backswimmers probably did not have the appropriate quantity and quality of food. Rodriguez-Castro (personal communication) observed that, under laboratory conditions with appropriate food such as mosquito larvae, *N. irrorata* and *Bueona scimitra* produce eggs continuously through the year.

Backswimmer oviposition rates varied in with the different artificial substrates tested. The most attractive material was the *Agave* sp. fibers (Fig. 2). Difference in oviposition substrate preference was consistent throughout the study. As can be noted on Fig. 2, the total number of eggs deposited on the different substrates was *Agave* sp. fibers, (310); nylon fibers (61); tree twigs, (31) and cotton thread (6). Statistical differences were found between these substrate preferences ($P < 0.05$). We thus conclude that the backswimmer exhibited a preference for *Agave* sp.fibers as an oviposition substrate. This may be due to it being the material most similar to that of natural sites where females lay eggs.

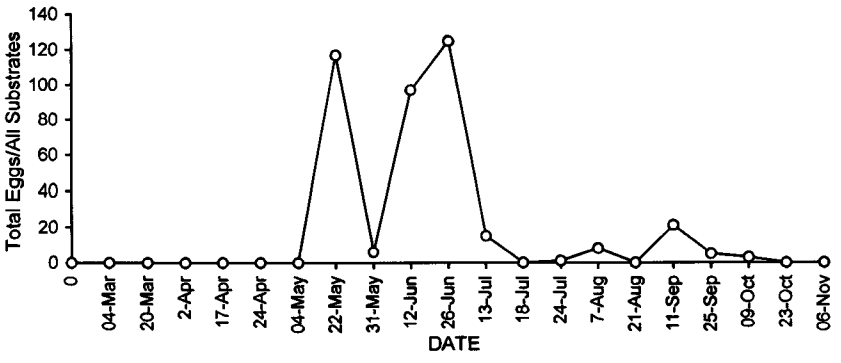

FIG. 1. Total number of eggs deposited by *Notonecta irrorata* females on all oviposition substrates during the period March to November 1996 in artificial pools at the Agricultural Field Station of ITESM, Apodaca, Nuevo León, México.

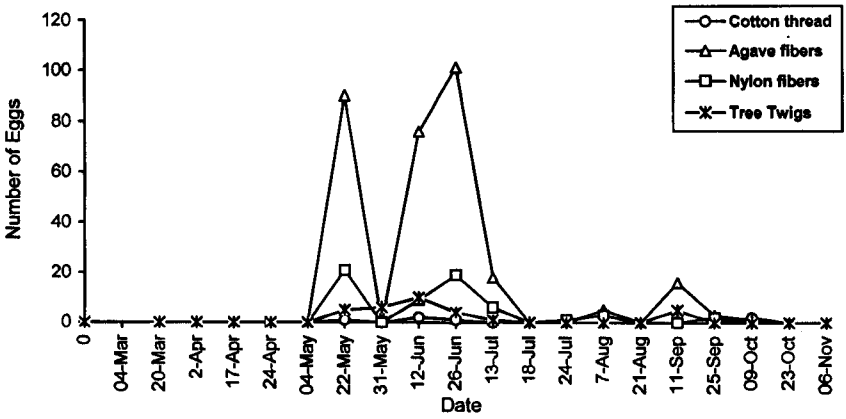

FIG. 2. Substrate preference by ovipositing *Notonecta irrorata* females during the period March to November 1996 in artificial pools at the Agricultural Field Station of ITESM, Apodaca, Nuevo León, México.

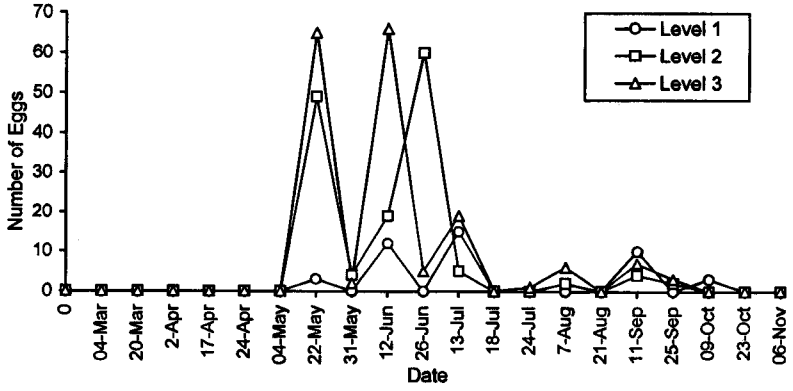

FIG. 3. Depth level preference by ovipositing *Notonecta irrorata* females during the period March to November 1996 in the Agricultural Field Station of ITESM, Apodaca, Nuevo León, México.

Females of *N. irrorata* usually selected the deepest portion of the strips located 30 cm below water surface for oviposition. A statistical difference was found among these depth level results ($P < 0.05$). As shown Fig. 3, the number of eggs at each depth was 49 eggs for the first level, 145 eggs for the second level, and 214 for third level. This study provides a baseline of information about *N. irrorata* and the possibility of mass rearing this notonectid species under artificial conditions, and a method to manipulate this aquatic predators. This information can improve the use of backswimmers as a biological control of mosquito larvae.

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FIRST RECORDS OF *STETHORUS HISTRIO* CHAZEAU (COLEOPTERA: COCCINELLIDAE) FROM THE UNITED STATES

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Beetles of the coccinellid 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. Because of this, several species of *Stethorus* have been used in concerted efforts at biological control of spider mites, especially *Tetranychus urticae* Koch. Some of these coccinellid species have been introduced, both intentionally and unintentionally in non-native localities (e.g., Gordon and Chapin 1983; Gordon and Anderson 1979, Hoy and Smith 1982). Recently, an Australian species, *Stethorus nigripes* Kapur, was collected in the Texas Panhandle in the United States (Gordon 1993). Its re-collection and its subsequent spread into adjacent Oklahoma and Kansas was documented by Pollock and Michels (2002). Our purpose here is to report the collection of *Stethorus histrio* Chazeau in Texas, representing the first authenticated record of this species from North America.

According to Gordon and Anderson (1979), *S. histrio* was originally described from La Réunion, Mascarene Islands, and is now known to occur in Australia, New Caledonia and Chile. Gordon and Anderson (1979) suspected that the presence of *S. histrio* in Chile is due to human transport, but there is no credible evidence of this. Gordon and Chapin (1983) expanded the known range of *S. histrio* to include Mexico (Yucatan). According to Houston (1980), *S. histrio* is quite widespread in Australia, being known from all states, including Tasmania. Specimens in the United States National Museum (N. Vandenberg, pers. comm.) are from Mexico, Paraguay, Chile, and Brazil. The Texas records reported here represent the first documented presence of *S. histrio* in North America, north of Mexico.

Specimens of *S. histrio* were collected at several localities in the Texas Panhandle during August 2001, through routine sampling of corn fields for species of *Stethorus* (see Pollock and Michels 2002 for a brief explanation of this project). Adults of *S. histrio* resemble closely those of *S. nigripes* Kapur, the latter of which was also recently documented as being very common in northern Texas and adjacent states (Pollock and Michels 2002). However, the larvae of the two species are rather distinctive; mature larvae of *S. nigripes* are conspicuously pink in color, while those of *S. histrio* are gray. It was the discovery of this difference that led the authors to suspect the presence of a species other than *S. nigripes*. Adults, larvae and pupae of *S. histrio* were collected from two entirely different locations. A large redbud tree (*Cercis canadensis*, Fabaceae) in a yard in Amarillo (Randall Co.), Texas, was moderately infested with tetranychid mites, among which were found larvae and adults of *S. histrio*. Identification of this species was confirmed by

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dissecting representative males and comparing the genitalia with the diagnostic figures in Gordon and Anderson (1979). The larvae collected with the adults were almost identical to those figured in Britton and Lee (1972) and Gordon and Anderson (1979).

The other situation in which specimens of *S. histrio* were found was in commercial corn fields near the town of Hart, Texas (southern Castro Co.). Routine sampling of cornfields in the Texas Panhandle had been underway through 2000 and 2001, and we found a field near Hart that was very heavily infested with mites, and which had numerous adults and larvae of *Stethorus*. The distinctively greyish larvae of *S. histrio* were quite common, as were adults and larvae of *S. nigripes*. Upon return to the laboratory, we noticed that we had collected three different species of *Stethorus* from a single corn field: *S. histrio*, *S. nigripes*, and the native *S. caseyi* Gordon & Chapin.

Prior to our research in the Texas Panhandle, *Stethorus* diversity in the Texas High Plains was thought to consist of the native species *S. punctum punctum* and *S. caseyi*. Based on the results presented in this paper and Pollock and Michels (2002), it is evident that *Stethorus* diversity is poorly understood, and may be richer than expected.

Species of *Stethorus* are well known from perennial habitats (e.g., orchards, ornamentals), but little is known about species diversity and ecology in annual habitats. Given the ability of these beetles to rapidly colonize mite-infested annual habitats, it is reasonable to assume that Stethorus has great potential as a biological control agent in annual cropping systems. Control of spider mites in annual crops, especially Banks grass mite in corn, is increasingly difficult as resistance to acaracides, specifically bifenthrin (Capture®), is becoming common. There is a need for further research to determine the number and range of species of *Stethorus* in the Texas Panhandle, and other parts of the Great Plains states. An understanding of the seasonal abundance, life history, and basic ecology of species of *Stethorus* in annual crops and perennial plant refuges is lacking and needs to be pursued.

Voucher specimens of the larvae and adults of all species of *Stethorus* collected during the course of this research were placed in the entomology collection at the Texas Agricultural Experiment Station Research and Extension Center at Bushland, TX.

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EFFECT OF SUBSTRATE ON *PAECILOMYCES FUMOSOROUSEUS*[1] MYCOSIS OF HOUSE FLY[2] LARVAE

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Larvae of house fly, *Musca domestica* L., often appear to be resistant to topical fungal infections (e. g., Mullens et al 1987, Steinkraus et al. 1990, Geden et al. 1995, Leucona et al. 1996). However, inoculated larvae and pupae were not maintained in microbe-rich substrates such as decaying organic material similar to their natural habitat in these studies, and this may be an important factor when trying to induce fungal infections in immature house flies. Shields et al. (1981) and Pereira et al. (1993) reported strong inhibition of *Beauveria bassiana* (Bals.) Vuill. in soil; however, this inhibition was removed after substrate sterilization. Species of *Aspergillus* and *Penicillium* are common soil fungi and are strong inhibitors of *Beauveria* (Shields et al. 1981, Majchrowicz et al. 1990). *Beauveria* conidia in soil were more than seven orders of magnitude more pathogenic to fire ants, *Solenopsis invicta* Buren, in sterile versus non-sterile soil (Pereira et al. 1993). Therefore, in this study our objective was to determine the effect of conidial concentration and incubation substrates upon mycosis of house fly larvae. The fungal strain we utilized, identified as *Paecilomyces fumosoroseus* (Wize) Brown and Smith (Samson 1974), was isolated from five house fly pupae showing a dense granular, pinkish-brown myceliar growth, collected by G. A. Vázquez-Jaime in urban Saltillo, Coahuila, México.

To optimize fungal activity (Brownbridge et al. 2001), *Paecilomyces* was inoculated on harvester ants, *Pogonomyrmex barbatus* (Smith), and then isolated from sporulating ant carcasses onto Sabouraud maltose agar plus 1% yeast extract (SMAY). Cultures (second transfer on SMAY) were grown at 25-27°C for three weeks under fluorescent light for conidia production. House fly larvae from wild populations were collected from a fermented bran paste (1 kg of wheat bran mixed with 500 ml of distilled water) placed on the ground for three days in a swine and cattle barn with a large *M. domestica* population. More than 99% of flies thus reared from this bran (n=130) were *M. domestica* (Dodge 1958)

[1]Hyphomycetes: Moniliales
[2]Diptera: Muscidae
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Paecilomyces fumosoroseus conidia were suspended in 0.025% Century™, an organosilicone surfactant (Union Carbide, Danbury, CT) in sterile distilled water, and filtered through nylon mesh. Conidial viability, determined microscopically from conidia incubated for 16 hours at 25°C on SMAY, was >98%. Third-instar house fly larvae, placed in a piece of nylon mesh (10 lines/mm) were dipped for five seconds in 20 ml of conidial suspensions in each of eight concentrations (conidia/ml) as follows: 1.64×10^8, 1.04×10^8, 1.64×10^7, 1.64×10^6, 1.64×10^5, and 1.64×10^4. Non-dipped larvae, and larvae dipped in water with surfactant only were included as controls. Three groups of 13-23 (mean=19) larvae were exposed to each conidial treatment, after which each larval group was incubated separately in 100-ml Petri dishes in one of three substrates: 1) sterilized moist filter paper, 2) a 1-cm layer of moistened non-sterile, fermented bran, or 3) a 1-cm layer of non-sterile moist cattle feces. Larvae were incubated at room temperature until pupation, after which pupae were extracted and placed on moist sterile filter paper in Petri dishes; fungal or adult fly emergence was recorded. Mortality values (as influenced by conidial concentration, incubation substrate and the two-factor interaction) were statistically analyzed using the logistic regression model from SAS 6.12 (SAS 1996).

Most treatments did not cause significant mortality of house fly larvae or pupae. The maximum mortality value (69%), and the only value significantly different from the controls ($P>0.021$) occurred in pupae dipped (as larvae) at the highest concentration (1.64×10^8 conidia/ml) incubated on sterile paper. Also, visible mycosis of pupae resulted only at the three higher spore concentrations, and only when larvae were incubated on sterile filter paper (5, 21, and 53% of pupae covered with fungal sporulation). Overall, conidial concentrations and the interaction concentration-substrate had a significant effect on mortality; overall, the effect of incubation substrate was not significant ($P>0.021$) probably due to only one concentration-substrate combination being so.

These results, like previous reports, indicate that house fly larvae are relatively recalcitrant to fungal infections. Phenomena possibly causing this resistance include the inhibitory effect of the larval substrate's microbial flora upon development of entomopathogenic fungi. When Pereira et al. (1993) applied conidia directly to fire ants and transferred them to sterile vs. non-sterile soil, they did not observe mortality differences. However, in the present report there was an effect of substrate on mortality regardless of direct application of spores to the insects' cuticle. Despite the fact that both Geden et al. (1995) and the present work made use of house fly-derived isolates, no patent infections were induced on mature larvae living in microbe-rich substrates. Conversely, *B. bassiana* conidia mixed in manure-sawdust medium caused high mortality of second-instar larvae (Watson et al. 1995); younger house fly larvae are likely more sensitive to fungal pathogens. House fly larvae can also be expected to have effective inherent antimicrobial defenses since they inhabit microbe-rich decaying organic matter. Perhaps less likely, physical removal of conidia from the cuticle during larval tunneling could cause low susceptibility to *P. fumosoroseus* in this work. Pathogenicity of this *P. fumosoroseus* strain to insects has been repeatedly shown against greenhouse whitefly, *Trialeurodes vaporariorum* Westwood (Sánchez-Peña and Vázquez-Jaime 1996), and *P. barbatus* (personal observation). These results indicate possible limitations to the use of *P. fumosoroseus* conidia as a house fly larvicide, even if high concentrations of viable, pathogenic inoculum are directly delivered to insects. Selection of highly virulent strains is a critical need.

Dr. Tadeusz J. Poprawski (deceased, USDA-ARS) confirmed the identity *of P. fumosoroseus*. Dr. Bradley A. Mullens (Univ. of California, Riverside) kindly commented on this manuscript. The statistical support of Dr. Tom Bohman, Nate Martin, Pat Truxillo and ACITS (University of Texas at Austin) is greatly appreciated. This

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ANALYSES OF COTTON LEAF CHARACTERISTICS EFFECT ON *BEMISIA TABACI* (HOMOPTERA: ALEYRODIDAE) BIOTYPE B COLONIZATION ON COTTON IN ARIZONA AND CALIFORNIA

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ABSTRACT

Cotton leaf characteristics and *Bemisia tabaci* (Gennadius) biotype B population density relationships were studied on 17 varieties of upland cotton, *Gossypium hirsutum* (L.), in Arizona and California from 1999 to 2002. Our objectives were to determine the effects of trichome density, leaf area, leaf perimeter, leaf perimeter-leaf area ratio, and okra-leaf vs. normal-leaf varieties on populations of *B. tabaci* in the field. The results showed that the density of branched stellate trichomes on underleaf surfaces was the primary factor influencing the varietal susceptibility to adult *B. tabaci*. Increased numbers of eggs and nymphs were found on hairy leaf Stonville (ST) 474 plants compared with smooth-leaf varieties. Leaf perimeter size and leaf perimeter-leaf area ratio were negatively related to *B. tabaci* numbers. Okra-leaf varieties had larger leaf perimeters and higher leaf perimeter leaf area ratios and fewer adults per leaf and immatures (eggs and nymphs) per cm^2 of leaf disk. Smaller leaves of normal, smooth leaf varieties may result in lower *B. tabaci* population densities.

INTRODUCTION

Bemisia tabaci (Gennadius) biotype B has been an economic pest in cotton, *Gossypium hirsutum* (L.), in the southwestern United States since 1991 (Natwick et al. 1995). Significant losses can occur because of reduced yields and lint contamination from honeydew excreted by adults and nymphs. A long-term solution to *B. tabaci* management that is environmentally and economically acceptable is needed. The development of *B. tabaci* resistant varieties by conventional plant breeding and selection are approaches that warrant increased attention for *B. tabaci* population suppression (Sippell et al. 1987). The adverse effects of resistant varieties on insect populations have been dramatic when successful host plant resistance has been accomplished (Wiseman 1999). Our previous studies identified cotton leaf trichome density, leaf thickness, and leaf shape as potential traits that may be incorporated into upland cotton breeding programs for the development of *B. tabaci* resistant varieties (Chu et. al. 1998, 1999, 2000, 2001, 2002). We report herein the results of a four-year study (1999-2002) on the impact of leaf perimeter sizes, leaf areas, and leaf perimeter-leaf area ratios as well as underleaf trichome densities on colonization of *B. tabaci* on cotton varieties in Arizona and California.

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MATERIALS AND METHODS

All varieties, but *G. thurberi* (Todaro), were upland cottons, *G. hirsutum* (Table 1). There were seven normal and ten okra-leaf varieties studied in the six field experiments from 1999 to 2002. All normal-leaf varieties were developed in the United States. All okra-leaf varieties, except *G. thurberi*, were developed in Australia. *G. thurberi* is a wild cotton found in at least eight counties in Arizona and Northern Mexico growing at 610 to 1,524 m elevations (Kearney and Peebles 1960). All varieties were smooth-leaf cottons except Stoneville (ST) 474 which is a hairy-leaf variety. The smooth-leaf varieties averaged about 20 or less stellate trichomes per cm^2 leaf disk from fifth node main stem leaves as compared with 120 per cm^2 leaf disk for the hairy leaf ST 474 variety. All varieties were not studied each year in each experiment because of the availability of seed (e.g., 93020-88-753, E0223, E0798, and Texas 121). The number of varieties studied per year ranged from four to ten.

TABLE 1. Normal- and Okra-Leaf Varieties Studied for Susceptibility to *Bemisia tabaci* Biotype B at Maricopa, AZ and Holtville, CA, 1999 - 2002.

	Leaf shape	Leaf type	Experiment location and year					
			Arizona			California		
Variety	O or N[a]	S or H	1999–1	2000-2	2001-3	2001-4	2002-5	2002-6
DP 20B	N	S[b]	X[c]	X	X	--	--	--
DP 50B	N	S	X	X	X	--	--	--
DP 5415	N	S	--	--	--	X	X	X
DP 90B	N	S	X	X	X	--	--	--
NuCOTN 33B	N	S	X	X	X	--	--	--
ST 474	N	H	X	X	X	X	X	X
Texas 121	N	S	--	--	--	--	--	X
E0223	O	S	--	X	X	--	--	X
E0798	O	S	--	X	X	--	--	X
E1028	O	S	--	X	X	--	--	--
G. thurberi	O	S	--	--	--	X	X	--
Siokra L23	O	S	X	X	X	X	X	X
Siokra I-4	O	S	X	--	--	--	--	--
FiberMax 819	O	S	X	--	--	--	--	--
FiberMax 832	O	S	X	--	--	--	--	--
89013-114	O	S	X	--	--	--	--	--
93020-88-753-	O	S	--	--	--	--	--	X
Total	N	--	5	5	5	2	2	3
	O	--	5	4	4	2	2	4

[a] N and O denote okra- and normal-leaf strains or cultivars, respectively.
[b] S and H denote smooth and hairy-leaf cultivar or strain, respectively.
[c] X denotes the varieties tested in experiments.

The six experiments were conducted in randomized complete block designs. Experiments 1, 2, and 3 were conducted at the University of Arizona Agricultural Research Center, Maricopa, Arizona, from 1999 to 2001; and Experiments 4, 5, and 6 were performed at the University of California Desert Research and Extension Center at Holtville, California, from 2001 to 2002. Some of the results from Experiments 1 and 2 were reported earlier (Chu et. al. 2002). The entire data set was analyzed for comparisons with Experiment 3 to study the input of leaf perimeter, leaf area, and leaf-

perimeter-leaf area ratios and leaf trichome densities on *B. tabaci*. There were four replicates for Experiments 1, 2, and 3; six for Experiments 4 and 5; and five for Experiment 6. Plots were eight rows wide for Experiments 1, 2, and 3; two rows wide for Experiment 4; and four rows wide for Experiments 5 and 6. Rows were 12.2-m long and 1-m apart with 3-m non-planted buffer areas between replicates in each experiment. Seeds were planted and watered for germination on 19 April 1999, 13 April 2000, 16 April 2001, 21 March 2001, 22 April 2002, and 22 April 2002 for Experiments 1 to 6, respectively. Plants emerged about two weeks later and were watered at 10- to 20-d intervals during the growing seasons. Plots were not treated with any insecticides except for diflubenzuron (1-(4-chlorophenyl-3-(2,6-difluorobenzoyl urea))) for the control of salt marsh caterpillars, *Estigmene acrea* (Drury) (Lepidoptera: Arctiidae) on 13 August 1999 during Experiment 1. Standard agronomic practices were followed for each experiment.

Densities of *B. tabaci* on cotton were estimated at 7-d intervals from 21 July to 6 October in 1999 for Experiment 1, from 10 July to 5 September in 2000 for Experiment 2, and from 2 July to 20 August 2001 for Experiment 3. On each sampling date, three plants per plot were selected at random for Experiments 1 and 2, and five plants per plot for Experiment 3. Leaves were picked from the first, third, fifth, seventh, tenth, and fifteen main stem nodes from Experiment 1, from the first to fifth and seventh nodes for Experiment 2, and from first to fifth nodes for Experiment 3. The plant main terminal was numbered one, and the nodes thereafter were numbered in descending order. The first node leaf measured ≥ 2.5-cm between the two largest leaf lobes. The fifteenth node positions were at the lowest point on the main stems at the time of sampling. A 2-cm^2 leaf disk was taken from the area adjacent to the middle of the leaf primary vein at the base of the leaf (Naranjo and Flint 1994). Numbers of eggs and nymphs were counted on the undersides of the leaf disks with the aid of a stereomicroscope. Adults per leaf were counted on the underleaf surfaces at the same leaf positions on the main stem nodes using the leaf-turn method described by Naranjo et al. (1995). Leaves were randomly selected from plants throughout each plot. Leaf area and leaf perimeter of each sampled leaf during the growing season were measured with a leaf area meter (CI-400 CIAS Image Analysis, CID, Inc., Vancouver, WA).

Densities of *B. tabaci* eggs and nymphs on plants were estimated from samples of ten plants selected at random in each plot bi-weekly from 5 July through 18 September 2001 for Experiment 4, and from June through mid-August in 2002 for Experiments 5 and 6. Leaf samples were picked from the fifth plant node as described. Eggs and nymphs were counted on single 1.5cm^2 leaf disks, as described previously. Adults per leaf were counted on ten, fifth node leaves also as described previously.

Seasonal mean numbers of *B. tabaci* eggs, nymphs, and adults were analyzed using ANOVA. When the treatment effects were significant, orthogonal comparisons were made between normal- and okra-leaf varieties with and without the hairy-leaf ST 474 variety data (Anonymous 1989). In addition, regression analyses were conducted comparing *B. tabaci* densities and trichome densities, leaf areas, leaf perimeters, and the leaf perimeter-leaf area ratios relationships using stepwise procedure for the analyses (SAS 2000). Numbers of eggs and nymphs per cm^2 leaf disk were combined into immatures for the analyses.

RESULTS AND DISCUSSION

Okra-leaf varieties had significantly fewer adults, eggs, and nymphs compared with normal-leaf varieties in all paired comparisons in the six experiments when the data for the hairy-leaf variety, ST 474, were included in the analyses (Table 2). However, this did not occur when the ST 474 data were excluded from analyses. It appears that the ST 474 hairy leaf variety data had a disproportionate influence on the mean densities of

adults, eggs, and nymphs on normal-leaf varieties. Butler et al. (1991) reported earlier that *B. tabaci* populations increased as the number of trichomes increased up to 70 trichomes per 13.7 mm^2.

TABLE 2. Seasonal Mean ± SE Numbers of *Bemisia tabaci* Biotype B Life Stages on All Normal- and Okra-Leaf Cottons at Maricopa, AZ, 1999-2001, Experiments 1-3, and Holtville, CA, Experiments 4-6, 2001-2002.

Experiment	Year	Treatment	No. adults/leaf	No./cm^2 leaf disk	
				Eggs	Nymphs
			ST 474 included		
1	1999	Okra	12.3 ± 0.9b[a]	29.7 ± 1.9b	10.7 ± 0.9b
		Normal	23.7 ± 1.9a	37.0 ± 3.6a	15.3 ± 1.8a
2	2000	Okra	3.6 ± 0.5b	6.0 ± 0.8b	2.4 ± 0.4b
		Normal	7.3 ± 0.8a	18.6 ± 2.6a	6.1 ± 0.9a
3	2001	Okra	7.6 ± 1.2b	6.9 ± 1.0b	1.5 ± 0.2b
		Normal	15.7 ± 2.3a	18.1 ± 4.2a	4.4 ± 1.0a
4	2001	Okra	1.9 ± 0.6b	1.1 ± 0.3b	0.6 ± 0.2b
		Normal	7.6 ± 0.7a	10.5 ± 2.0a	6.0 ± 1.2a
5	2002	Okra	0.3 ± 0.1b	0.2 ± 0.6b	0.2 ± 0.5b
		Normal	2.8 ± 0.5a	2.8 ± 0.6a	2.9 ± 0.5a
6	2002	Okra	1.8 ± 1.0b	0.5 ± 0.4b	1.2 ± 0.5b
		Normal	7.1 ± 1.2a	2.3 ± 0.4a	3.6 ± 0.6a
			ST 474 not included		
1	1999	Okra	12.3 ± 0.9b	29.7 ± 1.9a	10.7 ± 0.9a
		Normal	21.0 ± 1.4a	31.3 ± 2.6a	12.0 ± 1.0a
2	2000	Okra	3.6 ± 0.5b	6.0 ± 0.8b	2.4 ± 0.4b
		Normal	5.8 ± 0.3a	13.6 ± 1.0a	4.4 ± 0.4a
3	2001	Okra	7.6 ± 1.2b	6.9 ± 1.0b	1.5 ± 0.2b
		Normal	11.1 ± 1.0a	9.1 ± 1.0a	2.3 ± 0.2a
4	2001	Okra	1.9 ± 0.6a	1.1 ± 0.3b	0.6 ± 0.2b
		Normal	5.6 ± 0.5a	4.4 ± 0.3a	2.3 ± 0.2a
5	2002	Okra	0.3 ± 0.1b	0.2 ± 0.1a	0.2 ± 0.1a
		Normal	1.2 ± 0.1a	0.4 ± 0.1a	0.7 ± 0.1a
6	2002	Okra	1.8 ± 0.2a	0.5 ± 0.1a	1.2 ± 0.1b
		Normal	2.7 ± 0.3a	1.0 ± 0.1a	1.4 ± 0.6a

[a] Means in a column for pair comparison in an experiment not followed by the same letters are significantly different by orthogonal comparison ($P = 0.05$). For ST 474 included, the F values were from 4.5 to 64.3 with $P < 0.01$ for Experiments 1 to 6 for adults, eggs, and nymphs, respectively. For ST 474 not included, the F values were 43.9, 0.2, and 2.3 with $P < 0.01$, $P > 0.05$, and $P > 0.05$ for Experiment 1; 22.9, 38.9, and 20.1 with $P < 0.01$, $P < 0.01$, and $P < 0.01$ for Experiment 2; 16.1, 5.8, and 22.8 with $P < 0.01$, $P < 0.05$, and $P < 0.01$ for Experiment 3; 1.6, 6.6, and 4.7 with $P > 0.05$, $P < 0.05$, and $P < 0.05$ for Experiment 4; 5.2, 0.6, and 0.8 with $P < 0.05$, $P > 0.05$, and $P > 0.05$ for Experiment 5; 2.7, 0.1, and 5.9 with $P > 0.05$, $P > 0.05$, and $P < 0.05$ for Experiment 6, for adults, eggs, and nymphs, respectively.

Regression analyses suggested that when all factors were considered, the equation accounted for 66.1 and 68.0% of the variation in numbers of adults per leaf and immatures per cm^2 of leaf disk, respectively (Table 3). Underleaf trichome density was the most important factor measured and accounted for 53.2 and 62.2% of the variation in

TABLE 3. Regression Analyses of Trichome Density, Leaf Area, Leaf Perimeter, and Leaf Perimeter-Leaf Area Ratio of Cotton Leaves and the Density of *Bemisia tabaci* Biotype B life stages at Maricopa, AZ, 1999-2002.

	Intercept		Trichome		Leaf[a] perimeter	Leaf perimeter-leaf area ratio[a]	Total R^2
Adults/leaf	19.366	+	0.107		—	10.102	--
Partial r^2	--		0.532		--	0.129	0.661[b]
Immatures/cm^2 leaf disk	38.426	+	0.318	—	0.379	--	--
Partial r^2	--		0.622		0.058	--	0.680[b]

[a] Leaf perimeter and leaf perimeter –leaf area ratio were not a significant factor in regression equation for adults/leaf and immature/cm^2 leaf disk, respectively.
[b] Denotes significant at $P = 0.001$.

numbers of adults per leaf and immatures per cm^2 leaf disk, respectively. Other authors also have reported that cotton varieties with high trichome density are more susceptible to *B. tabaci* colonization compared with smooth-leaf varieties (Butler et al. 1991, Norman and Sparks 1997, Chu et al. 2000). High trichome density is associated with increased boundary layer humidity on leaf surfaces (Burrage 1971), which may be important for *B. tabaci* in desert condition habitats. Leaf perimeter-leaf area ratio accounted for 12.9% of the variation in numbers of *B. tabaci* adults per leaf. Large leaf perimeter-leaf area ratios are usually associated with smaller leaf areas that allow better air circulation in the plant canopy and possibly less boundary layer humidity under desert conditions and thus a more unfavorable habitat for the *B. tabaci* adults. Regression analyses also indicated that leaf perimeter was significantly (5.8%, F=19.6 with $P < 0.001$) related to the variation in numbers of *B. tabaci* immatures (Table 2).

These results reemphasize the importance of the smooth leaf cotton character for reducing *B. tabaci* colonization. Okra-leaf cotton has also been suggested as a source of resistance to *B. tabaci* and twospotted spider mite colonization (Wilson 1994, Jenkins 1999, Chu et al. 2002). Since almost all upland cottons planted in the United States are normal-leaf varieties, when *B. tabaci* is an economic pest, decreasing leaf size of the normal-leaf cotton to increase leaf perimeters and leaf perimeter-area ratios might be an additional effective strategy to reduce *B. tabaci* colonization.

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AERIAL MOVEMENTS OF THE COTTON APHID, *APHIS GOSSYPII* GLOVER (HOMOPTERA: APHIDIDAE), AND DISCOVERY OF MALE MORPHS IN THE TEXAS HIGH PLAINS

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ABSTRACT

Aerial dispersal of the cotton aphid, *Aphis gossypii* Glover, was monitored with an Allison-Pike® insect suction trap at the Texas Agricultural Experiment Station, Lubbock, Texas in 1989, 1992, 1993 and 1994. This suction trap collected a single male *A. gossypii* on 2 December 1992, the first confirmed report of the male form of *A. gossypii* in the Texas High Plains. A combined total of 13 male morphs were collected by the suction trap and from cotton plants near the trap site. In an effort to establish a method of predicting infestations in cotton, cotton aphid suction trap collections were compared to field densities in a nearby cotton field. Results indicated that suction trap collections during the growing season were not predictive of cotton aphid field population densities. However, numbers of cotton aphids collected by suction trap in November may be predictive of spring field population densities.

INTRODUCTION

The cotton aphid, *Aphis gossypii* Glover, was first described by Townsend Glover in 1876 (Palmer 1952). Two common names (i.e., cotton aphid and melon aphid) are currently accepted for *A. gossypii*. *A. gossypii* is a widely studied pest of interest to many producers and researchers. Infestations by the aphid in cotton may be considered an intermittent problem; in some years the aphid is a secondary pest (Leser 1994), while in other years, years in which widespread outbreaks occur, the aphid is a severe and economically significant pest (Leser et al. 1992). Currently, there are no accurate methods of predicting the density to which aphid populations will develop in cotton fields.

The life cycle of the cotton aphid in the Texas High Plains, as well as across most of the insect's range, is complex and not fully understood (Kring 1972). Most of the many variations in the life cycle of *A. gossypii* are known to occur in the Texas High Plains, where it is thought to consist primarily of viviparous, parthenogenetic females (Slosser et al. 1989). A holocyclic stage has been recorded in Connecticut where cotton aphids used *Catalpa bignonioides* Walter (common catalpa) and *Hibiscus syriacus* L. (rose of Sharon) as primary hosts (Kring 1959). O'Brien et al. (1990) collected four oviparous and two male cotton aphids from cotton regrowth at the Delta Branch Experiment Station in Stoneville, Mississippi, on 20 November 1988. These authors also reported a total of six males and eight oviparous females in the National Collection of Insects at Beltsville, Maryland. The possibility of a holocyclic stage has not been examined in the High Plains.

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The distribution of the cotton aphid is worldwide, mainly due to its broad host range (Blackman and Eastop 1984). Cotton aphids are highly polyphagous, utilizing plant species belonging to 89 families (Ebert and Cartwright 1997). They are known to transmit over 50 plant viruses (Kranz et al. 1977). O'Brien et al. (1992) collected cotton aphids from 24 non-cultivated host plants representing 18 plant families in Mississippi. The majority of these host plants occurred while cotton was present. Cotton aphids were collected from at least two of these host plants during every month of the year and overwintered on three species: evening primrose, *Oenothera speciosa* Nuttall; henbit, *Lamium amplexicaule* L.; and dock, *Rumex* spp. A survey of cotton aphid host plants has not been performed in the Texas High Plains, but many of the documented hosts are common in this area.

Cotton aphids appear to exhibit a form of aestivation in the Texas High Plains. Small, yellow forms are observed under unfavorable (hot and dry) conditions during the summer. These forms may not grow or reproduce until conditions become more favorable (Kring 1959, Kranz et al. 1977). Both winged and wingless forms are common in the Texas High Plains. The exact cause of production of alate forms is not fully understood, but it seems to be primarily associated with daylength (Slosser et al. 1989). Loxdale et al. (1993) stated that production of alates is "an environmentally-induced expression of genotype" and that not all winged forms actually disperse.

Although cotton aphid outbreaks in the Texas High Plains are believed to be aided by large influxes of aphids from more southerly locations, this has never been documented. Long-range dispersal of cotton aphids is poorly understood, perhaps due to their small body size. Aphid flights over great distances may not occur by choice. Aphids may develop large populations in locales where suitable host plants are available and subsequently be carried by prevailing winds (Loxdale et al. 1993). The exact impact of these immigrants on Texas High Plains cotton aphid field populations is unknown.

There is a paucity of information on the life cycle of the cotton aphid and the impact of influxes of aphids from more southerly locations in the Texas High Plains. The objectives of this study were to investigate the aerial movement of the cotton aphid throughout the year, to relate these findings to field population densities, and to examine the possibility of a holocyclic life stage in the Texas High Plains.

MATERIALS AND METHODS

Aerial cotton aphid populations were monitored at the Texas Agricultural Experiment Station, Lubbock, Texas, by using an Allison-Pike® insect suction trap (Allison and Pike 1988) in 1989, 1992, 1993 and 1994. Specimens were captured in pint jars containing a mixture of ethylene glycol (10%) and either ethanol or water (90%). The trap was constructed from a 1.5-m section of 38-cm polyvinyl chloride (PVC) pipe connected to a 6-m section of 30-cm PVC pipe using a coupler. Suction was created by a 30-cm electric fan powered by a 38-watt electric motor mounted under the collection cone and jar in the lower portion of the trap. The trap sampled approximately 570m^3 of air per hour from 8.3m above the soil surface. Surveys were conducted weekly from June through October in 1989, April through December in 1992 and 1993, and from March through December in 1994. All aphids were extracted from these samples, counted, and stored in 70% ethanol for identification. Cotton aphids were then separated from other species. Voucher specimens were placed at the Texas Tech University Museum.

Cotton aphid seasonal average data (number of aphids per leaf per week) from untreated, irrigated cotton were obtained from experimental plots adjacent to the suction trap site in 1992-1995 in an effort to establish the relationship between cotton aphid abundance in the field and numbers of cotton aphids collected by the suction trap. Cotton

aphids were sampled weekly in the field by counting aphids on three full-sized leaves picked from the upper, middle, and lower third of each of ten plants (total 30 leaves). Sampling was conducted from 15 July to 25 August each year.

RESULTS AND DISCUSSION

Suction trap collections of cotton aphids showed that the timing of occurrence of the first cotton aphid, seasonal activity patterns, and the cessation of the aerial activity in the Texas High Plains varied among years. Nevertheless, the peak flight activity occurred in mid- or late August in all four years of the study (Fig. 1 A-D). Peak activity periods were generally of short durations, lasting 1-2 weeks, except in 1992.

The first cotton aphid collected in 1989 was on 8 July, and the last cotton aphids were collected on 17 September. Peak flight activity in 1989 occurred during the week of 25 August with 1,982 cotton aphids captured by the trap (Fig. 1A).

In 1992, the first cotton aphid was collected on 13 May, and this was the only specimen collected in 1992 between 15 April and 21 July. Flight activity increased in late July and the peak activity was recorded on 12 August with 305 aphids captured per trap per week (Fig. 1B). Cotton aphids were captured throughout the month of August at the rate of approximately 300 aphids per trap per week. Cotton aphid flight activity in 1992 ceased on 2 December with one male specimen of the cotton aphid captured on that date.

The first collection of a cotton aphid in 1993 occurred on 30 June. Numbers then increased sporadically to a peak of 40 aphids per trap per week on 25 August (Fig. 1C). Cotton aphid numbers continued to vary from week to week, reaching 24 per trap on 17 September. The last cotton aphid was collected on 25 November and sampling was discontinued on 21 December.

In 1994, the first collection of a cotton aphid occurred on 26 May. No other cotton aphids were collected until 21 July (Fig. 1D). Numbers then began to increase, and peaked at 636 aphids in the trap on 25 August. Flight activity declined the following week, and the aphid numbers captured by the trap declined gradually to one aphid on 24 September. Only seven cotton aphids were collected between 24 September and 28 October, but a total of 321 were collected between 6 November and 2 December. During this period, the highest weekly count of 131 occurred on 17 November (Fig. 2). No cotton aphids were collected by the suction trap after 2 December, and the last sample was collected on 29 December.

The earliest collection of a cotton aphid by the suction trap within the four years of the study was on 13 May 1992. The date of the first appearance of a cotton aphid in suction trap samples did not appear to be predictive of field populations. Peak cotton aphid collections by the suction trap typically occurred during the second or third week of August. The dates of peak field populations ranged from late July to late August, overlapping the period of high suction trap counts. The highest untreated field population density was recorded in 1993 (Fig. 1C), when trap collections were lowest. Therefore, trap collections during this period do not appear to be predictive of field infestations.

The increase in cotton aphid collections by the suction trap in late July appeared to represent an increasing immigration of cotton aphids from more southerly locations and increased aerial movement of cotton aphids in the Lubbock area. However, it is also possible that the localized aphid infestations in pre-squaring cotton in the Lubbock area might have produced some alate forms in late June and early July and these aphids could also have been caught on suction traps. Increases in aphid collections by the trap in mid- to late August and early September appeared to represent local cotton aphid dispersal.

A second peak in cotton aphid collections was noted in late October to mid-November (Fig. 2). This peak appeared to represent movement of aphids out of mature

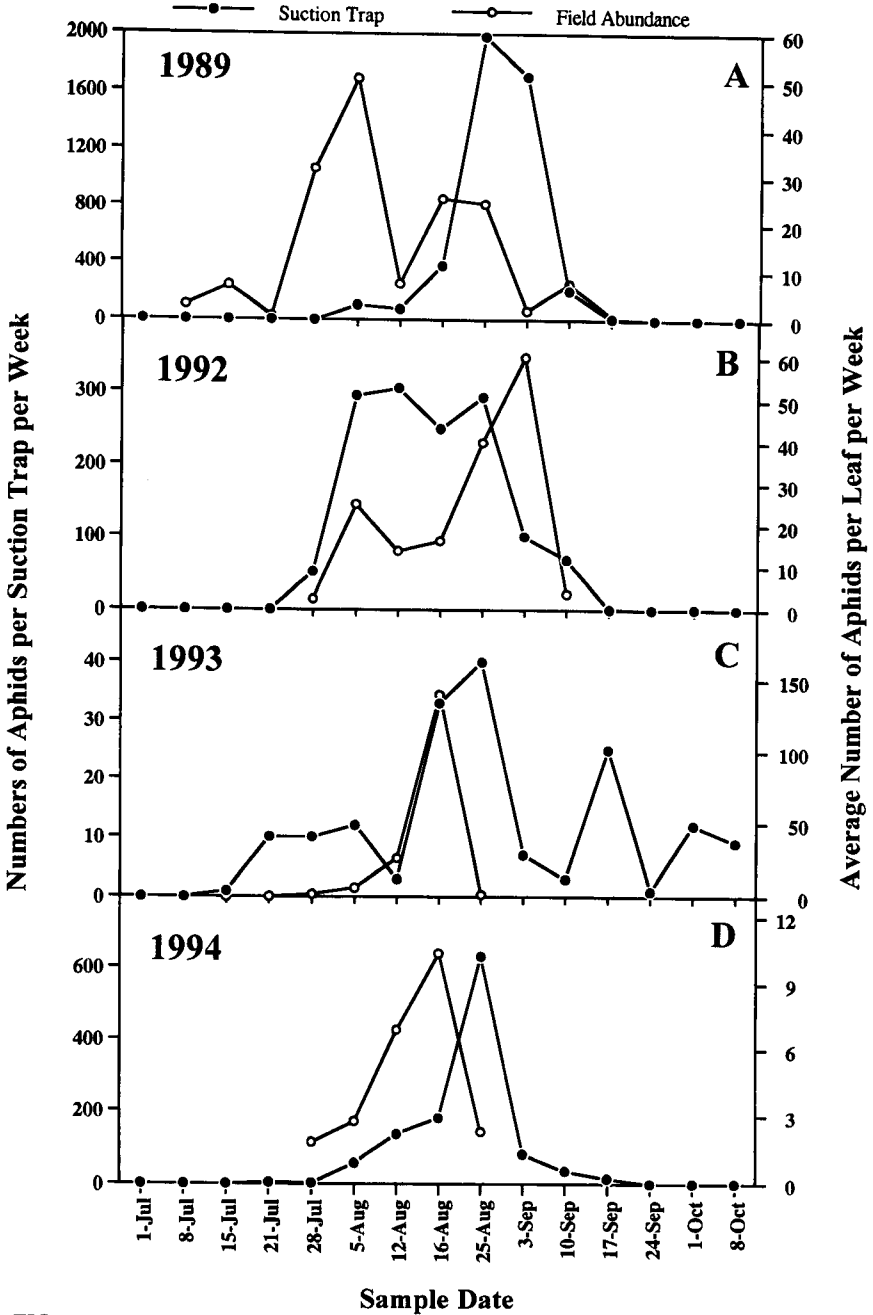

FIG. 1. Numbers of cotton aphids collected per week by a suction trap and mean numbers of aphids per leaf in untreated cotton plots, Lubbock, Texas, 1989, 1992-1994.

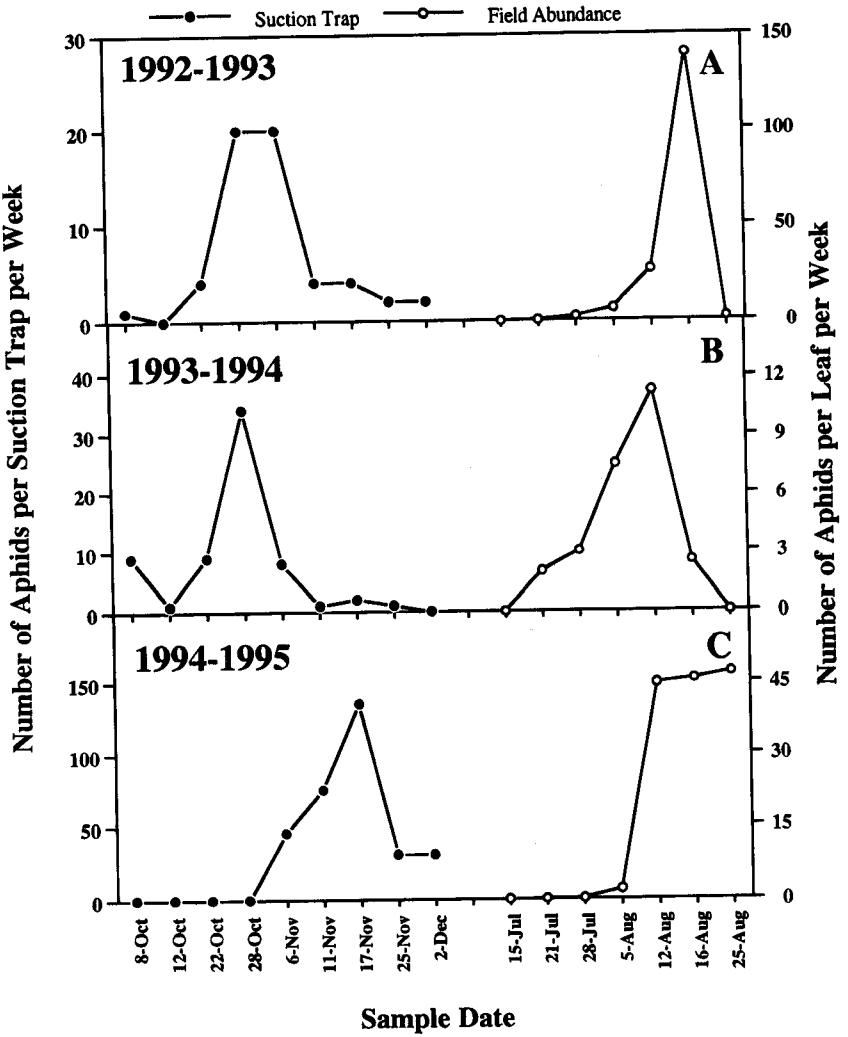

FIG. 2. Numbers of cotton aphids collected per week by a suction trap during the fall and mean numbers of cotton aphids per leaf in untreated cotton plots during the following summer. Lubbock, Texas, 1992-1995.

cotton, which was no longer nutritionally suitable. These cotton aphids were believed to be searching for a suitable overwintering host.

The first male cotton aphid recorded in the Texas High Plains was collected by the suction trap on 2 December 1992. A total of seven male cotton aphids were captured in the suction trap over the four year study period (Table 1). All males found in suction trap samples were collected in November and early December. In addition, a total of six male cotton aphids were collected in November of 1994 from cotton plants which had been grown in a caged environment. Identification of these aphids was confirmed by Dr. S. E. Halbert (Florida Department of Agriculture, Gainesville, Florida). The production of males in the fall implies the existence of a sexually reproductive overwintering generation. However, no oviparae were found on cotton or in suction trap samples. A single specimen of male *A. gossypii* was collected by a suction trap near Parma, Idaho, on 12 October 1987 (S.E. Halbert, unpublished data). Other confirmed findings of male cotton aphids in the United States are shown in Table 1. All of these males were collected between 9 October and 20 November, and collection sites were widely distributed throughout the United States. Therefore, production of males in November and early December appears to be related to shorter daylength.

The purpose of male cotton aphid production is believed to be for sexual reproduction with oviparous females. Although no oviparae were found in this study, a holocyclic life cycle likely exists in the Texas High Plains. Sexual reproduction would allow genetic recombination to occur and increase the size of the gene pool. Further studies would be required to identify possible overwintering host plants and examine the impact of overwintering aphids on field populations.

TABLE 1. Known Collections of Male *Aphis gossypii* in the United States to Date.

Survey Site	Survey Date	Host Plant	Male Specimen
Greenbush, MA[1]	18 Oct. 1949	strawberry	1
Washington, DC[1]	09 Nov. 1962	rose of Sharon	1
Washington, DC[1]	10 Nov. 1964	rose of Sharon	2
Albuquerque, NM[1]	09 Oct. 1969	catalpa leaves	2
Parma, ID[2]	12 Oct. 1987	suction trap	1
Stoneville, MS[1]	20 Nov. 1988	cotton (regrowth)	2
Lubbock, TX	02 Dec. 1992	suction trap	1
Lubbock, TX	08 Nov. 1994	cotton	6
Lubbock, TX	17 Nov. 1994	suction trap	4
Lubbock, TX	02 Dec. 1994	suction trap	2

[1]O'Brien et al. (1990).
[2]S. E. Halbert, unpublished data.

If overwintering cotton aphids influence early season field populations, aphids collected by a suction trap in November may have a predictive value. Trap collections were terminated early in 1989, so it is not possible to determine the impact on 1990 infestations. The November trap collections in 1992 and 1993 were relatively low, and the 1993 and 1994 field infestations were light, except for 16 August in 1993 (Fig. 2A, 2B). The November trap numbers in 1994 were approximately three-fold those of the previous two years, and unusually high cotton aphid field population densities were present in 1995; the field infestation in 1995 remained high throughout August (Fig. 2C). These data indicate that there exists a relationship between the November suction trap

catches, the potential overwintering population, and the level of field infestation the following summer in the Texas High Plains. However, this information is inconclusive at this time due to limited amount of data. The year-to-year variation in abundance of potential overwintering host plants, perhaps a function of late season rainfall, may be more important than numbers of late season flying aphids, as caught in the suction trap. In addition, winter severity may also impact winter survival of cotton aphids. Additional studies are needed to capture year-to-year variation in the relationship between overwintering populations and the resulting population dynamics the following growing season.

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CONTROL OF GREEN PEACH APHIDS *MYZUS PERSICAE*[1] WITH LADY BEETLES *HARMONIA AXYRIDIS* ON CHILE *CAPSICUM ANNUM*[2] IN THE GREENHOUSE

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ABSTRACT

Chile plants, *Capsicum annum* L., in greenhouse cages were inoculated with lady beetles, *Harmonia axyridis* (Pallas), to green peach aphid, *Myzus persicae* (Sulzer), ratios of 1:20, 1:40, 1:80, 1:160, 1:320, and 1:640. Live aphids were counted every two days on each plant in each cage for 10 days. Changes in aphid density were statistically significant in all six treatments at $p \leq 0.05$. Treatment ratios of 1:20, 1:40, 1:80, 1:160, and 1:320 gave 95% or better control of *M. persicae* after 10 days. The mean percentage aphid reduction at a 1:640 ratio fell to 85.69% after 10 days but still provided significant aphid density decreases during the 10 day test period.

INTRODUCTION

Chile, *Capsicum annum* L. (Solanaceae), has been grown in New Mexico for 400 years (Bosland et al. 1997). A variety of pests damage chile both in the field and greenhouse; however, the green peach aphid *Myzus persicae* (Sulzer) is one of the most important. Mackauer and Way (1976) reported that the green peach aphid is the most common aphid pest in U. S. greenhouses. This aphid also vectors many plant viruses (Kennedy et al. 1962, Harris and Maramorosch 1977, Matthews 1991) and is resistant to a large number of insecticides (Georghiou 1986, Quaclia et al. 1993). Due to pesticide resistance, escalating cost of chemical control, and environmental concerns, interest in biocontrol strategies is growing.

Biological control can be defined as the use of one organism to decrease the population of another organism (Huffaker and Messenger 1976). It is often not necessary to eliminate all pests, but only to reduce their numbers below an economic threshold (Dent 1993). Predators are distributed among 20 insect orders and are categorized by taxonomic affiliation (Hodek 1973, Canard et al. 1984, Gilbert 1993,) or feeding strategy, monophagy versus polyphagy (Hagen et al. 1976).

Lady beetles are one of the most formidable aphid predators (Hagan and van den Bosch 1968, Hodek 1973). The coccinellid *Harmonia axyridis* (Pallas) is an introduced species from the Far East (Timberlake 1943, Chapin and Brou 1991) and is established in the southern U. S.

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(Chapin and Brou 1991). This species is highly polymorphic and may live up to three years (Hodek 1973, Knodel and Hoebeke 1996).

In this study, *H. axyridis* was evaluated as a biocontrol agent for *M. persicae* in the greenhouse. The research specifically investigated the optimal predator/prey ratio required for aphid control within a specified period of time.

MATERIALS AND METHODS

This research was conducted at the Fabian Garcia Research Center, New Mexico State University. Adult *H. axyridis* were imported from Georgia to establish a laboratory colony. Young progeny from this colony were used to carry out the experiments. A laboratory colony of *H. persicae* was propagated from local insects, and was reared on potted chile plants in two greenhouse cages. Seeds of *C. annum* "Nu Mex Big Jim" were grown in 2.5-cm preformed peat pots until the three-leaf stage. The healthiest seedlings were transplanted into 12-cm plastic pots containing peat moss and pearlite. All plants were fertilized with a complete water soluble nitrogen-phosphorus-potassium fertilizer (20-20-20) and irrigated daily. All plants were sprayed with Bayleton[7] fungicide [1-(4-chlorophenoxy)-3-3-dimethyl-1-(1 H-1,2,4 triazol-1-yl)-2 butazone (Bayer Corp. Kansas City, MO.)] at a rate of 1.52 ml active ingredient per 3.78 L of water once every two weeks to prevent the growth of powdery mildew, *Leveillula taurica* (Lev.). Plants were grown under a 15-hour light:9-hour dark photoperiod. After three months (March to June), the plants averaged 36.5-cm in height. The number of leaves per plant ranged from 68 to 41-cm, averaging 53.5-cm.

Each experimental unit consisted of four individually potted chile plants enclosed in a screened cage. Before the start of the experiment, each pot of chile was covered at the base of the plant with a small mesh nylon net to prevent insects from falling onto the soil of the pot. Actual number of *H. axyridis* to *M. persicae* per treatment were: (1) 4:80, (2) 4:160, (3) 4:640, (5) 4:1280 and (6) 4:2560. The experiment was run as a completely randomized design. Each treatment was replicated three times (three cages containing four individually potted chile plants). Controls were established for each treatment (two cages containing four individually potted chile plants, but no *H. axyridis*). Cages for treatments and controls were randomly assigned. The mean number of *M. persicae* surviving the predation of *H. axyridis* were determined every 2 days for a 10-day period. At the end of the 10-day period, all *M. persicae* were removed from each cage and the cages were inoculated with the indicated numbers of predators and hosts for the next treatment.

Mean *M. persicae* densities and Standard errors were calculated for the treatments and controls. A dummy variable regression (Draper and Smith 1966) was used to evaluate the effects of the variables time, replication, and time by replication interactions. This analysis provides estimates for the parameters represented by the variables. The analysis estimated the intercepts and slopes for each treatment and further estimated the difference between the slope of the controls and the slope of the treatments. Only five cages containing chile plants were available for this experiment, thus the treatments were run sequentially. Significant differences were determined by combining treatment days 2, 4, 6, 8 and 10 using a one-way analysis of variance multiple range test.

RESULTS AND DISCUSSION

Table 1 presents the mean density of *M. persicae* per plant in the three replication cages and two control cages of each treatment as well as the mean percentage aphid reduction for all six ratio treatments.

TABLE 1. Mean Number of *M. persicae* (± SE[a]) Counted per Plant at Two-Day Intervals over 10 Days in Six Different *H. axyridis* : *M. persicae* Treatments on Greenhouse Grown Chile Plants, N.M.S.U., Las Cruces, New Mexico,1997.

Variable (Day)	Control Mean ± SE	Treatment Mean ± SE	Mean Percentage *M. persicae* Reduction
		Treatment 1 (1:20)	
2	47.88 ± 2.29	10.30 ± 2.76[b]	78.43
4	85.25 ± 2.12	12.92 ± 4.05[b]	84.85
6	118.75 ± 13.96	8.83 ± 4.63[b]	92.57
8	158.75 ± 11.66	4.92 ± 1.46[b]	96.91
10	204.75 ± 14.85	1.67 ± 0.72[b]	99.19
		Treatment 2 (1:40)	
2	97.13 ± 3.36	17.42 ± 2.52[b]	82.06
4	141.88 ± 13.61	11.25 ± 1.89[b]	91.89
6	199.88 ± 20.33	6.33 ± 1.66[b]	99.96
8	257.25 ± 19.09	4.33 ± 0.63[b]	98.31
10	311.13 ± 2.65	1.75 ± 0.25[b]	99.43
		Treatment 3 (1:80)	
2	141.75 ± 1.74	23.25 ± 0.90[b]	83.59
4	200.38 ± 1.24	12.67 ± 4.30[b]	93.67
6	280.00 ±12.73	7.75 ± 0.66[b]	97.23
8	347.88 ±11.49	5.08 ± 0.72[b]	98.53
10	423.00 ±14.85	2.00 ± 0.90[b]	99.52
		Treatment 4 (1:160)	
2	268.88 ±15.38	53.08 ± 4.07[b]	80.25
4	336.38 ± 5.48	31.67 ± 1.38[b]	90.58
6	399.88 ± 5.48	22.17 ± 0.52[b]	94.45
8	485.88 ± 8.66	16.25 ± 2.25[b]	96.65
10	574.50 ±29.34	6.42 ± 1.84[b]	98.88
		Treatment 5 (1:320)	
2	486.38 ± 15.03	166.92 ±12.49[b]	65.67
4	590.75 ± 27.58	94.75 ±16.02[b]	83.96
6	667.88 ± 58.51	64.33 ±10.62[b]	90.36
8	759.88 ± 50.73	50.17 ± 4.80[b]	93.39
10	829.38 ± 38.01	38.50 ± 7.19[b]	95.35
		Treatment 6 (1:640)	
2	816.88 ± 25.28	421.83 ± 64.56[b]	48.36
4	891.63 ± 0.53	372.00 ± 54.19[b]	58.27
6	1022.88 ±16.79	291.83 ± 58.32[b]	71.46
8	1108.63 ± 3.71	228.67 ± 36.29[b]	79.37
10	1197.63 ± 8.31	171.25 ± 26.96[b]	85.69

[a]Standard Error

[b]denotes significance between treatment and control at $p \leq 0.05$ using a one-way analysis of variance.

Fig.1 summarizes the estimated daily mean *M. persicae* density changes in the treatment and control cages of all six treatments. Declines in aphid density are a measure of mortality, presumably due to predation, while increases are a measure of aphid natality. In Treatments 1, 2, 3, 4, 5, 6, the estimated consumption rate by one *H. axyridis* was 2.5, 3.8, 2.5, 10.9, 30.1, and 64.4 per day, respectively, while the average number of *M. persicae* increased at a rate of 38.7, 54.3, 71.0, 75.9, 85.5 and 97.8 per day, respectively, in the control cages.

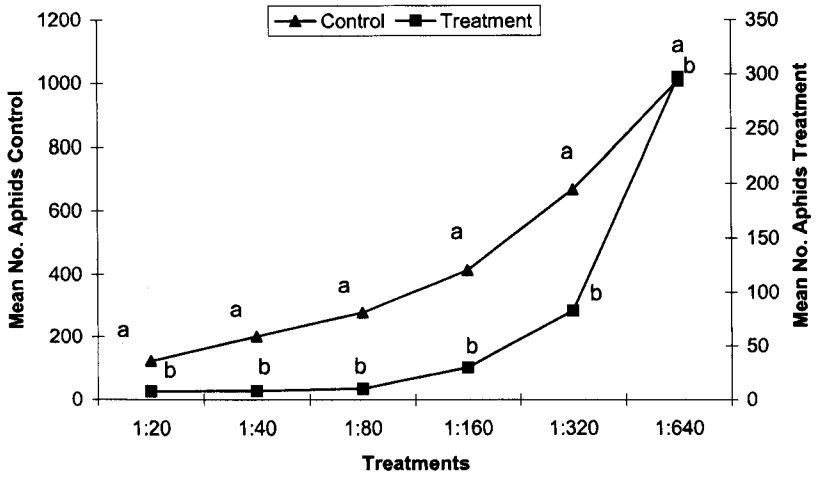

FIG. 1. Mean density of *M persicae* on chile plants after 10 days exposure to *H. axyridis* at different densities on Greenhouse Grown Chile Plants, N.M.S.U., Las Cruces, New Mexico,1997.

The greatest *M. persicae* consumption rate was observed during the first two days of each treatment. This may be due to the 24-hour fast imposed on *H. axyridis* before each treatment began. Aphid reduction was greater than 95.00% for the 1:20, 1:40, 1:80, 1:160, and 1:320 ratios but fell to 85.69% at 1:640 ratios, respectively, perhaps due to the overwhelming number of *M. persicae* per *H. axyridis*.

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COMPARATIVE SUSCEPTIBILITY OF EUROPEAN AND AFRICANIZED HONEY BEE [1] ECOTYPES TO SEVERAL INSECTICIDE CLASSES

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ABSTRACT

Three insecticide classes (pyrethroid, carbamate, and organophosphate) were assayed for comparative toxicity to European honey bees, *Apis mellifera* L. and Africanized honey bees, *Apis mellifera scutellata* Lepeletier. Ecotype verification was achieved through utilization of ecotype-specific isoenzyme analyses. The candidate insecticides tested are commonly used in crops in the geographical ranges of both European and Africanized honey bees. The European honey bee ecotype was significantly more tolerant of cyfluthrin (pyrethroid) than the Africanized honey bee ecotype, likely due to the preceeding decade of pyrethroid use in managed colonies to control the parasitic mite, *Varroa destructor* Anderson and Trueman. Both ecotypes of bees were equally susceptible to malathion (orgahophophate) and carbofuran (carbamate) insecticides. Discussion is given as to development of relative insecticide tolerance in European honey bees.

INTRODUCTION

The European honey bee, *Apis mellifera* L., is the dominant honey bee ecotype occurring the U.S., with distribution throughout the entire 50 states. With its discovery in Texas in 1990 (Sugden and Williams 1990), the Africanized honey bee, *Apis mellifera scutellata* Lepeletier, has now spread throughout the entire southwestern U.S. (Sheppard and Smith 2000). Both ecotypes in the region now forage in concert, pollinating not only agricultural crops in the process, but countless wild plant species as well. Both ecotypes are now considered sympatric throughout the southwestern U.S., with no efforts to eradicate the Africanized honey bee deemed feasible.

In field cropping situations in the southwestern U.S., European honey bees and Africanized honey bees are exposed to many agricultural chemicals during field foraging and pollination activities. The exposure of honey bees to insecticides used in these crops

[1] Hymenoptera: Apidae
[2] Disclaimer: 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.

is a major problem implicated in colony losses around treated fields. Recommendations have been made to lessen exposure of honey bees to toxic insecticides (Atkins et al. 1981), but actual application practices, such a timing of sprays, are not always feasible. Many studies have been conducted on the toxicity of insecticides on European honey bees (Johansen et al. 1957, Anderson and Atkins 1968, Stoner et al. 1985, Cox and Wilson 1987, Wilson et al. 1988), but none have addressed the comparative toxicity of insecticides to European versus Africanized honey bees in the U.S. Danka et al. (1986) compared toxicity of several insecticides on European versus Africanized honey bees in South America in the mid 1980's, characterizing honey bee ecotypes based on locality of colonies. These tests were conducted prior to the widespread and intensive use of the synthetic pyrethroid insecticide class in European honey bee colonies for control of the parasitic mite *Varroa destructor* Anderson and Trueman and prior to movement of Africanized honey bees into the U.S., where its exposure to in-hive pesticide use has been minimal.

Given these considerations of the widespread presence of Africanized honey bees in agricultural areas of the southwestern U.S. and the decade-long use of synthetic pyrethroid insecticide within European honey bee colonies for *V. destructor* control, we undertook the present study to investigate comparative toxicity of three classes of commonly used agricultural insecticides on European versus Africanized honey bees. Such toxicity data may provide indications of potential pollination disruption by either ecotype in the southwestern U.S., with implications on appropriate insecticide selection by farmers for use in crops while honey bees are present in fields.

MATERIALS AND METHODS

Colonies of European and Africanized honey bees were maintained in separate apiaries, ca. 20 miles apart, near Weslaco, Texas. European honey bee colonies were obtained as commercial packages produced in central Texas and subsequently established at Weslaco on previously unused wax foundation and hive equipment. European honey bees from colonies containing marked and clipped queens were verified as non-Africanized, as described below and used for tests, with no acaricide treatment in hives prior to test initiation. Suspected Africanized honey bees, originally collected from swarm traps along the Rio Grande River near Hidalgo, Texas, were from colonies that had received no pyrethroid acaricide treatment in colonies during the preceeding four years. Laboratory verification of individual colony ecotype and laboratory toxicity assays were conducted in late 2000- early 2001.

Isozyme bioassays were used to verify the ecotype identification of colonies used in tests (Nunnamker and Wilson 1981, Spivak et al. 1988). For each colony, 22 adult worker females were collected and frozen at -70° C until processing the following week. Samples were removed from the freezer and kept chilled as thoraces were removed individually from each bee. These were singly homogenized in 1.5 µl eppendorf tubes in distilled water and centrifuged for 10 min at 12,000 rpm in a refrigerated micro centrifuge. Pre-packaged mini PHAST gels were then loaded with 0.4 µl of supernatant per lane and separated using isoelectric focusing techniques. The co-occurrence of malate dehydrogenase and hexokinase bands were considered verification of Africanization for that colony; simultaneous absence of both markers, along with identification of an original marked queen at hive inspection, were considered confirmation of a European colony.

For each ecotype, brood was collected from three to four selected colonies confirmed as either European or Africanized, brought to the laboratory, and placed in an

incubator overnight in order to collect newly-emerged adult worker females. Emerged bees of European or Africanized verification were collected the next morning, pooled as to ecotype, and then held in cages for 24 hours at 28°C and 50% RH before test initiation, during which they were freely fed 50% sugar syrup and distilled water.

Unaesthestised bees were then individually treated topically on the dorsal thorax with technical grade insecticides dissolved in acetone. Chosen insecticides were cyfluthrin (pyrethroid), malathion (organophosphate), and carbofuran (carbamate). All technical grade materials were obtained from Chem Services Inc. (West Chester, PA 19381) and were of >95% purity. Stock solutions of serial dilutions were prepared by dissolving each insecticide in HPLC-grade acetone; both solutions and technical grade materials were stored at -20°C. Individual bees were treated with 1 µl of known concentration or with 1 µl of acetone as a control using a pre-calibrated microapplicator (Burkard, Rickmansworth, UK). Ten bees each were treated with each concentration or acetone and placed as groups in small screened cages. Five concentrations plus a control treatment were tested for each insecticide. Treated bees were held for 24 h at 28°C in darkness with syrup and water and then assessed for mortality. The criterion for mortality was inability to move when gently prodded. Modeling trials have demonstrated that 120 bees total, tested for a chemical for dose-mortality estimations, are sufficient to obtain accurate dose-mortality estimates (Robertson et al. 1984). In our tests, we replicated each insecticide trial per ecotype three separate times, for a total of 180 bees pooled from three-four colonies, verified as to ecotype, tested for each insecticide. Dose-mortality lines for each insecticide were analyzed by probit analysis POLO (LeOra Software) in order to estimate lethal dose values, confidence intervals, and slopes and standard error of means of slopes. Confidence intervals were compared between ecotypes at the LD_{50} levels; non-overlap of confidence intervals was considered demonstration of significant difference between ecotypes in response to a particular insecticide. For each chemical, equivalency of slopes was determined by non-overlap of two times the standard error of the mean.

RESULTS AND DISCUSSION

Results of isozyme analyses of sample bees from colonies used in tests confirmed the identification of ecotypes as either European or Africanized colonies. Those colonies established from swarms collected in South Texas were clearly Africanized as demonstrated by high frequencies of both isozyme markers, seen upon assay completion. Colonies established from packages with marked and clipped queens revealed the absence of the two characteristic marker isozymes, confirming their non-Africanized status. Selected colonies were used, as described above, for all subsequent insecticide assays, ensuring the continued verification of non-Africanized European honey bees by the presence of marked queens.

Both malathion and carbofuran were equally toxic to European honey bees and Africanized honey bees, as evidenced by overlap of 95% confidence intervals at the LD_{50} levels (Table 1). Response to cyfluthrin was significantly different between the two ecotypes, with European bees exhibiting a greater tolerance to this pyrethroid compound than Africanized bees (Table 1). This greater tolerance by European honey bees may be due to the preceeding decade of pyrethroid use in managed European colonies to control the honey bee parasitic mite *V. destructor*. Because *V. destructor* has been considered to have severely reduced feral populations of the European honey bee in the U.S. (Hoopingarner et al. 1992), selection pressure for pyrethroid tolerance in European honey bees likely occurred with little infusion of unexposed susceptible phenotypes from the wild.

TABLE 1. Response of European and Africanized Honey Bees to Topically Applied Insecticides; Values Given are Expressed as μg Insecticide per Bee.

Treatment	Ecotype	n	LD_{50} (95% CI)[a]	LD_{90} (95% CI)	slope (±SEM)
Malathion	AHB	180	0.123 (0.093-0.190)	0.279 (0.183-0.706)	3.602 (0.652)
	EHB	180	0.193 (0.167-0.218)	0.292 (0.254-0.371)	7.114 (1.184)
Cyfluthrin	AHB	180	0.011 (0.008-0.018)*	0.030 (0.024-0.043) *	3.109 (0.555)
	EHB	180	0.036 (0.020-0.051)	0.070 (0.050-0.202)	4.521 (0.669)
Carbofuran	AHB	180	0.033 (0.027-0.038)	0.057 (0.048-0.076)	5.400 (0.974)
	EHB	180	0.039 (0.034-0.045)	0.062 (0.053-0.082)	6.388 (1.132)

[a] Values followed by an asterisk are significantly different for that insecticide between ecotypes.

In contrast, Africanized honey bees are rarely maintained in managed colonies in the southwestern U.S., with little intense selection pressure by pyrethroid pesticides to control *V. destructor*, opposite to what is experienced by European honey bee colonies. Slope values for each insecticide tested, comparing ecotypes, were not significantly different, indicating similar homogeneity of response by both ecotypes to each insecticide. In testing carbamate and organophosphate susceptibility after eight years of observations, Anderson and Atkins (1968) found no change in tolerance by European honey bees to these insecticide classes. Tucker (1980), however, was able to demonstrate increased tolerance of worker European honey bees after selection for nine generations with carbaryl.

Danka et al. (1986) found in their studies of Africanized and European honey bees in Venezuela, with location of swarms used as designation of Africanized colonies, that Africanized honey bees were generally more tolerant than European honey bees toward three of the four classes tested, results they found surprising given the smaller size of Africanized honey bees. They discuss the role of degree of cuticular sclerotization as a possible explanation for susceptibility differences; in their study, they used newly emerged bees that were less than 24-h old. In our toxicity trials, however, we used adults that were at least 24-h old after emergence and so perhaps were more sclerotized than adults used by Danka et al (1986). Also, in our studies, we used populations of European and Africanized honey bees that likely received similar exposure to agricultural chemicals during the preceeding generational years, other than pyrethroids used in managed European colonies, and so may explain the greater similarity of response to carbamate and organophosphate insecticides tested in our study. The additional step of definitively confirming colonies as Africanized or European, as we did in our study, may also explain the differences in responses we saw compared to those seen by Danka et al (1986).

In the southwestern U.S., the primary ecotype found existing as feral colonies is the Africanized honey bee, with European honey bees virtually absent from the wild. This appears to be due, however, to differential increased ability of Africanized honey bees to withstand infestations of *V. destructor* (Rubink et al 1995), while European honey bees are more severely affected and colonies are rapidly killed by *V. destructor*

infestations. There are no field data to indicate that the greater presence of feral Africanized honey bees in the southwestern U.S., as well as in much of Latin America, compared to European honey bees, is due to any greater tolerance of the Africanized ecotype to insecticides. It is also important to state that, while statistically significant differences were seen between ecotypes in our study, as well as in Danka et al (1986), such differences in dose-mortality values would be minor compared to the much higher rates of insecticide use in field pest control settings. Insecticides are used in kilogram quantities per hectare, rates most likely much higher than what we used to determine dose-mortality estimates in our study. In order for selection for ecotype resistance to occur in a field setting, there must obviously be mortality of susceptible individuals, but also a concomitant survival of tolerant individuals. The insecticide rates used in field crop settings to which either ecotype would be exposed would seem not to allow survival of slightly tolerant individuals due to the excessive rates bees would experience. It is also important to note that differentials in European honey bee reproductive fitness has been demonstrated for European honey bees exposed to fluvalinate (pyrethroid) pesticide used in managed colonies to control *V. destructor*: Rinderer et al (1999) demonstrated that drones exposed to fluvalinate in managed colonies at normal usage rates lived significantly shorter than non-exposed drones and had minor reproductive fitness disadvantages (i.e., clear selection pressure for tolerance) and thus allowed for "selection" by queens of potentially more tolerant drones for production of offspring with resultant greater tolerance to pyrethroid pesticides. Such selective advantage by potentially tolerant drones may explain the greater tolerance of European honey bees to the pyrethroid insecticide tested in our study compared to Africanized honey bees in our study that did not receive such a level of selection within colonies. Additionally, only non-reproductive worker bees are typically exposed to high doses of field agricultural insecticides, possibly explaining further why resistance to organophosphate and carbamate insecticides has not arisen in either ecotype. The reason that European honey bees have not developed resistance to organophosphates in particular, given the use in the past 5 years of an acaricide in this class in managed hives, is unknown. As usage of this class, and potentially of other classes of pesticides within hives continues, managed European honey bees may exhibit increased tolerance to these compounds. Because development of pesticide resistance in organisms is difficult to predict timewise, only continued testing will reveal if such resistance will actually occur.

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SUITABILITY OF FORMIC ACID TO CONTROL *VARROA DESTRUCTOR*[1] AND SAFETY TO *APIS MELLIFERA*[2] IN THE SOUTHWESTERN U.S.[3]

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ABSTRACT

Formic acid in a gel form was tested in south Texas for its efficacy in controlling the parasitic honey bee mite, *Varroa destructor* Anderson and Trueman, and its safety for use in southern conditions. Formic acid gel gave significantly better control than untreated colonies, but such control was significantly less than standard treatments of fluvalinate and coumaphos. The formic acid gel treatment also resulted in significant, but not excessive, mortality of adult honey bees compared to untreated controls. This was particularly seen as temperatures consistently rose above ca. 24°C. Queen mortality was also observed in colonies treated with formic acid. Discussion is given on integration of the formic acid gel pack into a *V. destructor* management plan for southwestern U.S. beekeepers.

INTRODUCTION

The varroa mite, *Varroa destructor* Andersen and Trueman, is one of the most serious parasitic pests attacking honey bees in the U.S. and on a worldwide basis (Shimanuki et al. 1992). This mite damages immature and adult honey bees by feeding on bee hemolymph and by transmitting viral pathogens. Left unchecked, a heavy infestation of *V. destructor* can weaken and kill an entire colony within ca. two years and cause eventual loss of most colonies within an apiary.

The primary means of controlling *V. destructor* since its discovery in the U.S. in 1987 (Anonymous 1987) has been through synthetic chemical acaricides. The pyrethroid fluvalinate (Apistan®) and the organophosphate coumaphos (CheckMite+®) have been the most commonly used EPA-approved compounds for *V. destructor* control in the U.S. Beginning in 1998, however, U.S. beekeepers in widely diverse regions began noticing decreased efficacy of fluvalinate in their operations, as evidenced by unexpectedly high mite numbers in treated hives. Such decreased efficacy was confirmed as resistance by *V. destructor* to this compound by Elzen et al. (1999). Beekeepers then turned to coumaphos, which provided excellent control of fluvalinate-resistant mites (Elzen et al.

[1] Mesostigmata: Varroidae
[2] Hymenoptera: Apidae
[3] Mention of a trade name does not constitute endorsement by the USDA

2000). Elzen and Westervelt (2002), however, confirmed that *V. destructor* has now become resistant to coumaphos in Florida, with likely spread to other regions of the U.S. as bee colonies are moved as part of normal migratory activities of beekeeping.

Beekeepers have now increasingly called for the availability of alternative compounds for the control of *V. destructor*. One such compound is formic acid, which has received approval for use as a gel formulation by the U.S. Environmental Protection Agency.

Research on the suitability of formic acid liquid and gel formulations has been conducted in the U.S., but almost exclusively in the northern regions of the U.S. (Feldlaufer et al. 1997, Calderone and Nasr 1999, Calderone 2000). Beekeepers in the southern U.S. have called for information on the suitability of formic acid for *V. destructor* control in the relatively warmer conditions of the south and associated effects on honey bees in treated colonies. We thus initiated the present study to provide beekeepers in the southwestern U.S. with data they would find necessary to consider the use of formic acid in their operations. Because the only EPA-approved usage of formic acid registered currently in the U.S. is the gel formulation, we chose to evaluate this formulation in conditions of the southwestern environment.

MATERIALS AND METHODS

In order to ensure the highest contact of gel pack vapors with mites, treatment was initiated when the majority of *V. destructor* were on adult bees, where formic acid vapors would most readily reach phoretic adults. For this reason, the trial was initiated on 3 January 2002, a general date during which colonies in south Texas are most broodless (<5 frames of brood per colony). Colonies were located at Weslaco, Texas, at a site at least 5 miles from other apiaries. Colonies were established in the spring of 2001 from commercially-obtained packages, installed in new equipment/foundation, and remained untreated for *V. destructor* until initiation of the present study. All colonies were queenright and contained ample stores of honey and consisted of a deep bottom brood chamber and one deep honey super.

Standard compounds consisted of 10% fluvalinate strips (Apistan®, obtained from Dadant; Paris, TX) and 10% coumaphos strips (CheckMite+®, obtained from Dadant; Paris, TX). In both cases, one strip of either treatment was inserted in the bottom brood chamber, as recommended by manufacturers (one strip per five frames of brood). The formic acid treatment consisted of 200 g of formic acid gel (obtained from Better Bee; Greenwich, NY) in vegetable baggies, each of which was cut open in a X configuration immediately before application. One pack was placed over the end of the top bars of the brood chamber, but not over the brood area. One-inch spacers were inserted between bottom and top chambers, to allow for air flow. Spacers were inserted in all other treatment and control colonies as well. The control treatment consisted of colonies receiving no strips or gel packs. Treatments were randomly assigned to colonies, with five to six colonies per treatment.

Effects of treatments on *V. destructor* control were assessed according to the method of Feldlaufer et al. (1997). Sticky boards, under screens, were inserted on the bottom board of each colony at the start of the trial. Treatments were then immediately applied. Boards were removed weekly and numbers of fallen mites were counted for that week. This was repeated for a total of three weeks and was considered the treatment period. A following 3-week evaluation was then begun in all colonies by removing previous treatments and inserting one coumaphos strip in the brood chamber as a final clean-up treatment. Weekly mite drop was assessed as described for the treatment phase. Final efficacy of treatments was calculated by summing the number of mites dropped

during the treatment and evaluation phases (total drop), dividing the number dropped during the treatment phase by the total drop, and multiplying this value by 100. These percentage efficacy values were arc-sine transformed and analyzed by ANOVA and means separation tests (LSD, P=0.05).

Effect of treatments on adult honey bee mortality was assessed by positioning standard dead bee traps at colony entrances during both the treatment phase and evaluation phase. Numbers of dead bees were counted at 3-day intervals and were removed from traps. For each date, mean numbers of dead bees for each treatment were compared by ANOVA and means separation tests (LSD, P=0.05).

Temperatures during the trial were obtained from a local weather station website service. Final queen status was assessed by noting any dead queens in dead bee traps during the treatment and evaluation phases.

RESULTS AND DISCUSSION

All treatments applied for *V. destructor* provided significant control compared to untreated colony results (Table 1). The efficacy of the formic acid gel pack, however, was significantly less than the standard treatments of fluvalinate or coumaphos. There was also wide variation in control with formic acid, with as low as 69.8% control and as high as 93.8% control for individual colonies. In contrast, ranges of control afforded by fluvalinate and for coumaphos were much narrower, with both providing consistent control greater than ca. 90%. However, such consistent control in other regions of the U.S. by fluvalinate and now coumaphos may be questionable, due to reports of resistance by *V. destructor* to these compounds (Elzen et al. 1999, Elzen and Westervelt 2002). Our results of overall 84.5% control with the formic acid gel pack is higher than the 70.3% control reported by Feldlaufer et al. (1997) for tests run in Maryland in the spring months. They also reported relatively high variability in mite control between individual colonies, as we observed in our study.

TABLE 1. Activity of acaricides in causing *V. destructor* mortality in treated colonies.

Treatment	Percentage Drop (±SE)[a]	Range(%)
Fluvalinate	95.0 (1.1) a	90.4-97.7
Coumaphos	95.8 (1.4) a	90.8-98.8
Formic Acid Gel	84.5 (4.7) b	69.8-93.8
Control	33.8 (7.4) c	11.7-54.1

[a]Values within a column followed by different letters are significantly different (ANOVA, LSD, $P < 0.05$).

Statistically significant mortality of adult bees in the formic acid treated colonies was observed, compared to untreated control results (Table 2), starting at the sixth day of the treatment period. While significant, mortality was not excessive. Prior to this time, the temperature maximum averaged 15.5°C. Temperatures after day 6 averaged 24.1°C. No significant bee mortality was seen for fluvalinate or coumaphos treated colonies, compared to untreated colony mortality, after the sixth day of treatment and warmer

TABLE 2. Mortality of Adult Honey Bees Exposed to Acaricides Under Field Condition

Treatment	Mean No. Dead Adults (±SE)[a]					
	4 Jan	7 Jan	10 Jan	14 Jan	17 Jan	23 Jan
Fluvalinate	21.2 (2.7) a	125.7 (37.5) a	82.7 (18.5) a	51.2 (11.2) ab	28.2 (6.8) a	35.3 (10.0) a
Coumaphos	24.8 (4.1) a	173.6 (47.9) a	75.0 (11.6) ab	40.2 (13.7) a	39.2 (8.6) a	37.6 (8.8) a
Formic Acid	31.0 (8.2) a	159.8 (55.4) a	112.4 (21.0) a	91.4 (24.8) b	62.0 (13.2) b	77.6 (17.7) b
Control	22.4 (9.0) a	145.6 (83.9) a	31.6 (4.9) b	38.2 (11.4) a	28.4 (8.9) a	35.8 (9.7) a

[a]Means within a column followed by different letters are significantly different (ANOVA, LSD, $P < 0.05$).

temperatures. Queen mortality was also seen in the formic acid replicates, with 20% queen mortality observed during the treatment phase of the trial.

Because beekeepers are facing loss of both fluvalinate and coumaphos as efficacious acaricides, there is naturally great interest in alternative compounds. The formic acid gel pack would be a logical choice, but our results show that variability in control from hive to hive in southwestern U.S. conditions would have to be tolerated. Such variability would limit the attractiveness of the gel pack in standard U.S. bee stocks, but may be significantly less important in newly developed bee stocks that exhibit tolerance to *V. destructor* infestations (Rinderer et al. 2001, Harbo and Harris 2001, Spivak and Reuter 2001). These stocks are capable of withstanding higher mite populations, with only occasional treatment necessary. The issue of worker and queen kill would be of consideration for southern beekeepers, where unpredictable fall and winter temperatures may increase and so have potentially negative effects in formic acid gel pack treated colonies. An alternative solution to this possibility, at least for migratory beekeepers, would be to apply treatments while colonies may be in cooler northern locations, before migrating to southern (and warmer) locations. Integration of tolerant bee stocks, colony location, and the formic acid gel pack may thus be a workable solution in dealing with *V. destructor* that are rapidly developing resistance to all standard treatments for its control.

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IDENTIFICATION OF THREE FORENSICALLY IMPORTANT BLOW FLY (DIPTERA:CALLIPHORIDAE) SPECIES IN CENTRAL TEXAS USING MITOCHONDRIAL DNA

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ABSTRACT

The morphological identification of three blow fly species common to central Texas, *Cynomyopsis cadaverina* (Townsend), *Calliphora livida* (Hall), and *Calliphora vicina* (Robineau-Desvoidy), was confirmed using restriction enzyme analysis of PCR amplified mtDNA fragments. An amplified region of the COI gene from each species was subjected to digestion by four restriction enzymes. Restriction fragment patterns distinguished the three species. The DNA sequence of the COI gene fragment from each species was determined, confirming the restriction enzyme analysis. Subsequently, mtDNA fragments from field collected samples were amplified and subjected to restriction enzyme analysis in order to test the validity and reliability of the identification method. For each species tested, it was demonstrated that restriction enzyme digestion of amplified mtDNA fragments could be used to identify different blow fly developmental stages to species, previously a problematic task for early stages, where only limited morphological characters are available.

INTRODUCTION

Each blow fly species (Diptera:Calliphoridae) has a particular developmental time. This makes them useful for forensic investigations, provided that collected samples can be correctly identified to species. However, species identification using morphological characters can be problematic, especially in early developmental stages. This is particularly relevant, as in many instances insect evidence collected at a crime scene is predominantly eggs and larvae. The larvae of some species are indistinguishable, especially the early instars (Debang and Greenberg 1989). Furthermore, to preserve specimens at the time of collection, they are often killed, preventing the specimens from being reared to adulthood for identification. Eggs pose an even greater problem as the morphologies of many species are indistinguishable and are also susceptible to desiccation so that the embryo may die before eclosion, making it impossible to determine the species. Improper species identification could lead to an

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incorrect estimate of the post mortem interval (PMI). Therefore, the ability to perform a molecular identification from any insect evidence is critical.

DNA identification, or DNA "fingerprinting", has recently been applied to the identification of insects of forensic importance (Black and Duteau 1997, Roehrdanz and Johnson 1996, Sperling et al. 1994, Vincent et al. 2000, Wells and Sperling 2001, Azeredo-Espin and Madeira 1996, Wallman and Donnellan 2001)). Mitochondrial DNA (mtDNA) is relatively resistant to degradation and is particularly well suited as a template for identification purposes. There are hundreds of mtDNA copies in each cell and the mutation rate of mtDNA is high enough to provide numerous sequence differences among closely related species (Sperling et al. 1994, Lee and Gaensslen 1990). Most importantly, mtDNA can be used for the identification of all insect life stages. It is important that tests and confirmations of molecular identification methods are performed for each geographical region. This will help overcome possible complications such as local fixation of particular polymorphisms, and enable the molecular evidence to be used more effectively in a local court of law.

Four blow fly species were chosen for this study, three "bluebottle" species, *Calliphora vicina* (Robineau-Desvoidy), *Calliphora livida* (Hall), and *Cynomyopsis (=Cynomya) cadaverina* (Townsend), and the black blow fly, *Phormia regina* (Meigen). The occurrence of these species was recorded during a field study conducted in College Station, TX and a rural study site near College Station, TX. The "bluebottle" species chosen for molecular identification were the only ones collected of those that have been recorded for the area. The prevalence of the bluebottle species during the winter and spring months in Brazos and Burleson Counties of central Texas and the differences in their developmental time makes these species perfect candidates for forensic applications. In addition to general difficulties with adult and pupal morphological identification keys, the identity of *C. vicina* and *C. livida* larvae cannot be resolved (Debang and Greenberg 1989). The black blow fly, *Phormia regina* (Meigen), was chosen for comparison because this species has been used in a previous mtDNA-based species identification study (Sperling et al. 1994). Sperling et al. (1994) PCR amplified a region of the COI mitochondrial DNA gene and digested the PCR product with four restriction enzymes to establish a method for distinguishing between three blowfly species, *Phormia regina*, *Phaenicia sericata*, and *Lucilia illustris*. Applying the same restriction enzymes utilized in the previous study (Sperling et al. 1994), we PCR amplified and digested a 348 base pair sequence of the COI mitochondrial DNA gene and identified restriction fragments on agarose gels. The results of these studies are presented, as well as an analysis of the DNA sequence of this gene region from the three bluebottle species.

MATERIALS AND METHODS

Individual wild caught fresh blow fly specimens from collections made at two field study sites (Texas A&M Mosquito Research Laboratory, College Station, Brazos County, Texas; 4km SSW Snook, Burleson County, Texas) were killed by snap freezing in liquid nitrogen and DNA was extracted following Black and Duteau (1997). Only the thorax of each adult specimen was used for DNA extraction, the head, abdomen and wings were saved as vouchers for morphological identification.

A region of the mitochondrial genome within the cytochrome oxidase subunit I gene (COI) was amplified using the Polymerase Chain Reaction (PCR). The PCR primers used in this reaction are described by Sperling et al. (1994) and amplification of a 348bp gene fragment. PCR was performed in an MJ Research® PTC-200 Peltier Thermal Cycler (MJ Research, Inc. Waltham, MA 02451). Ten microliters (µl) of DNA

from each specimen was used in a 40µl total reaction volume (0.5µl Taq DNA polymerase [Promega, Madison, WI 53711-5399], 4.0µl thermophilic magnesium free 10X buffer [Promega,], 4.0µl 25mM $MgCl^{2+}$ Buffer, 4.0µl 2.5mM dNTP mix [Promega], 20pmoles COI (5') primer, 20pmoles COI (3') primer, and ddH20 to 40µl per sample). A preincubation at 94°C for 5 min. was followed by 30 cycles of denaturation at 94°C for 30 seconds, annealing at 45°C for 30 sec., and extension at 72°C for 30 seconds. The procedure was terminated with a final extension at 72°C for 5 minutes.

For restriction fragment analysis, 10µl of the PCR products were digested in a 20µl total volume according to the directions specified by Promega. The resulting products were separated on a 2% agarose gel and imaged using a UVP BioDoc-It system (UVP, Cambridge, UK). For DNA sequence analysis, the PCR products were gel purified and cloned using the pGEM®-T Easy Vector System I (Promega) according to the instructions provided by the manufacturer. Miniprep DNA was prepared according to the instructions provided with the QIAprep® Spin Miniprep Kit (QIAGEN Inc., Valencia, CA 91355). Following DNA purification, 5µl reactions (2µl Big Dye Mix, 10pM of primer, 400ngrams of DNA template, and ddH20 to 5µl) were prepared for DNA sequencing following the ABI PRISM™Big Dye™ Terminator Cycle Sequencing Ready Reaction Kit (PE Applied Biosystems, Alameda, CA 94501). After a preincubation at 94°C for 3 minutes, and 30 cycles of 94°C for 30 seconds, 50°C for 30 seconds, and 60°C for 1 minute, each reaction was run through a Micro Bio-Spin® P-30 Tris Chromatography Column (BIO-RAD, Hercules, CA 94547) to remove unincorporated nucleotides. Samples were dried down and submitted to the Gene Technologies Laboratory (Texas A&M University) for processing. DNA sequence analysis and restriction enzyme mapping was performed with the Vector NTI® Suite of programs (Informax®, North Bethesda, MD 20852).

RESULTS AND DISCUSSION

Fig. 1 shows the pattern of restriction fragments generated by four different enzyme digestions of the COI mtDNA gene fragment for each species. Species identification can be made with the restriction enzyme digestion. *EcoRV* does not distinguish between any of the species. The *DdeI* pattern identifies *C. livida* from the rest of the species and the *DraI* pattern identifies *P. regina*. Lastly, the *HinfI* pattern can distinguish between *C. cadaverina* and *C. vicina* (Table 1).

Cloned COI PCR products from two additional individuals of each of the three bluebottle species were subjected to DNA sequence analysis. Fig. 2 shows the alignment of one DNA sequence from each species. While single base pair polymorphisms were identified between members of the same species (data not shown), these changes did not occur at the chosen restriction enzyme recognition sites. In a previous study by Vincent et al. (2000), amplified mtDNA from the COI gene of *Phaenicia* spp. specimens was analyzed by restriction enzyme digestion to determine if the restriction enzymes used in the study by Sperling at al. (1994) correctly identify species. In that study, samples sizes were small (n=3,4, or 5), and the results showed that polymorphisms in members of the same genus and species made the specimens indistinguishable from each other. Here, PCR amplified products from the COI gene of a further 23 individuals of each species were subjected to digestion by all four restriction enzymes. Identical restriction fragment patterns were observed for all individuals within each species, for all enzymes tested (data not shown). Egg and larval

samples from each species also returned the same results (data not shown), confirming the utility of this method for species identification of all life stages of these blow flies.

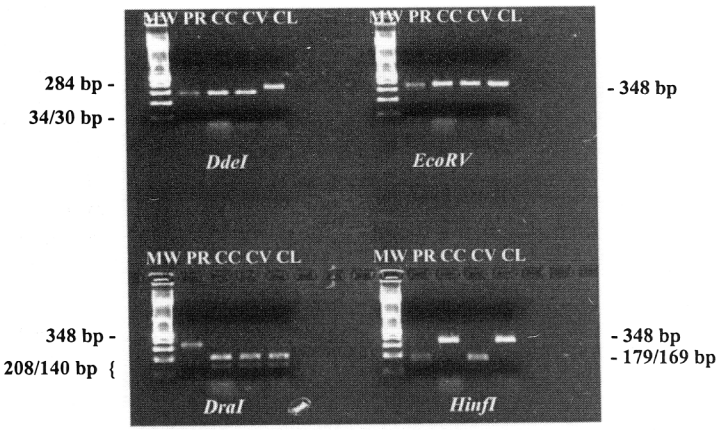

FIG.1. 2% agarose gel showing restriction fragment patterns following restriction enzyme digestion of 348bp COI PCR product from each species. MW= Molecular Weight (100bp ladder, Promega), PR= *P. regina*, CC= *C. cadaverina*, CV= *C. vicina*, and CL= *C. livida*. See Table 1 for the fragment sizes produced by each restriction enzyme.

TABLE 1. Fragment Sizes Produced by the Restriction Enzyme Digestion of the 348bp COI PCR Gene Product from Each Species, by Four Different Restriction Enzymes, as Listed.

	Enzyme Produced Fragment Size			
Species	Dde I	Eco RV	Dra I	Hinf I
C. cadaverina	284, 64	348	208,140	348
C. vicina	284,34,30	348	208,140	179,169
C. livida	348	348	208,140	348
P. regina	284,64	348	348	179,169

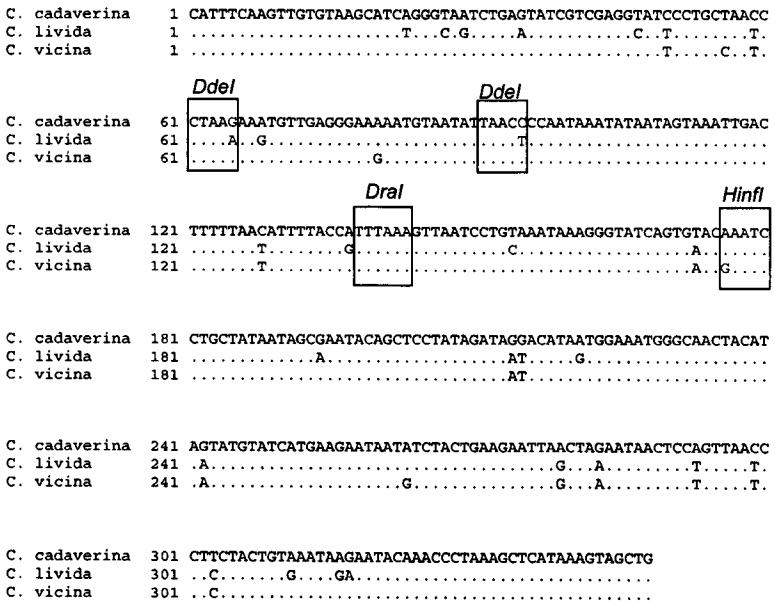

FIG. 2. Sequence differences within the 348bp COI gene fragment of each bluebottle species (GenBank Accession Numbers AY036971 - *C. cadaverina*; AY036969 - *C. livida*; AY036970 - *C. vicina*). Only sequence differences are shown. Restriction enzyme recognition sites are highlighted with boxes.

In conclusion, the mtDNA identification procedure used in this study was reliable for positively identifying the four species tested. However, the results of this study should not be used as a definitive tool for identification without first examining all available morphological characteristics as well as geographic and seasonal data. Without taking into account these other sources of information, it may be possible to make a misidentification, particularly if further surveys reveal species that share the same or similar restriction enzyme digestion profiles for this mtDNA region.

More surveys and experimental testing must be performed to enable other closely related species of blow flies occurring in the central Texas area to be distinguished. The genus *Phaenicia* is an excellent candidate for further validation of these methods. In a recent field study performed in central Texas (Tenorio et al. 2003), two species from this genus were catalogued in the same geographical area. These species are closely related and share many of the same morphological characteristics. They typically occur during different seasons of the year but could easily be misidentified because of a lack of suitable keys for identification purposes. For example, some members of the *Phaenicia* genus occurring in Texas are indistinguishable even as adults. Further research will assist the validation of an entomological approach to the estimation of post mortem intervals, geographic location of death, and recent history of the individual.

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USING COLD-STORED OR OVERWINTERING *ACERIA MALHERBAE* NUZZACI (ACARINA: ERIOPHYIIDAE), A GALL-FORMING ERIOPHYIID MITE, FOR INFESTATION OF FIELD BINDWEED

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ABSTRACT

Clippings of *Aceria malherbae* Nuzzaci infested field bindweed, *Convolvulvus arvensis* L., were used to successfully infest field bindweed in a greenhouse for up to 70 days after the clippings had been collected and stored at either 2 and 4°C. Percentage of plants infested was significantly different between mites collected from the two sites (34.5% from Moore County and 16.5% from Carson County, Texas), and the ability of mites to infest plants decreased significantly as the number of days in storage increased. No significant difference in percentage of plants infested was found between years (27.8% in 1999 and 23.2% in 2000) or storage temperature (23.5% at 2°C and 27.5% at 4°C). Overwintering *A. malherbae* from field bindweed rootstock infested 66.7 and 75% of the field bindweed grown in a greenhouse, and it took an average of 24.5 (± 6.6) and 21.4 (± 4.5) days for the bindweed plants to begin showing damage symptoms in 1999 and 2000, respectively.

INTRODUCTION

Field bindweed, *Convolvulus arvensis* L., a perennial weed that competes with other plants for water and nutrients, infests 32 field crops in more than 40 countries, reducing crop yield, crop quality, and land value (Holm et al. 1977). Field bindweed was reported in Virginia in 1739, and soon spread along the Atlantic seaboard. German and Russian immigrants introduced field bindweed to the Great Plains as a contaminant in wheat seed in the 1870s (Holm et al. 1977). Field bindweed infests almost 250,000 ha of farmland in Texas and causes economic losses of more than $50 million each year (Bean and Wiese 1990). It can reduce yields of broadcast-seeded cereal crops by 20-50% and of row crops by 50-80%. The annual cost attributed to field bindweed control and its impact on crops in the Texas Panhandle is $10-60 per ha (Bean and Wiese 1990).

Field bindweed is found in dry to moderately moist soils and can survive long periods of drought, growing best in fertile but also rocky soils (Holm et al. 1977). It is best adapted to semiarid regions west of the Mississippi River. The taproot of field bindweed can a reach depth of 30 m or more, and laterally spreading roots and rhizomes make control difficult (Swan and Chancellor 1976). The seeds are extremely hard, impervious to water, and can remain viable in the soil for more than 30 years (Weeler and Degennaro 1984).

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Because of their small size and hard coat, the seeds are spread by birds or wind or as a contaminant in crop seed or manure. Manure containing field bindweed seed is a problem in areas such as the Texas High Plains because feedlots sell manure to farmers as a fertilizer.

Aceria malherbae is one of eight species of eriophyiid mites, and one of four species in the genus *Aceria*, that attack field bindweed in Greece, Italy, Spain, and southern France (Rossenthal 1983). *A. malherbae* was imported from Greece as a biological control agent of field bindweed (Boldt and Sobhian 1993). Quarantine of *A. malherbae* at the USDA-ARS Insect Quarantine Facility at Temple, Texas, in 1988 involved testing for host preference. The mites were tested on plants of the family Convolvulaceae, to which field bindweed belongs, including sweet potato (*Ipomoea batatas* (L.) Lam.), ivy-leaf morning-glory (*I. hederacae* Jacq.), tall morning-glory (*I. purpurea* (L.) Roth), heavenly-blue morning-glory [*I. tricolor* Car., referred to as *I. rubrocaerulea* Hook. in Boldt and Sobhian (1993)], and hedge false bindweed (*Calystegia sepium* (L.) R. Br.). No evidence of feeding was found on any plant tested. Therefore, Boldt and Sobhian (1993) concluded that *A. malherbae* was closely linked to the physiology of the host and had a restricted host range.

A. malherbae feeds along the center of the upper surface of field bindweed leaves, causing the leaves to fold and fuse along the middle vein (Rossenthal 1983). As feeding progresses, the plant cells hypertrophy, causing the leaves to thicken and develop a rough surface. Clear protuberances or papillae, termed microgalls by Boldt and Sobhian (1993), are produced by the plant cells and cause the leaves to seem grainy or mealy. These galls can also occur on the stems, rhizomes, buds, and deeper roots. Heavily-infested plants are covered with microgalls, becoming deformed and chlorotic. The mites retard the growth of and eventually kill the bindweed plant. *A. malherbae* also interferes with vegetative growth, flowering and seed production. The mites overwinter on field bindweed roots and attack new roots and shoots in the spring.

Approximately 5,800 *A. malherbae* were released in May and 9,000 were released in June 1989 on field bindweed at the Texas A&M University Agricultural Research and Extension Center at Bushland, Texas (Boldt and Sobhian 1993). The mites survived for three consecutive years and were considered established in the Texas Panhandle in 1992. The mites were slow colonizers, moving only 125 m per year from the original site. In the fall of 1992, 35-38% of the leaves of plants surrounding the initial release site were infested. In the winter of 1992, plants were dug up and each rhizome was found to be infested with an average of 78-90 mites. It is not known if *A. malherbae* is dispersed by wind, as are other Eriophyiidae, or distributed on field bindweed by some other mechanism, such as movement from the roots and foliage of one plant to the next.

In 1998, a site where *A. malherbae* had been released east of Amarillo, Texas, was inadvertently mowed. Three weeks after the initial release at the site, field bindweed infested with *A. malherbae* was found more than 100 m from the release site. The mites were thought to have been distributed on clippings from the mowing and had moved off the clippings onto noninfested field bindweed. Because of these observations, we hypothesized that a practical and economical way to distribute the mite to new sites might be to store refrigerated clippings of mite-infested field bindweed. Viability of mites on refrigerated clippings was assessed in this experiment. In addition, since *A. malherbae* overwinters on field bindweed roots, we assessed whether mite-infested rootstock of field bindweed collected in the fall could be used as an infestation source.

MATERIALS AND METHODS

Since 1998, the entomology program has been involved in an area-wide project to redistribute *A. malherbae* throughout the Texas Panhandle. Field bindweed sites in Carson and Moore counties that were part of this redistribution program and heavily infested with *A. malherbae* were selected to provide mite-infested field bindweed clippings for this research.

The field bindweed at each site was mowed on 15 October 1999 and 25 July 2000, and the clippings were collected in a bag attached to the mower. Clippings were taken to a greenhouse, and 250 ml of clippings were placed in 946-ml paper bags. Approximately 100 bags from each site were stored at 2 and 4°C in Percival I30-BLL environmental chambers (Percival, Boone, Iowa). Four bags were removed weekly from each environmental chamber, and clippings from an individual bag were distributed over a mite-free field bindweed plant growing in a 15.2-cm diameter plastic pot. Field bindweed plants, which had been germinated in the greenhouse, were at the four-leaf stage when infested. Clippings were placed on plants on the same day the clippings were collected from the field (Day 0) and on new plants every 5-7 days thereafter until 11 sets of plants had been infested in 1999 and 10 sets of plants had been infested in 2000. The plants were observed visually for damage (microgalls, leaf distortion and chlorosis) caused by mites every 3-5 days for three months, beginning three days after the plants were infested. Plants showing damage symptoms were considered infested and counted as such. The data were analyzed using the Probit Procedure available in the SAS (1999) computer program and the results of the χ^2 tests associated with this procedure were used to determine significant differences between field sites, years, temperatures and lengths of time mites were in storage.

Root samples were taken from mite-infested field bindweed sites in the fall of 1999 and 2000 to determine whether mite-infested rootstock of field bindweed could be used as a source for infesting field bindweed with *A. malherbae*. Three to four roots were taken approximately 1.2-m deep from the soil. The roots were examined for mites; approximately 50 mites were taken from infested rootstock and placed onto a field bindweed plant growing in a 15.2-cm plastic pot. Four plants were infested in this manner at three different dates, giving a total of 12 replications. The foliage was examined every 3-5 days for signs of mite damage, beginning three days after the plants were infested. The experiment ended after three months. Analyses consisted of conventional averages and standard error calculations.

RESULTS AND DISCUSSION

Average percentages of field bindweed plants infested varied by the different combinations of sites, years, and temperatures (Table 1). By the end of the experiment, 10- 50% of the plants were infested when exposed to clippings of field bindweed infested with *A. malherbae*. Variability in percentage of plants infested could have been caused by differences in the number of *A. malherbae* on the clippings of field bindweed and perhaps the quality of the clippings themselves. Mowing field bindweed at the nurseries was, uneven because of rough ground and variable plant height. The number of *A. malherbae* collected probably depended on the percentage of ground covered and the abundance of mite-infested field bindweed plants. We did not count individual *A. malherbae*. Counting eriophyiid mites is labor and time intensive, and the purpose of the experiments was to determine whether clippings of field bindweed infested with *A. malherbae* could be stored for future infestation of field bindweed plants. Chi-square tests, used to determine significant differences between the percentages of plants infested by *A. malherbae* collected from the two sites during the two years and stored at different temperatures indicated that there were because of rough ground and variable plant height. The number of *A. malherbae* collected no significant differences by year or storage temperature in the ability of *A. malherbae* to infest field bindweed (Table 2). There was a significant difference between locations ($\chi^2 = 16.57$, $p = 0.05$) and the length of time that *A. malherbae* were stored ($\chi^2 = 27.63$, $p = 0.05$). Time in storage results form a gradient based on number of days and a binary assessment of infestation (1=infested, 0=noninfested), rather than a specific comparison between locations, years, or temperatures. Therefore, only the relevant test results for time in storage are presented in Table 2, and more detailed results are presented in Table 3. The negative

TABLE 1. Percentage Infestation of Greenhouse Field Bindweed Inoculated with Aceria malherbae-Infested Bindweed Clippings from Two Locations in the Texas Panhandle, Stored at Two Temperatures.

Year	n	Storage temperature °C	Percentage infestation	SE[a]
		Moore County		
1999	48	2	22.7	6.1
1999	44	4	50.0	7.6
2000	52	2	40.4	6.9
2000	52	4	25.0	6.1
		Carson County		
1999	38	2	21.1	6.7
1999	40	4	17.5	6.1
2000	40	2	10.0	4.8
2000	40	4	17.5	6.1

[a] Standard error.

TABLE 2. χ^2 Tests Between Location, Year, Storage Temperature, and Time in Storage.

Comparison	Percentage infestation	SE [a]	Estimate	χ^2	SE for χ^2	Prob. > χ^2
			Location			
Moore	34.5	6.7	1.05	16.57	0.26	0.0001
Carson	16.5	5.9				
			Year			
1999	27.8	6.6	-0.07	0.07	0.25	0.7848
2000	23.2	6.0				
			Temperature (°C)			
2	23.5	6.1	-0.05	0.04	0.25	0.8325
4	27.5	6.5				
Time	na	na	-0.03	27.63	0.01	0.0001

[a] Standard error.

estimate, or coefficient, indicates that successful infestation decreased as time in storage increased.

The site in Carson County was infested in 1998, while the site in Moore County was infested in early 1999. Clippings from the site in Moore County resulted in an overall infestation of 16.5% of the field bindweed plants in the greenhouse compared to 34.5% with clippings from the site in Carson County. *A. malherbae* was established at the site in Carson County almost a year longer than at the site in Moore County and would be expected to

TABLE 3. Days in Refrigerated Storage and Average Days to Infest Field Bindweed by *Aceria malherbae* Collected from Two Sites in the Texas Panhandle in 1999 and 2000.

1999, 2°C			1999, 4°C			2000, 2°C			2000, 4°C		
	Avg. days			Avg. days			Avg. days			Avg. days	
Days [a]	to infest	SE [b]	Days	to infest	SE	Days	to infest	SE	Days	to infest	SE
					Moore County						
4	--- [c]	---	4	8.5	1.5	3	15.3	2.8	3	---	---
7	11.0	1.0	7	11.0	0.6	8	15.0	0.0	8	---	---
14	33.0	0.0	14	26.0	3.9	14	---	---	14	21.7	2.3
19	28.0	0.0	19	12.8	1.8	23	17.0	0.0	23	11.5	5.5
22	25.0	0.0	22	---	---	28	15.0	5.0	28	21.0	0.0
35	---	---	35	12.0	0.0	34	13.0	1.0	34	10.5	4.5
47	6.0	0.0	47	5.0	1.0	36	15.7	5.0	36	19.3	2.3
50	9.5	1.5	50	11.0	0.0	40	16.5	1.5	40	---	---
54	7.0	0.0	54	6.3	0.7	49	21.0	0.0	49	21.0	0.0
57	---	---	57	12.0	0.0	58	8.0	4.0	58	---	---
59	10.0	0.0	59	---	---	70	12.0	0.0	70	16.0	0.0
Avg.	16.2			11.6			14.8			17.3	
SE	3.8			2.0			1.1			1.8	
					Carson County						
3	13.0	7.0	3	---	---	4	---	---	4	26.0	0.0
6	---	---	6	23.0	9.0	11	17.0	2.0	11	19.0	0.0
9	12.0	2.0	9	14.0	0.0	15	15.0	0.0	15	12.5	2.5
16	---	---	16	---	---	23	---	---	23	22.0	0.0
23	5.0	0.0	23	27.5	5.5	31	---	---	31	32.0	0.0
28	22.0	6.0	28	18.5	9.5	37	---	---	37	---	---
31	25.0	0.0	31	---	---	45	14.0	0.0	45	14.0	0.0
44	---	---	44	---	---	53	---	---	53	---	---
56	6.0	0.0	56	---	---	63	---	---	63	---	---
59	---	---	59	---	---	66	---	---	66	---	---
Avg.	13.8			20.8			15.3			20.9	
SE	3.3			2.9			0.9			3.0	

[a] Days mite-infested clippings were refrigerated before being applied to noninfested field bindweed

[b] Standard error.

[c] Mites failed to become established, not included in average.

result in greater infestation of the field bindweed plants. However, the Carson County site was mowed in October, and the Moore County site was mowed in July. At the Carson County site, A. *malherbae* may have either left the plant or moved down onto the roots by October, but may have still been on the foliage at the Moore County site in July.

We originally hypothesized that the longer *A. malherbae*-infested clippings were stored, the less likely they were to infest field bindweed because of declining clipping

freshness, growth of saprophytic fungi and *A. malherbae* moving off the clippings. In general, this was true, although the number of days it took for the noninfested bindweed plants to become infested by the mite-infested clippings was not highly variable (Table 3). Regardless of the site, year, or temperature, fewer field bindweed plants were infested by *A. malherbae* as the number of days that the mites were in storage increased (Fig. 1, 2). Percentage of plants infested after being exposed to clippings from the site in Moore County (Fig. 1) was less than that from exposure to clippings collected from the site in Carson County (Fig. 2). At most, 50% of field bindweed plants in the greenhouse were infested when exposed to *A. malherbae* on refrigerated clippings from Moore County while as many as 75% of plants were infested when clippings from the site in Carson County were refrigerated for as many as 54 days. Again, this could be because of the time of year the clippings were taken.

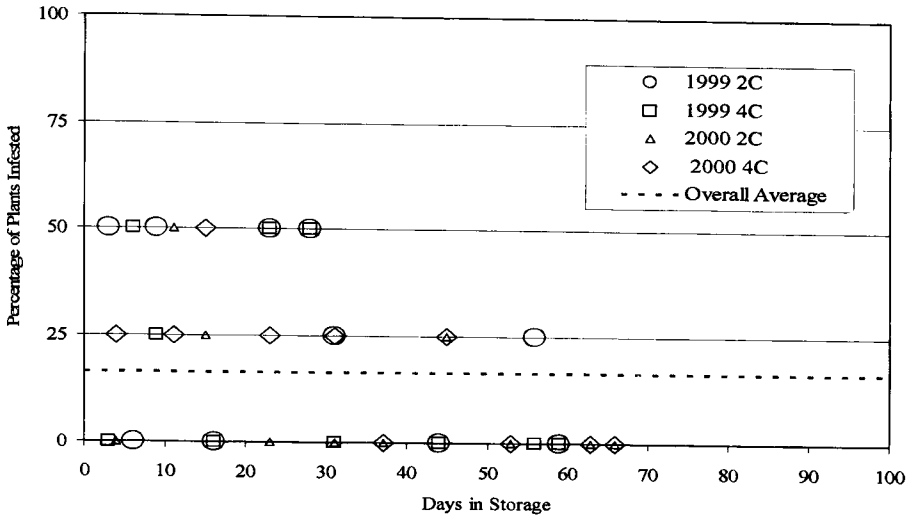

FIG. 1. Percentage of field bindweed plants infested with *A. malherbae* after exposure to mite-infested clippings of field bindweed from Moore County, Texas, in 1999 and 2000 versus number of days the clippings were stored at 2 or 4°C.

In 1999, *A malherbae* from clippings from the site in Moore County remained viable for at least 59 and 57 days when the clippings were stored at 2 and 4°C, respectively (Table 3). In 2000, *A. malherbae* from clippings from this site remained viable for at least 70 days when the clippings were stored at 2 and 4°C. In 1999, *A. malherbae* from infested clippings stored at 2°C from the site in Moore County took an average of 16.2 $\pm$ 3.8 days to infest field bindweed and an average of 11.6 $\pm$ 2.0 days to infest field bindweed when the clippings were stored at 4°C. In 2000, *A. malherbae* from infested clippings from the site in Moore County that were stored at 2°C took an average of 14.8 $\pm$ 1.1 days to infest field bindweed, and an average of 17.3 $\pm$ 1.8 days to infest field bindweed when stored at 4°C.

In 1999, clippings from Carson County harbored live mites for 56 and 28 days when stored at 2 and 4°C, respectively (Table 3). In 2000, *A. malherbae* remained viable for 45 days when the infested clippings from this site were stored at 2 or 4°C. In 1999, *A. malherbae* from infested clippings stored at 2°C from the site in Carson County took an average of 13.8 $\pm$ 3.3 days to infest field bindweed, and an average of 20.8 $\pm$ 2.9 days to infest field bindweed when the clippings were stored at 4°C. In 2000, *A. malherbae* from infested clippings that were stored at 2°C from Carson County took an average of 15.3 $\pm$ 0.9

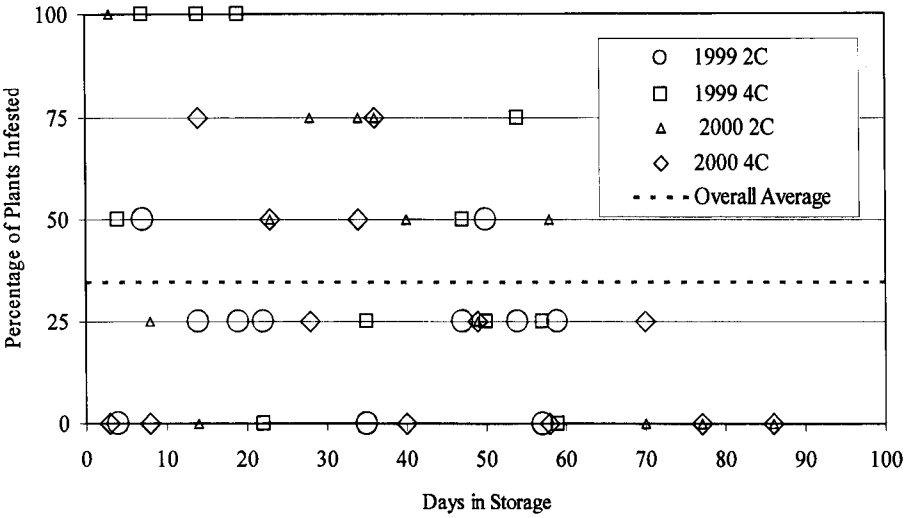

FIG. 2. Percentage of field bindweed plants infested with *Aceria malherbae* after exposure to mite-infested bindweed clippings from Carson County, Texas, in 1999 and 2000 versus number of days clippings were stored at 2 or 4°C.

days to infest field bindweed, and an average of 20.9 ± 3.0 days to infest field bindweed when the clippings were stored at 4°C.

The experiments in 1999 and 2000 to determine if overwintering mites collected from field bindweed rootstock could infest greenhouse-grown field bindweed resulted in infestation rates of 66.7 and 75.0% and averages of 24.5 (± 6.6) and 21.4 (± 4.5) days for the bindweed plants to begin showing damage symptoms, respectively.

The results of this research indicated that mowing *A. malherbae*-infested field bindweed and using the clippings as a source for infestation is a practical way to redistribute this mite as a biological control agent. If mite-infested field bindweed clippings are stored at either 2 or 4°C and used in a redistribution program, the infestation rates observed would be acceptable, especially if the clippings were used within one month. However, for commercial application the infestation rate is low, and additional quality control would be needed to ensure greater rates of infestation. Low rates of infestation after exposure to mite-infested clippings are probably related to variations in the abundance of mites, suitability of mowed plants to harbor *A. malherbae*, and variations in the efficiency of the mower. Greater infestation rates during longer periods in storage could probably be obtained if *A. malherbae* were uniformly distributed in fields from which the field bindweed clippings were collected and the bindweed was actively growing. Mower blade type, blade sharpness, and mowing height also need to be considered. The ability to use overwintering mites from field bindweed rootstock as an infestation source is of value because mites could theoretically be collected at any time of the year from roots to provide *A. malherbae* colonies for use in a greenhouse or laboratory.

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NUCOTN 33B® AND DELTA AND PINELAND COTTONS: PINK BOLLWORM (LEPIDOPTERA: GELECHIIDAE) INFESTATIONS AND CRY1AC TOXIC PROTEIN IN OVERWINTERED AND SEEDED COTTONS WITH BIOASSAY MORTALITIES OF OTHER LEPIDOPTEROUS LARVAE.

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ABSTRACT

NuCOTN 33B® (Bt) and Delta and Pineland (DPL 5415) cottons were grown in furrow and furrow plus drip irrigated cotton plots in Arizona. Cry1Ac toxic protein produced in Bt cotton was measured with commercially available enzyme-linked immunosorbent assay (ELISA) kits. Irrigation type had no effect on Cry1Ac measured in cotton leaves or bolls. First-instar, laboratory-reared pink bollworm (PBW), *Pectinophora gossypiella* (Saunders), larvae placed on Bt cotton bolls collected in field throughout the season died (100%) irrespective of decreasing Cry1Ac toxic protein levels in late season bolls. PBW field infestations averaged over 2.5 live larvae per (DPL 5415) immature green boll compared with no live larvae in Bt immature green bolls. Results were similar for PBW infestations of open mature bolls on Bt and DPL 5415 whole cotton plant samples. Two unexplained PBW larval exit holes occurred in open mature Bt bolls. These could have been on plants from non-Bt contaminated seed in Bt seed lots. In bioassays, using laboratory-reared cabbage looper (CL), *Trichoplusia ni* (Hübner), larval mortality percentages decreased with decreasing Cry1Ac content measured in Bt leaves. Cry1Ac protein was expressed in cotton bolls and leaves from overwintered Bt plants. PBW and tobacco budworm (TBW), *Heliothis virescens* (L.), larval mortalities averaged 99 and 100% (overwintered and 2002 seeded Bt cotton), respectively, compared with averages of 40 and 3%, respectively, on DPL 5415 bolls and leaves (overwintered and 2002 seeded cotton).

INTRODUCTION

Transgenic cottons (Bt) with the Bollgard® gene producing the insecticidal protein of *Bacillus thuringiensis* Kurstaki (Berlinger) have been widely grown commercially in Arizona since 1997 (Sims et al. 2002). Pink bollworm (PBW), *Pectinophora gossypiella* (Saunders), larvae have been very susceptible to the toxic protein in the field. Annual widespread and intensive monitoring has shown <1% cotton boll infestations (Flint et al. 1996, Simmons et al. 1998, Flint and Parks 1999, Sims et al. 2002). Tobacco budworm (TBW), *Heliothis virescens* (F.), cotton leafperforator, *Bucculatrix thurberiella* Busck, saltmarsh caterpillar, *Estigmene acrea* (Drury), cabbage looper (CL), *Trichoplusia ni* (Hübner) and beet armyworm, *Spodoptera exigua* (Hübner), have also been reduced in Bt field plots or shown to be susceptible in laboratory bioassays (Wilson et al. 1992; Flint et

al. 1995; Watson 1995; Ellsworth et al. 1995a,b, 1996; Henneberry et al. 2001b). The net result has been an average decline in Arizona insecticide use. Frisvold et al. (2000) reported that growers adopting Bt technology averaged $15,000 per farm more income compared with those not adopting the technology. Concerns for loss of effectiveness due to potential contamination of Bt seed lots with non-Bt seed (Flint and Parks 1999), loss of Bt effectiveness or non-expression of the toxin in late season (Ellsworth et al. 1995a, Greenplate et al. 1998) and Bt resistance development (Patin et al. 1999, Gould and Tabashnik 1998) have not materialized. Bt cottons continue to be highly efficacious for season-long PBW management in the field commercially and in research plantings (Henneberry and Forlow Jech 2000, Henneberry et al. 2001a, Marchosky et al. 2001). Development of resistance remains an issue since highly resistant PBW strains have been selected under laboratory conditions (Bartlett 1995, Patin et al. 1999, Sims et al. 2001, Sims et al. 2002). The difference between field and laboratory selection for Bt resistance remains unexplained. Continuing study is essential to improve our knowledge of pest and beneficial insect-Bt cotton interactions and to preserve the efficacy of Bt cotton for as long as possible. Many factors appear to affect Cry1Ac protein expression in cotton plant tissue. Results by Greenplate (1999) suggested some environmental influence, but no specific causes were identified. In 2001, our field and laboratory studies with Bt cotton were conducted to relate crystalline protein content estimates in bolls to PBW mortalities and in leaves to CL mortalities from Bt cotton grown under furrow alone or furrow plus drip irrigations to simulate differences in plant stress between irrigations. Delta and Pineland (DPL) 5415 cotton (Delta and Pineland, Scott, MS) served as a control. In 2002, we determined PBW and TBW mortalities and Cry1Ac amounts in overwintered Bt cotton and annual seeded Bt cotton. Bt and DPL 5415 (control) cotton yields were estimated in 2001.

METHODS AND MATERIALS

2001 Field Plots. DPL 5415 and NuCOTN 33B® (Bt) (Monsanto Co., St. Louis, MO) cotton seeds were planted on 13 April at the Western Cotton Research Laboratory, Phoenix, AZ. There were four whole plots of each cultivar. Each whole plot was 16 rows wide by 19-m long. Split plots were 8 rows wide by 19-m long. Split-plot treatments (two) were (1) furrow irrigations at 14-day intervals that supplied about 12.7cm of water and (2) furrow irrigation plus supplementary T-tape (T-system International, Inc., San Diego, CA) drip irrigation. Supplementary drip irrigations were 102 liters of water per hour per day per 31m of row. The experiment was replicated four times. The T-tape drip 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.

Male PBW moth catches. PBW male moths were sampled by placing a Delta trap (Flint and Merkle 1983), baited with one gossyplure (Hummel et al. 1973) impregnated rubber stopper (100 µl), in each quadrant of the field. Traps were located at the tops of cotton plant canopy. They were collected and replaced weekly at canopy level with new traps. Glossyplure-impregnated rubber stoppers were replaced every other week.

Laboratory bioassays. PBW and CL larvae used were from the Western Cotton Research Laboratory colonies reared on artificial diets described by Bartlett and Wolf (1985) and Henneberry and Kishaba (1966), respectively.

For CL bioassays in 2001, Bt and DPL 5415 leaves were picked at random from each plot on 19 June, 10 July, 14 August and 25 September. Leaves were trimmed to fit in 15.0cm diameter x 1.5cm deep plastic petri dishes lined on the bottom with moist filter paper. Five first-instar CL larvae were placed in each dish containing a Bt or DPL 5415 leaf. Living and dead larvae were counted after seven days.

On 11 July, 15 August and 18 September 2001, firm, immature green Bt and DPL

5415 bolls (21 to 28 days-old) were picked from plants in each plot. Bolls (5) were placed individually in 5cm diameter x 6.5cm tall cylindrical polyethylene containers. Five, first-instar PBW larvae were placed on each boll. Bolls were examined on day 7 following infestation with the aid of a microscope. PBW larval entrance holes in the carpel walls were counted. Bolls were then dissected, and all living and dead larvae and their developmental stages were recorded.

ELISA. The amounts of Cry1Ac protein in Bt cotton leaves and bolls were determined on all sampling dates using enzyme-linked immunosorbent assays (ELISA). Controls were DPL 5415 leaves and immature cotton bolls. Tissue samples were taken from the same plant parts used for larval infestations. Materials, sample preparations, solutions, extractions, dilutions, negative controls, and assays were as described in the Cry1Ab/Cry1Ac plate kit (Envirologix, Inc., Portland, ME). Samples were 0.6cm boll pieces that were weighed and placed in 1.5cm microcentrifuge tubes. Leaf samples, also weighed, were 0.6cm diameter, circular leaf punches. Samples were homogenized in extraction buffer with a fitted pestle or metal drill bit.

Field Infestations. On 28 September and 14 October 2001, 25 immature green cotton bolls were picked at random from each furrow and furrow plus drip irrigated plot to determine PBW infestations in Bt and DPL 5415 cotton plots in the field. Bolls were examined for PBW exit holes and 25 bolls from each plot were placed in ventilated plastic boxes and held in a screenhouse. Bolls were dissected and examined for PBW larvae after two weeks.

A second estimate of PBW boll infestations developing under field conditions was made using whole cotton plant sampling. On 28 November 2001, five whole plants from each plot were picked to examine mature open and immature green bolls from fruiting branches on each plant node for evidence of PBW infestation. Mature open bolls were examined for PBW larval exit holes. Immature green bolls were examined for exit holes and were dissected and examined for the presence of larvae. The data were summarized for bolls on fruiting branches at plant nodes 6-10, 11-15, 16-20, 21-25, 26-30, and 31-35.

Cotton Yields. Cotton yields in furrow and furrow plus drip irrigated Bt and DPL 5415 cottons were estimated by picking all open bolls on 21 November 2001 from 4-m of row in each replicated plot of each cultivar. Numbers of bolls were recorded. Seed cotton was removed from the bolls by hand, processed in a laboratory gin and the seed and lint weighed.

2002 Field Plots. DPL 5415 and Bt cotton seeds were planted in seven rows wide by 18-m long plots at the same location as 2001 on 29 April 2002. Overwintered volunteer plants of Bt and DPL 5415 cotton from 2001were found randomly occurring in the experimental field. Both Bt and DPL 5415 overwintered volunteer cultivars had been defoliated on 1 November 2001 and stalks cut on 15 January 2002. We tagged the overwintered volunteer plants and incorporated them into the current years experiment to compare Cry1Ac toxic protein levels in leaves and bolls from overwintered volunteer plants with the toxic protein levels in cotton leaves and bolls from plants arising from seed planted in 2002. The experiment was conducted as a completely random design with four observations of volunteer and annual seeded cotton of each cultivar. Standard grower practices were used during the 2002 season and cottons were irrigated every 14 days from 3 May to 27 August, 2002.

Male PBW Moth Catches. One glossyplure-baited trap as described was placed in the middle of the field and three traps equidistant from each other in each quadrant of the field. Traps were replaced and baits renewed as described for 2001.

Laboratory bioassays. On 29 August and 24 September 2002, firm immature green bolls (21 to 28 days-old) were picked from plants of volunteer and seeded Bt and DPL 5415 cottons. Bolls were placed individually in 5cm diameter x 6.5cm tall polyethylene containers. Five, first-instar PBW larvae were placed on each boll. Bolls were examined

on day 7 following infestation with the aid of a microscope. PBW larval entrance holes in the carpel walls were counted. Bolls were then dissected, and all living and dead larvae were recorded.

For TBW, larvae were from the Western Cotton Research Laboratory colony and reared on artificial diet described by Henneberry and Kishaba (1966). We picked leaves from four plants of each volunteer and 2002 seeded Bt and DPL 5415 cotton plot on 28 June, 12 August and 16 September 2002. Two leaves from each plant were trimmed to fit in 15.0cm diameter x 1.5cm deep plastic petri dishes lined on the bottom with moist filter paper. Five, first-instar TBW larvae were placed on leaves in each petri dish. Living and dead larvae were counted after 3 days.

ELISA. The amounts of Cry1Ac protein in Bt cotton leaves and bolls on all sampling dates were determined to compare amounts of the toxic protein in overwintered volunteer plants and in cotton plant parts from seeds planted in 2002. Controls were DPL 5415 leaves and immature cotton bolls. Tissue samples were taken from the same plant parts used for larval infestations. Sample sizes were as previously described. ELISA materials, sample preparations, solutions, extractions, dilutions and assays were as previously described.

Data were analyzed using ANOVA and means separated using the methodology of least significant differences following significant F tests or using Student's "*t*" tests for paired treatment comparisons (MSTAT-C 1989). For both tests, differences with probabilities of ≤ 0.05 or ≤ 0.01 were considered significantly different.

RESULTS

2001 Male PBW moth catches. Moths caught in glossyplure-baited traps increased in August, decreased slightly in early September, and peak numbers occurred in early October, followed by a leveling off and sharp decrease in late November (Fig. 1A). The data confirm the presence of PBW adult season-long activity in the field during boll development and maturation.

Laboratory bioassays. There were no significant effects of furrow irrigation compared with furrow plus drip irrigation for either cultivar for living or dead PBW or CL larvae, percentage mortalities, PBW entrance holes or other insect criteria (F values for 1,33 df in all cases were < 1.68; $P > 0.05$). Thus the data were combined for all further statistical analyses of 2001 PBW and CL data. Cry1Ac insect toxic protein found in leaves from furrow irrigated plots (1.05, 0.24, 0.40 and 6.18 ppm on 19 June, 10 July, 14 August and 25 September, respectively), were not significantly different compared with leaves from furrow plus drip irrigated plots (0.86, 0.074, 0.54 and 0.23 ppm, respectively) (F values 1, 14 df=1.14, 0.79, 1.52, 1.23, respectively. $P > 0.05$)

Average CL larval mortalities were 81, 63, 32, and 6% for Bt larvae sampled 19 June, 10 July, 14 August and 25 September, respectively (Table 1). Corresponding average Cry1Ac toxic protein amounts (ppm) measured were 0.96, 0.69, 0.47 and 0.18, respectively. The regression, $y=3.12+66.6x$, $r^2=0.99$ was significant ($P < 0.05$, where x and y are dose-mortality relationships). Larval mortalities feeding on DPL 5415 were 3, 13, 3 and 9% on 19 June, 10 July, 14 August and 25 September, respectively.

For boll samples taken on 11 July, 15 August, 18 September, Cry1Ac determination (ppm) were 0.13 vs 0.14, 0.11 vs 0.11 and 0.05 vs 0.04 ppm, respectively, for furrow alone compared with furrow plus drip irrigation (t=0.59, 0.20 and 1.60, respectively, 62 df, $P>0.05$). Irrigation type effects on Cry1Ac toxic protein determination in bolls were not significant on any sampling date. Average PBW larval mortalities over all dates were shown in Table 2. No laboratory-reared PBW larvae survived beyond the first instar after placement on Bt bolls. On dissection of DPL 5415 bolls, there were 0.03, 0.25 and 0.35 living first-instar, second through fourth instar and pupae per boll,

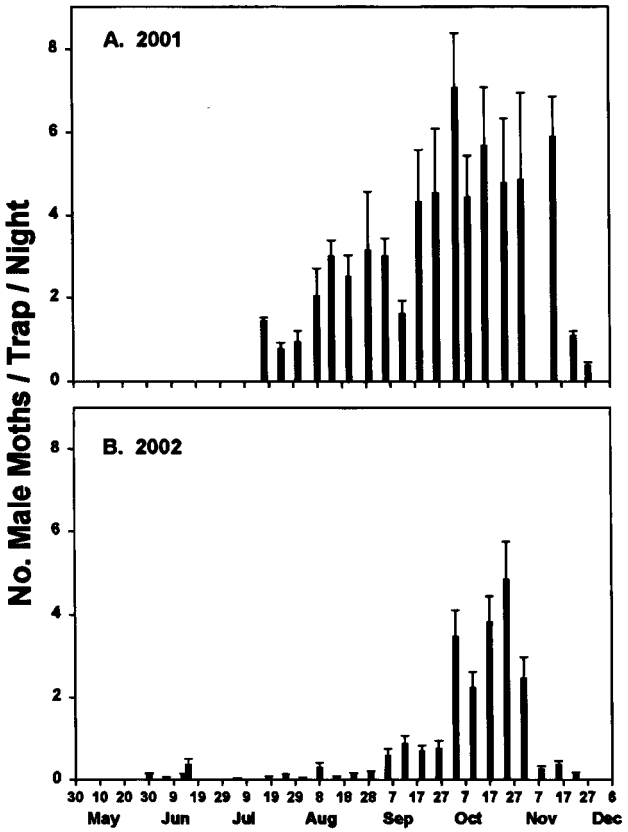

FIG 1. Mean (± SE) numbers of pink bollworm male moths caught per trap per night in split-plot plantings of NuCOTN33B® and Deltapine 5415 cottons.

Table 1. Mean (± SE) Percentages of Laboratory-Reared Cabbage Looper (CL) Larval Mortality Following Seven Day Feeding Periods on NuCOTN 33B® (Bt) and DPL 5415 Leaves From Field Plots.

Sampling date	No.[a] Samples	Bt % Mortality	Cry1Ac (ppm)	DPL 5415 % Mortality
June 19	8	81.11 ± 4.55	0.96 ± 0.09	2.52 ± 1.30
July 10	16	62.50 ± 8.43	0.69 ± 0.06	13.24 ± 2.18
August 14	16	32.23 ± 5.28	0.47 ± 0.05	2.92 ± 1.12
September 25	16	5.59 ± 0.18	0.18 ± 0.04	9.41 ± 2.94

[a] Means of 4 replications, 2 to 4 observations per replication. Five CL larvae per observation.

TABLE 2. Mean (± SE) Number of Laboratory-Reared Living (L) and Dead (D), Pink Bollworm (PBW) Larvae, Living Pupae and Percentages of Larval Mortality Following Bioassays in Field Grown NuCOTN 33B® and DPL 5415 Cotton Bolls.

	PBW instar No./boll[a]					
	1		2 to 4			
Cultivars	L	D	L	D	Pupae/boll	% Mortality
NuCOTN 33B®	0.00 (0.00) b	2.13 (0.23) b	0.00 (0.00) b	0.00 (0.00) b	0.00 (0.00) b	100.0 (0.00) a
DPL 5415	0.03 (0.00) a	1.47 (0.16) a	0.25 (0.05) a	0.01 (0.01) a	0.35 (0.00) a	68.7 (0.00) b
F, 1, 33 df	6.64	7.73	40.78	4.26	15.50	69.92
P	≤ 0.05	≤ 0.05	≤ 0.05	≤ 0.05	≤ 0.05	≤ 0.05

[a] Four replications, 20 bolls per replication, 5 first-instar larvae per boll on 11 July, 15 August, and 18 September. Means in a column not followed by the same letter are significantly different $P \leq 0.05$. Method of least significant differences.

respectively. The average Cry1Ac content for the three boll collection dates was 0.10 ± 0.01 ppm (not tabulated). Cry1Ac amounts ranged from 0.148 ppm in early-season to 0.024 ppm in late-season

Field Infestations. Immature cotton bolls picked at random from each plot in the field on 28 September and 24 October had no living PBW larvae in Bt bolls and 2.06 dead larvae per boll compared with no dead larvae in DPL 5415 bolls and 2.63 living larvae per boll (Table 3). There was no significant difference between cultivars for the number of PBW entrance holes per boll. Individual larvae may make more than one entrance hole before entering a cotton boll (Henneberry and Jech 2000).

TABLE 3. Mean (± SE) Numbers of Pink Bollworm (PBW) Larvae per Field Infested Immature Green NuCOTN33B® or DPL 5415 Cotton Boll Sampled on 28 September and 14 October 2001.

		Larvae per boll[a]		
Cultivars	No. bolls	Live	Dead	Entrance Holes
NuCOTN 33B® (Bt)	400	0.00 ± 0.00 b	2.06 ± 0.53 a	5.25 ± 1.21 a
DPL 5415	400	2.63 ± 0.71 a	0.00 ± 0.00 b	8.88 ± 1.84 a
F, 1, 21 df	--	13.26	49.3	3.23
P	--	≤ 0.05	≤ 0.05	> 0.05

[a] Means of 4 replications, 2 observations per replication in a column not followed by the same letter are significant by different $P \leq 0.05$. Method of least significant differences. Sampled 28 September and 24 October, 2001.

For whole plant samples overall, there were more open bolls per cotton plant in furrow plus drip irrigated compared with furrow irrigated plots (Table 4). More PBW

TABLE 4. Mean (±SE) Numbers of Open Cotton Bolls per Plant per Node on Whole Plant Samples of Deltapine 5415 and NuCOTN 33B® Cottons.

	Number of[a]		
Cultivars/Irrigation	Open bolls	PBW Larvae Per Boll	Infested Bolls (%)[a]
NuCOTN B33® (Bt)[b]			
Furrow	3.53 ± 0.35 a	0.01 ± 0.01 b	0.17 ± 0.17 b
Furrow plus drip	3.86 ± 0.33 a	0.03 ± 0.02 b	0.46 ± 0.34 b
Deltapine 5415			
Furrow	4.23 ± 0.42 a	0.04 ± 0.02 b	1.36 ± 0.79 b
Furrow plus drip	3.17 ± 0.41 a	0.46 ± 0.12 a	11.61 ± 3.49 a
F, (*P*), df = 1, 95	2.46 (0.05)	14.79 ($P \leq 0.05$)	11.15 (≤ 0.05)
Main effects			
Cultivar			
Bt[b]	3.70 ± 0.23 a	0.02 ± 0.01 b	0.32 ± 0.19 b
DPL 5415	3.70 ± 0.30 a	0.25 ± 0.07 a	6.48 ± 1.94 a
F, (*P*), df = 1, 95	0.00 (> 0.05)	20.88 (≤ 0.05)	19.63 (≤ 0.05)
Irrigation			
Furrow	3.35 ± 0.27 b	0.03 ± 0.01 b	0.75 ± 0.40 b
Furrow plus drip	4.04 ± 0.27 a	0.24 ± 0.07 a	6.03 ± 1.90 a
F (*P*) df = 1, 95	8.78 (≤ 0.05)	17.70 (≤ 0.05)	13.94 (≤ 0.05)

[a] Means of 4 replications within the same column and category not followed by the same letter are significantly different $P \leq 0.05$. Method of least significant differences.
[b] No exit holes, all dead larvae.

larvae per boll occurred in furrow plus drip irrigated plots compared with furrow only irrigated DPL 5415, but not Bt plots. There was an average of 0.25 PBW larvae per DPL 5415 boll compared with 0.02 PBW larvae per Bt boll. The few PBW found in Bt bolls occurred on fruiting branches at nodes 6 to 10 and 11 to 15 (Table 5). No PBW larvae were found in DPL 5415 cotton bolls from fruiting branches on nodes 6 to 10, but generally thereafter, larvae per boll increased with increasing node number except for late in the season when larvae per boll decreased (nodes 26-35). The highest percentages of DPL 5415 bolls infested occurred for bolls on fruiting branches at nodes 31 to 35. Data is not tabulated, but no PBW infestations occurred in Bt or DPL 5415 immature green bolls from fruiting branch nodes 6 to 25 shown in Table 5. No infested immature green Bt bolls were found at nodes 26 to 30 and 31 to 35 compared to 5.6 and 40.0% infestation for DPL 5415 immature green bolls from the same nodes.

TABLE 5. Mean (± SE) Numbers of Mature Open Cotton Bolls and Pink Bollworm Larvae per Boll at Different Plant Nodes on NuCOTN B33® (Bt) and Deltapine (DPL) 5415 Cottons.

Cultivar/ Plant Nodes	Number of[a] Open bolls/ Node Series	PBW larvae/boll	Bolls Infested (%)
NuCOTN B33® (Bt)			
Nodes			
06-10	4.08 ± 0.28	0.05 ± 0.03 b-d	0.96 ± 0.63 cd
11-15	4.80 ± 0.37	0.05 ± 0.05 cd	0.08 ± 0.93 cd
16-20	3.80 ± 0.35	0.00 ± 0.05 d	0.00 ± 00.00 d
21-25	5.00 ± 0.56	0.00 ± 0.00 d	0.00 ± 00.00 d
26-30	3.38 ± 0.33	0.00 ± 0.00 d	0.00 ± 00.00 d
31-35	1.13 ± 0.26	0.00 ± 0.00 d	0.00 ± 00.00 d
DPL 4515			
Nodes			
06-10	3.98 ± 0.29	0.00 ± 0.00 d	0.00 ± 0.00 d
11-15	5.05 ± 0.40	0.05 ± 0.05 cd	0.74 ± 0.73 cd
16-20	4.10 ± 0.67	0.25 ± 0.12 bc	4.69 ± 2.36 bc
21-25	5.58 ± 0.64	0.65 ± 0.26 a	11.55 ± 4.07 a
26-30	2.88 ± 0.47	0.30 ± 0.18 b	7.22 ± 4.08 a-c
31-35	0.60 ± 0.29	0.25 ± 0.18 bc	20.67 ± 11.00 ab
F, df = 5, 69	0.62	3.98	3.43
P	> 0.05	≤ 0.05	≤ 0.05

[a] Means of 4 replications, 5 whole plants per replication in a column not followed by the same letter are significantly different. Method of least significant differences.

Cotton Yields. Numbers of cotton bolls and cotton seed and lint weights were numerically but not statistically greater for Bt compared with DPL 5415 cotton (Table 6). Numbers of cotton bolls and higher cotton seed and cotton lint weights were significantly greater for furrow plus irrigated plots compared with furrow irrigation.

2002 Male PBW Moth Catches. In 2002, fewer PBW male moths were caught in gossyplure-baited traps compared with 2001 (Fig. 1B). Less than one moth per trap per night was caught through mid-September followed by a rapid increase to a peak of five moths per trap per night in late October and a sudden decline to about three tenths of a moth per trap per night in early November.

Laboratory Bioassays. No live TBW larvae survived following three-day feeding periods on leaves picked from overwintered or 2002 seeded Bt plants (Table 7) compared

TABLE 6. Mean (± SE) Numbers of Open Mature Cotton Bolls and Cotton Seed and Lint Weights per Four Meters of DPL 5415 and NuCOTN 33B® Cotton Cultivar in Furrow and Furrow Plus Drip Irrigation Plots.

Cultivars/Irrigation	No. bolls/[a] 4 m row	Grams of cotton (per 4 m of row)[a] Seed	Lint
NuCOTN B33® (Bt)			
Furrow	624 ± 68 a	1190 ± 105 a	711 ± 61 a
Furrow plus drip	760 ± 34 a	1571 ± 87 a	975 ± 54 a
DPL 5415			
Furrow	454 ± 72 a	817 ± 13 a	547 ± 90 a
Furrow plus drip	720 ± 46 a	1441 ± 89 a	991 ± 62 a
F[b] (*P*)	1.07 (> 0.05)	1.05 (> 0.05)	1.38 (> 0.05)
Main effects			
Cultivars	692 ± 44 a	1380 ± 96 a	843 ± 63 a
Bt			
DPL 5415	586 ± 64 a	1128 ± 139 a	769 ± 98 a
F[c] (*P*)	2.81 (> 0.05)	4.43 (> 0.05)	0.93 (> 0.05)
Irrigation			
Furrow	539 ± 56 b	1003 ± 105 b	629 ± 59 b
Furrow plus drip	740 ± 28 a	1506 ± 630 a	983 ± 38 a
F [c] (*P*)	10.31 (≤ 0.05)	17.68 (≤ 0.05)	21.3 (≤ 0.05)

[a] x 1000 approximate per acre equivalents, means of 4 replications within a column in the same category not followed by the same letter are significantly different ($P \leq 0.05$). Method of least significant differences.
[b] df - 3, 9; *P* = probability.
[c] df = 1, 9.

TABLE 7. Mean Numbers of Laboratory-Reared Living and Dead Tobacco Budworm Larvae and Percentage Mortalities After Three-Day Feeding Periods on NuCOTN 33B® (Bt) and Deltapine (DPL) 5415 Cotton Leaves from 2001 Overwintered and 2002 Seeded Cottons.

Cultivar/ Treatment[a]	TBW Larvae Live	Dead	% Mortality	Cry1Ac Toxic Protein(PPM)[b]
Cultivar Effect				
Bt	0.00 b	3.98 a	100.00 a	0.379 -
DPL	4.13 a	0.10 b	2.40 b	0.000 -
F =[c]	1974.20	1423.30	2386.10	- -
P =	0.000	0.000	0.000	- -
2002 Seeded vs Volunteer Effect				
Seeded	2.10 a	2.02 a	51.98 a	0.416 a
Volunteer	2.02 a	2.06 a	50.42 a	0.342 a
F =	0.46	0.01	1.43	- -
P =	> 0.05	> 0.05	> 0.05	- -

[a] Means of 4 replications, 3 observations per replication in a column in the same group not followed by the same letter are significantly different.
[b] t = 0.502, 22 df, NS.
[c] df = 1, 33.

with over 97% TBW larval survival following the same feeding period on overwintered or 2002 seeded DPL 5415 leaves. Amounts of Cry1Ac toxic protein found in leaves from overwintered Bt plants was not significantly different compared with amounts in leaves from 2002 seeded plants.

For PBW larvae, more dead and fewer live larvae were found in Bt bolls from overwintered or 2002 seeded cotton plants compared with bolls from overwintered or 2002 seeded DPL 5415 plots (Table 8). The differences in Cry1Ac toxic protein content in bolls of overwintered volunteer Bt cotton compared with 2002 seeded cotton were not statistically significant.

TABLE 8. Mean Numbers of Laboratory-Reared Living and Dead Pink Bollworm Larvae and Percentage Mortalities After Seven-Day Feeding Periods in NuCOTN 33B® (Bt) and Deltapine (DPL) 5415 Cotton Bolls from 2001 Overwintered and 2002 Seeded Cottons.

Cultivar/ Treatment[a]	PBW Larvae			Cry1Ac Toxic Protein(PPM)[b]
	Live	Dead	% Mortality	
Cultivar Effect				
Bt	0.01 b	1.57 a	99.81 a	0.320 -
DPL	1.00 a	0.65 b	40.20 b	0.000 -
F =	61.72	19.17	63.71	- -
P =	≤ 0.05	≤ 0.05	≤ 0.05	- -
2002 Seeded vs Volunteer Effect				
Seeded	0.49 a	1.23 a	71.18 a	0.332 a
Volunteer	0.52 a	0.99 a	68.83 a	0.307 a
F =	0.05	1.29	0.08	- -
P =	> 0.05	> 0.05	> 0.05	- -

[a] Mean of 4 replications, 2 observations per replication in a column not followed by the same letter are significantly different.
[b] t = 0.309, 14 df, NS

DISCUSSION

Bt cottons continue to maintain high levels of efficacy for season long PBW control in Arizona (Sims et al. 2002). The author's statewide monitoring of PBW susceptibility to Cry1Ac since 1997 shows higher levels of susceptibility to Bt toxic protein in 2000 compared with 1997. In our studies, Cry1Ac amounts in leaf and fruiting body tissues as determined with commercial ELISA kits decreased as the season progressed. PBW larval mortality in all field sampled bolls was 100% at the lowest level of Cry1Ac measured. Two PBW exit holes, found in mature open bolls, were probably from plants from non-Bt seed that may occur as contaminants in Bt seed lots (Flint and Parks 1999). Cry1Ac toxic protein amounts in overwintered Bt cotton leaves and fruiting forms were not significantly different compared with amounts measured in cotton leaf and boll tissue from cotton seed from the current year. Low toxic protein extraction efficiency and interference of other chemicals not showing insecticidal activity may have influenced our quantifications of the toxic protein (Greenplate 1999) comparisons. However, the insect bioassays provide evidence that sufficient levels were expressed by overwintered Bt plants that no difference in efficacy against PBW or TBW larvae were detected compared with tissues from 2002 seeded cotton. Cotton is a perennial plant and how long the plants continue to express Cry1Ac protein after sequential periods of dormancy and regrowth is unknown.

Cabbage looper larvae are less susceptible to the Bt toxic protein than PBW or TBW (Henneberry et al. 2001b) and larval mortality percentages decreased late in season and appeared to be related to lower Cry1Ac contents in the leaf tissue. The results suggest

a dose response reaction for this species that could result in reduced control late in the season. However, our results need verification since CL mortalities were recorded after seven-day feeding periods. CL mortalities have been determined to increase with increasingly longer feeding periods on Bt cottons (Henneberry et al. 2001b).

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DAMAGE, SURVIVAL, AND PARASITISM OF *ANTHONOMUS EUGENII* (COLEOPTERA: CURCULIONIDAE) ON PIQUIN PEPPER IN NORTHERN MEXICO

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"Piquin" pepper (*Capsicum annuum* L. var *aviculare* Dierb.), a wild pepper with small fruits, is being considered as a new crop alternative for northeastern Mexico because of its increasing demand (Rodríguez-del-Bosque et al. 2002). Although several insect species, including the pepper weevil (*Anthonomus eugenii* Cano), have been reported feeding on piquin, damage is insignificant in most cases, especially in its natural habitat (Gaona et al. 2002). However, an unusual high infestation by *A. eugenii* was detected in an experimental plot of piquin pepper during the spring of 2003 in northern Tamaulipas, Mexico, probably as a result of populations moving from infested jalapeño plants into an untreated adjacent plot.

We present a first report on the pepper weevil injury and survivorship on piquin. Field and laboratory studies were conducted at the INIFAP-Campo Experimental Rio Bravo, near Rio Bravo, Tamaulipas. A 0.5-ha experimental plot of piquin pepper planted in September 2002, was surveyed for *A. eugenii* damage symptoms (Garza 2001) in 20 plants selected randomly on 9, 16, and 20 May 2003. Percentage damage was estimated by dividing the number of damaged fruit by the total number of fruit, and multiplying by 100. During each sampling date, 150 randomly selected fruit showing weevil damage symptoms were placed individually in 30-ml plastic cups to observe survival of *A. eugenii* under laboratory conditions (27°C, 70% RH). Adult emergence of *A. eugenii* or parasites was recorded every 24h for 30 days. Piquin fruit not producing emerging weevils or parasites were dissected and observed using a steromicroscope to detect other mortality factors.

During the period of field samplings (9-20 May), average minimum and maximum temperatures were 24.5° and 34.9°C, respectively, with a daily average of 71.7% RH. First, second and third samplings showed 6.9, 19.3 and 31.8% of piquin fruit damaged by *A. eugenii*, respectively; infestation rates never observed in the wild. Extensive samplings of piquin pepper in its natural habitat in the northeastern Mexican states of Coahuila, Nuevo León and Tamaulipas during 2001 and 2002 did not show noticeable damage symptoms by *A. eugenii* (L.A.R.B, unpublished data), suggesting substantial damage to piquin may occur only in disrupted (agricultural) areas.

In the laboratory, fate of *A. eugenii* collected from infested piquin fruit in the field (n = 450) was as follows: 31.6% emerged as adults (Fig. 1); 4.8% reached adulthood, but failed to emerge; 33.1% died as immatures (larvae or pupae), most likely due to desiccation; 24.8% were parasitized; and in 5.7%, mortality causes could not be determined. All *A. eugenii* adults emerged singly, in contrast to multiple feeding and emergence in larger pepper fruits (Garza 2001).

Parasites emerging from *A. eugenii* included *Catolaccus hunteri* (Crawford), *Eurytoma* sp., *Eupelmus* sp., and *Bracon* sp., with 15.2, 6.9, 1.8 and 0.9% parasitism rates, respectively. Total parasitism in the present study was higher than in other reports for

peppers with larger fruits (Riley and Schuster 1992, Mariscal et al. 1998, Cortez et al. 2002), probably as a consequence of a differential thickness of the fruit pericarp, a physical barrier to parasitoids (Riley and Schuster 1992).

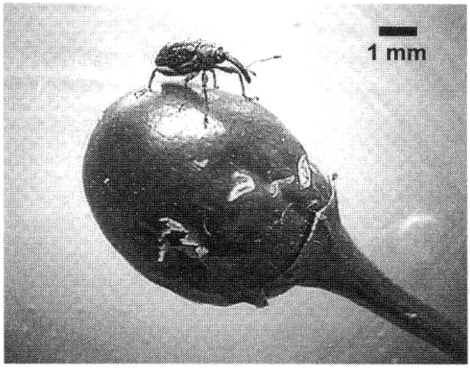

FIG. 1. *Anthonomus eugenii* emerging from an exit hole (bottom) of a piquin pepper fruit. Rio Bravo, Tamaulipas, Mexico. May, 2003.

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