DEVELOPMENTAL RESPONSE OF *LYSIPHLEBUS TESTACEIPES*[1] TO TEMPERATURE

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ABSTRACT

The number of days required for development of *Lysiphlebus testaceipes* (Cresson) from egg to adult emergence from the aphid mummy was measured at 10, 14, 18, 22, and 26° C, respectively, using the greenbug, *Schizaphis graminum* (Rondani), as the parasite's host. The threshold temperature for development was 6.6° C (*SE* = 0.33), while the degree-day requirement for development was 170 DD (*SE* = 14.5). The threshold temperature of *L. testaceipes* was 3.5° C higher than the average of threshold temperatures of other aphidiid parasitoids of cereal aphids for which published estimates are available, and 1.8° C higher than the highest threshold temperature reported for these species. The degree-day requirement for development of *L. testaceipes* was 100 DD lower than the average degree-day requirement for other aphidiid cereal aphid parasitoids.

INTRODUCTION

Lysiphlebus testaceipes (Cresson) (Hymenoptera: Aphididae) is a solitary endoparasitoid of many aphid species, including several economically important pests of cereals (Carroll and Hoyt 1986, Stary et al. 1988). *L. testaceipes* is an important biological control agent of the greenbug, *Schizaphis graminum* (Rondani), the most important aphid pest of cereals in several Southern Plains states. The parasitoid occasionally maintains greenbug populations below the economic threshold in wheat and sorghum (Kring et al. 1985, Rice and Wilde 1988, Patrick and Boring 1990). For example, Texas pest management guidelines advise that control measures be delayed in wheat fields infested with greenbugs when 15% or more of greenbugs have been mummified by *L. testaceipes*. This allows farmers to determine whether greenbug populations will be controlled by the parasitoid (Patrick and Boring 1990). Although *L. testaceipes* can sometimes suppress greenbug populations, the environmental conditions under which effective biological control occurs are not well understood. Additional knowledge of the biology and ecology of *L. testaceipes* may enhance the predictability of effective control and permit development of more refined pest management guidelines.

This study was initiated to provide basic information on the biology of *L. testaceipes*. In particular, we determined the relationship between temperature and immature development

[1]Hymenoptera: Aphididae

of *L. testaceipes* and estimated the parameters of a linear temperature-dependent development function.

MATERIALS AND METHODS

L. testaceipes used in this study were obtained from greenbugs in wheat fields in north-central Oklahoma. A laboratory colony was maintained at 16L:8D, 22 ± 0.5 °C, and 50-60% RH in a plant growth chamber for 3-4 generations prior to initiating the study. To establish an experimental replicate, an artist's brush was used to place 50 1st-3rd instar greenbugs on barley, *Hordeum vulgare* L., seedlings growing in a 10-cm diameter plastic pot. The potted seedlings were covered with a vented, clear plastic, cylindrical cage (10-cm diameter × 30-cm height). After allowing the aphids to settle for 4 h, an aspirator was used to transfer six active female parasitoids from the stock colony to the cage. After 4 h, parasitoids were removed from the cage, and the caged plants were placed in growth chambers maintained at 10, 14, 18, 22, or 26° C (all ±0.5° C), respectively, 16L:8D, and 50-60% RH. Plants were checked daily and mummies were removed, placed in a plastic petri dish (5 cm diameter × 1.4 cm height), and returned to the plant growth chamber. Petri dishes were shaded from direct light in order to minimize heat build-up within them. Mummies were examined daily for adult emergence. The number of days required from oviposition until adult emergence was recorded. Four experimental replicates were established at each temperature.

The threshold temperature and degree-day requirements for development from egg to adult emergence were estimated from a linear regression of development rate (y) against temperature (x):

$$y = a + bx$$

Development rate is the reciprocal of development time (1/ development time in days). The developmental threshold is the intercept of the temperature (x) axis, and development time in degree-days is the reciprocal of the regression coefficient (Campbell et al. 1974). Standard errors of the estimates of the developmental threshold and degree-day requirement were calculated using formulae presented in (Campbell et al. 1974).

RESULTS AND DISCUSSION

The time required for *L. testaceipes* to develop from egg to adult emergence from the mummy decreased as temperature increased from 49.1 days at 10° C to 9.3 days at 26° C (Table 1). A linear regression equation provided a good fit to the relationship between temperature and development rate ($r^2 = 0.99$). The parameters of the regression equation are $a = -0.0386$ ($SE = 0.000498$) and $b = 0.00587$ ($SE = 0.0000317$). The threshold temperature for development, calculated from the regression parameters, was 6.6° C ($SE = 0.33$), while the corresponding degree-day requirement for development was 170 DD ($SE = 14.5$).

TABLE 1. Average Number of Days Required for Development of *Lysiphlebus testaceipes* from Oviposition to Adult Emergence from the Mummy (Standard Error in Parentheses).

	Temperature (°C)				
	10	14	18	22	26
Number reared	104	69	68	64	70
Development time	49.1 (3.08)	24.1 (1.13)	15.2 (0.54)	10.6 (0.53)	9.3 (0.54)

The estimate of the developmental threshold for *L. testaceipes* in our study was lower than the 7.5° C as reported by Tang and Yokomi (1995) who studied development of *L. testaceipes* on the black citrus aphid, *Toxoptera citricida* (Kirkaldy), at constant temperatures ranging from 15 to 30° C. The degree-day requirement for development of *L. testaceipes* was also lower than their estimate of 213 DD.

Developmental thresholds for *L. testaceipes* from both studies were substantially greater than estimates for other aphidiid parasitoids of cereal aphids from studies conducted in plant growth chambers under controlled temperatures. Estimates of the developmental threshold for *Aphidius matricariae* Haliday were 1.4 and 4.5° C (Bernal and Gonzalez 1993, Miller and Gerth 1994). Estimates for *Diaeretiella rapae* (M'Intosh) ranged from 2.1 to 4.8° C (Campbell et al. 1974; Bernal and Gonzalez 1993, 1995; Elliott et al. 1995b). Elliott et al. (1995a) estimated the developmental threshold of *Aphidius colemani* Viereck at 2.8° C. Numerous factors could account for the differences in parameter estimates among studies, including differences in the range of temperatures used in the studies, differences in developmental responses to temperature among species and geographic populations of the same species, differences in experimental protocol, and sampling and observation errors. In spite of the potential for variation it is noteworthy that the threshold temperatures reported for other species average 3.5° C lower than our estimate for *L. testaceipes* and 4.4° C lower than the estimate reported by Tang and Yokomi (1995). On the other hand, our estimate of the degree-day requirement for development of *L. testaceipes* was lower than all but one of the estimates reported for the above mentioned aphidiids and was 100 DD lower than the average of estimates reported for these species. The estimate by Tang and Yokomi (1995) was also lower than all but one estimate and was 57 DD lower than the average for the other species.

Honek and Kocourek (1990) correlated data on developmental responses of numerous insect species to average annual temperatures in the region of origin of the species. They found that species from warmer climates generally required higher developmental thresholds and lower degree-day requirements for development than insects from cooler climates. However, this generalization did not hold true all species. A possible explanation for the exceptions they encountered was that temperatures during active growth of a species were more directly related to developmental responses than average temperature conditions for the entire year (Honek and Kocourek 1990). *L. testaceipes* actively parasitizes hosts from spring through autumn (Rogers et al. 1972, Carroll and Hoyt 1986). The relatively high developmental threshold and low degree-day requirement of this species suggests it may be best adapted to warm climates and may be most effective during late-spring and summer in the Southern Plains region. Adaptation of developmental response within an insect species to geographic variation in climate has been reported (Honek and Kocourek 1990). It is unknown, however, whether *L. testaceipes* from different climates exhibit developmental responses similar to those we studied.

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PARASITISM OF SEVERAL APHID SPECIES BY *APHELINUS ASYCHIS*[1] (WALKER) AND *APHELINUS ALBIPODUS*[1] HAYAT AND FATIMA

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ABSTRACT

Aphelinus asychis (Walker) and *A. albipodus* Hayat and Fatima from locations in Asia and Africa (hereafter referred to as strains) were tested for their abilities to develop from egg to mummy and from mummy to adult stages in 16 common aphid species. Strains of *A. asychis* from China, Kazakhstan, and Morocco developed to the mummy stage in 15, 14, and 15 aphid species, respectively. There was no difference in mean adult emergence rate between strains. An ordinal rating system of none, low, and high was used to compare the extent of mummy formation among strains. Mummy formation ratings were significantly correlated for comparisons of strains from China and Kazakhstan (r = 0.68) and China and Morocco (r = 0.63), but not for strains from Kazakhstan and Morocco (r = 0.40). Correlations of ratings for strains from proximate geographical locations were no larger than for strains from more widely separated locations. There was no difference in mean adult emergence rate among strains of *A. albipodus* from China (Altai, Pingluo, and Urumqi) and Caucasus (Buddenousk). Strains from Altai, Urumqi, Pingluo, and Buddenousk developed to the mummy stage in 13, 12, 12, and 10 species, respectively. Mummy formation ratings for all pairs of strains were significantly correlated. The largest correlation, 0.83, was for the comparison of the Urumqui and Altai strains, which were closest together geographically. The smallest correlations, ranging from 0.45-0.60, were for comparisons involving the most distant (Buddenousk) strain with Chinese strains.

INTRODUCTION

Aphelinus asychis (Walker) and *A. albipodus* Hayat and Fatima are native to the Old World and are solitary endoparasitoids of aphids. These species were imported into the United States from Asia, Europe, and Africa for classical biological control of the Russian wheat aphid, *Diuraphis noxia* (Mordvilko). One or both species were recently reported to have established in Texas (Michels and Whitaker-Deeberg 1993), Colorado (Elliott et al. 1995), and Washington (Tanigoshi et al. 1995, Pike et al. 1997).

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Most aphid parasitoids are polyphagous (Stary and Rejmanek 1981). This is true of *A. asychis* that has been recorded from numerous hosts including cereal aphid species (Thompson 1953, Jackson and Eikenbary 1971, Rogers et al. 1972, Gordh 1979). The range of hosts of *A. albipodus* is not known, but this species probably has several hosts (Michels and Whitaker-Deerberg 1993, Pike et al. 1997). These parasitoids may be important in biological control of the Russian wheat aphid and other economically important cereal aphids, such as the greenbug, *Schizaphis graminum* (Rondani).

Biological traits of a parasitoid species, including the species it parasitizes, sometimes vary among geographical populations (hereafter referred to as strains) (Messenger and van den Bosch 1971). Availability of hosts in other crops and in non-arable land may be critical to long-term survival and effectiveness of aphid parasitoids in an annual crop such as wheat, *Triticum aestivum* L. (Ehler and Miller 1978, Wissinger 1997). This study was conducted using *A. asychis* and *A. albipodus* obtained from widely separated locations in Asia and Africa. Our objectives were to determine if the two species would parasitize 16 aphid species common to cultivated and non-cultivated plants in the Southern Great Plains, and if differences existed among strains of each species in their ability to successfully parasitize the aphids.

MATERIALS AND METHODS

Laboratory colonies of 16 aphid species were established from aphids collected from the field in central Oklahoma. Each of the 16 species was maintained on the host plant species from which it was originally obtained, or on a suitable alternative species. Barley, *Hordeum vulgare* L., was used as host for the Russian wheat aphid; the greenbug; the English grain aphid, *Sitobion avenae* (Fabricius); the western wheat aphid, *Diuraphis tritici* (Gillette); the bird cherry-oat aphid, *Rhopalosiphum padi* (L.); the corn leaf aphid, *R. maidis* (Fitch); and the yellow sugar cane aphid, *Sipha flava* (Forbes). Common sunflower, *Helianthus annuus* L., was used for *Aphis helianthi* Monell; the Oleander aphid, *Aphis nerii* Boyer de Fonscolombe; and *Dactynotus helianthicola* Olive. Red clover, *Trifolium pratense* L., was used for the clover aphid, *Therioaphis trifolii* (Buckton). Faba bean, *Vicia faba* L., was used for the pea aphid, *Acyrthosiphon pisum* (Harris). Cotton, *Gossypium hirsutum* L., was used for the cotton aphid, *Aphis gossypii* Glover. Cabbage, *Brassica oleracea* L., was used for the cabbage aphid, *Brevicoryne brassicae* (L.). Canola, *Brassica napus* L., was used for the turnip aphid, *Lipaphis erysimis* (Kaltenbach), and the green peach aphid, *Myzus persicae* (Sulzer). Seedlings of the species used to maintain an aphid colony were used as experimental plants.

Laboratory colonies of *A. asychis* were established from individuals collected in 1991 and 1992 in wheat fields near Pinglou, China; Dmitrievka, Kazakhstan; and Sette, Morocco. Laboratory colonies of *A. albipodus* were established from individuals collected during the same years in wheat fields near Altai, Pingluo, and Urumqi, China and Buddenousk, Caucasus. All strains of both species were tested for their ability to parasitize and develop to adulthood on the 16 aphid species. We were interested in the ability of geographical strains of the two parasitoids to parasitize the 16 aphid species. The number of aphid mummies that formed for each experimental replicate was measured. Also measured was the percentage of adults that emerged from mummies.

Each aphid species was exposed to each geographical strain of parasitoid. This was accomplished by placing 75 small- to medium-sized nymphs of the particular aphid species on an appropriate host plant growing in a 10-cm-diameter pot, and covering the plant with

a cylindrical plastic cage (5.8 by 30 cm). The aphid nymphs were allowed to settle for 4 h, after which six mated female parasitoids were introduced into the cage. The caged plants were maintained at 21 ± 4° C under artificial light (16L:8D) in an insect rearing room. After 24 h the adult parasitoids were removed, and the caged plant was returned to the rearing room. Plants were watered every 1-2 d and inspected each day for the presence of mummies. All mummies that formed on a plant were removed, placed in a plastic petri-dish (5 by 1.4 cm), and returned to the rearing room. Mummies were checked periodically to determine the number of adults that emerged. One to three such replicates for each of the 16 aphid species were established for each strain of *A. asychis* and *A. albipodus*. If <10 mummies formed in a replicate an additional replicate was established. This process was continued until ≥10 mummies formed or a total of three replicates were established. This was done to minimize the chance that an aphid would be mistakenly classified as a poor host or non-host for a strain. A replicate in which ≥10 mummies were achieved by a strain was considered evidence of ability to successfully parasitize the particular host and the test was not repeated. If ≥10 mummies were not produced after three replicates, the replicate with the highest number of mummies was used as a measure of the ability of the strain to parasitize a particular aphid species.

Although 75 aphid nymphs were placed on each plant in an experimental replicate, the number of nymphs that successfully established on the plant varied greatly among aphid species. For that reason, the number of mummies formed could be misleading for comparing aphid species as hosts to parasitoids. Therefore, we rated aphid species according to the following system: no mummies formed in any replicate, <10 mummies formed in the best replicate, and ≥10 mummies formed in the best replicate. For purposes of statistical analysis the ratings were expressed on a numeric scale with 0 corresponding to none, 1 corresponding to <10, and 2 corresponding to ≥10 mummies formed. The percentage of mummies from which an adult parasitoid emerged for a particular aphid species was calculated from the total number of mummies formed in all replicates.

Correlation of mummy ratings among strains were calculated using Kendall's τ rank correlation coefficient (Daniel 1978). Correlation coefficients were tested for significance with the probability of a type-one error set at $\alpha = 0.05$. Differences among strains in mean percentages of adult emergence from all hosts for which mummies formed for all strains were tested using the Kruskal-Wallis test (Daniel 1978) with $\alpha = 0.05$.

RESULTS AND DISCUSSION

Mummy formation ratings and percentages of adult emergence from mummies of the 16 aphid species are listed in Table 1 for three strains of *A. asychis*. Mummies formed for 14 aphid species for the Demitrievka strain compared to 15 species for the Pingluo and Sette strains. Less than 10 mummies of *T. trifolii* and no mummies of *S. flava* formed for any strain. For *D. helianthicola*, no mummies formed for the Kazakhstan strain, while ≥ 10 mummies formed for the Pingluo and Sette strains. Differences in ratings among strains also occurred for *A. pisum*, *A. gossypii*, and *D. tritici*. Adult emergence from mummies was high for all strains (Table 1). Mean percentage adult emergence from aphid species for which mummies formed for all strains did not differ among strains ($\chi^2 = 3.84$; df = 2; $P = 0.15$).

Mummy formation ratings for *A. asychis* were correlated for the Pingluo and Demitrievka strains and the Pingluo and Sette strains, but not for the Demitrievka and Sette strains (Table 2). Although there were differences among strains in mummy formation ratings and percentages of adult emergence for the 16 aphid species, correlation analysis

TABLE 1. Mummy Formation Rating and Percentages of Adult Parasitoid Emergence for 16 Aphid Species Exposed to *Aphelinus Asychis* from Three Geographical Locations in China, Morocco, and Kazakhstan.

	Demitrievka, Kazakhstan		Pingluo, China		Sette, Morocco	
Aphid species	Mummy rating[a]	% adults	Mummy rating	% adults	Mummy rating	% adults
A. helianthi	H	70	H	100	H	94
A. nerii	H	98	H	100	H	99
R. padi	H	93	H	100	H	94
B. brassicae	H	86	H	100	H	95
R. maidis	H	95	H	98	H	95
A. gossypii	H	86	H	100	L	75
S. avenae	H	97	H	100	H	96
S. graminum	H	96	H	100	H	100
M. persicae	H	100	H	57	H	88
A. pisum	L	100	H	86	H	100
D. helianthicola	N	-	H	95	H	95
D. noxia	H	98	H	100	H	100
T. trifolii	L	50	L	75	L	75
L. erysimis	H	100	H	100	H	92
D. tritici	L	100	L	100	H	100
S. flava	N	-	N	-	N	-

[a]H, ≥10 mummies formed; L, < 10 mummies formed; N, no mummies formed.

TABLE 2. Kendall's τ Rank Correlations for Mummy Formation Ratings for Pairwise Comparisons of *Aphelinus asychis* from Three Geographical Locations in China, Morocco, and Kazakhstan.

	Sette, Morocco	Pingluo, China
Demitrievka, Kazakhstan	0.40	0.68*
Sette, Morocco		0.63*

*Significant correlation ($P < 0.05$).

provided no clear relationship between these measures for particular hosts and the geographical proximity of *A. asychis* strains.

A. albipodus parasitized fewer of the 16 aphid species than *A. asychis*. Depending on strain, mummies formed for 10-13 aphid species and adults emerged for 9-13 species (Table 3), compared to 14-15 species for which mummies formed and adults emerged for strains of *A. asychis*.

The four *A. albipodus* strains were similar with respect to the aphid species they parasitized (Table 3). The strains had identical mummy formation ratings for seven aphid species. However, there were some notable differences in ratings among strains. For example, three strains had a high rating for *A. helianthi* and *S. avenae*, but no mummies formed for the Buddenousk strain. *A. nerii* had a low rating for the Altai strain but was not parasitized by other strains.

TABLE 3. Mummy Formation Ratings (rating) and Percentages of Adult Parasitoid Emergence (%) for 16 Aphid Species Exposed to *Aphelinus Albipodus* from Four Geographical Locations in China and Caucasus.

	Altai, China		Urumqi, China		Pingluo, China		Buddenousk, Caucasus	
Aphid species	rating[a]	%	rating	%	rating	%	rating	%
A. helianthi	H	98	H	100	H	100	N	-
A. nerii	L	100	N	-	N	-	N	-
R. padi	H	100	H	100	L	100	L	93
B. brassicae	L	45	L	100	L	0	L	0
R. maidis	H	95	H	100	L	100	L	93
A. gossypii	H	100	L	69	L	100	L	56
S. avenae	H	100	H	100	H	100	N	-
S. graminum	H	100	H	100	H	100	H	84
M. persicae	H	100	H	94	H	100	H	93
A. pisum	L	100	N	-	L	100	L	50
D. helianthicola	N	-	N	-	N	-	N	-
D. noxia	H	100	H	78	H	100	H	100
T. trifolii	N	-	L	100	N	-	N	-
L. erysimis	H	100	H	100	H	97	H	100
D. tritici	H	100	H	100	H	97	L	100
S. flava	N	-	N	-	N	-	N	-

[a]H, ≥10 mummies formed; L, < 10 mummies formed; N, no mummies formed.

Percentage of adult emergence from mummies also appeared to differ among strains. No adult parasitoids of the Pingluo and Buddenousk strains emerged from *B. brassicae* mummies, but 100% of *B. brassicae* mummies of the Urumqi strain produced an adult parasitoid. Adult parasitoids emerged from 56 to 100% of *A. gossypii* mummies depending on strain. Statistical analysis of mean percentage of adult emergence from host species that exhibited either a low or high mummy formation rating yielded no evidence that percentage adult emergence differed among strains ($\chi^2 = 4.09$; df = 3; $P = 0.25$).

TABLE 4. Kendall's τ Rank Correlations for Mummy Formation Ratings for Pairwise Comparisons of *Aphelinus Albipodus* from Four Geographical Locations in China and Caucasus.

	Urumqui, China	Pingluo, China	Buddenousk,Caucasus
Altai, China	0.83*	0.79*	0.55*
Urumqui, China		0.75*	0.45*
Pingluo, China			0.60*

*Significant correlation ($P < 0.05$)

Kendall's tau rank correlations of mummy formation ratings were significant for all pairwise comparisons of strains (Table 4). Correlations among pairs of strains from geographically proximate locations were larger than for pairs of strains from widely

separated locations. The largest correlation, 0.83, was between the Urumqui and Altai strains, which were closest together geographically (approximately 300 km), whereas the smallest correlations were for comparisons involving the Buddenousk strain and a Chinese strain. Buddenousk is approximately 2,500 km from the nearest Chinese location (Urumqui) and about 4,000 km from the most distant location (Pingluo).

Parasitoids of aphid pests of winter wheat may often require a sequence of host aphids to maintain populations in the Southern Great Plains (Rogers et al. 1972, Eikenbary and Rogers 1974). These aphids live on plants in non-arable land and fields of summer crops. Movement by *A. asychis* and *A. albipodus* from cereal aphids in wheat fields to hosts in other plant communities may occur regularly in the Southern Great Plains because cereal aphid populations frequently decrease to nearly undetectable levels during summer and early-autumn (Eikenbary and Rogers 1974, Elliott et al. 1998). Our study indicates there are no physiological or behavioral barriers to reproduction or survival of *A. asychis* and *A. albipodus* on a wide range of prospective host aphid species. Both *A. asychis* and *A. albipodus* seem to be polyphagous, a trait that may enhance their value as biological control agents in the dryland winter wheat cropping systems in the Southern Great Plains. Both species exhibit the breadth in host range required to survive in agricultural landscapes during summer in the absence of a summer diapause, which has not been demonstrated for either species.

Elliott et al. (1994a, 1994b) compared the acceptability and suitability to native and exotic (Syrian) *Diaeretiella rapae* M'Intosh of a group of aphid species similar to that used in the present study. *A. asychis* strains parasitized 87-94 % and *A. albipodus* parasitized 56-81% of potential hosts in the present study, while *D. rapae* parasitized only 50-62% of potential hosts. Differences among species were caused partly by differences in parasitization of legume-feeding aphids. All *A. asychis* strains parasitized *A. pisum*, and two of three strains parasitized *T. trifolii*. Three strains of *A. albipodus* parasitized *A. pisum*, but mummy formation ratings for this host were low, and only one strain parasitized *T. trifolii*. *D. rapae* did not parasitize either species. Parasitism of three common sunflower-feeding aphids (*A. helianthi*, *A. nerii*, and *D. helianthicola*) also accounted for differences among species. These three aphid species were parasitized by most strains of *A. asychis*. *Aphis helianthi* was parasitized by three of four strains of *A. albipodus*, but *A. nerii* and *D. helianthicola* were not parasitized by most strains of *A. asychis*. The three aphids were not parasitized by *D. rapae*.

Ability to parasitize legume- and sunflower-feeding aphids may be advantageous to *A. asychis* as a biological control agent in the annual dryland cropping systems of the Southern Great Plains. Sunflowers are an important component of the flora of disturbed and non-arable lands and are also a component of the weedy flora of winter wheat fields (Rogers et al. 1982). Sunflowers have seasonal phenologies that complement winter wheat because they actively grow during summer and early autumn when cultivated winter wheat is absent (Rogers et al. 1972). Aphids feeding on various sunflower species provide a potential reservoir for parasitoids during periods when cereal aphids are rare or absent from grain crops (Rogers et al. 1972, Eikenbary and Rogers 1974).

The value of *A. asychis* and *A. albipodus* as biological control agents of cereal aphids in the dryland winter wheat cropping system that dominates the Southern Great Plains is uncertain (Michels and Whitaker-Deeberg 1993, Elliott et al. 1995). Our observations suggest both species, particularly *A. asychis*, could be effective biological control agents because of their ability to parasitize several common aphids, a trait that could contribute to development of large numbers of parasitoids to colonize aphids infesting winter wheat fields. Obviously other factors, such as seasonal phenology, dispersal ability, and host

habitat recognition and acceptance are critical to a parasitoid's success (Ehler and Miller 1978, Wissinger 1997). For example, Bernal and González (1993, 1996) found temperature thresholds for development of *A. asychis* and *A. albipodus* immatures were several °C higher than that of the Russian wheat aphid. This difference could limit the effectiveness of the parasitoids against this important aphid pest of dryland wheat and barley, especially in early spring when the Russian wheat aphid increases to economically damaging numbers (Elliott et al. 1998). These aspects of the biology of *A. asychis* and *A. albipodus* warrant investigation.

ACKNOWLEDGMENT

We thank Keith Hopper, Dominique Coutinot (USDA, ARS European Parasite Laboratory); Larry Ertle, Ken Swan, and Joe Tropp (USDA, ARS Beneficial Insects Research Laboratory; Keith Pike and Lionel Tanigoshi (Washington State University); Frank Gilstrap and Leslie McKinnon (Texas A&M University); and Dan González and Marcella Waggoner (University of California, Riverside) for overseas collection, quarantine, and laboratory rearing of the parasitoids used in this study. We also thank Kris Giles and Tom Royer (Oklahoma State University) for reading the initial draft of the manuscript and providing many useful ideas for improvement.

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SEASONAL INFESTATION BY PINK BOLLWORM, *PECTINOPHORA GOSSYPIELLA* (SAUNDERS), OF TRANSGENIC AND NON-TRANSGENIC CULTIVARS OF COTTON, *GOSSYPIUM HIRSUTUM* L., IN CENTRAL ARIZONA

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ABSTRACT

Bolls from transgenic cotton, NuCOTN 33 (Delta and Pine Land Co, Scott, MS) or Paymaster 1560 BG (Paymaster Cotton, Lubbock, TX) containing the Bollgard™ gene (Monsanto Co., St. Louis, MO) were compared to parental non-transgenic cultivars DPL-5415 and Paymaster 1560 in central Arizona in 1996 and 1997. Bolls were collected from paired transgenic and non-transgenic fields in Queen Creek, Buckeye, Gila Bend, Maricopa, Marana, and Eloy. Approximately equal numbers of bolls were collected from the edges of each field on a weekly to biweekly schedule from July through October. One collection only was made in October at Marana (1996, 1997) and Eloy (1997). Infestations of pink bollworms were less than 10% in all fields through August in both years. Infestations increased to 100% in several non-transgenic fields in October. Infestations of pink bollworms found in transgenic cultivars were less than 1% in all fields except for a single field in 1997 where 6.1 pink bollworms per 100 bolls were found. Overall percentage pink bollworm infested bolls for 1996 was 0.0004% in transgenic cultivars (n = 33,350 bolls) compared with 34.19% in non-transgenic cultivars (n = 33,850 bolls). In 1997 the percentages were 0.0038% in transgenic cultivars (n = 36,650 bolls) including 61 pink bollworms from 1000 bolls in field pair G compared with 48.32% (n = 35,100 bolls). From these numbers it is apparent that pink bollworm remains a significant pest of cotton in central Arizona. We conclude that cultivars of transgenic cotton retained efficacy for control of pink bollworm through 1997.

INTRODUCTION

Transgenic lines and cultivars of cotton, *Gossypiuim hirsutum* L., that carry a gene encoding an insecticidal protein (Cry 1Ac) derived from *Bacillus thuringiensis* var. *kurstaki* (Berliner) (Bollgard™ gene, Monsanto Co., St. Louis, MO) have a high level of efficacy against larvae of the pink bollworm, *Pectinophora gossypiella* (Saunders) (Wilson et al. 1992, Flint et al. 1995, Flint et al. 1996). Transgenic cotton was first commercially planted in Arizona in 1996 and 1997. The principal cultivar planted in both years was NuCOTN 33 (Delta and Pine Land Co., Scott, MS). Paymaster 1560 BG (Paymaster Cotton, Lubbock, TX) was planted in one of our test fields in 1997. Both of these transgenic cultivars contain the same Bollgard™ gene.

The development of resistance to Bollgard ™ cotton is a concern to growers and technology producers. Growers of transgenic cotton are required by contract to plant at least 20% of their total acreage to non-transgenic cotton. The non-transgenic cotton may be sprayed with conventional insecticides but not BT. A seldom practiced Monsanto Co. option requires 4% of the total acreage to be planted to non-transgenic cotton and remain untreated by any insecticide. These refuge acres are intended to delay the evolution of resistance to Cry 1Ac (Tabashnik 1997). Measurement of susceptibility of the pink bollworm to transgenic cotton containing Bt toxin protein is central to monitoring the evolution of resistance in this insect.

Our objectives were to provide baseline efficacy data for transgenic cotton in central Arizona and to look for development of resistance in areas where transgenic cotton was grown on the same or closely adjacent fields during 1995-1997. We first measured susceptibility of the pink bollworm to commercial transgenic cotton in 1995 (Flint et al. 1996). In that year, transgenic cotton containing Cry 1Ac was grown for seed increase in several central Arizona locations. Our results for this first year of commercial production provided baseline information for comparisons with efficacy in subsequent years. We report here on infestations of pink bollworm in transgenic cotton in 1996 and 1997 at the same locations tested in 1995.

MATERIALS AND METHODS

Transgenic cotton, NuCOTN 33, or Paymaster 1560 BG, containing the Bollgard™ gene was planted in commercial fields in central Arizona in 1996 and 1997. Fields of transgenic cotton were paired with non-transgenic fields in Queen Creek, Buckeye, Gila Bend, Maricopa, or Marana. Paired fields were usually adjacent or within 0.5 km of each other. Paymaster 1560 BG was planted in a field plot test (3 ha plots) near Eloy in 1997. The parental cultivars for NuCOTN 33 and Paymaster 1560 BG are DPL 5415 and Paymaster 1560, respectively. The parental cultivars were paired with their transgenic counterparts in most of our tests. The field locations were consistent with those in our 1995 tests (Flint et al. 1996) in each area because the same growers cooperated in these tests. All fields were 20 ha or greater. Planting times, fertilization, insecticide use, and cultural practices varied among growers but were similar for each pair of fields. Insecticide treatment specifically for pink bollworm were withheld from transgenic cotton fields; however, transgenic cotton fields were treated for insect pests not susceptible to transgenic cotton. These pests include the silverleaf whitefly, *Bemisia argentifolii* Bellows and Perring, and lygus, *Lygus hesperus* Knight. Many growers consider their use of insecticides to be proprietary information. We did not consider these records necessary for our study.

Variable numbers of firm green bolls (2-3 weeks old) were collected from fields on a weekly or biweekly schedule July through October. Collection dates depended on irrigation and spray schedules. Sometimes only a single field pair was sampled on a given date. Both fields of each pair were always sampled together. Collections were taken from the edges of each field on each sample date rather than walking through the fields. Collections from edges facilitated picking large numbers of bolls since many fields were impassable by August. In our experience, edges of cotton fields often have greater infestations of pink bollworms than the interior of the field (unpublished data). Bolls collected through 1 October were incubated in lots of 50 bolls per ventilated plastic box and held at 27± 2°C and ambient humidity in a storage room. Pink bollworm larvae, pupae, and adults were counted after 2 weeks of incubation. From 15 September to 1 October, bolls were placed in paper bags after incubation in boll boxes. Bags of bolls

were held in a screened outdoor insectary for hand dissection. All bolls picked after 1 October were held in the insectary only. These methods were used because pink bollworm larvae enter diapause within bolls in increasing numbers during September, and these larvae can only be counted by hand dissection of bolls. Virtually all pink bollworm larvae are in diapause in October and there is no need to incubate these bolls. Dissection of stored bolls was completed by mid-January in 1996 and mid-November in 1997.

The field pairs for both years are identified by letters rather than identifying growers. Field pairs for the same grower and area have the same letter identification both years. Field pairs with the same letter identification were on the same or nearby ground both years.

RESULTS

1996. Very few pink bollworm larvae were found in any of the fields until September (Table 1). Thereafter, two of the non-transgenic fields became heavily infested. The non-transgenic field of field pair B averaged 1.2 and 2.9 larvae per boll in September and October, respectively (total of 5886 pink bollworms in 3250 bolls). A total of 6 larvae were found in bolls from the transgenic field of field pair B in the same period. This pair of fields, to our knowledge, was not sprayed after mid-September. However, in contrast to 1995 (Flint et al. 1996), there were abundant bolls left on plants in October. In 1996, the entire farm was planted with NuCOTN 33 except the single field of DPL-5415 that was our control. In 1996, the grower opted for a longer growing season than in 1995. The other conspicuous example of heavy late season boll infestation was field pair F. In the only sample collected from these fields in October (1,100 bolls each), the non-transgenic cotton field averaged 3.4 larvae per boll. The sample from the transgenic cotton field yielded no larvae, but about 10% (estimated) of the bolls had some visible internal damage due to pink bollworm feeding. Prior to dissection, 500 of the bolls from each field of field pair F were examined for exit holes. The transgenic bolls had seven exit holes, one per boll. The non-transgenic had 181 exit holes; some bolls had multiple exit holes. Thus, some larvae were able to complete development and exit bolls in the transgenic cotton field. Additional damage occurred when larvae were unable to complete development but caused the feeding damage we observed. It was our observation at the time of boll collection that the non-transgenic field had very few remaining bolls on the plants (our estimate was 1 boll per 10 m of row). The field of transgenic cotton had numerous bolls remaining, several per plant. These two fields reflected management practices that took into account the late season advantages of transgenic cotton (but increased the chance of selection for resistance). Other paired fields had much lower infestations rates of pink bollworm. The numbers of pink bollworms found as a percentage of the total bolls collected in 1996 were 0.0004% (n = 33,350 bolls) for NuCOTN 33 and 34.19% (n = 33,850 bolls) for DPL-5415.

1997. Characteristically, very few pink bollworms were found in boll samples until September (Table 2). The non-transgenic cotton of field pair A become heavily infested in September and October. This pair of fields was located approximately 3.2 km miles from the 1996 location of field pair A. The grower terminated irrigation early in the non-transgenic field in 1997 and did not attempt to control pink bollworm from mid-September onward. Early termination of irrigation reduced the number of susceptible bolls in October. However, an average of 1.86 pink bollworms per boll were found in the 1,200 bolls collected in October. The transgenic cotton of field pair A, located less than 0.2 km from the heavily infested non-transgenic cotton fields, had a total of 1 pink bollworm in 1,200 bolls. The non-transgenic cotton of field pair D also sustained heavy infestations in October with an average of 1.76 pink bollworms per boll. This non-transgenic (non-

TABLE 1. Boll Infestations of Pink Bollworm (PBW) in NuCOTN 33 and DPL-5415 in Paired Fields in Central Arizona, 1996.

Field pair	Cultivars	No. bolls sampled and no. PBW found for indicated month								Total	
		Jul		Aug		Sept		Oct			
		Bolls	PBW	Bolls	PBW	Bolls	PBW	Bolls	PBW	Bolls	PBW
A	NuCOTN 33	800	0	1,500	0	2,300	2	1,400	0	6,000	2
	DPL 5415	800	8	1,500	38	2,400	97	1,400	187	6,100	330
B	NuCOTN 33	800	0	1,600	0	2,050	0	1,200	6	5,650	6
	DPL-5415	800	3	1,600	20	2,050	2466	1,200	3,420	5,650	5,909
C	NuCOTN 33	800	0	800	0	1,600	0	400	0	3,600	0
	DPL-5415	800	5	800	5	1,600	13	600	204	3,800	227
D	NuCOTN 33	200	0	3,600	0	5,400	3	600	0	9,800	3
	DPL 5415	0	0	3,600	4	5,200	704	1,200	1	10,000	709
E	NuCOTN 33	1,200	1	1,800	1	2,700	1	1,500	2	7,200	5
	DPL 5415	1,200	0	1,800	6	2,700	420	1,500	203	7,200	629
F[a]	NuCOTN 33	--	--	--	--	--	--	1,100	0	1,100	0
	DPL 5415	--	--	--	--	--	--	1,100	3,768	1,100	3,768
Totals	NuCotn 33	--	--	--	--	--	--	--	--	33,350	14
	DPL-5415	--	--	--	--	--	--	--	--	33,850	11,572

[a]Paired commercial fields sampled 30 October.

TABLE 2. Boll Infestations of Pink Bollworm (PBW) in Transgenic and Non-Transgenic Cotton in Paired Fields in Central Arizona, 1997.

Field pair	Cultivars	No. bolls sampled and no. PBW found for indicated month									
		Jul		Aug		Sept		Oct		Total	
		Bolls	PBW	Bolls	PBW	Bolls	PBW	Bolls	PBW	Bolls	PBW
A	NuCOTN 33	1,600	4	2,200	0	1,900	13	1,200	1	6,900	18
	DPL 5415	1,600	10	2,200	164	1,900	2,948	1,200	2,236	6,900	5,358
B	NuCOTN 33	2,220	0	1,600	0	1,000	1	1,400	6	6,200	7
	SureGro 125	2,220	11	1,600	9	1,000	230	1,400	472	6,200	722
C	NuCOTN 33	2,200	0	2,200	0	1,750	8	1,200	3	7,350	11
	Germain 9230	2,200	0	2,200	19	1,700	886	1,200	951	7,300	1,856
D	NuCOTN 33	1,300	1	2,750	0	4,100	5	600	0	8,750	6
	DPL 5415c	700	0	2,850	4	4,100	1,807	600	1,056	8,250	2,867
E	NuCOTN 33	0	0	1,900	1	1,200	0	1,350	29	4,450	30
	DPL 5415	0	0	1,900	4	1,200	142	1,350	434	4,450	580
F[a]	NuCOTN 33	--	--	--	--	--	--	1,000	2	1,000	2
	DPL	--	--	--	--	--	--	1,000	2,770	1,000	2,770
G[b]	Paymaster 1560 BGa	--	--	--	--	--	--	1,000	61	1,000	61
	Paymaster 1560 BGb	--	--	--	--	--	--	1,000	6	1,000	6
	Paymaster 1560	--	--	--	--	--	--	1,000	2,742	1,000	2,742
Totals	Transgenic	--	--	--	--	--	--	--	--	36,650	139
	Non-transgenic	--	--	--	--	--	--	--	--	35,100	16,962

[a] Paired commercial fields sampled 18 October.

[b] Collections from a large field plot test (ca. 3 ha per plot). In Paymaster 1560 BGa there were 61 larvae in 22 bolls, five bolls contained single larvae. For paymaster 1560 BGb, six bolls contained single larvae. Bolls were collected 17 October.

[c] Monsanto Roundup™ ready transgenic cultivar.

Bollgard™) cotton field was planted with a cultivar that contained a gene for resistance to Roundup™ herbicide (Monsanto Co., St Louis, MO). No pink bollworms were recovered from the transgenic cotton of field pair D in October. Field pair F was located in the exact same fields as the 1996 test, and the 1997 results were quite similar. In 1997, the non-transgenic cotton field averaged 2.77 pink bollworms per boll in the single sample of 1,000 bolls collected 18 October. We recovered two diapause larvae from the comparable sample of transgenic bolls of field pair F.

Two transgenic plots are listed for field pair G: 1560 BGa and 1560 BGb (separated by several hundred meters). A single non-transgenic cotton plot was sampled for comparison. Plot 1560 BGa produced 61 pink bollworms per 1000 bolls. This particular plot contained 20% non-transgenic plants planted together in one section of the plot. The remaining plants, from which we sampled, were presumably transgenic. However, only five of the hand-dissected bolls contained a single larvae. The other 17 bolls contained 56 larvae, a substantial multiple infestation of each of these bolls. For this reason we doubt that these bolls were from transgenic plants. The other transgenic plot, 1560 BGb, had six infested bolls with a single larva in each. We had no methods to test for the insecticidal protein in presumed transgenic plants.

Damage from larval feeding was observed in 1997 in transgenic cotton bolls from fields adjacent to heavily infested non-transgenic fields. We did not document the percentages of bolls showing visible damage, but in some cases it was clear that damage was sufficient to prevent boll opening. Visibly damaged bolls appeared to have been infested by several pink bollworm larvae, none completing development to diapause larvae. Pimples in the carpel wall, feeding channels in the carpel wall, and small necrotic areas in the lint were visible signs of pink bollworm attack. Damage to transgenic bolls was a late season result in the fields we observed.

The 1997 percentage pink bollworms of transgenic bolls was 0.0038% (n = 36,650 bolls) compared with 48.32% for non-transgenic bolls (n = 35,100 bolls).

DISCUSSION

Our current results indicate that the Bollgard™ gene incorporated into DPL-5415 (NuCOTN 33) or Paymaster 1560 (1560 BG) provided nearly complete control of late instar pink bollworm larvae in central Arizona cotton fields in 1996 and 1997. This was our conclusion for 1995 observations (Flint et al. 1996). The central question is whether a decrease in efficacy is discernible over the 1995-1997 period. There was an overall 12-fold increase in pink bollworms in transgenic cotton bolls and a four-fold increase in non-transgenic bolls between 1995 and 1997. However, if the 56 pink bollworms found in multiple infested bolls of Paymaster 1560 BGa are removed from the 1997 totals, the increase becomes seven-fold. This is a comparison of 0.3 and 2.3 pink bollworms per 1,000 bolls for 1995 and 1997, respectively (Table 3). The percentage of non-transgenic seed included in transgenic seed is not published but is suggested to be 1% or more by Monsanto Co. We should expect ten bolls per 1,000 to be infestable by pink bollworm if this is an accurate percentage. In fact, we seldom found ten pink bollworms per 1,000 bolls in samples from transgenic fields. The percentages (and variations in percentages) of non-transgenic plants in fields of transgenic cotton needs to be accurately defined.

The economic effect of damaged bolls in transgenic cotton fields is unknown. Damage to transgenic bolls due to feeding by early instars of the pink bollworm was noted in early experimental lines (Wilson et al. 1992, Ellsworth et al. 1995, Flint et al. 1995, Watson 1995) and in 1996-97. Pink bollworms do not avoid transgenic cotton. It appears to us that damage to bolls is related to the numbers of larvae that attack them.

TABLE 3. Comparison of Pink Bollworm Infestation of Transgenic and Non-Transgenic Cotton Bolls, 1995-1997.

Year	Calculated pink bollworms/1000 bolls		Total bolls
	Transgenic	Non-transgenic	
1995[a]	0.34	118.01	78,240
1996	0.42	341.86	67,200
1997	2.26[b]	483.24	74,750

[a] Flint et al. 1996

[b] 56 pink bollworms found in multiply infested bolls in the plot of Paymaster 1560 BG a not included in the calculation.

Bolls from transgenic cotton in fields adjacent to heavily infested non-transgenic cotton fields suffer damage. These multiple infested transgenic cotton bolls develop mold and may not open fully. However, multiple infestations and damage to transgenic cotton bolls is a late season result. Thus, the numbers of damaged bolls and their effect on yield should be minor since these late season bolls constitute a small part of the harvest.

We do not believe that a significant increase in pink bollworm resistance to the Bollgard™ toxin is shown in our 1995-1997 data. Expectation of resistance is justified. Bartlett (1997) makes cogent arguments for the need for resistance management practices beyond the current refugia strategies. Bartlett also argues for extensive survey to locate founder resistant populations. Tabashnik (1997) notes the lack of field-testing (and the difficulty of doing so) of the various strategies to delay the development of resistance to the Bollgard™ toxin. Tabashnik (1997) foresees the development of resistance to the Bollgard™ toxin by some pest Lepidoptera within a few years.

The circumstance that supports the rapid development of resistance by pink bollworm is the wider use of transgenic cotton plants. The pink bollworm is uniquely suitable for development of resistance to transgenic cotton. The pink bollworm successfully develops only in cotton and migrates extensively, early and late in the season (Noble 1969). Late season production of transgenic cotton with concomitant increased numbers of pink bollworms provides greater selective pressure for resistance. Also, the resistance gene in the pink bollworm may be inherited as a co-dominant factor (Bartlett 1997). The hectarage planted in transgenic cotton in Arizona increased dramatically during the 1995-1997 period. In 1996, 21,200 ha of transgenic cotton were planted in Arizona. In 1997 the number of hectares increased to 80,800, more than half of Arizona's total hectarage (C. Gniffke, personal communication). Increasing pink bollworm infestation of non-transgenic cotton fields provides large numbers of insects for selection for resistance in adjacent transgenic cotton fields. Tabashnik et al. (1997) suggest that two strategies may have merit for delaying the evolution of resistance in pink bollworm: bigger refuge hectarages or alternating transgenic and non-transgenic cotton between years. However, testing these strategies in large areas under commercial conditions is difficult. The opportunity to take effective action to delay evolution of resistance in pink bollworm is passing.

ACKNOWLEDGMENT

We thank cooperating growers and their pest control advisors for their help in conducting these tests. We also thank our laboratory personnel who collected, incubated and dissected bolls: Francis Arvallo, Melani Charney, Rochelle Christensen, Jim Holmes, Jason Jones, Kristin Kunkle, Maria Panter, and Michelle Rodebaugh. Others who assisted in one or several aspects of the study are Larry Antilla, Tim Dennehy, and Rick Webb. We could not have completed these studies without their assistance.

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EFFECT OF NEW COTTON INSECTICIDE CHEMISTRIES, TEBUFENOZIDE, SPINOSAD AND CHLORFENAPYR, ON *Orius insidious* AND TWO *Cotesia* SPECIES

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ABSTRACT

Three new insecticide chemistries recommended for cotton, tebufenozide (Confirm™), spinosad (Tracer™) and chlorfenapyr (Pirate™), were evaluated for their effects on survival of two key beneficial insects, the predaceous insidious flower bug, *Orius insidiosus* (Say)(Heteroptera: Anthocoridae), and the parasitic wasp of many lepidopteran pest larvae, *Cotesia marginiventris* (Cresson) (Hymenoptera: Braconidae). The closely related species *Cotesia plutella* (Kurdjumov) (Hymenoptera: Braconidae) was also evaluated. The mortalities of insects exposed for 24 or 48 h, respectively, to fresh dried foliar residues of these insecticides were compared in the laboratory. Mortality of *O. insidiosus* on Confirm™ and Tracer™ treated leaves was low, ≤ 18%, and similar to that of the control. Karate™ was the most toxic, killing > 87% of adult *O. insidiosus* after 24h. Pirate™ was intermediate in toxicity, killing 21-55% of *O. insidiosus*. Residues of Confirm™ also were nontoxic to adult *C. plutella*. However, Tracer™ and Pirate™ residues were toxic to this parasitoid and to *C. marginiventris*. Pirate™ was more toxic than Tracer™ to these two parasitoids. Therefore, under the conditions of this study, Confirm™ and Tracer™ were less harmful than Pirate™ for all species tested. A simple toxicity rating system for insecticide selectivity to beneficial insects is discussed.

INTRODUCTION

Natural enemies are a key component for the management of lepidopteran cotton pests such as the beet armyworm, *Spodoptera exigua* (Hübner), and fall armyworm, *Spodoptera frugiperda* (J. E. Smith); outbreaks of these pests are thought to be in part due to the use of non-selective insecticides (Croft 1990, Hagen et al 1971, Luckmann and Metcalf 1994). The braconid wasp *Cotesia marginiventris* (Cresson) is an important parasitoid of armyworm and heliothine larvae (Rubertson et al. 1994). The relative abundance of *C. marginiventris* among all parasitoids of bollworm, *Helicoverpa zea* (Boddie), and budworm, *Heliothis virescens* (F.), larvae is approximately 10% in South Texas (J. Benedict, unpublished data). Further, a closely related species, *Cotesia plutella* (Kurdjumov), is an important parasitic wasp for the control of lepidopteran larvae (loopers) in Cruciferae. In cotton, the predator *Orius insidiosus* (Say) feeds on aphids and mites, and is also a key factor in the mortality of lepidopteran eggs in the field (Sansone et al. 1997). Adults and nymphs of *O. insidiosus* and cocoons and adults of *C. plutella* are commercially available (Hunter 1994). These

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commercially available beneficial insects may be used for biocontrol in augmentation programs or as one component of integrated pest management (IPM) programs; therefore, knowledge of their susceptibility to insecticides is useful in selecting specific insecticide chemistries for IPM programs. Three new chemistries with novel modes of action are commercially available for use in the control of lepidopteran pests in cotton: spinosad (Tracer™) (Sparks et al. 1996, Thompson et al. 1995), chlorfenapyr (Pirate™), and tebufenozide (Confirm™). Briefly, spinosad acts on the nicotinic acetylcholine receptor (Salgado 1997, 1998; Salgado et al. 1998); chlorfenapyr uncouples respiration from ATP production (Treacy et al. 1994, Salgado 1997); and tebufenozide is an ecdysone mimetic, binding to the ecdysone receptor causing premature initiation of molting in lepidopteran larvae (Wing 1988, Wing et al. 1988, Dhadialla et al. 1998). The Texas Agricultural Extension Service suggests that Tracer™, Karate™ and Confirm™ be used for control of certain lepidopteran pests of cotton in Texas (Huffman et al. 1996, Knutson et al. 1998, Norman et al. 1998, Sansone et al. 1998).

The objective of the research reported herein was to determine the toxicity against several beneficial insects of fresh residues on cotton leaves of Confirm™, Pirate™, and Tracer™ compared with a commonly used synthetic pyrethroid, Karate™. The long term goal of our research is to rank insecticides registered for use in cotton for their selectivity towards several beneficial insects in order to provide information and recommendations for selectivity of insecticides used in IPM programs.

MATERIALS AND METHODS

Adults of *Orius insidiosus* (Say) were purchased from IPM Laboratories (Locke, NY), and cocoons of *Cotesia plutella* (Kurdjumov) (Hymenoptera: Braconidae) were purchased from Biofac Crop Care (Mathis, TX). *Cotesia marginiventris* (Cresson) (Hymenoptera: Braconidae), originally obtained from Dr. B. Vinson in this Department, was reared in the laboratory, using second-instar tobacco budworms, *Heliothis virescens* (F.), as a host. Parasitized larvae were maintained individually, using 12-well tissue culture dishes. Upon adult emergence, wasps were transferred to plexiglas ventilated cages and fed diluted honey; adults of *O. insidiosus* were fed cotton aphids. Adults of *O. insidiosus* were tested within one week of eclosion, and wasps within 1-4 days of eclosion.

The insecticides evaluated were: Tracer™ [4 lb AI/gal, spinosad (spinosyn A and D); Dow Elanco, Hesperia, CA]; Pirate™ [3 lb AI/ gal, chlorfenapyr, 4-bromo- 2- (4-chlorophenyl) - 1 - etoxymethyl- 5-trifluoromethylpyrrole-3-carbonitrile; Cyanamid, Wayne, NJ], Confirm™ (2 lb AI/gal, tebufenozide; Rohm & Haas, Philadelphia, PA) and Karate™ (1 lb AI/gal, lambda-cyhalothrin; Zeneca Ag Products, Wilmington, DE). Insecticides were applied at the manufacturer's recommended rate for cotton as follows: Confirm™, 0.125 lb AI/acre; Tracer™, 0.073 lb AI/acre; Pirate™, 0.2 lb AI/acre and Karate™, 0.025 lb AI/acre.

Cotton plants (*Gossypium hyrsutum* L.; variety CAB-CS) were grown in 20-gallon pots, four plants per pot, in the greenhouse. Plants used for assays were 6-7 nodes, approximately 11-inches high from the cotyledon (node 0). Plants were grown throughout the course of the experiment to ensure availability of plants of similar size.

Potted plants were treated in a simulation-aircraft precision sprayer chamber, calibrated to simulate an aircraft application at 5 gallons/acre (Bouse et al. 1970). In the chamber, the tops of the plants were precisely situated at 41.5 inches from the nozzle by means of an adjustable screen table, while the sprayer moved at a speed of 5 mph. The

nozzle was 800ZE and provided an average droplet size of 306 μm or 25 drops/cm^2 ; the pressure was set at 30 psi. Water sensitive cards (Teejet; Wheaton, IL) were placed at the average height of the plants to control for homogeneity of droplet size during application. Insecticide treatments were applied on three separate days, constituting three replications. Insecticide solutions (250 ml) were freshly prepared on the day of application by weighing the formulations, except for Karate™ which was measured by volume. The insecticide treatments, as well as controls, were applied in a random manner. On each day, three pots were used per insecticide treatment and control with water. Pots were individually sprayed to avoid pseudoreplication. After spraying, plants were arranged in a completely randomized design. The assays were performed on three typical sunny summer days; treatments were applied in the morning. Insects were tested on 2h-old and 24h-old insecticide residues, as well as controls. Plants were allowed to dry for two or 24 hours outdoors in a well ventilated area, simulating natural cotton crop conditions following aerial application. The terminal and three most apical leaves, were placed on filter paper inside ventilated petri dishes of 14.5 cm in diameter x 2.5 cm. The leaves were kept turgid for several days by keeping the cut end of the stem inside an Eppendorf tube containing a small piece of cotton soaked in sterile sugar-water, and sealing the top of the tube with Parafilm™ around the stem. From each treated pot, the terminal and three apical leaves from three plants were randomly selected, for a total of nine petri dishes per insecticide treatment per day. Five insects per petri dish were utilized. A total of 135 insects were tested per treatment, corresponding to three replications of 45 (5 x 9) insects each per bioassay. Bioassays of *C. marginiventris* were replicated three times with a total of 85 adults for Tracer™ and 91 adults for Pirate™. Assays with Confirm™ and Karate™ were not performed due to an insufficient number of adult *C. marginiventris* of similar age. Insects were exposed for 24 or 48 h to 2-h-old or 24-h-old foliar residues of the commercial formulations or water-treated controls. Insects were maintained in a Percival™ insect incubator at 24°C and 14L:10D light cycle until the end of the testing period. Mortality was recorded as the end point. Percentage mortalities were transformed arcsine $\sqrt{}$ % and analyzed by analysis of variance; means were compared by least significant differences (LSD) [$P \leq 0.05$]. The SAS computer program version 6.03 was used for the statistical analysis (SAS Institute 1988). Mortalities were combined because there was no effect of dishes within treatments. Insecticides were ranked as harmless (1= <25% mortality), slightly harmful (2= 25-50% mortality), moderately harmful (3= 51-75% mortality), and harmful (4= >75% mortality).

RESULTS AND DISCUSSION

Results of the foliar residue bioassays (Tables 1-4) provide a measure of gross efficacy and selectivity of the commercial formulations assayed on two different orders of insects. Exposure of *O. insidiousus* for 24 or 48 h to 2-h-old (Table 1) or 24-h-old (Table 2) residues of Confirm™ or Tracer™ did not cause a statistically significant increase in mortality over control insects. Pirate™ toxicity for *O. insidiosus* was significantly higher than that of Tracer™ and Confirm™, causing 52% and 44% mortality after 48 h of exposure, for both 2-h- and 24-h-old residues, respectively (Table 1). Residue age (ie., 2 h or 24 h) had little influence on percentage mortality of *O. insidiosus* for any insecticide.

As expected, Karate™ residues, both 2-h- and 24-h-old, were highly toxic within the first 24 h of exposure causing greater than 85% mortality (Table 1). The percentage mortality after 24 h or 48 h of exposure to both, 2-h- and 24-h-old residues did not change substantially (compare Tables 1 and 2) for all insecticides tested, indicating that the

TABLE 1. Percentage Mortality of *Orius insidiosus* Exposed for 24 or 48 hours to 2-h-Old Insecticide Spray Residues on Cotton Leaves, Test 1.

Insecticide	lb AI/acre	% observed mortality after exposure for 24 hours	48 hours[a]
Control		3.6 ± 2.2 c	7.4 ± 2.4 c
Confirm	0.125	5.3 ± 1.8 c (1.7 %)	7.8 ± 2.1 c (0.4%)
Tracer	0.073	4.6 ± 2.0 c (1.03 %)	16.3 ± 3.9 c (9.6%)
Pirate	0.2	28.2 ± 5.1 b (25.5 %)	55.9 ± 8.1 b (52.4%)
Karate	0.025	86.0 ± 4.1 a (85.5 %)	94.8 ± 2.4 a (94.4 %)
LSD (0.05)		9.3	10.8
F test (P)		0.0001**	0.0001**

[a]Means in columns followed by different letters are significantly different ($\alpha = 0.05$), Fisher protected LSD. *P* value(s) followed by an asterisk (**) indicates a highly significant F test ($P \leq 0.001$). Groups of 675 insects were used for the 24 h and 48 h exposure tests, respectively. Numbers in parenthesis are percent corrected mortality (Abbott 1925).

TABLE 2. Percentage Mortality of *Orius insidiosus* Exposed for 24 or 48 hours to 24 h-Old Insecticide Spray Residues on Cotton Leaves, Test 2.

Insecticide	lb AI/acre	% observed mortality after exposure for 24 hours	48 hours[a]
Control		4.4 ± 1.6 c	13.3 ± 3.8 c
Confirm	0.125)	4.5 ± 1.6 c (0.1)	16.2 ± 8.5 c (3.3)
Tracer	0.073	3.1 ± 1.2 c (0)	17.8 ± 2.2 c (5.2)
Pirate	0.2	21.3 ± 2.8 b (17.7)	51.9 ± 3.3 b (44.5)
Karate	0.025	87.1 ± 5.0 a (86.5)	100.0 ± 0.0 a (100)
LSD (0.05)		8.0	16.4
F test (P)		0.0001**	0.0001**

[a]Means in columns followed by different letters are significantly different (a = 0.05), Fisher protected LSD. *P* value(s) followed by an asterisk (**) indicates a highly significant F test ($P \leq 0.001$). Groups of 675 insects were used for the 24 h and 48 h exposure tests, respectively. Numbers in parenthesis are percent corrected mortality corrected (Abbott's formula).

effectiveness or Pirate™ and Karate™ was similar during the first two days (compare Table 1, 24 h vs. Table 2, 24 h), and apparently during the first three days (Table 2, 48 h). Therefore, the release of beneficial insects may be unwise within the first 3 days following applications of Pirate™ or Karate™.

The effects of the insecticides on *C. plutella* are shown in Table 3. In contrast to the results with *O. insidiosus*, exposure to 2-h-old Tracer™ residues caused significant mortality (42.8%) after 24 hours, and Karate™ did not cause mortality (7.5%) above controls.

We investigated the low toxicity of Karate™ observed in this bioassay by means of an additional foliar dip assay in which similar plant tissues were immersed in various concentrations of Karate™ from 1,800 ppm to 10 ppm. Tissues were air dried for 2 h in the laboratory, and insects were exposed to these fresh residues. In all cases, Karate™ caused

TABLE 3. Percentage Mortality of Adults of the Braconid Parasitic Wasp *Cotesia plutella* Exposed for 24 hours to 2 h-Old Insecticide Spray Residues on Cotton Leaves.

Insecticide	lb AI/acre	Corrected (Abbott's) mortality after 24 hours	% mortality after 24 hours (± SEM)[a]
Control			0.7 ± 0.7 c
Confirm	0.125	3.7	4.4 ± 1.9 c
Tracer	0.073	42.8	43.2 ± 9.3 b
Pirate	0.2	78.45	78.6 ± 8.3 a
Karate	0.025	7.5	8.2 ± 3.1 c
LSD (0.05)			14.6
F test (P)			0.0001

[a]Means followed by different letters are significantly different ($P \leq 0.05$) based on Fisher protected LSD.

100% mortality after 24 h. Therefore, we attribute these differences in toxicity to a repellent effect of Karate™ and the ability of wasps to avoid the sprayed surface of cotton tissues. The bioassay arena that we used can be considered an assay with choice because insects were able to survive exposure to a highly toxic compound. Fresh residues of lamda-cyhalothrin are also highly toxic to another parasitic hymenopteran, *Aphidius rhopalosiphi* (Jansen 1996). The repellent effect of lamda-cyhalothrin is known (Tomlin 1990); however, repellency has not been observed when mixed with pheromone for control of *Spodoptera littoralis* Boisduval (De Souza et al. 1992).

Dried residues of Tracer™ caused up to 50% mortality of *C. plutella*. Thus, the statement by Peterson et al. (1996) that several genera of beneficial insects are tolerant to this compound should be cautiously considered. These authors indicated that a *Cotesia* spp. was among the active beneficial arthropods observed in Tracer™ -treated cotton during 1995.

Pirate™ was highly toxic to *C. plutella* and it does not appear to have a repellent effect. Results of Pirate™ and Tracer™ toxicity on *C. marginiventris* using foliar spray assays indicate that both insecticides are highly toxic to this species (Table 4), with about 84-96% mortality above the control. Additionally, *C. marginiventris* is more sensitive to these insecticides than *C. plutella* (Tables 3 and 4). Tillman (1996) reported 100% mortality of *C. marginiventris* with topically applied Pirate™ and found that this species is only relatively more tolerant to three insecticides (oxamyl, thiodicarb and acephate) out of 14 tested.

Our results indicate that even closely related species of natural enemies respond differently to the same compound; therefore, generalizations on toxicity of a compound to insects of a particular genus based on the results obtained with one species should be viewed with caution. The disruption of parasitic hymenopterans, mainly represented by *Microplitis demolitor,* by Tracer™ was reported in cotton field studies in Australia, and the authors also cautioned against broad statements on the selectivity of spinosad to all beneficial insects (Murray and Lloyd 1997).

Percentage mortalities of *O. insidiosus* exposed to Tracer™ and Pirate™ were similar to those reported by Elzen et al. (1998). Results with Pirate™ contrasted with those of Wiley et al. (1995) who reported that Pirate™ has low to moderate impact on beneficials. Foliar residue bioassays on soybean with hemipteran predators also showed a higher toxicity of chlorfenapyr over spinosad at 24, 48 and 72 h (Boyd and Boethel 1998).

TABLE 4. Percentage Mortality of Adults of *Cotesia marginiventris* Exposed for 24 hours to 2 h-Old Insecticide Spray Residues on Cotton Leaves.

Insecticide	lb AI /acre	Corrected (Abbott's) % mortality	% mortality after 24 hours[a]
Control			12.38 + 3.06 b
Tracer	0.073	83.7	85.71 ± 7.04 a
Pirate	0.2	95.8	96.31 ± 2.38 a
LSD	0.05		12.56
F test (P)			0.0001

[a]Means followed by different letters are significantly different ($P \leq 0.05$) based on Fisher protected LSD.

Our findings support previous reports that tebufenozide is harmless to slightly harmful to beneficial or non-target arthropods, including parasitic wasps (Addison 1996, Brown 1996, Dadhialla et al. 1998). However, tebufenozide was found to decrease peak egg production in the following generation of tebufenozide exposed lacewings (Rumpf et al. 1998) and to have sublethal effects on *Trichogramma praetiosum*, reducing the time of development (Consoli et al. 1998). It was not harmful for nymphal development and oviposition of *Orius laevigatus* (Fieber) (Vandeveire et al. 1996).

The insecticides were ranked according to percentage mortality observed utilizing the system explained above. Ranks were Confirm™ and Tracer™ (1 = Harmless), Pirate™ (2 = Moderately harmful), and Karate™ (4 = Harmful) for *Orius insidiousus;* Confirm™ (1), Tracer™ (2), and Pirate™ (4) for *Cotesia plutella*; and both Tracer™ and Pirate™ (4) for *C. marginiventris*.

The results of our study reflect the effects of dried insecticide residues and not those of direct spray applications. Additional measurements of rate of parasitism and predation in insecticide treated fields would provide a more complete assessment of insecticide effects. Similarly, behavioral responses, such as the observed avoidance of Karate™ treated plants by *C. plutella*, should be considered when interpreting the results. Consequently, ranking systems of insecticides for use in IPM must reflect the average of negative effects among all the key beneficial insect species in cotton.

ACKNOWLEDGMENT

We thank Dr. Kirk (USDA, College Station, TX) for allowing the use of the spray chamber, Dr. K. El-Zik (TAMU) for providing cotton plants and greenhouse space, S. House (USDA, College Station, TX) for training with the spray chamber and S. Deike for technical assistance. Dow Elanco, American Cyanamid, Rohm and Haas, and Zeneca Ag. Products generously provided insecticides. This research was supported in part by the Extension Service IPM Mini-Grants Program to the authors and the Texas Agricultural Experiment Station.

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WEEDS AND CULTIVATED HOSTS OF THE SILVERLEAF WHITEFLY *BEMISIA ARGENTIFOLII* [1]BELLOWS AND PERRING IN BAJA CALIFORNIA SUR, MEXICO.

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ABSTRACT

The silverleaf whitefly, *Bemisia argentifolii* Bellows and Perring was found on 73% of the cultivated plants and on 66% of the weeds hosts observed. The most important cultivated hosts were *Brassica spp. Cucurbita spp*, and *Solanum melongena.* Among the weeds hosts, the most preferred were *Flaveria trinervia, Malva parviflora,* and *Sphaeralcea emoryi*. Families of plants in descending order of preference were Compositae, Solanaceae, Leguminosae, Cucurbitaceae, and Brassicaceae. The families Amaryllidaceae, Poaceae, and Aizoaceae did not host this insect pest. On both weeds and cultivated plants there was a significant preference of the silverleaf whitefly for foliar plants.

INTRODUCTION

Whiteflies are one of the most important groups of insect pests worldwide (King and Saunders 1984, Liu et al. 1993). The silverleaf whitefly (SLWF), *Bemisia argentifolii* Bellows and Perring, has become one of the most destructive pests on several crops in Mexico. This pest status has been attributed to its high virus transmission; a few individuals are enough to considerably reduce yield and quality, often up to 100% (Sifuentes et al. 1991, Fishpool and Burban 1994).

In 1991, the SLWF damaged important crops in Mexico such as cantaloupe, watermelon, cotton, and sesame in the Mexicali, Baja California, and San Luis Rio Colorado, Sonora, valleys. Losses were estimated at approximately $20 million (Martínez 1995). Damage was produced not only by feeding of high populations of SLWF but also by contamination of fiber and fruits (e.g., sooty mold growing in honey dew excreted during the process of feeding). SLWF has become a common problem in other areas of Mexico, and it is now reported in 16 states where it has damaged soybeans, cotton, peppers, cantaloupe, cucumber, squash, tomato, beans, watermelon, lettuce, sweet potato, radish, peanuts, poinsettia, and other crops (Pacheco 1985, Ortega 1990, Sifuentes et al. 1991).

In addition to the crops mentioned above, there are some uncultivated plants that also are hosts to the SLWF. These plants may be associated with the crops, developing either within the crop or at the field borders, and are usually considered weeds (Ortíz 1988, Roditakis 1990, Costa et al. 1991). In México, Avilés (1991) reported 27 host plants of the SLWF in Yucatán;16 of these had virus symptoms.

In Baja California Sur, there are approximately 76,000 ha dedicated to agricultural production. The SLWF has become an important insect pest in many of these crops, so it is a priority to obtain data that contributes to the establishment of strategies for whitefly management.

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The objective of this study was to identify those plants that will serve as hosts for the complete development of this insect pest, including cultivated crops and weeds present in the agricultural valley near La Paz.

MATERIALS AND METHODS

The agricultural valley of La Paz is near the southwest part of the city of La Paz in Baja California Sur (100 22.5' W, 42° 2.5 N), with an elevation 30-40 m above sea level. This valley is represented by two agricultural communities, Chametla and El Centenario. The agricultural area is 15,000 ha. Most of the fruits and vegetables consumed by people of La Paz and neighboring villages are supplied from these farms.

Plant collections were made during 1993 to 1995 from crops or associated vegetation found within, at the edges or around the crop, and in irrigation furrows. Those plants that had fourth-instar nymphs, commonly known as pupae, were considered hosts of the SLWF because their presence indicated the plant provided enough nutrition to the insect to complete its development.

The plants collected were prepared and identificated. Pupae of the SLWF were collected from the plants. Some were kept in 70% alcohol for further examination, others were mounted on microscope slides, for taxonomic separation (Gill 1991, Ortega and Garza 1993, Bellows et al. 1994). To confirm the species identification, samples of adults and nymphs were sent to the National Center of Reference in Mexico (INIFAP), which is part of the Plant Health Service.

To estimate host preference of the SLWF, we used a scale based on the relative density (RD) of pupae on each plant. We used (-) when pupae were never found, 1 for 1-10, 2 for 11-20, 3 for 21-30, 4 for 31-40, and 5 for 41 or more individuals (Roditakis, 1990). The plants were classified as glabrous (-), slight pubescent (+), pubescent (++), and highly pubescent (+++). Finally, we recorded the regional name of the plant and its growth habits: shrub (Ab), tree (Ar), annual herb (Ha), or perennial herb (Hp).

RESULTS AND DISCUSSION

Whiteflies in the *Bemisia* genus have been reported from more than 600 host plants (Mound and Halsey 1978, Butler et al. 1986, Gill 1992, Ravisankar 1993) and *Bemisia argentifolii* has been cited on more than 500 host plants (Wool and Greenberg 1990, Machain and Lopez 1996). In our study we collected 30 weed species (12 families) and 26 cultivated plants (11 families). *Bemisia tabaci* has been the most widely studied species in the whitefly complex. Mound and Halsey (1978) reported 63 host families, with the most important being Cucurbitaceae, Leguminosae, Malvaceae, Solanaceae, and Vervenaceae. For *Bemisia argentifolii*, there are fewer reports; however, it is believed to have a host range similar to that of *Bemisia tabaci* (Brown et al. 1995).

In our study, most of the host plants were herbaceous with 67% being annuals, 20% perennials, and the remainder shrubs and trees. The plant growth habits are important because those plants that have foliage all year provide food, and shelter to the insect even though there is no suitable crop for development. The uncultivated host plants are a refuge for the insect and could be the primary inoculum for the cultivated plants in an agricultural area.

The species of plants studied are listed in Tables 1 and 2. The growth habit of plants, density of foliar structures, the maximum number of setae present on the pupae, and the relative density of pupae per leaf are presented in both tables.

The weeds were mainly Dicotyledoneae except for three (Table 1). Most (56%) had foliar structures ranging from glabrous to highly pubescent. From the total number of plants observed, 67% were positive for the presence of pupae of *Bemisia argentifolii*. Those with the

TABLE 1. Weeds associated with Horticultural Crops and Relations to *Bemisia argentifolii* in the Area of Chametla and El Centenario in Baja California Sur.

Scientific Name	Regional Name	Plant growth [a]	Foliar structure [b]	Maximum number of setae in pupae [c]	Pupae density [d]
Abutilon californicum	malva	Hp	+++	-	0
Amaranthus palmeri	quelite	Ha	++	4	3
Ambrosia ambrosioides	chicura	Ha	-	2	2
Ambrosia confertiflora	estafiate	Hp	++	8	1
Atriplex barclayana	chamizo	Sh	+++	-	0
Cenchrus ciliaris [e]	zacate buffel	Hp	+++	-	0
Chenopodium album	chuale blanco	Ha	++	6	3
Chenopodium murale	chuale	Ha	+++	-	0
Chloris virgata [e]	pata de gallo	Hp	-	-	0
Convolvulus arvensis	gloria de la mañan	Ha	+++	8	3
Convolvulus sagitatus	-	Hp	-	1	1
Cucumis dipsaceus	melón de coyote	Ha	++	6	4
Datura discolor	toloache	Ha	++	8	4
Flaveria trinervia	-	Ha	-	1	5
Helianthus annuus	girasol	Ha	+++	8	1
Malva parviflora	malva	Hp	+	4	5
Nicotiana glauca	levantate Don Jua	Sh	-	1	1
Physalis greenei	tomatillo	Ha	-	1	2
Pithecellobium dulce	guamuchil	Tr	-	-	0
Polygonum argyrocoleon	-	Ha	-	1	2
Ricinus communis	higuerilla	Sh	-	1	1
Rumex maritimus	lengua de vaca	Ha	++	-	0
Salsola kali	chamizo	Ha	-	-	0
Setaria adherens [e]	-	Ha	-	-	0
Sisymbrium altissimum	-	Ha	-	1	1
Sonchus oleraceus	endivia	Ha	+	2	3
Sphaeralcea emoryi	malva	Ha	+++	8	5
Trianthema portulacastrum	verdolaga silvestre	Ha	-	-	0
Verbesina encelioides	girasol	Ha	+++	6	2
Xanthium strumarium	huizapolón	Ha	++	7	2

a. Sh = Shrub, Tr= Tree, Ha= Herbaceus annual, Hp= Herbaceus perennial.
b. (-)= Glabrous, (+)= Low pubescent, (++)= Pubescent, (+++)= High pubescent
c. Pairs of large seta present in the dorso-caudal area of
d. Density : 0= None; 1= Very low; 2= Low; 3= Medium; 4= High; 5= Very high.
e. Monocotyledons.

highest density were *Flaveria trinervia*, *Malva parviflora*, and *Sphaeralcea emoryi*, followed by *Cucumis dipsaceus*, *Datura discolor*, *Amaranthus palmeri*, *Chenopodium album*, *Convolvulus arvensis*, and *Sonchus oleraceus*.

Flaveria trinervia is widely distributed in agricultural commercial plots. It has the capacity to support very large population densities of whiteflies. This plant is commonly found among other species or vegetable varieties such as cabbage, lettuce, broccoli, tomato, and pepper. The cryptic habit of *F. trinervia* makes it difficult to eliminate by weeding and thus the plant remains a reservoir of the insect.

M. parviflora and *S. emory* are host plants that also support large populations of whiteflies and are abundant in the agricultural area. However, they are eliminated by the grower in the weeding process, because they are more evident as weed problems.

There were some plants on which no whiteflies were found even though they were in the same agroecosystem (Tables 1 and 2); they represent an option for more studies because they

could have some inhibiting mechanism or other characteristic that could open new possibilities for whitefly management. Ravisankar (1993) reported that some monocotyledons did not allow the development of whiteflies when confined to microcages. The whieflies died soon after being introduced to the cages, without feeding or reproducing. When cotton was used as a host plant, the insect reproduced successfully.

TABLE 2. Cultivated Plants in the Agricultural Area of Baja California Sur, during the Winter and Fall 1993-1995, Agricultural Seasons and their Relation to *Bemisia argentifolii*.

Scientific Name	Common name spanish	Foliar structure [a]	Maximum number of setae in pupae [b]	Pupae density [c]
Allium cepa	cebolla	-	-	-
Allium sativum	ajo	-	-	-
Apium graveolens	apio	-	-	-
Beta vulgaris	betabel	-	-	-
Brassica oleracea	col	-	1	5
B. oleracea	coliflor	-	1	4
B. oleracea var. botrytis	brocoli	-	1	4
Capsicum annuum	chile	-	1	2
Citrullus vulgaris	sandía	+++	8	3
Citrus sinensis	naranjo	-	1	1
Coriandrum sativum	cilantro	-	-	-
Cucumis melo	melón	+++	8	5
Cucumis sativus	pepino	+++	7	5
Cucurbita pepo	calabaza	+++	8	3
"	calabacita	+++	8	4
Daucus carota	zanahoria	+	4	2
Ficus carica	higuera	++	6	2
Lactuca sativa	lechuga	-	1	3
Lycopersicon esculentum	tomate	++	6	4
Medicago sativa	alfalfa	+	2	2
Mentha spicata	yerbabuena	+	3	2
Ocinum basilicum	albahacar	-	1	2
Phaseolus vulgaris	frijol	-	1	3
Raphanus sativus	rábano	++	6	2
Solanum melongena	berenjena	++	8	5
Vicia faba	haba	-	-	-

a. (-)= Glabrous, (+)= Low pubescent, (++)= Pubescent, (+++)= High pubescent

b. Pairs of large seta present in the dorso-caudal area of

c. Density : 0= None; 1= Very low; 2= Low; 3= Medium; 4= High; 5= Very high.

The relationship between whiteflies and cultivated host plants has been well documented (Pacheco 1985, Roditakis 1990, Gill 1992, Arnal et al. 1993). In Baja California Sur, Cortez (1994) reported a list of cultivated host plants of *Bemisia tabaci* in the Santo Domingo valley that were observed throughout the year. For *B. argentifolii*, no information was given. This report represents the first study that identifies host plants for this species (Table 2). The most important crops where *B. argentifolii* was found were cantaloupe, watermelon, cucumber, cabbage, cauliflower, broccoli, tomato, and chili pepper. Crops that were not infested by the SLWF were corn, onion, garlic, and celery. Martínez (1995) reported eggs and first-instar larvae of SLWF in early stages of corn development, but the insect did not develop to complete its

entire life cycle. It is possible that nutrients in the corn plant are not adequate for development of the SLWF.

The great variety of host plants for the SLWF in Baja California Sur corroborated that this insect is a polyphagous species, with host plants similar to those reported for *Bemisia tabaci.* Most of them belong to the Dicotyledoneae that have a wide distribution in this agricultural area. The host plants found in this area also have been reported in other agricultural zones (Greathead 1986, Roditakis 1990, Gill 1991, Natwick et al. 1993, Ravisankar 1993, Fishpool and Burban 1994).

The SLWF and *B. tabaci* have reached the status of primary pests mainly because of their opportunistic characteristic of adaptation to different host plants and to their rapid development (Brown et al. 1995). The insect also changes the morphology of its immature stages according to the structural characteristics of the host, such as the degree of pubescence, allowing better adaptation to the substrate (Brown et al. 1995). The variety of host plants determines its longevity, fertility, and population development. Even the behavior of egg laying is influenced by the host plant; *Trialeurodes vaporariorum* prefers glabrous leaves (Collman and All 1980), but *B. tabaci* and *B. argentifolii* prefer to lay their eggs on pubescent leaves (Costa et al. 1991).

ACKNOWLEDGMENT

The authors acknowledge the collaboration of J.L. Leon, R. Domínguez and M. Domínguez in the identification of plant material obtained in this study. Also we appreciate the support given by F. Cota and A. Cota in the collection of this material. Thanks to Dr. Ellis Glazier for his editing of the English. This study was financially supported by Consejo Nacional de Ciencia y Tecnología, Secretaría de Educación Pública and by the Centro de Investigaciones Biológicas del Noroeste, S.C. through financial assignment to the proyect AGR-3 "Biología y Toxicología de Plagas Agrícolas en el Noroeste de México".

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SPRAY-DRIED MICROENCAPSULATED *BACILLUS THURINGIENSIS* FORMULATIONS FOR THE CONTROL OF *EPILACHNA VARIVESTIS* MULSANT[1]

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ABSTRACT

A new sprayable microgranule formulation of *Bacillus thuringiensis* subsp. *kumomatoensis* (strain C-9) was evaluated for the control of the Mexican bean beetle *Epilachna varivestis* Mulsant. Strain C-9 was isolated from dead *E. varivestis* in a *Phaseolus vulgaris* L. field in Durango, México. A spore and crystal complex of the C-9 strain were active against *E. varivestis* larvae in bioassays using treated bean plants (LC_{50} of 642 µg/ ml), and against *Trichoplusia ni* (Hübner) larvae using artificial diets (LC_{50} of 219 µg/ml). The sprayable powder (microgranule formulation), consisting of cornstarch (CS), nixtamalized corn flour (NCF), vegetable oil, powdered sugar, 2-propanol, malachite green, and formaldehyde, was prepared using a spray-dry process. Shelf life of formulated *B. thuringiensis* maintained over 90% of its original activity after 24 months of storage. A field test demonstrated that treatments of formulated *B. thuringiensis* controlled *E. varivestis* significantly better than treatments with unformulated *B. thuringiensis*. This study demonstrated that *B. thuringiensis* can potentially be formulated by spray drying, based on ingredients, which would help to improve its activity as a biopesticide under field conditions.

RESUMEN

Se evaluó un nuevo formulado microgranular asperjable con la cepa C-9 de *Bacillus thuringiensis*, para el control de la conchuela del frijol *Epilachna varivestis* Mulsant. La cepa C-9 fue aislada de larvas muertas de *E. varivestis*, encontrada en un cultivo de frijol en el estado de Durango, México. La cepa C-9 mostró actividad tóxica contra larvas de *E. varivestis* en bioensayos, usando plantas de frijol tratadas con el complejo esporas-cristales (LC_{50} de 642 µg/ml), y contra larvas de *Trichoplusia ni* usando dietas artificiales (LC_{50} de 219 µg/ml). El polvo asperjable (formulación microgranular) se preparó con los ingredientes almidón de maíz, harina de maíz nixtamalizado, aceite vegetal, azúcar pulverizada, 2-propanol, verde de malaquita y formaldehído, y se procesó empleando el método de secado por aspersión. Estudios de vida de anaquel del formulado mostraron que se conservó arriba de un 90% de la actividad original

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de *B. thuringiensis* después de mantener el formulado almacenado por 24 meses a temperatura ambiente. Pruebas de campo demostraron que los tratamientos donde se aplicó el formulado con *B. thuringiensis* controlaron significativamente mejor a *E. varivestis* que los tratamientos con *B. thuringiensis* sin formular. Este trabajo demuestra que es factible mejorar la actividad bioinsecticida de *B. thuringiensis* bajo condiciones de campo, al formularlo con ingredientes naturales, usando la técnica de secado por aspersión.

INTRODUCTION

The potential of *Bacillus thuringiensis* subsp. *kumamotoensis* (strain C-9) spray-dried formulations based on starch and nixtamalized corn flour for application to bean crops against the Mexican bean beetle, *Epilachna varivestis* Mulsant, (Coleoptera: Coccinellidae) in Mexico was investigated. *E. varivestis* is an important pest of snap bean (*Phaseolus vulgaris* L.), soybean (*Glycine max* Merrill), and lima bean (*P. lunatus* L.), causing serious economic loss (Kogan 1971, Kauffman and Flanders 1985, Own and Brooks 1986). Biological control techniques used against *E. varivestis* include annual inoculative releases of the parasitoid wasp, *Pediobius foveolatus* Crawford, which was reported to suppress *E. varivestis* pest populations in soybeans (Kauffman and Flanders 1985, Bernhardt and Shepard 1978). Microbial control of *E. varivestis* using *B. thuringiensis* (Cantwell and Cantello 1982) or *Nosema* sp. (Brooks et al 1985, Brooks 1986), or both microbial and parasitoid control agents (Cantwell et al 1985, Own and Brooks 1986) have been reported.

The current dominance of *B. thuringiensis* in the microbial insecticide market has resulted in diverse formulation technologies (Burges 1998). To maintain its commercial success, *B. thuringiensis* products must advance beyond current technology, such as palatability and keeping the toxin in the feeding zone of the target pest when exposed to adverse environmental conditions (Gillespie et al 1994, Mulla 1990). Residual toxicity has been improved by avoiding inactivation of spores and crystals by ultraviolet (UV) rays or wash-off by rain (Akiba 1991).

Recently, starch- and flour-based film-forming materials (McGuire et al 1996, Behle et al 1997), and starch- and flour-based *B. thuringiensis* sprayable formulations (Tamez-Guerra et al 1996) have been developed for lepidopteran pests to address issues of feeding stimulation and protection from environmental degradation. Even though UV degradation is considered the main reason for loss of activity in the field, there are other properties to consider. For example, rheological properties of the mixture to be spray-dried are important for industrial production (Rao 1992). The manufacturing process must not reduce the insecticidal activity. The final product must maintain activity when stored at room conditions (Burges 1998). The product's particle size must be sufficiently small to be applied by conventional spray equipment (McGuire et al 1996). After promising laboratory results (Tamez-Guerra et al 1996), field tests are the best way to compare the spray-dried formulations with the unformulated bioinsecticide as well as with currently recommended chemical insecticides.

MATERIALS AND METHODS

B. thuringiensis strain C-9 (subsp. *kumomatoensis*) was obtained from the International Collection of Entomopathogen Bacilli at Facultad de Ciencias Biológicas, Universidad Autónoma de Nuevo León, México (FCB-UANL, MX). This strain was originally isolated in Durango, México. Spores and crystals used in this study were produced as described by Tamez-Guerra et al (1998).

E. varivestis founders were supplied by Centro Interdisciplinario de Investigación para

el Desarrollo Integral Regional, Instituto Politécnico Nacional, Vicente Guerrero, Durango, Mexico (CIIDIR-IPN, MX.). *E. varivestis* was reared using *Phaseolus vulgaris* L. var. peruano bean plants. Plants were grown in plastic pots (30-cm diameter) in a glasshouse. *E. varivestis* adults and larvae were reared on potted plants inside a cage held in a rearing room (~20°C, 55 ± 5% RH). First- and second-instar larvae of *E. varivestis* were used in all bioassay and field tests. *Trichoplusia ni* (Hübner) neonate larvae were supplied by FCB-UANL, Mexico. This colony was reared using an artificial diet as reported by Tamez-Guerra et al (1996). *T. ni* neonates were used in all bioassays.

The ingredients used to produce microgranules were selected based on availability, cost, and functionality. All products, except malachite green and formaldehyde, were available in Mexican grocery stores. Five formulations made with various ratios of cornstarch (CS) (Maizena, Monterrey, NL, MX.), and nixtamalized corn flour (NCF) (Maseca, Guadalupe, NL, MX.) at CS:NCF ratios of 4:0, 1:3, 1:1, 3:1 and 0:4 were compared. The CS:NCF mixture (100 g) was combined with 280 ml of distilled water, 100 g of powdered sugar (Valle Verde, Santa Catarina, NL, MX.), 20 ml of vegetable oil (Capullo, Monterrey, NL, MX.), 120 ml of 2-propanol (Capullo, Monterrey, NL, MX.), 0.1 g of malachite green oxalate salt (Sigma, St. Louis, MO.), and 0.5 ml of 37% formaldehyde (Sigma, St. Louis, MO.) (Tamez-Guerra et al 1996). For laboratory experiments, 3 g of *B. thuringiensis* (spore-crystals) per each 97 g of total solids was added to the mixture to reach a 3% spore-crystals product. For field experiments, the formulation with CS:NCF at a ratio of 1:1 was made with 2% and 4% *B. thuringiensis*.

Because rheological properties affect spray-drying characteristics, different mixtures were subjected to rheological tests of viscosity and shear rate following the methodology described by Rao (1992). Viscosity and shear rate were measured with a Brookfield RVF viscometer (Brookfield Engineering Laboratories, Inc., Stoughton, MA) at 2 and 20 rpm spindle rotation. A 60% sucrose standard was used as a value correction factor. Variable speed viscosity measurements were taken immediately, 30 min and 2 hrs after initial mixing to determine if the fluid type was Newtonian or non-Newtonian. The viscosity value is most accurate when it ranges between 20 and 500 cp. Non-Newtonian fluids give unequal values at different times and speeds.

Microgranule formulations were made using a Niro atomizer (Microcorp, Columbia, MD) spray dryer. The spray-dry conditions included 10 ml/min flow rate, an inlet temperature of 130°C, an outlet temperature of 65 ± 5°C, and an air pressure of 4 kg/cm^2 (Tamez-Guerra et al 1996).

Since moisture content of any natural product can influence biodegradation, the microgranulated formulations were tested for moisture at 0, 0.5 and 2 years after the granules were made. The formulations were stored in glass jars (not sealed) at room temperature (~25°C, 45%RH). Moisture content of each formulation was determined based on the average of three samples from each formulation with a Mark 2 Moisture Analyzer (Onmimark, Temple, AZ). Particle size of various formulations consisting of CS:NCF at ratios of 4:0, 1:3, 1:1, 3:1 and 0:4, and the same preparation at 4:0, 1:1, and 0:4 without spray-drying were determined with a Coulter Multisizer (Coulter Co. Miami, FL). Approximately 40-50 µg of each sample were diluted in 20 ml of Isoton II (Coulter Co.; Miami FL) to a final concentration of 2-2.5 µg/ml. The sample size was 500 µl, using an aperture size (orifice) of 140 µm. The data were analyzed using an AccuComp software version 4.2 (AccuComp, Hialeah, FL). Scanning microscopy (JSM-6400V, Jeol; Peabody, MA) micrographs were taken of the five formulations tested for particle size to determine the shapes and dimensions of the granules after spray drying.

A leaf bioassay was used to determine the LC_{50} of the unformulated and spray-dry formulated *B. thuringiensis* strain C-9 against first- and second-instar larvae of *E. varivestis*. Leaf disk sections (33-cm diameter) were cut from bean leaves and placed individually in plastic

petri dishes (Falcon 50 x 9 mm, Becton Dickinson and Co., Lincoln Park, NJ) containing one filter paper (Whatman No. 1, 42.5 mm diameter, Whatman International Ltd., Maidstone, England) per dish. To determine the LC_{50}, each disk was treated individually with 100 µl of solution containing concentrations of 0, 50, 100, 200, 500, or 1000 µg of spore-crystals complex/ml, and allowed to dry. Five disks were treated for each concentration. Each treated leaf disk was infested with three larvae. The dishes were maintained in a culture room at 25 ± 3°C and 65 ± 5% RH. After three days of incubation, one untreated bean leaf was placed in each dish. Live and dead larvae were counted after seven days. The LC_{50} was determined using probit analysis (SAS Institute 1989).

The LC_{50} of *B. thuringiensis* strain C-9 against *T. ni* was determined by a diet incorporation bioassay. Spore-crystal *B. thuringiensis* complex was added to warm and liquefied artificial diet at concentrations of 15, 25, 50, 100, 250 and 500 µg/ml of diet. Plastic cup containers with 5 ml artificial diet plus *B. thuringiensis* complex were used to test the insecticidal activity against *T. ni* neonates. Seventy-five larvae, three replicates of 25 larvae each, were exposed to determine mortality at each dose (McGuire et al 1997). Containers were maintained at 28 ± 5°C and 65 ± 5% of RH. Mortality was recorded after 8 days of feeding.

To determine if spray drying reduced activity of the *B. thuringiensis* strain C-9 against *E. varivestis*, unformulated and 3% spore-crystals complex formulated with CS and NCF at ratios of 1:1 were tested. Leaf disk bioassays against *E. varivestis* treated with 100 µl of solutions at concentrations of 0.5 and 1.0 mg/ ml of *B. thuringiensis* spore-crystal complex, as described above, were tested. Bioassays consisted of 15 larvae, five replicates with three larvae per dose. Diet incorporation bioassay against *T. ni* was compared using 50 and 500 µg/ ml of *B. thuringiensis* spore-crystal complex. Unformulated and microgranulated product were also assayed for insecticidal activity after six months and two years of storage at room temperature (~25°C, 45%RH).

A field experiment was established at Vicente Guerrero, Dgo, at CIIDIR-IPN, MX, using *P. vulgaris* var. peruano. Peruano beans were planted in rows separated by 0.66 m in early July 1994 and furrow irrigated as needed. The field was naturally infested with *E. varivestis*. Treatments were applied two months after planting, using a randomized complete block design with six treatments and four replications. Each plot was six rows wide by 10 m long with 120-150 bean plants/row. Treatments consisted of unformulated and formulated *B. thuringiensis* C-9 strain' spore-crystals complex at rates of 2% and 4%, carbaryl (1-naphthy methylcarbamate, Sevin WP at 5%), and an untreated control. The 2% powder was applied at 160 g/ha, the 4% unformulated powder was applied at 320 g/ha, and the 2% and 4% formulations were both applied at 8 kg/ha. Carbaryl was applied at a recommended rate of 7 kg/ha. All treatments were sprayed at a rate of 100 l/ha. Eggs, larvae, pupae and adults were counted on eight randomly selected plants from each plot once each weak after application.

RESULTS

A mixture of 100 g of CS with 20 ml of vegetable oil and 280 ml of warm water (60-70°C) was too viscous too measure. After adding 100 g of powdered sugar, the viscosity of the mixture decreased to 11.2 centipoises (cps). The same mixture with NCF substituted for CS had a viscosity of 1,434.3 cps. A third mixture, with equal parts of each flour (ratio 1:1), gave a viscosity value of 49.2 cps. Only the mixture with NCF produced a non-Newtonian (pseudo-plastic) fluid type because measurements were 976.4 and 1,434.3 after 30 min and 2 hrs, respectively. Different amounts of vegetable oil, added for feeding stimulation, were also tested in the mixture for changes in viscosity. By increasing the amount of oil, increases in the viscosity values of the mixture were observed. The viscosity values with equal amounts of CS and NCF

matrix ingredients and variable quantities of vegetable oil were 4.2 cps with 5 ml, 5.5 cps with 10 ml, 12.0 cps with 40 ml, and 14.2 cps with 50 ml.

Low quantities (5-10 ml) were not sufficient to coat the granules and it was observed that the *B. thuringiensis* spore-crystal complex dispersed quickly in water. Large amounts (40-50 ml) caused clumping of the microcapsules. Therefore, the optimum quantity of vegetable oil was 20.0 ml.

The moisture content of all microgranulated *B. thuringiensis* formulations immediately after spray drying averaged 2.3% (Table 1). Formulations containing the same or higher amounts of CS than NCF had lower moisture levels. After six months, the microgranulated formulations had an average of 5.0% moisture. After two years, formulations averaged 5.4% moisture.

TABLE 1. Moisture Content (%) of *Bacillus thuringiensis* Microgranulated Formulations.

Ratio		Time after production (years)		
CS	NCF	0.0	0.5	2.0
1	0	2.0	5.1	5.2
0	1	2.4	5.0	5.4
1	3	3.0	5.2	5.5
3	1	2.1	4.8	5.4
1	1	2.0	4.9	5.6
	Average [a]	2.3	5.0	5.4

[a]Average of all formulations, three samples per batch. Means in the same column are not significantly different from each other (LSD, 0.05).

Microgranules containing more CS than NCF had smaller particles (Table 2). For the *B. thuringiensis* formulation mixture containing a 1:1 ratio of CS:NCF, the particle size of the mixture before spray drying was comparable with the particle size of the spray dried formulation. The particle size of the spray dried formulation with a 4:0 ratio of CS:NCF as the matrix was smaller than the formulation with a 0:4 ratio. Microgranules containing a 3:1 ratios of CS:NCF were smaller than the 1:3 ratio of CS:NCF formulation (Table 2).

The scanning electron microscope micrographs of *B. thuringiensis* spray dried formulations, showed mostly smooth surface spherical particles, especially in microgranules containing more CS than NCF. The sizes of the microgranules were similar to those determined by the Coulter Multisizer. Also, no free *B. thuringiensis* spores or crystals in the spray-dried formulations were observed in micrographs, indicating entrapment of the active ingredient (Fig. 1).

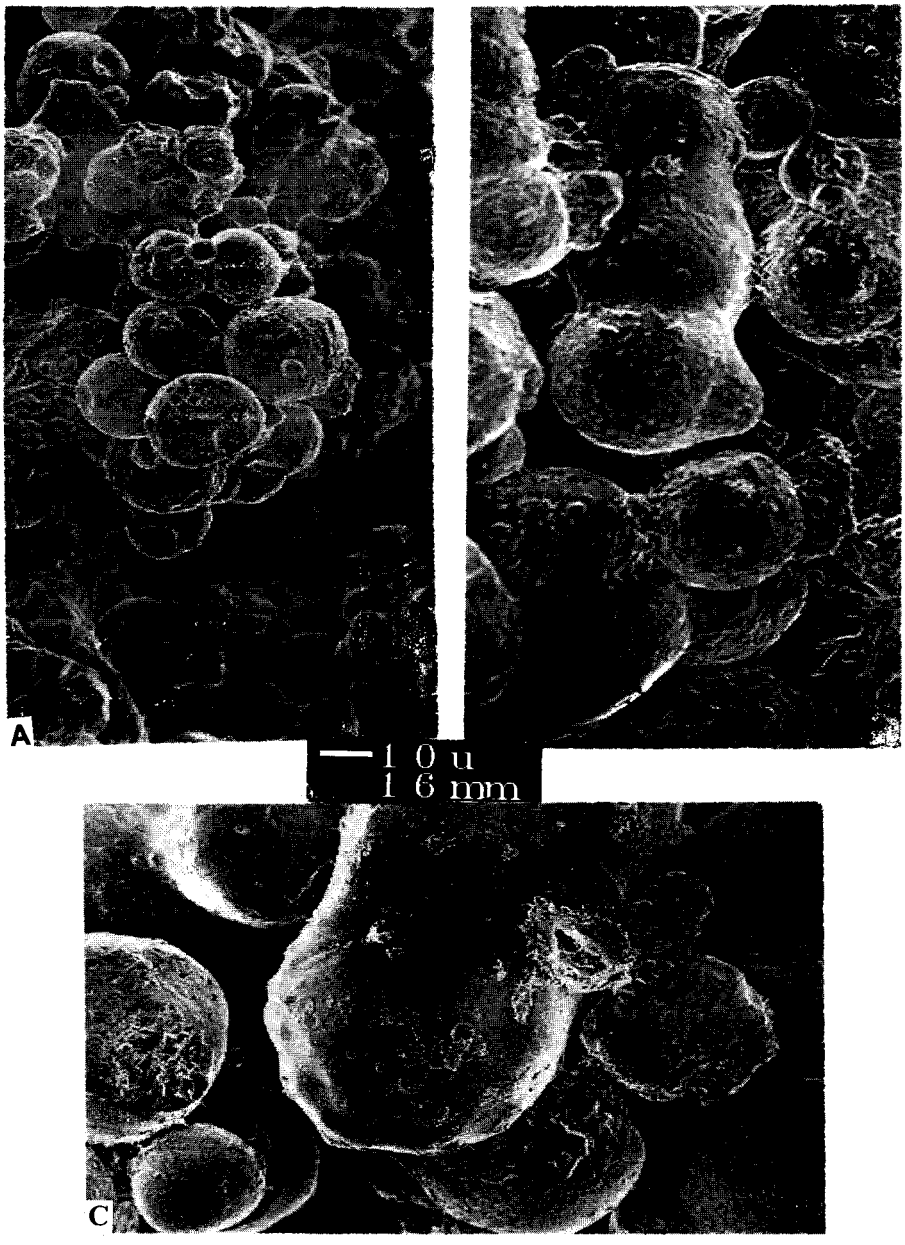

FIG. 1. Scanning electron microscope micrographs of spray-dried formulations of 3% *B. thuringiensis* spores-crystals. A) microgranules containing CS only; B) microgranules containing NCF only; C) microgranules containing a 1:1 ratio of CS:NCF.

TABLE 2. Particle Size of Ingredients and *Bacillus thuringiensis* Spray Dried Formulations Measured with a Coulter Multisizer.

Microgranules matrix ingredients[a]	Ratio of CS:NCF	Mean (μm)	95% C. L. (μm)[c]	S. D. (μm)[d]
Cornstarch (CS)	4:0	12.0	11.9-12.1	5.2
Nixtamalized corn flour (NCF)	0:4	9.9	9.7-10.1	7.9
CS:NCF[b]	1:1	25.8	25.5-26.1	13.3
CS formulation	4:0	11.9	11.8-11.9	5.3
NCF formulation	0:4	25.1	24.9-25.2	14.2
CS:NCF formulation	1:1	27.0	26.7-27.2	14.8
CS:NCF formulation	1:3	40.1	40.0-40.2	8.9
CS:NCF formulation	3:1	31.4	31.2-31.5	14.8

[a]One hundred grams of matrix ingredients total amount in formulations containing: 100.0 g powder sugar, 20 ml vegetable oil, 120 ml isopropyl alcohol, 280 ml distilled water, 0.5 ml formaldehyde, and 0.1 g malachite green oxalate salts.
[b]Particle size of the formulated mixture, before spray dried.
[c]95% C. L. = 95% confidence limit.
[d]S. D. = standard deviation.

TABLE 3. Percentage Mortality of *E. varivestis* and *T. ni* Larvae Exposed to *B. thuringiensis* Strain C-9, Unformulated and Formulated at 3% (1:1 CS:NCF Formulation) after Storage at Room Temperature[a].

Storage time (years)	*Epilachna varivestis*[b]			*Trichoplusia ni*[c]		
	Dose (mg/33 mm² leaf)	Unform	Form 3%	Dose (μg/ml)	Unform	Form 3%
0.0	1.0	66.6 a	66.6 a	500	69.3 ab	77.3 a
	0.5	23.3 bcd	26.6 bc	50	23.4 d	24.0 d
	0.0	6.6 cd	6.6 cd	0.0	1.3 e	1.3 e
0.5	1.0	53.3 a	46.6 ab	500	62.6 bc	72.0 ab
	0.5	19.9 cd	23.3 bcd	50	25.3 d	22.7 d
	0.0	0.0 d	0.0 d	0.0	6.6 e	6.6 e
2.0	1.0	53.3 a	53.3 a	500	57.3 c	66.6 abc
	0.5	16.7 cd	26.6 bc	50	18.7 d	19.5 d
	0.0	6.6 cd	6.6 cd	0.0	2.6 e	2.6 e

[a]Mean in the same column with same letter are not significantly different from each other (LSD, 0.05).
[b]Bioassays with treatments applied to bean leaf disks (100-ml/disk leaf dose). Means are the average of five replicates, three larvae/replication. $F = 53.21$; df = 17, 53; S. E. = 5.4; critical value for comparition (CVC) = 11.0.
[c]Diet incorporation bioassays on artificial diet. Means are the average of three replicates, 25 larvae/replication. $F = 6.37$; df = 14,74; S. E. = 12.4; CVC = 24.8.

The LC_{50} of our formulation product for C-9 strain versus *E. varivestis* using the bean leaf bioassay was 642 µg/ml ($X^2 = 2.48$, Slope = 2.77 ± 0.19, 95% C.I. from 396 to 728 µg/ml). LC_{50} versus *T. ni* using a diet incorporation bioassay was 229 µg/ml ($X^2 = 4.46$, Slope = 2.58 ± 0.22, and 95% C.I. from 198 to 264 µg/ml.

Formulations of *B. thuringiensis* strain C-9 gave similar insecticidal activity value when compared to the unformulated bioinsecticide (Table 3). Based on highest dose-response bioassay, neither formulated or unformulated samples lost insecticidal activity against *E. varivestis* or *T. ni* after two years storage at room temperature (Table 3).

Carbaryl and formulated and unformulated *B. thuringiensis* treatments were compared for control of *E. varivestis* in naturally infested bean fields. All life stages were present in the field at the time of the application. Only two treatments, with formulated *B. thuringiensis* at 4% and carbaryl, significantly reduced the insect population compared with the untreated control ($F = 0.05$) (Table 4).

TABLE 4. Number of *Epilachna varivestis*/Plant in Bean Plots Treated with Carbaryl and Biological (Formulated and Unformulated *B. thuringiensis*) Insecticides.[a]

Treatments	Egg mass	Larval Instar				Pupae	Adult
		1st	2nd	3rd	4th		
Powder 2%	3.28 bc	19.14 d	4.42 cd	3.37 b	1.87 b	2.25 bc	2.57 a
Powder 4%	2.62 b	4.00 b	5.25 d	2.11 a	1.77 b	0.88 a	5.62 b
Form 2%	3.62 c	9.87 c	3.87 c	3.33 b	1.88 b	2.88 c	10.12 d
Form 4%	1.37 a	2.12 b	2.12 b	1.25 a	1.11 ab	0.77 a	7.25 c
Sevin	0.75 a	0.62 a	0.37 a	1.46 a	0.87 a	1.65 b	4.34 b
Control	2.87 b	9.50 c	3.90 c	3.34 b	1.90 b	3.90 d	10.15 d
F	18.76	36.00	19.08	9.25	2.40	27.19	30.31
Df	5, 186	5, 186	5, 186	5, 186	5, 186	5, 186	5, 186
LSD (0.05)	0.7215	3.1724	1.1303	0.9153	0.8152	0.6495	1.5675
SE Comp	0.3657	1.6081	0.5730	0.4640	0.4132	0.3292	0.7946

Average of four weekly counts of eight randomized plants per treatment after treated; values in a column with the same letter are not significantly different. LSD (0.05).

Counts after 8 days application allowed observations of the effect of the treatments on the pest. Although there were no differences among some treatments and the untreated control, numbers of larvae and pupae were higher 8 days after the application in the untreated control compared to the treated plots (Figs. 2 and 3).

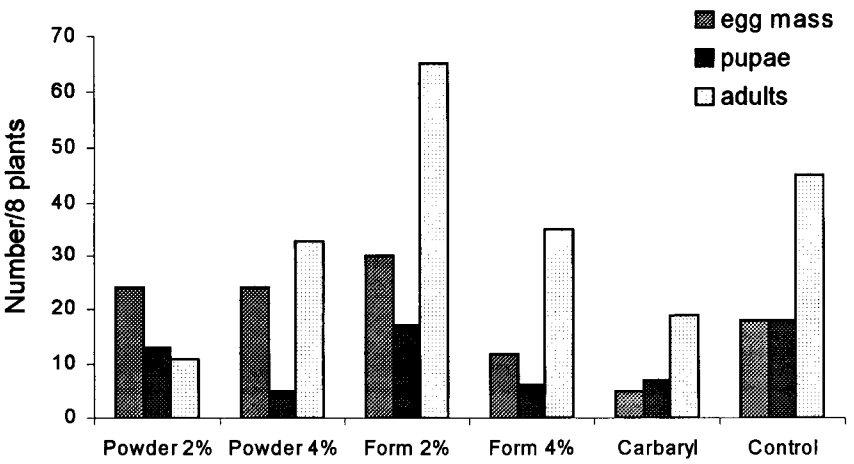

FIG. 2. Number of different stages of *E. varivestis* on bean crop 8 days after treatment with carbaryl and biological (formulated and unformulated *B. thuringiensis*) insecticides. Average of four replicates; total number per eight random plants.

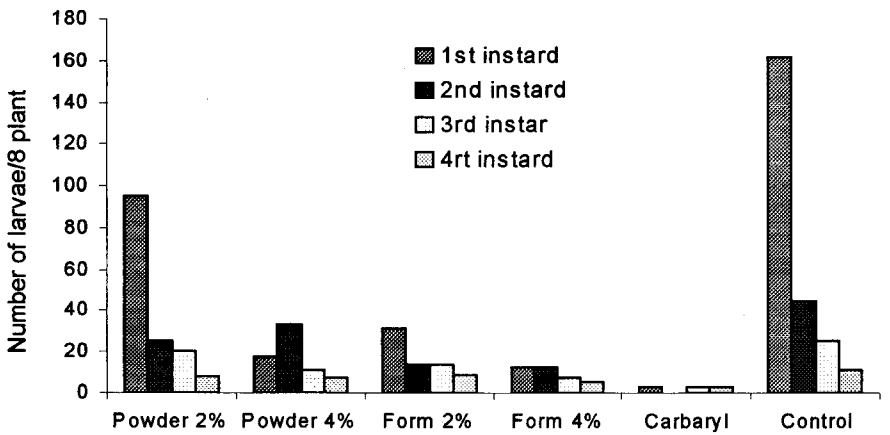

FIG. 3. Number of different instar larvae of *E. varivestis* on bean crop 8 days after treatment with carbaryl and biological (formulated and unformulated *B. thuringiensis*) insecticides. Average of four replicates; total number per eight random plants.

DISCUSSION

Since the 1960's, encapsulation of *B. thuringiensis* has been studied as a protection system to suppress the loss of activity by UV irradiation or rain wash-off and to improve feeding stimulation. Zehnder et al (1992) observed that encapsulated *B. thuringiensis* formulations had higher resistance to inactivation by rainfall and successful control of coleopteran pests when compared with unformulated *B. thuringiensis*.

Spray drying is a well-recognized process for encapsulation of food or chemicals (Young 1986). Identifying rheological properties such as viscosity of mixtures can minimize the energy costs of drying (Rao 1992). We found a decrease in viscosity of the mixture containing 100% of CS after adding sugar. We observed that the viscosity of the CS mixture began low (11.2 cps) while the viscosity of NCF was high (1,434.3 cps). The mixture with NCF only had a non-Newtonian behavior. High viscosity or non-Newtonian properties in a mixture are undesirable for spray drying. A suitable water solubility and viscosity (Newtonian behavior) was found by using equal parts of CS and NCF as matrix ingredients, with a viscosity of 49.2 cps.

It was also observed that the moisture content in five spray dried formulations increased over time during the first six months after preparation, but not after that (Table 1), which is associated with improved shelf life. The particle size values of various spray-dried formulations showed that all formulations had a particle size between 9.9 and 40.1 μm, which facilitated spraying of microgranules using conventional field spray equipment. CS-NCF based microgranulated formulations with higher CS amounts had smaller particle sizes compared with NCF-based formulations (Table 2). Microgranules containing CS will disperse in water better than microgranules with NCF, resulting in smaller particle size. This could provide better plant coverage when sprayed and improve the biopesticide efficacy (Smith et al 1978). For formulations with only NCF as an encapsulating matrix, the microgranules clumped together in the spray tank.

The formulation based on CS and NCF at a 1:1 ratio was a suitable material for spraying, and cheaper than the formulation with only CS. The CS cost is approximately $0.50/lb ($1.00/kg) whereas the NCF cost is $0.12/lb ($0.20/kg).

Micrographs revealed no free *B. thuringiensis* spores or crystals in the spray-dried formulations. This is consistent with previous results and similar spray-dried microgranulated formulations where *B. thuringiensis* activity was tested against *Ostrinia nubilalis* (Tamez-Guerra et al 1996). Cotton plants bioassays under simulated solar conditions demonstrated that spray dry formulations gave better solar protection to *B. thuringiensis* compared to mixtures of the same ingredients that were not spray dried (Tamez-Guerra et al 1996).

As for shelf life, *B. thuringiensis* activity in this formulation showed no loss of insecticidal activity when stored at room temperature (28°C average) for 2 years.

The field experiment was performed in early July, in a furrow-irrigated field, which facilitated the development of the *E. varivestis* population (Kitayama et al 1979). We then determined the effects of treatment on all the pest's life stages. Formulated treatments with 4% spore-crystals provided better control of *E. varivestis* when compared with treatments of 2% formulated, and 2% and 4% unformulated *B. thuringiensis* (Table 4). The numbers of pupae of *E. varivestis* were lower in all the *B. thuringiensis* treated plots versus the untreated control. Only the number of adults showed a different pattern compared with the other stages, where the lowest number of adults was observed in the 2% powder treated plots. This was probably due to the mobility of adults among plots.

As a product, the microgranulated formulation obtained by this spray-dry process demonstrated that the insecticidal activity was conserved for 2 years under room storage conditions, which together with the moisture content are good indications of the shelf life of the

product. It may then be possible to use this methodology for bioinsecticide production. However, to use this process successfully, more research focused in adherent material suitable for using in the spray dry technique is required.

ACKNOWLEDGMENT

Sincere thanks and appreciation are given to Robert W. Behle (USDA-ARS Peoria IL.) and Ricardo Gomez (FCB-UANL, Monterrey MX) for their excellent critical reviews of the manuscript. We also thank Michael R. McGuire (USDA-ARS Peoria IL) for support of a portion of this research. This research was supported in part by CONACYT No. 3559-N9311.

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EFFECT OF *EUPHORBIA HIRTA*[1] NECTAR AND ITS COMPONENT SUGARS, UPON THE SURVIVAL OF BETHYLID PARASITOIDS OF THE COFFEE BERRY BORER[2]

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ABSTRACT

In the south of Mexico, attempts to control severe infestations of the coffee berry borer *Hypothenemus hampei* Ferrari (Coleoptera: Scolytidae), by means of two imported African parasitoids, *Cephalonomia stephanoderis* Betrem and *Prorops nasuta* Waterston (Hymenoptera: Bethylidae), have been less than satisfactory. The lack of a suitable alternative food source in the field, such as floral nectar, could be one reason for this result. Both parasitoids have been shown to feed upon the nectar of *Euphorbia hirta* L. (Euphorbiaceae) under laboratory conditions. In these experiments, females of *C. stephanoderis* and *P. nasuta* survived up to 55 and 17 days, respectively, when provided daily with a fresh *E. hirta* flower. Survival time with diluted honey was 41 and 8 days maximum and in the case of the control (no food or water), 7 and 4 days, respectively. Qualitative analysis of the nectar showed the presence of saccharose, fructose and glucose. Parasitoids, isolated in test tubes, were daily given drops of 1M, 2.5M and 5M solutions of the three sugars (5M, saccharose and glucose only) until death. Saccharose, at a concentration of 2.5M, gave the best survival rates for both parasitoids, up to 59 and 28 days for *C. stephanoderis* and *P. nasuta*, respectively.

INTRODUCTION

The struggle to control the coffee berry borer (CBB), *Hypothenemus hampei* Ferrari, continues in most coffee growing areas of the world. In the case of Mexico, the pest was found for the first time in Soconusco in 1978 (Baker et al. 1989), and attempts are being made to develop integrated and biological control techniques as cost effective and environmentally friendly alternatives to the extensive use of the insecticide endosulphan.

In 1985, the first consignments of the parasitoids *Cephalonomia stephanoderis* Betrem and *Prorops nasuta* Waterston (Hymenoptera: Bethylidae) were imported from Africa and held in quarantine in England. The importation was carried out without a detailed evaluation of the bethylids in their natural habitat. The first shipment arrived in Soconusco in 1988 (Barrera et al. 1990a), and further importations of both parasitoids have followed. The bethylids have been successfully reared in the laboratory and several thousand have been released into coffee plantations of the area.

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[1]Euphorbiaceae

[2]*Hypothenemus hampei* (Coleoptera: Scolytidae)

These parasitoids have been employed as biological control agents of the CBB in several countries and, in some cases, follow-up studies on the level of establishment and degree of CBB control achieved have been carried out. There has been more work done on *C. stephanoderis* and the results of various studies, although in some instances promising, show that survival of the parasitoids and the degree of control of the CBB is quite variable and often negligible (Barrera 1994, Benavides et al. 1994, Delgado et al. 1990, Dufour et al. 1995, Portillo and Bustillo 1995, Sponagel 1994). One of various factors that could be responsible for this poor performance is the lack of a suitable non-host food source.

Wolcott (1942) first suggested that the introduction of beneficial insects might be more successful if all factors essential to the optimum development of the insects were considered. He proposed that for too long entomologists had tended to focus only on the parasitic stage and neglected other aspects of the life history of the insect. Increasing interest is now being shown in the relationship between insect natural enemies and non-host foods, such as floral and extrafloral nectar, honeydew and pollen, with respect to natural enemy performance. The subject has been amply reviewed by Jervis and Kidd (1996) who discuss the general importance of wild flowers as sugar sources for adult parasitoids to fuel activities such as flight, mating, egg production and host finding. They (and more recently Dyer and Landis 1996) cite several studies in which survival, percentage parasitism and number of offspring produced are higher with a greater availability of non-host foods (also referred to as alternative foods). The importance of non-host foods is not restricted to those parasitoids that as adults do not feed on their host. Leius (1960), working with three hymenopterous parasitoids of Lepidoptera, found that even when the adults fed on host body fluids, both sexes also fed upon nectar.

Umbelliferous flowers are frequently cited as being particularly attractive and beneficial to hymenopterous parasitoids (e.g., Leius 1960). Alvarez and Reyes (1987), working in Nuevo Leon, found a total of 28 species of hymenopterous parasitoids feeding upon the nectar produced by six of the commonest weeds in the area. In that study, the most frequently visited weeds were *Ricinis communis* L. (Euphorbiaceae) and *Helianthus annus* L. (Compositae). Wolcott (1942) described how the parasite *Larra americans* Saussure, introduced to Puerto Rico from Brazil to control the mole cricket *Scapteriscus vicinus* Scudder, became established only in the presence of two weed species, *Borreria verticillata* (Rubiaceae) and *Hyptis atrorubens* (Labiatae). The bethylid *Goniozus nephantidis* (referred to as *Parasierola nephantidis* in the article) was found feeding on the nectar of *Justicia jendurosa* (Acanthaceae) in Gujerat in India (Yadav 1985). Syme (1975) cites Gulii (1963) who suggested the planting of nectiferous plants, including *Euphorbia villosa* Waldst and Kit and *E. macrocerus* F. (Euphorbiaceae), in order to increase the efficiency of the European pine shoot moth *Rhyacionia buoliana* (Schiffermueller).

To be truly beneficial to these insects, nectiferous flowers must be detectable at some distance by the parasitoids. Wackers and Swaans (1993) showed that *Cotesia rubecula,* a parasite of *Pieris* sp., responded positively to various flower odors in a Y- tube olfactometer. The flower odors were highly attractive both to starved insects and to those satiated on sugar water. This attraction was deemed to be an innate response which would enable inexperienced parasitoids to locate floral nectar. Olfactory responses of female parasitoids to the odors emanating from flowers may be determined by the number of eggs in the ovaries and thus indirectly by the stage in the reproductive cycle and age of the female (Shahjahan,1974).

Various complex relationships exist in which carbohydrates, as a component of the insect diet, affect the utilization of dietary proteins and the overall balance of the diet. The effects of dietary omissions may not show themselves in the test generation and may affect the different life stages of the insect in different ways (Johansson 1964).

Extensive work has been carried out on the sugar content of nectars; Baker and Baker (1979), for example, analysed the floral nectar of 387 species of plants and found that in virtually all cases, the nectar contained sucrose, glucose and fructose. The nectar of some species also contained dilute solutions of other monosaccharides and oligosaccharides. For some plant families a common trend, in terms of sugar content, can be observed, but in many cases no such trend is distinguishable (Rowley 1976).

Both floral and extrafloral nectar contain amino acids, which could play an important role in the nutrition and development of adult parasitoids. Extrafloral nectar is often distinct in composition from that of its floral nectar; most extrafloral nectars are reported as having a full complement of protein-building amino acids as well as other, non-protein amino acids (Rogers 1985).

Barrera et al. (1993) observed maximum survival of females of *C. stephanoderis* of 76 days, with an average of 38.23 days, when given a diet of pure honey and maintained at a temperature of 26 ± 1°C. With honey diluted with distilled water (1:1), the maximum survival was 70 days, with an average of 33.87 days. More recent tests carried out on females of *C.. stephanoderis* (derived from the same original population) with a diet of 1:1 diluted honey, showed a maximum survival considerably less than this (Barrera 1989).

Diluted honey, which contains mainly glucose and fructose (Santiesteban 1994), is the conventional diet for the emerging parasitoids in the laboratory, both before release in the field or being used for further rearing, but preliminary tests for a separate series of experiments with *P. nasuta* showed that this parasitoid survives better on a diet of sucrose or saccharose.

Euphorbia hirta L. (Euphorbiaceae), is one of several weeds mentioned by Cenicafé (1990) as suitable for selection in coffee plantations in Colombia as a non-invasive ground cover. The presence of a selected herbaceous layer with shallow roots and a creeping habit contributes to the prevention of erosion and weed suppression during the establishment and life of a plantation, without competing with the coffee crop. The abundance and species composition of beneficial insects, be they parasites, parasitoids or predators is affected by the nature of the herbaceous layer as a provider of nectar, alternative hosts and refuge sites.

From preliminary experiments, in which individual *C. stephanoderis* in test tubes were presented with flowers of various herbaceous plants commonly found in the coffee plantations of the region of Soconusco, Chiapas, Mexico, definite nectar feeding was observed only in the case of *E. hirta* (A. Damon, unpublished). *P. nasuta* was later also found to feed on the nectar of *E. hirta.* This paper presents the initial stage of an investigation into the relationship between *C. stephanoderis* and *P. nasuta* and nectar, in the hope of finding a suitable nectar source that can be provided in the coffee plantations in Soconusco to improve the level of control of the CBB achieved by these parasitoids.

MATERIALS AND METHODS

All experiments were carried out in a small room without climate control at ECOSUR (El Colegio de la Frontera Sur, before, CIES). This brought the experiment closer to natural conditions and those under which the coffee producers of the region rear their own parasitoids on site. Maximum and minimum temperatures were measured daily throughout the experiment. Humidity was measured constantly during the first 7 weeks of experimentation.

Qualitative analysis of the nectar of Euphorbia hirta. Gas chromatography was used to qualitatively analyze the sugar content of *E. hirta* nectar. Fifty flower heads were washed with methanol; the resultant solution was placed in a vial and then dried under a flow of

nitrogen at 75°C. Trimethylsilyl derivatives of sugars were formed by addition of 100µl of TRI-SIL ® Z (trimethylsilylimidazol [TMSI] in pyridine, Pierce Chemical Company, Rockford, IL). The vial was capped, shaken and heated at 75°C for at least 15 minutes. Samples (0.5µl) were injected into a gas chromatograph (Varian Model 3600) equiped with a flame ionization detector and a 30m x 0.250mm capillary column DB-17. The carrier gas was helium at 20ml/min. The temperature of the column was programmed from 125°C (1-min hold) to 250°C (1-min hold) at 5°C/min. The peaks were identified with previously established standards. (Sweeley et al. 1963).

Survival of C. stephanoderis and P. nasuta with the nectar of Euphorbia hirta and comparison with diluted honey. In the rearing unit at ECOSUR, approximately 300 parasitoides were placed with coffee berries infested with CBB at a rate of 1:1 or 1:2 into plastic containers. For the experiment, parasitoids were collected as they emerged; numbers of males and females were not determined at this stage. Bethylid parasitoids undergo sibling mating and most of the female parasitoids collected in this way would have mated, either within the coffee berry, before emergence, or in the containers. One hundred and fifty individuals of each parasitoid, *C. stephanoderis* and *P. nasuta,* were placed individually in test tubes. The tubes were sealed with fine gauze held in position with a section of plastic tubing. The tubes were divided into three groups of fifty for each parasitoid, each group being maintained separately on plastic trays, set above soapy water to prevent access by foraging ants. The first group served as the control; no food was given. The parasitoids in the second group were given a small streak of honey, applied to the gauze seal of the tube every day. The honey was diluted 1:1 with water and was changed regularly (diluted honey ferments rapidly at ambient temperatures). The fifty parasitoids in the third group were each given a fresh flower of *E. hirta* daily.

Every day, between 1300 and 1600 hours, flowers were collected from surrounding wasteland. Individual flower heads were excised and placed with the cut stem on clean filter paper to absorb the flow of latex. The cut stems dried within five minutes and each flower head was then checked under a stereomicroscope for the presence of nectar. After removing the previous flower head, one flower with nectar was placed in each of the tubes containing parasitoids in the third group. At the same time, the parasitoids in the second group were given a streak of diluted honey.

A daily record of mortality was kept. All tubes were revised before the parasitoids were fed. Dead parasitoids were dissected under a stereomicroscope to determine sex.

Statistical analysis was carried out pooling female and male parasitoids together. The data were analyzed using the Median Test and the Kruskal-Wallis Test (Lee 1980) to make a comparison between the survival curves of the three treatments. The Median Test was used where the results showed a substantial difference between the means and medians.

Survival of C. stephanoderis and P. nasuta with the component sugars of the nectar of Euphorbia hirta (glucose [administered as dextrose], saccharose and fructose). Three series of experiments were carried out, each using a different molarity (1M, 2.5M and 5M) of the three sugars. In the final series, with 5M solutions, only two sugars, dextrose and saccharose, were tested because of a lack of supply of fructose.

The numbers of parasitoids used in each series depended upon availability (Table 1). The experiment was set up as previously described with the bethylids maintained in test tubes. Sugar solution was given every afternoon as a streak applied to the gauze seal. The sugar solutions were stored in a refrigerator at 4°C to prevent fermentation.

As before, the tubes were revised every day before feeding, a daily record of mortality was kept, and dead parasitoids were dissected under a stereomicroscope to determine sex.

TABLE 1. Numbers of Parasitoids Used in the Experiments.

Series	Molarity	C. stephanoderis No. parasitoids per treatment	C. stephanoderis Total	P. nasuta No. parasitoids per treatment	P. nasuta Total
1	1	30	90	50	150
2	2.5	100	300	100	300
3	5	58	116	100	200

In this experiment, there proved to be a greater and highly variable number of males in the population so the statistical analysis relates only to the females parasitoids. The few parasitoids that escaped were excluded from the experiment. The data from series 1 and 2 were analyzed using the Median Test and the Kruskal-Wallis Test (Lee 1980) to make a comparison between the survival curves of the three treatments. The Median Test was used where the results showed a substantial difference between the means and medians. However, in the case of series 3 (5M solutions of dextrose and saccharose), the logrank test for the difference between two treatments was used.

RESULTS AND DISCUSSION

During the period of experimentation, daily temperatures ranged from 25-30°C. The humidity, measured constantly during the first 7 weeks, ranged from 45-85%.

Qualitative analysis of the nectar of Euphorbia hirta. The nectar of *E. hirta* contained saccharose, glucose and dextrose. Quantitative analysis was not possible due to the very small quantities of nectar produced which could not be suctioned up even by the smallest micropipette.

TABLE 2. Survival, in Days, of *C. stephanoderis* and *P. nasuta* on Diets of *E. hirta* Nectar, Diluted Honey and Without Food (Control).

Treatments	Mean[a]	Median	SD
	C. stephanoderis		
Nectar	24.92 a	26.00	15.47
Honey	11.44 b	11.00	5.20
Control	4.75 c	4.76	1.45
	P. nasuta		
Nectar	10.02 a	10.00	4.51
Honey	3.92 b	4.00	1.63
Control	3.76 b	4.00	1.17

[a] Different letters signify that treatments were significantly different at confidence level 0.05.

Survival of C. stephanoderis and P. nasuta with the nectar of Euphorbia hirta and comparison with diluted honey. The results are presented in Table 2 and Figs. 1 and 2. Both *C. stephanoderis* and *P. nasuta* survived significantly longer when fed nectar from a fresh *E. hirta* flower daily than diluted honey and the control (no food or water). Maximum survival observed was 55 and 17 days, respectively. The diluted honey diet and control gave

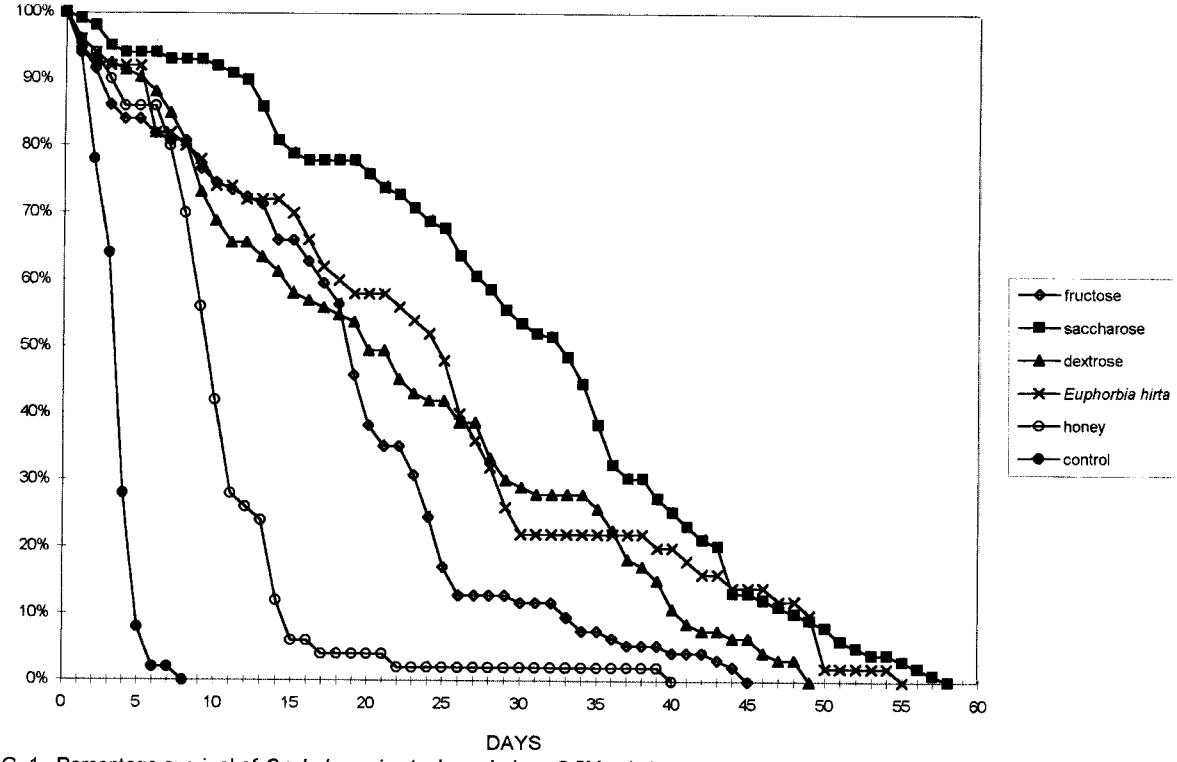

FIG. 1. Percentage survival of *Cephalonomia stephanoderis* on 2.5M solutions of various sugar diets.

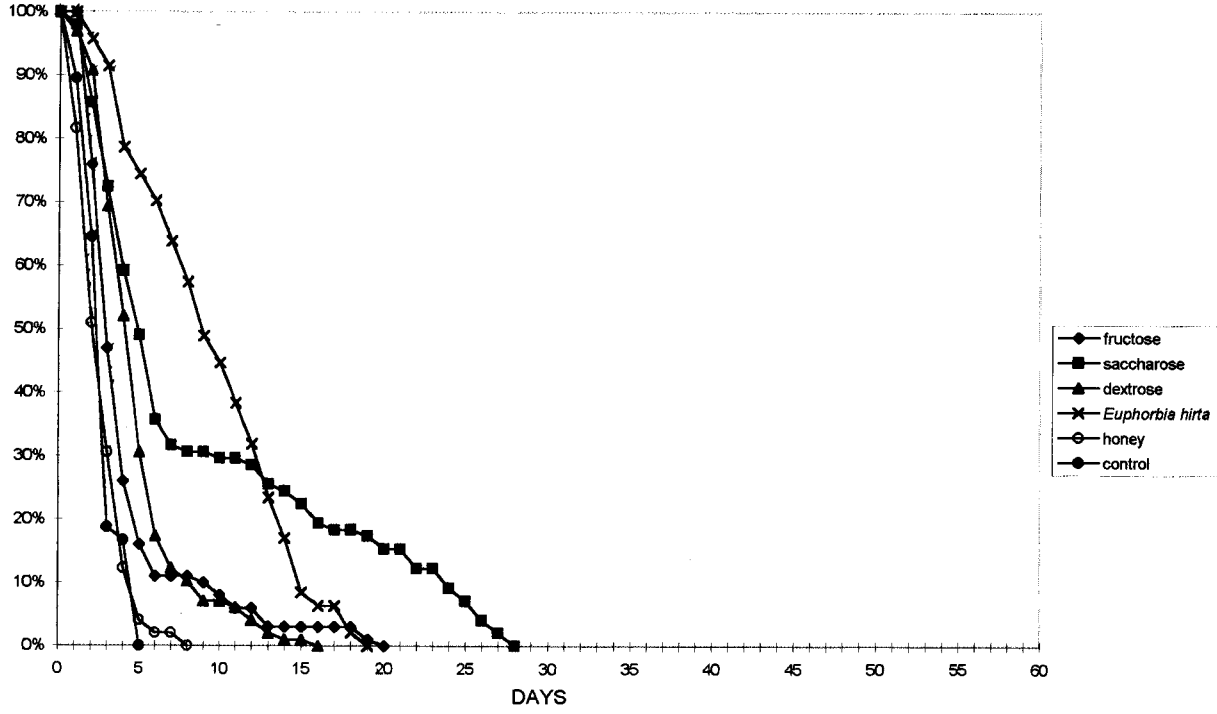

FIG. 2. Percentage survival of *Prorops nasuta* on 2.5M solutions

maximum survival times of 41 and 8 days and 7 and 4 days, respectively. The diluted honey diet gave significantly longer survival times than the control for *C. stephanoderis,* but survival of *P. nasuta* was poor on the honey diet and not significantly different from the control.

Survival of C. stephanoderis and P. nasuta with the component sugars of the nectar of Euphorbia hirta; (glucose [administered as dextrose], saccharose and fructose). The response of *C. stephanoderis* to the three sugars at the lower concentration of 1.0M was not significantly different. *P. nasuta* survived significantly longer on the diet of 1.0M saccharose (Table 3).

TABLE 3. Survival, in Days, of *C. stephanoderis* and *P. nasuta* on 1.0M Solutions of Fructose, Saccharose and Glucose.

Treatments	Mean[a]	Median	SD
	C. stephanoderis		
Fructose	3.59 a	3.00	2.38
Saccharose	4.12 a	3.00	8.01
Glucose	3.29 a	2.50	2.43
	P. nasuta		
Fructose	2.65 a	1.00	3.77
Saccharose	4.69 b	2.00	6.04
Glucose	1.50 b	1.00	0.56

[a] Different letters signify that treatments were significantly different at confidence level 0.05.

All treatments at concentrations of 2.5M, for both parasitoids, were shown to be significantly different to one another (Table 4). At this concentration, saccharose gave a much greater survival rate than the two monosaccharides. Maximum survival time was 59 and 28 days for *C. stephanoderis* and *P. nasuta*, respectively. Maximum survival times for fructose and dextrose were 45 and 20 days and 49 and 16 days, respectively. The results of the tests with 2.5M solutions are included in Figs. 1 and 2.

Only two sugars were tested at the concentration of 5M (Table 5). A significant difference was observed in the case of *C. stephanoderis*; saccharose at 5M gave better survival than glucose 5M. No significant difference was observed in the case of *P. nasuta.*

For both parasitoids, relatively longer maximum survival times were achieved with 2.5M solutions of the disaccharide saccharose. According to the means, however, nectar was a better diet for *P. nasuta* than the 2.5M saccharose. For *C. stephanoderis*, survival times on the diet of nectar were very similar to those with 2.5M saccharose. *P. nasuta* did not survive as long as *C. stephanoderis* on the nectar diet, but mean survival on nectar was greater than all other treatments. Weaker solutions (1.0M) were not effective; survival was uniformly poor and lower than the control. Stronger, 5M solutions were observed to be generally less favorable than 2.5M solutions, particularly for *C.stephanoderis*. Male parasitoids have been observed feeding on the nectar of *E. hirta* and results from the few males in these experiments suggest that their survival too may be extended when non-host foods are available.

TABLE 4. Survival, in Days, of *C. stephanoderis* and *P. nasuta* on 2.5M Solutions of Fructose, Saccharose and Glucose.

Treatments	Mean[a]	Median	SD
	C. stephanoderis		
Fructose	18.76 a	19.00	11.14
Saccharose	30.62 b	33.00	14.10
Glucose	22.02 c	20.00	14.10
	P. nasuta		
Fructose	4.47 a	3.00	3.59
Saccharose	8.91 b	5.00	8.14
Glucose	5.08 c	5.00	2.81

[a] Different letters signify that treatments were significantly different at confidence level 0.05.

TABLE 5. Survival, in Days, of *C. stephanoderis* and *P. nasuta* on 5M Solutions of Fructose, Saccharose and Glucose.

Treatments	Mean[a]	Median	SD
	C. stephanoderis		
Saccharose	17.89 a	19.00	12.58
Glucose	12.76 b	12.00	11.22
	P. nasuta		
Saccharose	6.67 a	5.00	4.80
Glucose	5.96 a	5.00	5.42

[a]Different letters signify that treatments were significantly different at confidence level 0.05.

Survival of *P. nasuta* on the honey diet was particularly poor, barely exceeding that of the control. However, neither parasitoid showed a favorable reaction to the honey, which contains mainly the monosaccharides fructose and glucose; whereas, significantly longer survival times were achieved with nectar and the disaccharide saccharose. It is therefore recommended that the substitution of saccharose solution (2.5M) for the conventional diet of diluted honey could be beneficial to the laboratory management of both parasitoids but particularly for *P. nasuta*.

P. nasuta did not survive particularly well on any of the diets. As discussed in previously reported references, this parasitoid is more difficult to manage than *C. stephanoderis*. Obviously, there must be one or several reasons for this, which may include factors relating to non-host foods, such as a requirement for proteinaceous components.

The variability observed in survival rates for a diet of *E. hirta* nectar could have been due to differences in concentrations and total amounts of nectar between individual flowers. Plant age and state of health, time of day, weather conditions and foraging by other insects prior to collection could all significantly affect quantity and quality of nectar.

It is probable that the dietary requirements and ability to assimilate non-host foods of these bethylids varies according to age and reproductive state, as has been demonstrated for

many other parasitoids. In this experiment the parasitoids were collected as they would be in order to to be released in the field. Occasionally a parasitoid may remain in the container for a longer period and "emerge" later; this can be considered rare, however, as by the end of the one-month life cycle all CBB in the container are usually dead or dying and the new generation is compelled to "emerge" (i.e., leave the container to search for new hosts). The variation is more likely to relate to the quality of a population of laboratory insects, such as this, that has been inbred over several generations without renewal or selection. Further comparisons should be made to wild populations, preferably from their places of origin in Africa.

All ecological and physiological factors must be fully understood if maximum benefit is to be obtained from the use of parasitoid biological control agents that feed upon non-host foods.

ACKNOWLEDGMENT

We would like to thank Godofredo Flores who carried out the majority of the daily revisions necessary for these experiments. The research was carried out at the laboratories of ECOSUR, Tapachula, as part of the Coffee Berry Borer Project under the direction of Dr. Juan-Francisco Barrera.

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SOUTHWESTERN ENTOMOLOGICAL SOCIETY

PERSPECTIVE

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A REVIEW OF AREA-WIDE MANAGEMENT OF *HELICOVERPA* AND *HELIOTHIS* (LEPIDOPTERA: NOCTUIDAE) WITH PATHOGENS (1987-1997)

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ABSTRACT

Research to develop improved methods of managing serious insect pests of Mississippi Delta crops, specifically cotton, by use of natural insect pathogens was begun in 1987 at the USDA, ARS, Southern Insect Management Research Unit (SIMRU) in Stoneville, MS. Previous research had shown that non-crop plants, particularly early-season weeds, were hosts for the tobacco budworm, *Heliothis virescens* (F.), and cotton bollworm, *Helicoverpa zea* (Boddie), prior to the presence of crop hosts. It was theorized that tobacco budworm and cotton bollworm populations could be managed by either controlling the insects on the weeds using insecticides, or by controlling the early season hosts themselves via herbicides or mowing. Since entomopathogens (microbial insecticides) are considered to be among the safest methods of insect control, research was begun to investigate their use in a management scheme. Positive results of small field and cage tests (prior to 1990) led to large area studies, beginning with a 25,900-ha test in 1990, and culminating in 81,000-ha tests in 1994 and 1995. Tests over smaller areas in 1996 and 1997 were designed to (1) evaluate the effectiveness of a commercially-prepared formulation of the virus sprayed twice at weekly intervals (1996) and (2) determine efficacy of a half-rate of the new virus formulation in an attempt to reduce costs (1997). Collective results of this 11-yr study indicate that virus application can be accomplished at a reasonable cost, and that such treatment consistently reduced the number of moths emerging from weed hosts by >70%. Herein, we present brief results of the long-term study that led to the present question of what the future holds for this project.

INTRODUCTION

Stadelbacher (1979, 1981) discussed the importance of several early-season wild and domesticated host plants of the tobacco budworm, *Heliothis virescens* (F.), and cotton bollworm, *Helicoverpa zea* (Boddie), in the Delta of Mississippi and their importance in the buildup of the first generation which subsequently invades cotton. The major early-season hosts of these insects, wild geranium, *Geranium dissectum* L. and *G. carolinianum* L., grow primarily in disturbed areas such as ditch banks, roadsides, and tilled but unplanted fields, and are widely distributed in the Delta. Seed of both species germinates from mid-October through

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early spring, and flowers and sets fruit from mid-April to late-May. Larval feeding is restricted to the immature fruit. In a study of larval and adult populations, Stadelbacher (1979, 1981) calculated that an average of 450,000 *Heliothis* larvae and 17,000 adults were produced per hectare (2.47 acres) of wild geranium, and theorized that wide-area control of the first larval generation could thus have a positive impact on the management of bollworm/budworm in row crops such as cotton. Additional research showed that: 1) only 3.5% of the overwintering *Heliothis/Helicoverpa* population survived to emerge as adults; 2) spring emergence of the overwintered population occurred 6 weeks before cultivated host plants were available; 3) surviving populations were restricted to and concentrated in early-season alternate host plants which occupied <5% of the total rural area; and 4) moths emerging from these wild hosts in early to mid-June moved into cotton (Knipling and Stadelbacher 1983, Snodgrass et al. 1991). The rationale for attacking bollworm/budworm populations during the first seasonal generation, and possibly the second, was addressed by Knipling and Stadelbacher (1983). Several methods for control of this first larval generation were examined, but due to the large-area control needed, the use of safe, natural control methods such as microbial agents was deemed most appropriate for further study.

The most promising of the microbial agents available for use against first larval generation bollworm and tobacco budworm larvae on weeds was believed to be the baculoviruses [nuclear polyhedrosis viruses (NPV) and granulosis viruses], due to their safety, relative stability, and virulence. The baculovirus from the cotton bollworm (*H*NPV) was the first to be registered by the Environmental Protection Agency for use on row crops, and the safety studies conducted prior to registration were considered the most in-depth ever on any insect pathogen or insecticide. This virus occurs naturally in the proposed test area and is known to infect only insects in the *Heliothis/Helicoverpa* genera. Although this fact has relegated it to relatively few commercial markets, this virus has a potential infectivity such that the LD_{50} may be as low as a single polyhedron (one virus particle) per bollworm/budworm larva (Burges 1981). The negative aspects of baculoviruses include their slow activity and problems related to in-field persistence of the virus.

Several reviews are available on various phases of this study (Hardee and Bell 1996a, 1996b, 1996c, 1997) but the following is a summary of research techniques, methods and materials, and general results for the entire 11-year study.

METHODS, MATERIALS, AND RESULTS

1987. Research by Bell (1988a) showed that an adult moth developed on approximately every 1-3 m of early-season wild hosts. A dye-marking method was used to first examine the effect of NPV applications on the emergence of this generation of adults in 1987. In that study, one-half of field plots in an area were treated with a blue dye and the other half was treated with a red dye mixture. In another area, one-half of field plots were treated with blue dye and the other half with red dye plus *H*NPV at a rate of 6.0×10^{11} polyhedral inclusion bodies (PIB)/ha. Evaluation of the effects was determined using pheromone-baited cone traps (Hartstack et al. 1979). The first marked adult was captured on 18 May and the last on 21 June. Both red and blue marked tobacco budworm and bollworm adults were captured from areas around fields treated with either blue or red dye without *H*NPV. Only blue-dye marked moths were trapped near fields treated with either the blue dye or red dye + *H*NPV. These results indicated a significant reduction in the number of budworms emerging from the virus-treated areas (Bell 1988b).

1988. A cage study was conducted during April and June to more accurately measure (although under caged conditions) the effectiveness of microbials in early-season control of

bollworm/budworm (Bell 1991). This test consisted of three replicates of each of four microbial treatments and an untreated control. The four treatments examined were: NPV in water, NPV in an aqueous 10% crude cottonseed oil formulation, NPV in a COAX®-dust formulation, and an insect growth regulator (IGR) (SAN415WG354) + *Bacillus thuringiensis* (Bt) Berliner (SAN8101) formulation supplied by Sandoz Crop Protection, Inc. (Des Plaines, IL).

Fifteen screen cages (plastic insect screen, 4 m x 8 m x 3 m tall) were erected over the early-season weeds, the canopy of which was mostly wild geranium, primarily *G. dissectum*, but also containing *G. carolinianum*. On 26, 27, and 29 April, each cage was infested with approximately 1,000 bollworm and 1,000 budworm larvae from our lab culture. Microbial treatments were applied on 2 May, when larvae were from 3-7 d old. All virus treatments were applied at a rate of 6 x 10^{11} PIB/ha, and the IGR + *Bt* was applied at 112 gm (AI)/ha + 1.1 kg/ha. The NPV-oil, NPV-water, and IGR-*Bt* treatments were applied in a volume equivalent to 46.8 l/ha using an atomizing sprayer held approximately 0.5 m above the plant canopy. The dust formulation was applied at a rate of 3.4 kg/ha using a small garden duster. Cages remained undisturbed from 2 May until 29 May, when the first adult was observed. Cages were then searched daily and numbers, species, and sex of moths were recorded. The first adult moth was captured 1 June, and the last on 15 June.

Virus treatments resulted in overall reductions in emergence of 80.7 to 91.2% (Bell and Scott 1989), with tobacco budworm emergence reduced 78.5 to 89.2% and cotton bollworm emergence reduced 71.4 to 100%. Although the application of virus in water resulted in the greatest level of control of both budworms and bollworms (89.2 and 100%, respectively), there were no significant differences due to formulation used. The overall number of moths per cage in the IGR + *Bt* treatment was significantly less than in the check. However, the level of control was significantly less than that obtained with any of the NPV treatments. These tests indicated that *H*NPV was effective in reducing bollworm/budworm emergence, and would be a good candidate for use in management systems where pests develop on early-season wild hosts.

1989. The success of a large-area program utilizing early-season control methods mandated that the results obtained by hand application be reproduced using aerial application. Thus, a study was undertaken to examine the effectiveness of aerially-applied virus in controlling the emergence of bollworm/budworm adults from early-season host plants (Bell and Hardee 1991). The test was conducted in Washington County, Mississippi, with aerially-applied treatments consisting of three levels of NPV: 0, 50, and 100 larval equivalents (LE)/ha [one LE equals 6 billion polyhedral inclusion bodies (PIB)]. Treatments were applied in water on 27 April; untreated control plots were covered with plastic sheeting during application. Following application, screen cages (4 x 8 x 4 m tall) were placed over each plot and secured. Cages searches were conducted daily after the first moth was observed, and moths were captured and removed. Numbers, species, and sex of moths were recorded.

The first adults were captured on 30 May and the last on 12 June. Budworm emergence was reduced 88% and bollworm emergence by 100% in plots treated with 100 LE/ha (Bell 1990), for a total average emergence reduction of 90%. The 50 LE rate caused reductions of 65% in budworms and 57% in bollworms, for an overall reduction of 64%. The mean total numbers of bollworm/budworm adults emerging all differed significantly from each other. The results of this test were therefore similar to those obtained when alternate host areas were treated by hand, indicating that a single aerially-applied *H*NPV treatment could result in a reduction in first-generation adult emergence of about 90%. However, a large-area test was needed to evaluate this technique as a management tool in the Mississippi Delta. Such a test was planned for 1990.

1990. The study consisted of aerially applying a viral insecticide (Elcar®, *H*NPV) to early-season alternate host plants of bollworm/budworm within a single area (16.7 x 16.7 km, 25,900 ha) at a time of maximum immature seed production in a majority of the wild geranium in the test area. Emerging adult populations would be compared to those in a similar, untreated area, separated by a 16.0 km buffer (Bell and Hayes 1994, Hayes and Bell 1994). The treatment area was in parts of Washington and Sunflower Counties in the Mississippi Delta. Spray coverage, infection rates of larvae on pre-season wild hosts, persistence of virus on plants, number and species of moths emerging from alternate hosts within the areas, and number and species of the first bollworm/budworm adult populations invading cotton were determined.

Since the treatment of a large, populated, non-crop area with a microbial pesticide was unprecedented, the reaction of the public to the project was of utmost concern. We believed, however, that an informed public would be an approving public. The strategy for the public information campaign was developed jointly by USDA scientists involved with the project, the USDA, ARS Information Office, Delta Council, and personnel of Sandoz Crop Protection, Inc. This successful information campaign was effective in minimizing possible problems associated with area-wide application of insecticides.

Virus was applied both by aircraft and by trucks equipped with mist blowers. Both methods of application were calibrated to deliver virus at a rate of 100 LE in 19 l of water/ha. Since the plan was to treat only alternate host habitats, pilots attempted to treat only field edges, borders, fallow fields, treelines, and other similar habitats. Water-sensitive cards were placed randomly among the weeds to determine spray coverage. Aerial application began on 24 April, a time believed to be about 7 days prior to maximum overall seed production in the *G. dissectum*. Aerial application was completed by 8 May, although much of the application was done during windy weather conditions not favorable to spot-spraying sites. A series of early spring weather fronts caused many farmers to delay tilling their fields, resulting in an unusual amount of weed areas where the appropriateness of treatment was questionable. Truck-mounted mist blowers (calibrated to deliver 20 l/ha along the side of the truck in a 18-m swath width at 8 km/h were used to treat along major roadways within the test area (560 km total) between 30 April and 4 May. Although these areas are known to be major sites for geranium (Stadelbacher 1982), they are more difficult to treat by aerial application, either because of vehicle traffic or nearby power lines.

Bioassays were made of field-collected material to estimate the efficiency of the spray application and viral persistence. To estimate the quantity of virus on the plants, *G. dissectum* terminals were cut at random from eight plots in the treatment area as soon as possible after application. Terminals (72) from each locations were fed to 6-d-old tobacco budworm larvae for 48 hr, after which larvae were placed on artificial diet. Larval mortality was recorded 10 days after the beginning of the feeding period. A final bioassay was conducted on 29 May to determine the residual virus on the weeds. Random samples of remaining wild geranium were collected and fed to larvae as before, with 144 larvae fed material from treated plots and 144 fed material from untreated area.

The methods used for the cage tests were similar to those used in the previous study. Untreated control plots were covered with plastic sheeting during application. Daily searches were conducted within the cages after the first budworm/bollworm moth was observed, and moths were captured and removed. Pheromone traps were used to monitor the relative abundance and fluctuations in the adult male populations in the treatment and control areas. In both areas, four trap locations (one trap/species/location) were identified near cotton fields within each 1.6-mi interval/(radius) from the center. Additional sampling extended to 8 km in 16 km in the treatment area, to present an assessment of migration on the treated area. Depending on availability of an accessible cotton field, attention was given to spacing trap

locations in different quadrants. Trap locations consisted of one trap/species placed at the edge of a cotton field along an accessible roadway; traps were separated by about 35 m. Because fields are frequently cultivated and roadsides are often mowed or burned, traps were placed near power poles and in other protected sites. Traps were routinely monitored from 1 April, which included the flight of the parental, F_1 and F_2 generations.

In addition to the estimation of the emerging population by adult trapping, attempts were made to estimate the number of adults by counting the egg deposition on early hosts and cotton within the test areas. This method required visually checking plants for a total of 30 min in each sample area and recording the numbers and relative populations of budworm/bollworm eggs.

When *G. dissectum* terminals were randomly collected from eight different plots immediately after aerial application and fed to 6-day-old tobacco budworm larvae for 48 hours, mortalities at 10 days averaged 75.1% (67.7% to 88.7%) compared to a background mortality of 9.3% when larvae were fed terminals from untreated areas. Although the mortalities of larvae fed treated terminals were significantly greater than those fed untreated terminals, the 75.1% mortality was less than that reported in a previous study. In that study, the treated area was an open field, and the application was made in broadcast fashion, not to a strip target area. Larval mortalities in terminal bioassays averaged 95.7%. The random samples tested here were taken from along tree lines, field borders, and other similar habitats, not from open fields. This lack of virus on the target hosts was also indicated by the numbers of droplets found on water-sensitive cards. A similar application rate and droplet size in the earlier test resulted in an average of 54.4 droplets per cm^2, whereas there was an average of 3.9 droplets per cm^2 in the present test, indicating a 93% reduction in droplets hitting the plants. When the larvae were fed terminals collected 1, 2, 3, 5, 7, and 9 days after treatment, viral mortality averaged 77, 69, 64, 57, 41, and 38%, respectively. Only 5% of larvae died from NPV when fed wild geranium collected from the treated area 18 days after all applications had been completed; samples from outside of the test area produced no viral mortality. Very little wild geranium remained viable by that date.

There was a 41% total overall average reduction in emergence in the treated cages (Table 1), indicating much less effect than in the previous tests where the least overall reductions were about 88%. In the previous study, however, moth emergence was reduced only by 64% when the virus dosage was halved (50 LE/ac). The present bioassay data indicated that aerial application resulted in only about 28% as much virus on the plants as in the previous study. If these indications were real, the 41% reduction in moth emergence in this study was reasonable. These data all indicated that deposition of virus on the target area was a major problem in this test, even in the cage test areas where application was somewhat easier than in other host plant habitats.

TABLE 1. Number of Moths Emerging from *G. dissectum* after Treatments with NPV (1990).

Treatment	No. moths per cage, mean ± SEM[a]		
	H. virescens	*H. zea*	Totals
Untreated	8.11 ± 1.31 a	1.44 ± 0.56 a	9.56 ± 1.23 a
Aerial application	5.00 ± 1.76 b	0.67 ± 0.37 ab	5.67 ± 1.75 b
LSD	1.47	1.08	3.32

[a]n=9 cages/treatment. Values within columns followed by the same letter are not significantly different ($P > 0.05$, Fisher's LSD).

Pheromone traps and egg counts were used to assess the degree of area-wide suppression achieved by early-season application of the *H*NPV. Eggs (F_2) were collected from cotton and other hosts to characterize the surviving reproductive populations. The effect of treatment was demonstrated by deviations in trap capture patterns within-year between treated and control plots and between years in the treated plot. Rates of increase (r_i) between generations were calculated by dividing the number of moths captured in one generation by the number from the previous generation. The results of these data (Bell et al. 1990) indicated that the single virus application reduced the adult budworm population emerging from early season alternate hosts by 25 to 38%, and the bollworm population by 19 to 31% in the 167-km^2 area. Overall budworm/bollworm adult emergence was reduced by 41% in cage test areas. Although the treatment failed to reduce the adult population as much as expected, the results were still encouraging. This reduction in the number of moths was understandable when all factors of the study were taken into consideration, primarily the lack of spray coverage. A secondary factor was the timing of the application. Although aerial application studies had been done in which the virus was applied to alternate host areas, the conditions of this test were different in that much of the application had to be done under windy conditions. Bioassays were very informative in indicating the presence of the virus on the plants. Based on that data, the reductions in insect populations were comparable with prior tests. Although timing was less a factor than spray coverage, a 21-day application period was considered too long for most seasons.

1992. A repeat of the 1990 study was planned for 1991, but the test was canceled due to severe flooding during the spring of that year. A similar but smaller test was planned for spring of 1992 in which the goal was improved spray coverage. Preliminary tests in 1991 showed that the addition of 4% soybean oil w/emulsifier increased the amount of material reaching the surface from a spray altitude of 17 m. These tests indicated that use of a crop oil additive and applying the spray as a "blanket" application would increase virus deposition on the early-season hosts.

The test site was 7,326 ha (4.8 km radius circle) in a 9.6 x 9.6 km area used as an untreated control during 1990 and 1991 tests. Data were collected to determine spray coverage, infection rates of larvae on pre-season wild hosts, persistence of the virus on the plants, and the number and species of moths emerging from alternate hosts within the treated area and compared to the surrounding untreated area.

The virus was applied at the time of maximum immature *G. dissectum* seed production (24, 25, 27, and 28 April) using four fixed-wing aircraft, each calibrated to deliver virus at a rate of 100 LE/ha in 9.3 l of water containing 4% crop oil spray additive. The lead aircraft was equipped with an electronic satellite guidance system, which recorded the aircraft's position relative to the earth's surface. The three other aircraft followed.

Bioassays were conducted on *G. dissectum* collected at various times from various locations within the treated area to estimate the efficiency of the spray application and the persistence of virus on the wild geranium. Six-day-old tobacco budworm larvae were allowed to feed on terminals for 48 h (60 larvae, four samples/location and time), after which they were then placed on clean artificial diet. Larval mortality due to virus was recorded 10 days after the beginning of the feeding period. The first samples were collected on 27 April and the final collected on 13 May.

Cage studies for evaluating the effectiveness of the virus application were as described previously. A total of 12 cages (six treated and six untreated control) were erected in pairs. The control plots were covered with plastic sheeting during application, as in previous tests. Searches were conducted daily within the cages after the first moth was observed, and the number and species of moths captured were recorded.

Pheromone traps were used to monitor the relative abundance of the adult male bollworm/budworm populations in the treatment area (center 4.8-km radius) and the untreated area (the area surrounding the treated area and 4.8-9.6 km from center of the test area). In both areas, 26 trap locations (1 trap/species/location) were identified near cotton fields, with eight to nine pairs within each 1.6 km interval/radius from the center (total area encompassed in a radius of 9.6 km). Traps were routinely monitored from 20 March to 31 August.

Allowing tobacco budworm larvae to feed for 48 h on *G. dissectum* terminals collected 0, 2, 3, 4, 6, 7, 8, and 11 d after treatment produced virus-induced mortalities of 83, 81, 60, 79, 75, 67, 60, and 33%, respectively. Samples collected in the treated area on May 13 (17 d after treatment) did not produce viral mortalities greater than samples from untreated areas. These results tend to verify the improved coverage obtained in this study compared to the 1990 study, since mortalities were consistent over the areas tested. The indicated persistence of the virus also compared well with that previously reported, again indicating roughly 47% of the original activity remaining 9 days after application as reported in previous tests.

Cage data indicated that the affected generation of adults (that generation developing as larvae on the wild hosts) began emerging on 3 June (bollworm) and 12 June (budworm). A total of 36 budworms and 13 bollworms emerged in the six untreated areas, compared to seven budworms and seven bollworms in the six treated areas. The cage data, therefore, indicated a significant reduction in budworm emergence (80.6%) in treated areas compared to untreated hosts (Table 2). Although the bollworm emergence was 46.2% less in the treated cages, the difference was not significant.

TABLE 2. Bollworm and Budworm Emergence from *G. dissectum* Aerially Treated with NPV[a] (1992).

	H. virescens	*H. zea*
Treated	1.17* ± 0.4	1.17 ± 0.7
Untreated	6.33 ± 1.7	2.21 ± 0.9

[a]Values represent numbers of moths emerging per cage (6 cages/treatment). Means denoted by * are significantly different from the control ($P = 0.05$).

Pheromone trap data indicated similar populations of both species within the treated and untreated areas until 3 June, after which there was an observed period of reduced response within the treated area, particularly the center 4.8 km radius or 9.6 km dia. Emergence of adult budworms in the cages indicated the population emerging from 12 through 26 June most likely developed as larvae on the early season hosts. During that period, budworm traps in the 9.6 km diameter treated area averaged 8.0 moths/trap/night (Table 3) compared to 11.4 moths/trap/night captured in the untreated area (5-10 km from the center of the test), a reduction of 29.8%. However, captures averaged 6.4 moths/trap/night in the middle 6.4-km diameter of the treated area, a reduction of 43.9% in that portion of the virus treated area compared to the surrounding untreated area.

The indicated reduction in budworms within the center of the test (43.9%) was an improvement over the results obtained in 1990, but the effectiveness indicated by trap data was not as good as that shown by the cage data. This difference may be explained by immigration of moths from outside the test area, reducing the indicated effectiveness of the virus application based on trap data. Red-dye-marked moths from another program were being released at least 13-km from our test area. Trap captures in part of our test area averaged up to 33% marked moths during the release period, indicating significant movement of moths into the area. It is,

therefore, reasonable to speculate that the actual reduction in budworm moth emergence from early season hosts was greater than ~43.9% shown by the adult captures.

TABLE 3. Mean Number of Budworms Captured by Pheromone Traps per Night within Treated and Untreated Areas of the Mississippi Delta in 1992.

	6/12	6/15	6/17	6/19	6/22	6/24	6/26	$\bar{x}$
Untreated Area[a]	6.58	14.79	14.83	15.96	9.22	9.92	8.23	11.36
Treated Area A[a,b]	4.83	11.36	10.65	11.83	5.92	6.12	5.31	8.00
Treated Area B[c]	3.35	10.00	7.92	11.35	4.49	4.42	3.26	6.40
Prob of sig. diff. between Treated A and untreated (X^2)[d]	0.72	0.47	0.98	0.22	0.76	0.88	0.76	

[a]The treated area (center 3-mi radius) received an aerial application of bollworm NPV on 24-28 April at a rate of ≈ 99 LE/ha.
[b]Data based on 26 traps in each area. Treated Area A = 4.8-mi radius area. Untreated area = 4.8 to 9.6 mi radius area surrounding treated area.
[c]Data based on 13 traps located in center 3.2 km radius of treated area (Treated Area B).
[d]Probabilities of significant differences based on frequency distributions (Chi-square test).

1994. An area-wide, early-season pest management test was conducted utilizing a 505-km^2 area, or twice the diameter of the 1990 test. Since no commercial virus was available, over 8 million cotton bollworm larvae were reared over a four-month period to produce enough EPA-labeled insect virus to treat 87,000 ha. Application of the virus to 79,000 ha was made from 28 April through 3 May using contracted private aircraft equipped with satellite global positioning systems. Although the virus and formulating materials were tested and shown to have no effect on catfish, spray nozzles were turned off over catfish ponds.

Evaluation of the effects of the virus application was accomplished using several methods: pheromone trapping in the treated area compared to traps in three similar untreated areas of the delta; cages placed over treated and untreated weed hosts as in previous tests; plant bioassays (feeding plant materials to 6-day-old larvae); and examining plants in selected fields within the treated area compared to the three untreated areas for differences in eggs or larvae.

Pheromone trap counts of budworm and bollworm moths in the treated area between June 10 and August 22 averaged 53% (17-91%) less than traps in three similar, untreated areas. A total of 195 tobacco budworms emerged in 12 cages over untreated areas compared to 80 emerging in cages over treated areas, a reduction of 59%. Four bollworm moths emerged in treated areas compared to 11 in untreated areas, a reduction of 64%. Virus-induced mortality of 57 larvae collected from weeds in two locations three days after treatment was 85%. Bioassay data showed that 76% of larvae fed treated terminals for 48 h (average of 22 samples of weed samples collected after application had dried, 30 larvae/sample) died from virus infection. The cost of virus production and application was about $449,162, or $5.54/ha. Although the adult trapping counts and infection data appeared to support the significant effect of virus in reducing bollworm and budworm populations in the area, reports on cotton scouting and a possible economic impact comparing the treated area to the untreated areas did not show any positive benefit. Furthermore, counts of larvae and eggs within the treated area were equal to the untreated areas.

1995. As in 1994, over 8 million cotton bollworm larvae were used to produce virus to treat over 81,000 ha and evaluate the effect of such treatment on the resulting populations of

bollworms and budworms. Laboratory bioassays of the virus used in both the 1994-1995 field seasons showed a product activity comparable to commercially available virus. The virus was likewise applied using contracted private aircraft with global positioning systems to treat the same area as in the 1994 test. In 1995, the virus was applied from 5 May through 10 May 1995, approximately 1 wk later than in 1994. Evaluation methods were similar to those used in 1994, with slight modifications. First, a total of 398 tobacco budworm larvae were collected at various times from weed hosts in 21 locations. Second, pheromone traps were placed in the treatment area plus the one check area which in 1994 had captures closest to the average of the three check areas used that year. Planned cage tests were lost due to circumstances of application. An evaluation based on examinations of cotton fields for presence of eggs and larvae was conducted.

The mortality of the collected larvae due to virus infection by date of collection is shown in Table 4. It was impressive that the larvae collected on 11 and 12 May were all infected (100% mortality due to virus). By the next week, the percentage of infected larvae decreased; but by that time, many of the infected larvae would have been dead and therefore would not have been collected. The persistence of virus over the following 10 days could indicate disease transmission from diseased and dying larvae to previously-uninfected larvae. In 1994, overall counts of tobacco budworm and bollworm moths in the treated area between 10 June and 22 August averaged 53% less than in traps in the untreated areas. During the same period in 1995, the overall counts averaged 66% less in the treated area (Table 5). We expected the effects of the early virus treatment to be expressed during the emergence of moths in the June generation. That is what was indicated by the 1994 *H. virescens* trap captures (7 June through 8 July). The 1995 trap data not only indicated the reduction in June, but may have also indicated continued reductions through the July population. Although we felt that the timing of our application was too late to cause reductions of the first population of *H. zea*, trap capture data suggest this was not the case. We found larvae easily during the period of 11-19 May, and the amount of virus on the plants from dying budworm larvae might have affected the bollworms if they were in a second generation at that time.

TABLE 4. Mortality Due to NPV in Budworm Populations Collected from Treatment Area. Virus Treatment Applied 5-10 May 1995[a]

Collection date	#Larvae collected	# Larvae killed by NPV	% NPV mortality
11 May	25	25	100
12 May	20	20	100
15 May	86	40	47
16 May	122	59	48
17 May	114	54	45
19 May	18	8	44
22 May	5	4	80
23 May	8	5	63
Total/overall	398	215	54

[a]Samples represent larvae collected from 21 of the 25 treated sectors.

TABLE 5. Pheromone Trap Counts, 20 Traps/Species/Treatment (1994-1995)

Dates	# Trap nites	Species	Treated		Untreated		% Reduction
			Total # moths	x̄ Moths/ trap/nite	Total # moths	x̄ Moths/ trap/nite	
					1994		
6/7-7/8	35	*H. virescens*	6,084	8.69	15,205	21.72	59.99
		H. zea	8,538	12.20	20,566	29.38	58.48
7/12-8/16	39	*H. virescens*	15,589	19.99	21,744	27.88	28.31
		H. zea	12,547	16.09	34,181	43.82	63.29
6/7-8/16	74	*H. virescens*	21,673	14.64	36,949	24.97	41.34
		H. zea	21,085	14.25	54,747	36.99	61.49
6/7-8/16		Total moths	42,758		91,696		53.37
					1995		
6/7-7/12	38	*H. virescens*	2,725	3.59	8,245	10.85	66.95
		H. zea	1,867	2.46	5,696	7.49	67.22
7/14-8/16	35	*H. virescens*	6,559	9.37	16,275	23.25	59.70
		H. zea	11,375	16.25	35,622	50.89	68.07
6/7-8/16	73	*H. virescens*	9,284	6.36	24,520	16.79	62.14
		H. zea	13,242	9.07	41,318	28.30	67.95
6/7-8/16		Total moths	22,526		65,838		65.79

1996. A circular treatment area with a 5.6 km radius was established at N 90° 48' 38" W 33° 19' 00" that encompassed approximately 9,972 ha. The HzNPV formulation used in this study consisted of Gemstar™ LC (biosys, Inc.) diluted in water with an equal volume of Soysurf™ (Sanders Seed Co., Cleveland, MS) and applied at an application volume of 2.33 l per ha. Aerial applications of virus were applied at a rate of 4.94 X 10^{11} occlusion bodies (OB's) per ha to the entire area on two separate occasions (30 April and 9 May) . These two dates were selected to coincide with the larval emergence of bollworms and tobacco budworms, respectively.

Twelve enclosure cages (26.8 m^2 x 1.8 m high) were set up in a randomized complete block design of three replicates with four treatments at two locations. Treatments within each replicate were an untreated control; early virus treatment only; late virus treatment only and both virus treatments. A single cage was used for each treatment and each cage was monitored daily for adult emergence from 20 May until 10 June. Earlier studies have shown that each enclosure cage isolated a representative sample of tobacco budworm and bollworm larvae. The number and species of moths emerging in each cage were recorded for data analysis.

Sampling transects ran from the study site center, bisecting the circle and extending for 8 km beyond the study site boundaries. Sampling sites were located every 1.6 km along the transects with two (one each for bollworm and tobacco budworm) standard cone traps (Hartstack et al. 1979) established at each site. Trap contents were sorted for identification and counted three times each week over the 12-wk period following virus application.

Overall adult emergence among all of the treatments in the study area was low, and this was attributed to the low moth populations observed in the area. The low rates of adult emergence precluded any statistical analysis of the data.

H. zea was the predominant species with >90 % in the control and treated area. Tobacco budworm moth captures in the center of the virus treatment area averaged 21 moths/trap/week versus 268 moths/trap/week in the untreated area during peak trap capture. This represented a 92% reduction in tobacco budworm moth captures. Trap counts for bollworms in the center of the virus treatment area were 9% lower than moth captures in the surrounding untreated area.

Results from the pheromone trap capture data suggest that the virus application was less effective at reducing bollworm moth emergence then tobacco budworm moth emergence. Timing of application or movement into the treatment area were considered to be the primary factors responsible for the observed lack of reduction in bollworm moth emergence in the treatment area. The pheromone trap sites reported for the treatment area were only 6 km from the border of the treatment area. Movement of 8 km and upwards to 19 km has been reported for *H. virescens* and *H. zea,* depending upon environmental conditions (Hayes 1991). This degree of movement could have a significant impact on any attempt to evaluate the success of the 1996 area-wide program using pheromone trap capture data.

1997. The impact of a lower virus application rate (ca. 20 larval equivalents) was evaluated during the 1997 field season. A circular treatment area was established near Bourbon, MS, with an 8-km radius that encompassed approximately 40,485 ha. Plots of wild geranium were established at two locations in the treatment area in a RCB design with four replicates of each treatment. Each replicate consisted of four plots (12 m^2) with plot borders mowed to facilitate cage installation following virus application. Two plots within each replicate were artificially infested with laboratory-reared neonate larvae (100 bollworm and budworm larvae) on four separate days prior to application. Treatments within each replicate consisted of an untreated naturally-infested control, a naturally-infested virus treatment, an artificially-infested control, and artificially-infested virus treatment. Each plot was covered with a cage composed of plastic insect screen (4m X 8m) after virus application.

The HzNPV formulation used in this study consisted of Gemstar™ LC (Thermo Trilogy, Inc.) diluted in water with an equal volume of cotton seed oil (PBSY) with an emulsifier (Quality Limited Products) and applied on 4-6 May 1997 at an application volume of 2.4 l per ha. Aerial applications of virus were applied at a rate of 2.47 X 10^{11} occlusion bodies (OB's) per ha to the entire area. Untreated control plots were covered with plastic during the virus application.

Data from the 1997 area-wide study have yet to be fully analyzed. Preliminary data indicate that overall coverage averaged 85% in the treated area. A reduction in overall adult emergence among the virus treated cages with a natural infestation averaged ca. 83%, whereas virus treated cages that had been artificially infested showed an 81% reduction in adult emergence. The trap count data have not been analyzed at this time.

DISCUSSION

All studies have shown that application of baculovirus (NPV) to early-season hosts of the tobacco budworm and cotton bollworm resulted in reduction in the numbers of adults that emerge from treated areas compared to adjacent untreated areas. This fact is demonstrated both by the significant reduction in the numbers of moths in cages placed over treated areas (compared to untreated areas), and by the infection rates of larvae collected from treated early-season weeds. Based on those results, we estimate that one or two properly timed NPV treatments with good coverage consistently resulted in viral-induced mortality of at least 70%.

In the largest tests (1994 and 1995), the rates of infection of larvae on the hosts as well as the reductions shown in moth emergence did not translate to a significant difference in the numbers of eggs/larvae on the cotton in the treated area compared to the surrounding untreated areas. During both years, numbers of adults during the crop season in the entire area of the study was relatively low. Much of the theory regarding the use of this pest management system is related to the distance and timing of moth flights. One indication of significant moth movement was the recapture in traps of several moths released in a sterile hybrid study (Laster et al. 1996) at least 13 km from the virus area. Mobility of the target pest makes evaluation of the success of suppression tactics difficult over large areas of the agroecosystem, and it will definitely play a part in the use of this method as a management tool.

Although the virus used in 1994-1995 was produced at this laboratory in cooperation with the USDA-ARS Gast Rearing Laboratory at Mississippi State University, the new commercial formulation performed well in 1996-1997. However, due to the current methods of virus production, any producer of NPV will need considerable lead time between the order for large amounts of the virus and the expected delivery date.

A grower-funded program for managing *Heliothis/Helicoverpa* has been discussed for the Mississippi Delta in 1998 that would encompass approximately 324,000 ha. A conservative estimate on the amount of cotton planted in this area would be approximately 91,000 to 101,000 ha of cotton although this amount may vary by 20% for a given year. The total grower contribution per cotton hectare can be calculated by determining the total cost of the program and dividing by the total amount of cotton planted in the treated area.

The estimated cost for aerial application of the virus would range from $1.24 to $1.73 per ha and the cost for the oil adjuvant would be approximately $1.06 per ha. Thus, the estimated total cost for the aerial application and oil adjuvant would not exceed $2.79 per ha. Virus costs would range from $4.94 to $6.18 per ha depending upon the quantity purchased. The estimated total cost for the program in the proposed area would be $8.97 per ha or $2,906,000. If we assume that the cotton acreage represents 31% of the total area in the treatment program then the estimated cost per hectare of cotton would be $28.70 ($11.61 per cotton acre).

In the Mississippi River Delta, noncropped areas consist mostly of disturbed margins of cultivated fields or the shoulders of roads. Wild geranium is very abundant in this relatively small noncropped area, and here it serves as an excellent host plant for the tarnished plant bug, and as the main host for the first generations of the bollworm and tobacco budworm. Small plot research has shown that bollworm and tobacco budworm populations can be reduced by as much as 97% with one mowing, and by 99% with one herbicide application (Knipling and Stadelbacher 1983). Mowing can reduce plant bug adult levels by 40% and nymphs by 79%, while a herbicide treatment can cause reductions of 65% for adults and 73% for nymphs. Both control methods have been tested only in small plots, and the effects of the treatments on pest and beneficial arthropods found in the noncropped areas has not been evaluated on large areas. Any supplemental mowing or herbicide treatments by growers would be an added benefit. However, aerial applications of herbicides in an area-wide program would not be environmentally acceptable. In addition, the length of time required to mow and apply one ground application of herbicide would be greater than the time required for heliothine development and growth of the wild plant hosts.

With the anticipated planting of large acreages of transgenic cotton in the future to control bollworm/budworm, a grower funded program to control the same pests is less likely to occur. This program, however, is available as a management tactic in the future if transgenic cotton does not perform satisfactorily. Additional benefits of area-wide sprays of virus may also include a reduction in numbers of these insects in corn, grain sorghum, and soybean.

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BEMISIA ARGENTIFOLII: ACTION THRESHOLDS, UPLAND COTTON YIELDS AND COTTON LINT STICKINESS IN THE IMPERIAL VALLEY, CALIFORNIA

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ABSTRACT

Studies were conducted in 1995 and 1996 in the Imperial Valley, California, to determine the effect of *Bemisia argentifolii* Bellows and Perring on upland cotton yields and honeydew lint contamination. Insecticide applications were initiated on upland cotton for *B. argentifolii* control at four and 15 adults per leaf-turn. Results showed that initiating chemical control at four adults per leaf-turn produced higher lint yields and less lint stickiness compared to initiating chemical control at 15 adults per leaf-turn. Higher yields and lower lint stickiness occurred at both treatment levels compared to untreated cotton. Initiating treatments at 15 adults per leaf-turn required two and three applications and initiating control at four adults per leaf-turn required five and six applications during 1995 and 1996, respectively. Economic returns based on insecticide costs and lint yield were highest when plots were treated at four adults per leaf-turn. Cotton lint stickiness was not considered in the analyses and discounts for sticky cotton could significantly reduce net monetary returns.

INTRODUCTION

The sweetpotato whitefly, *Bemisia tabaci* Gennadius, was first reported on cotton at Gila Bend, AZ in the 1920's (Russell 1957) and was of sporadic economic importance in Arizona and Southern California through the 1980's (Butler and Henneberry 1989). In the late 1980's, differences in biology and whitefly isozyme banding patterns suggested that a new biotype, sweetpotato whitefly Strain B had been introduced in the area (Brown et al. 1995). Bellows et al. (1994) described strain B as a new species, *B. argentifolii* Bellows and Perring. Crop loss values in Imperial County, California were estimated to exceed $328 million during 1991 to 1994 (Birdsall et al. 1995). In Arizona and California, a total of 224,291 ha of cotton were reported infested with the new species in 1996 and lint yield losses were estimated at 24,891 bales (Williams 1997). In addition to reduced yields, cotton lint stickiness that occurs as a result of insect honeydew lint contamination is a major problem in the textile industry. Honeydew contaminated lint sticks to high speed machinery and makes lint difficult or impossible to process. The problem is worldwide in scope and sticky cotton is subject to discounts of up to 18 cents per kilogram (Anonymous 1996).

Chemical control has been the most common method of protecting cotton yields and preventing lint stickiness. In an effort to reduce insecticide use and costs, conserve

natural enemies and delay or prevent insecticide resistance development, action threshold levels of two to eight adults per leaf-turn have been suggested for initiating chemical control for *B. tabaci* in Sudan, Thailand, and India (Stam et al. 1994, Mabbett et al. 1980, Sukhija et al. 1986) and for *B. argentifolii* in the United States (Ellsworth and Meade 1994; Ellsworth 1995; Ellsworth et al. 1996, 1997; Naranjo et al. 1998). Most studies suggest no significant yield differences for action thresholds between five to ten adult whiteflies per leaf but significant reductions in yield for 20 or more adults per leaf (Yee et al. 1998; Naranjo et al. 1998). We conducted studies in 1995 and 1996 at Brawley, CA, to compare the effects of action thresholds of four, or 15 *B. argentifolii* adults per leaf-turn on cotton lint yields and cotton lint stickiness.

MATERIALS AND METHODS

The studies were conducted at the USDA, ARS Irrigated Desert Research Station at Brawley, CA. Upland cotton, *Gossypium hirsutum* L., cv. Deltapine 5461, seeds were planted and irrigated for germination on 13 March 1995 and on 11 March 1996. The experimental designs were randomized complete blocks with eight and nine replicates for 1995 and 1996, respectively. Each plot was four rows wide and 18.3-m long. Rows were spaced 1-m apart. There were four unplanted rows between plots and 9.1-m fallow alleys to separate blocks. This partially isolated small-plot methodology has been used successfully in comparison of insecticide treatment effects on *B. argentifolii* (Chu et al. 1993).

Insecticide treatment was initiated when the number of whitefly adults were four or 15 adults per leaf-turn on 5th main stem leaves from terminals. The four adults per leaf treatment level was developed in earlier threshold studies that maximized lint yields (Chu et al. 1999). The 15 adults per leaf infestation level was the highest economic injury level reported by Naranjo et al. 1996. Untreated plots were controls. The insecticide mixture used for whitefly control was fenpropathrin (Danitol®, Valent Corp. Walnut Creek, CA) at 0.22 kg AI/ha and acephate (Orthene®, Valent Corp. Walnut Creek, CA) at 0.56 kg AI/ha (Akey et al. 1991, Chu et al. 1993, Ellsworth and Meade 1994). Foliar applications were applied in 212 liters of water/ha at 759 kPa pressure with a tractor mounted spray boom designed to improve underleaf coverage. There were three nozzles per row. One nozzle was positioned above and directed downward to the tops of plants. A nozzle on a drop on each side of the row was directed toward the plants and provided bottom and middle leaf coverage.

The effect of insecticide treatments on *B. argentifolii* densities was determined by weekly plant sampling. Adults were sampled using the leaf-turn method discussed by Naranjo and Flint (1995). Also a 3.8 cm^2 leaf disk was taken from each of 10 leaves picked per plot to evaluate treatment effects on eggs and nymphs (Naranjo and Flint 1995). A total of 12 and 14 adult weekly samples, respectively, were taken during 1995, beginning on 21 May, and in 1996, beginning on 14 May. In 1995, plants were defoliated and seed cotton was harvested on 21 and 29 August, respectively. In 1996, plants were defoliated and seed cotton was harvested on 14 and 27 August, respectively. For both years, seed cotton was hand picked from two 4-m areas in randomly selected rows to determine lint yields. The effect of *B. argentifolii* control on lint stickiness at harvest was determined using the thermodetector method described by Perkins and Brushwood (1995).

Data for *B. argentifolii* densities for all sampling dates were averaged for the season and analyzed with ANOVA using the MSTAT-C program (1989). Lint yields and thermodetector counts were also analyzed using ANOVA. Seasonal whitefly means and

means for lint yields and thermodetector counts were separated with Student-Neuman-Keul's Multiple Range Test. Economic returns in relation to insecticide control costs, were calculated using $71/ha/application ($31.15, 12.39, 27.8 for fenpropathrin, acephate, and ground application fee, respectively, personal communication, Rockwood Chemical Co., Brawley, CA, 1996) and assumed lint price of $1.54 per kg.

RESULTS AND DISCUSSION

During 1995, five insecticide applications were made (14 and 28 June, 12 and 19 July, and 2 August) at four adults per leaf and three applications (28 June, 12 and 26 July) at 15 adults per leaf. In 1996, six applications were made (15 and 29 May, 12 and 26 June, and 16 and 31 July) at four adults per leaf and two applications (5 June and 3 July) at 15 adults per leaf.

Adult, egg and nymph population densities in untreated plots remained low through 20 June, thereafter, increasing to peak populations for adults on 11 July, and for eggs and nymphs on 1 August (Fig. 1A, 1B, 1C). Adults, eggs and nymphs fluctuated similarly during the season but at lower population levels in insecticide-treated plots. Seasonal average numbers of adults, eggs and nymphs were significantly lower in insecticide treated plots compared with untreated plots. Plots treated at four adults per leaf had significantly lower populations compared with plots treated at 15 adults per leaf (Table 1).

Higher densities of *B. argentifolii* adults, nymphs, and eggs occurred earlier in the season in 1996 (14 May to 18 June) compared to the same periods of time in 1995 (Fig. 1D, 1E, 1F). This probably occurred because of adult migration into the experimental plots from nearby whitefly parasite refugia. Less adult migration from the refugia to the experimental plots occurred after 18 June. Following the early adult peak population on 18 June, densities of adults decreased in untreated plots through 29 July and increased thereafter to over 30 per leaf-turn on 13 August. Densities of eggs and nymphs in untreated plots also decreased from early peaks on 11 and 25 June, respectively, but increased from 2 July to 13 August for eggs and from 16 to 23 July for nymphs. After two applications of the fenpropathrin-acephate mixture to plots with the 15 adults per leaf, densities of adults, eggs and nymphs were significantly reduced to about half of those found in untreated plots (Fig. 1D, 1E, 1F). Seasonal average densities of adults, eggs and nymphs in plots treated at four adults per leaf were significantly lower compared with numbers in plots treated at 15 adults per leaf (Table 1).

Lint yields were 2.9X and 1.9X greater in 1995 and 1996, respectively, when plots were treated at 15 adults per leaf compared with untreated plot yields (Table 2). When plots were treated with four adults per leaf, lint yields were 3.3X and 2.6X greater in 1995 and 1996, respectively, compared with untreated plot yields. Thermodetector counts of harvested cotton lint were 44 and 65 in untreated plots, respectively, for 1995 and 1996, compared with 32 and 49 in plots treated at 15 adults per leaf and to 16 and 24 for plots treated at four adults per leaf. Thermodetector counts of 16 and 24 are moderate levels of lint stickiness (Perkins and Brushwood 1995) and counts of 32 and 49 indicate heavy lint stickiness. In both cases, this would not be acceptable lint quality for processing at the textile mill. Blending sticky cotton lint with non-sticky cotton lint has been practiced to reduce the problem. Cotton of medium stickiness blended at proportions of not more than 10 to 15% sticky to non-sticky of lint has been shown to result in lint acceptable for processing (Gutknecht 1988; Gutknecht and Fournier, 1998). High levels of lint stickiness probably occurred in 1995 because the last scheduled insecticide applications were on 2 August for 4 adults per leaf and 26 July for 15 adults per leaf plots. In 1996, last insect-

Table 1. Seasonal Mean[a] Numbers of Silverleaf Whitefly Eggs, Nymphs, Adults on Leaf Samples from Untreated Plots and Cotton Plots Insecticide-Treated[b] When 4 or 15 Adults Per Leaf-Turn Were Used at Treatment Threshold. Brawley, CA, 1995 and 1996.

Treatment level	No. application	Eggs/cm^2 leaf disk	Nymphs/cm^2 leaf disk	Adults/ leaf turn
		1995		
Untreated	0	20.9 ± 2.3 a	7.6 ± 0.9 a	21.9 ± 2.2 a
15 adults/leaf	3	10.0 ± 0.9 b	3.8 ± 0.6 b	12.7 ± 1.4 b
04 adults/leaf	5	05.4 ± 0.4 c	1.7 ± 0.2 c	07.5 ± 0.9 c
		1996		
Untreated	0	33.4 ± 2.4 a	14.5 ± 0.9 a	16.0 ± 1.0 a
15 adults/leaf	2	17.6 ± 1.9 b	07.7 ± 0.7 b	11.1 ± 0.8 b
04 adults/leaf	6	05.3 ± 0.6 c	01.9 ± 0.2 c	05.0 ± 0.5 c

[a] Means ± SE in a column with different letters differ significantly (Student-Neuman-Keul's Multiple Range Test, $P = 0.05$).
[b] Fenpropathrin-acephate mixture.

Table 2. Cotton Lint yields, Thermodetector Counts and Economic Returns from Untreated Cotton Plots and Cotton Plots Insecticide-Treated When Four and 15 Adults Per Leaf-Turn Occurred. Brawley, CA, 1995 and 1996.

Treatment level	Application	Lint kg/ha	Thermodetector counts	Income in dollars: Gross	Income in dollars: Minus insecticide cost
			1995		
Untreated	0	0426 ± 49 c[a]	44 ± 3 a	0656	0656
15 adults/leaf	3	1239 ± 74 b	32 ± 4 b	1908	1695
04 adults/leaf	5	1410 ± 42 a	16 ± 2 c	2171	1816
			1996		
Untreated	0	0595 ± 32 c	65 ± 6 a	0916	0916
15	2	1133 ± 96 b	49 ± 6 b	1745	1603
04	6	1572 ± 60 a	24 ± 8 c	2421	1995

[a] Means ± SE in a column with different letters differ significantly (Student-Neuman-Keul's Multiple Range Test, $P = 0.05$).

icide applications were made on 31 July for four and 3 July for 15 adults per leaf plots. Plots were not defoliated until 221 August 1995 and 14 August 1996. Thus, open bolls were exposed to *B. argentifolii* populations for 19 and 14 days in 1995 and 1996, respectively. This probably compromised the effects of insecticide treatments for reducing cotton lint stickiness. The results indicate that the time lapse between the last insecticide

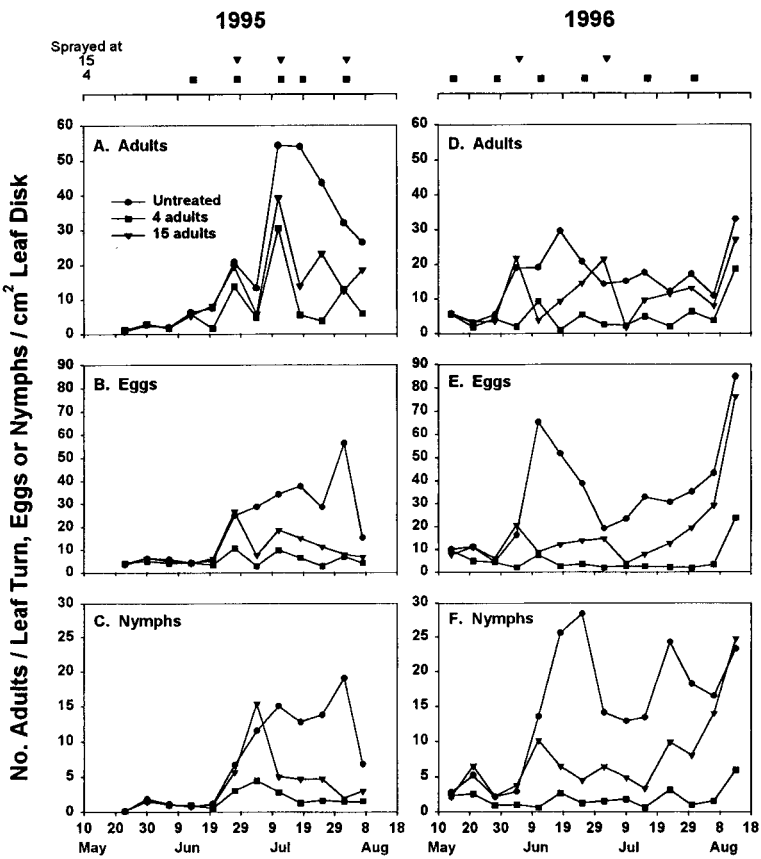

Figure 1. Seasonal distribution of whitefly adults, eggs and nymphs in untreated cotton plots (A and D) and in cotton plots insecticide-treated when four and 15 adults per leaf-turn (B, C, E and F) occurred, Brawley, CA, 1995 and 1996.

application and the defoliation application is an important consideration in protecting cotton lint from honeydew contamination.

Insecticide control of *B. argentifolii* did protect cotton yields. On the basis of yield alone, crop values for untreated were $656/ha in 1995 compared to $1,695 and $1,816/ha for the 15 and four adults per leaf treatments, respectively. In 1996, crop values for untreated, 15 and four adults per leaf treatments were $916, $1,603 and $1,995, respectively. Other pests were not a significant factor and the major impact on yields was the whitefly infestation levels. In this analysis, a possible discount for sticky cotton lint is not considered. Discounts of 18 cents per kilogram of lint could significantly reduce net monetary return. If whiteflies are a serious problem, it is assumed that irrigation

termination, defoliation as soon as possible and early harvest will be practiced to minimize late-season buildup of honeydew on the lint of open bolls (Henneberry et al. 1998).

The fenpropathrin-acephate mixture used in our studies effectively reduced whitefly populations. The same mixture has been shown less effective for *Bemisia* control in Arizona (Dennehy and Williams 1997). However, in Imperial Valley, California, Castle et al. (1996) monitored whitefly susceptibility to the mixture from 1992 to 1994 and concluded that no tolerance for the mixture had occurred in the area.

The four adults per leaf treatment level in our studies is in the range of two, six, and six to eight per leaf in Thailand (Mabbett et al. 1980), the Sudan (Stam et al. 1994), and India (Sukhija et al. 1986), respectively. Our adult threshold also agrees with previous reports from Arizona and California. Ellsworth and Meade (1994) in Arizona suggested that the whitefly treatment level for protecting cotton yield was between one and ten adults per leaf and later refined to the level between five and ten adults per leaf (Ellsworth 1995). More recently, these thresholds have been further defined as three to five adults per leaf for conventional insecticide chemistry (Ellsworth et al. 1996) and three to five adults plus 0.5 to 1.0 large nymph per leaf disk for insect growth regulators (Ellsworth et al. 1997). The results of a two-year regional study at five locations, two each in California and Arizona, and one in Texas, in 1994 and 1995, also showed that treatment levels of five to ten adults per leaf were optimal as opposed to 2.5 or 20 adults per leaf. Net economic return was generally highest when treatments were initiated at five to ten adults per leaf (Naranjo et al. 1998). Yee et al. (1998) reported similar results for protection of cotton yields and also suggested that treatment thresholds of five to ten adults per leaf resulted in reduced honeydew production.

Implementation of action thresholds in crop production systems is a major step toward ecologically oriented integrated pest management systems. Based on our results and those of other investigations the whitefly action threshold for cotton yield production appears well established.

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INFLUENCE OF FLUORESCENT BRIGHTENERS ON THE FIELD ACTIVITY OF THE CELERY LOOPER[1] NUCLEOPOLYHEDROVIRUS

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ABSTRACT

A range of application volumes were tested in the field to produce Calcofluor white M2R residue concentrations on cotton leaves comparable to those found in earlier tests to be optimal for virus enhancement (i.e., $\geq$ 30 $\mu g/cm^2$) as well as UV protection (i.e., $\geq$ 2 $\mu g/cm^2$). Spectrophotometry of leaf washes on the day of application showed that the range of resultant residues fell short of that required for enhancement, but did include the level required for UV protection. However, subsequent washes to measure the field persistence of M2R alone showed that the concentration of M2R residues dropped off rapidly so that it was completely ineffectual by 3 days post-application. This was confirmed in bioassays of leaf samples when the addition of the nuclear polyhedrosis virus (Af*M*NPV) isolated from the celery looper, *Anagrapha falcifera* (Kirby), to the M2R tank mix resulted in mortality not significantly different from that caused by application of the virus alone. A similar fluorescent brightener, Blankophor BBH, was also found not to have a significant effect on the field performance of Af*M*NPV, even when combined with a flour/sucrose formulation. Also, a comparison of the field activity of in vivo- and in vitro-propagated Af*M*NPV preparations revealed no significant difference between the two.

INTRODUCTION

A number of multi-nucleocapsid nucleopolyhedroviruses (*M*NPV) are infectious to *Helicoverpa zea* (Boddie), but few are highly virulent to this pest (Vail et al. 1978, 1982), although most of the same isolates are highly infectious to *Heliothis virescens* (F.). Because both species can be economic pests of cotton and often populations are mixed, the low activity of these viruses toward *H. zea* has limited their potential as microbial control agents for use on cotton. Hostetter and Puttler (1991) described an *M*NPV (Af*M*NPV) isolated from the celery looper, *Anagrapha falcifera* (Kirby), that has

[1]Lepidoptera: Noctuidae

relatively high virulence to both species. The cross infectivity of Af*M*NPV to other cotton pests such as the pink bollworm, *Pectinophora gossypiella* (Saunders), beet armyworm, *Spodoptera exigua* (Hübner), and cabbage looper, *Trichoplusia ni* (Hübner), provided a stimulus to develop this virus as a microbial control agent for cotton as well as other crops (Vail et al. 1992, 1993). However, its relatively low persistence in the field was recognized as an impediment to its commercial development.

While investigating compounds that might provide UV protection, Shapiro (1992) identified the enhancement effect of stilbene brighteners on the activity of the nucleopolyhedrovirus (NPV) isolated from the gypsy moth, *Lymantria dispar* (L.). By adding brighteners, Shapiro and Robertson (1992) reported reductions in the LC_{50} of this NPV of up to 1,800-fold. Similar increases in NPV activity were reported by Hamm and Shapiro (1992) for the fall armyworm, *S. frugiperda* (J. E. Smith). Because of this unique enhancement effect, the use of fluorescent brighteners (FB) in biological control was patented (U.S. Patent No. 5,124,149) (Shapiro et al. 1992).

In laboratory trials that followed, enhancement in activity of homologous as well as heterologous viruses against *L. dispar* (Shapiro and Dougherty 1994) and *H. zea* (Shapiro and Vaughn 1995) resulted from the addition of a FB to virus inocula. Zou and Young (1996) reported enhancement of the *Pseudoplusia includens* (Walker) NPV of approximately 15- and 1,600-fold for first- and second-instar *P. includens*, respectively. Similar studies conducted by Vail et al. (1996) with Af*M*NPV showed that enhancement of activity for *T. ni*, *H. virescens*, *H. zea* and *S. exigua* varied from 2.9- to 13.6-fold for LC_{50} values and 3.7- to 16-fold for LC_{95}s. These studies also showed that when given a choice between water- and FB-treated diets, larvae preferred the water-treated diet.

Studies of enhancement by fluorescent brighteners under field conditions soon followed for agricultural pests. Vail et al. (1993) showed an extension of the time for 50% loss of original Af*M*NPV activity from 5.5 to 11.5 days on cotton foliage at high viral dosages. However, similar extension of activity was not noted at lower application rates. In these studies the action of FB appeared to be mainly that of a UV protectant. Hamm et al. (1994) conducted field tests with brighteners to enhance activity of *S. frugiperda* NPV in corn. They reported increased mortality attributable to increases in polyhedral inclusion bodies (PIB) and FB concentrations as well as spray volume, but also noted that the FB appeared to be acting principally as a protectant. Zou and Young (1996) conducted field studies with FB for the *P. includens* NPV. These studies showed that 0.3-1.0% brightener significantly enhanced viral activity, but less than in the laboratory.

The present studies were conducted in the San Joaquin Valley, CA, to further elucidate the enhancement/persistence activity of brighteners with Af*M*NPV as a possible microbial control agent for lepidopterous pests of cotton. The primary objective in 1993 was to determine optimal tank mix concentrations and application volumes of Calcofluor white (M2R) to be used in the field. We were also interested in measuring field persistence of M2R alone because visually detectable "fluorescence" appeared to diminish quickly.

MATERIALS AND METHODS

Procedures used in the present tests were similar to those described by Vail et al. (1992, 1993). All tests were conducted with cotton (variety: Acala) at Five Points in the

southern San Joaquin Valley, CA. The range of M2R residue concentrations that we were interested in testing began with a calculated 2 $\mu g/cm^2$ of leaf surface area at the low end because in 1992 that concentration resulted in apparent UV protection of the virus (Vail et al. 1993). The high end of the range was 30 $\mu g/cm^2$, also calculated, because in the laboratory that concentration was found to be optimal and most economical for enhancement of viral activity (Vail et al. 1996).

Preliminary tests in 1993 eliminated the option of using M2R concentrations higher than 1% because the viscosity of the tank mix that developed in the pressurized spray tank made delivery impossible. Also, using a tank pressure of 2.8 kg/cm^2 as a constant, we pre-tested various Conejet® nozzles (Spraying Systems, Wheaton, IL) at various speeds (durations) to give a series of spray volumes that were calculated to yield the required range of M2R residues (2, 6, 15, and 30 $\mu g/cm^2$) on the leaf surface. Three Conejets® were used per row; one on top and one drop on each side of the row.

Mean hourly solar radiation and air temperature were recorded at a weather station adjacent to the field plots. A randomized complete block design (three replications) was used in which single row plots were 3.05 m long with in-row and lateral buffer zones as described by Vail et al. (1992). A tank mix of 1% (wt/vol) M2R was applied at four different volumes as summarized in Table 1. On days 0, 1, 3, 7, 10 and 14 post-application, 25 leaves were collected from each plot. A Model 3100 leaf area meter (Li-Cor, Lincoln, NE) was used to measure surface areas (note: readings were multiplied by 2 so that upper and lower surfaces were accounted for) of the leaves which were then washed by tumbling for 10 min in a vaned gallon jar in 1 L of wash solution (Vail et al. 1989). The solutions were then examined against known standards in a Hitachi Model 100-20 spectrophotometer (Hitachi, Tokyo, Japan) with the operating wavelength of 340 nm to determine M2R residue concentrations. Over time these readings were indicative of the field persistence of M2R alone. Once the applications were repeatable, a single virus concentration of 1.2×10^{12} occlusion bodies (OBs)/ha was added to the M2R tank mix at each application rate. Three additional leaves per plot were sampled and assayed against *H. zea* as described by Vail et al. (1992, 1993). All virus preparations, in the form of OBs, were bioassayed before application in the field and found to be within the activity limits described by Vail et al. (1996).

In 1995 cotton trials, Blankophor BBH (Pacific Division of Keystone Aniline Corporation, Santa Fe Springs, CA), a commercial-grade stilbene fluorescent brightening agent, was used in place of M2R. It was used at a concentration of 0.25% (wt/vol) in tank mixes with or without a 1% (wt/vol) solution of a pregelatinized white flour/sucrose formulation (#62191) provided by M.R. McGuire (USDA, ARS, NCAVR, Peoria, IL). A test of the influence of the brightener was combined with a comparison of the field activity and persistence of formulated and non-formulated in vivo- and in vitro-propagated Af*M*NPV. The in vivo preparation was produced as described by Vail et al. (1992) and the in vitro-produced virus was provided by biosys®, where it was propagated in a 10 L bioreactor culture of serum-free Tn5B1-4 cells ("High Five"; Invitrogen). Both were standardized as mentioned above. OBs were applied at a rate of 7.4×10^{11}/ha. Triton X-100 at a concentration of 0.06% (vol/vol) was used as an adjuvant in all treatments. The application rate of 94 L/ha was common to all treatments. A randomized complete block design was used with plot size and arrangement similar to those used in 1993. Data were analyzed using PROC GLM with the BON option (SAS Institute 1994).

RESULTS AND DISCUSSION

In 1993 the M2R residues (Table 1) that were originally targeted were not reached as closely as calculated, probably due to run-off from the high volume of spray or to evaporation. The range that resulted, as measured by spectrophotometry of the initial (day 0) leaf washes, did include what was assumed to be required for UV protection based on 1992 observations (i.e., $\geq$ 2 $\mu g/cm^2$) (Vail et al. 1993). However, even the highest application rate was not concentrated enough to provide optimal enhancement levels (i.e., $\geq$ 30 $\mu g/cm^2$) as determined in laboratory studies (Vail et al. 1996).

Of possibly greater significance was the finding that M2R residues dropped off very rapidly (Fig. 1) so that by day 3 post-application, there was not enough remaining on the cotton leaves to have any effect on virus activity. This was confirmed when Af*M*NPV was applied with M2R and leaf assays resulted in larval mortality that was not significantly different for the entire range of application rates, including the virus treatment without M2R (Fig. 2). Throughout the test, mortality in M2R treatments without virus was not significantly different from that in the water-blank control, and by day 10 all treatments were equal to the controls (Table 2).

The weather conditions likely to affect field persistence of virus and additives are shown in Fig. 3. Cumulative incoming solar radiation for the three M2R persistence trials was 393.86, 321.21 and 354.91 MJ/m^2, respectively. Hourly incoming solar radiation, averaged over the 14 days of the trials (daylight hours), was 2.02, 1.90, and 1.82 MJ/m^2, respectively. Mean hourly temperatures were 25.1, 23.6, and 24.7°C, and relative humidities averaged 55.7, 55.9, and 46.6% for the three consecutive 14 day trials. No precipitation was recorded throughout the tests.

In 1995 the fluorescent brightener, Blankophor BBH, did not cause a significant difference in the field performance of Af*M*NPV (Table 3). Combining the brightener with the pregelatinzed white flour/sucrose formulation in tank mixes improved its effect somewhat, although not significantly except on day 7. This was probably due to the fact that the formulation made the leaves more palatable, a situation that became apparent during an earlier study on cabbage (unpublished data). There was no significant difference in the field activity of in vivo- and in vitro-propagated virus.

In contrast to the approximate 66% retention of FB residues through a period of 4.5 mo on oak tree foliage reported by Webb et al. (1994), our studies indicate that after only 14 days FB residues on cotton foliage were only about 20% of the original amount applied. However, the improvement in percentage mortality of third- and fourth-instar gypsy moths in the field (Webb et al. 1994) was only 1.5-fold as opposed to the approximately 1700-fold enhancement in the laboratory reported by Shapiro and Robertson (1992). Also, Zou and Young (1996) reported high levels of enhancement in the laboratory ($\approx$5-fold) with 0.1% FB on second-instar *P. includens*, but when applied on soybean in the field, enhancement was much lower ($\approx$2-fold).

The dichotomy of these findings could be attributed to several factors: (1) the difficulty in providing adequate concentration of the brightener as well as coverage in the field, (2) the breakdown of virus and adjuvants depending on the chemical and physical characteristics of the plant species to which they are applied (i.e., soybean versus cotton) (McLeod et al. 1977, Young et al. 1977), and (3) in the case of applications to control forest pests, the reduction in loss of effective brighteners on the foliage may be provided by overstory protection. All these results point to the fact that, especially in the case of cotton, there is still much more research needed to improve the efficacy and persistence

TABLE 1. Nozzle Size, Duration and Volume of Spray, and M2R Residues for Cotton Applications with Tank Pressure Constant at 2.8 kg/cm^2, Five Points, CA, 1993.

Conejet nozzles (3)	Duration (seconds/plot)	Spray volume (L/ha)	M2R residues (μg/cm^2)	
			Calculated	Actual $\pm$ SEM
TX-SS8	20	1590	30	21.2 $\pm$ 2.2
TX-SS8	10	795	15	10.9 $\pm$ 0.9
TX-SS4	9	359	6	5.1 $\pm$ 0.5
TX-SS4	3	94	2	1.4 $\pm$ 0.1

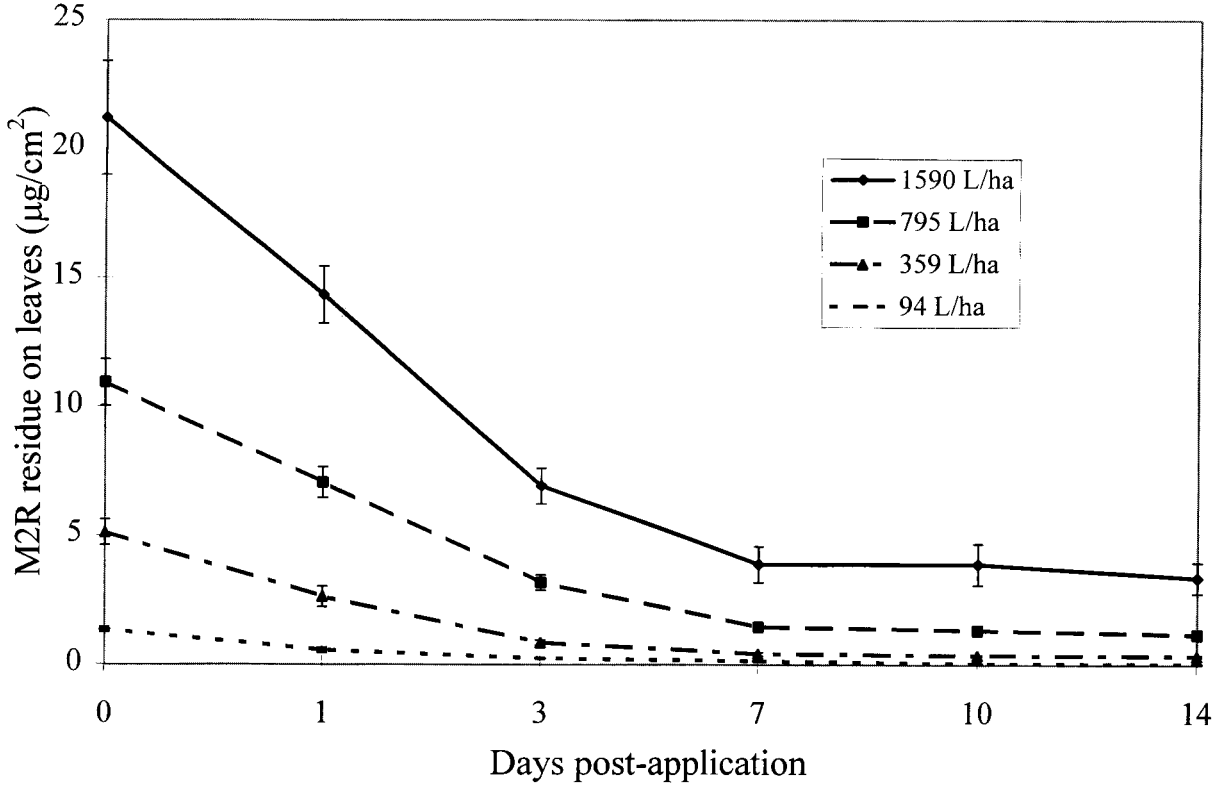

FIG. 1. Persistence of M2R on cotton leaves at given days post-application, Five Points, CA, 1993.

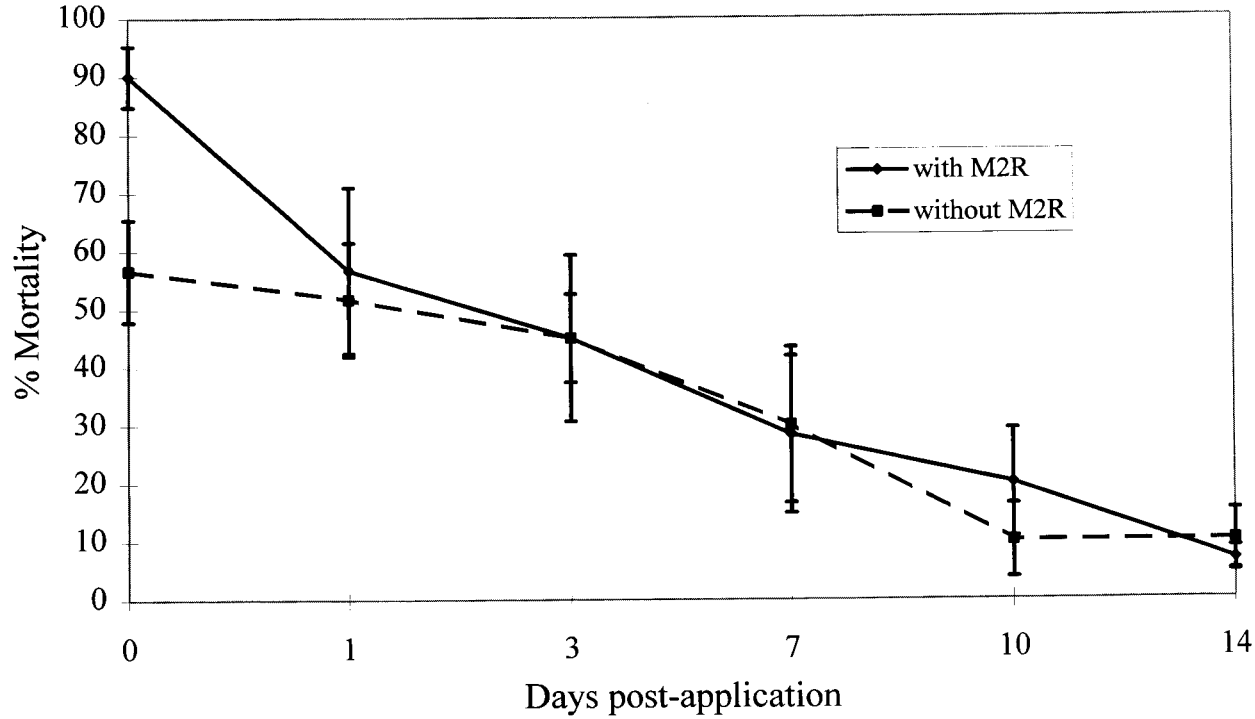

FIG. 2. The effect of M2R on persistence of Af*M*NPV as indicated by the % mortality of *H. zea*, Five Points, CA, 1993.

TABLE 2. Average Percentage Mortality of *Helicoverpa zea* Exposed as Neonatal Larvae to Cotton Leaves Treated with M2R with or without Virus at Given Days Post-Application, Five Points, CA, 1993.

Treatment($\mu g/cm^2$)	Percentage mortality ± SEM[a]					
With Af*M*NPV:	Day 0	Day 1	Day 3	Day 7	Day 10	Day 14
21.2	76.7 ± 5.6 abc	68.3 ± 7.0 a	62.9 ± 10.1 a	37.5 ± 10.3 a	23.3 ± 8.0 a	11.7 ± 4.0 a
10.9	86.7 ± 4.9 ab	76.7 ± 9.6 a	56.7 ± 11.2 a	30.0 ± 7.8 ab	20.0 ± 9.3 a	15.0 ± 5.6 a
5.1	90.0 ± 5.2 a	56.7 ± 14.3 a	45.0 ± 7.7 a	28.3 ± 13.5 ab	20.0 ± 9.3 a	6.7 ± 2.1 a
1.4	65.0 ± 6.7 bc	60.0 ± 9.3 a	50.7 ± 8.4 a	30.0 ± 9.3 ab	18.3 ± 6.0 a	6.7 ± 2.1 a
0	56.7 ± 8.8 c	51.7 ± 9.8 a	45.0 ± 14.3 a	30.0 ± 13.4 ab	10.0 ± 6.3 a	10.0 ± 5.2 a
Without Af*M*NPV:						
21.2	11.2 ± 7.0 d	0.0 ± 0 b	8.3 ± 3.1 b	0.0 ± 0.0 b	0.0 ± 0.0 a	8.3 ± 5.4 a
10.9	6.7 ± 2.1 d	0.0 ± 0 b	8.7 ± 3.4 b	6.7 ± 4.9 ab	0.0 ± 0.0 a	3.3 ± 3.3 a
5.1	13.3 ± 2.1 d	1.7 ± 1.7 b	7.0 ± 2.2 b	3.3 ± 2.1 ab	0.0 ± 0.0 a	6.7 ± 4.9 a
1.4	10.0 ± 3.7 d	0.0 ± 0 b	3.3 ± 2.1 b	1.9 ± 1.9 b	0.0 ± 0.0 a	5.0 ± 5.0 a
0	8.5 ± 4.8 d	1.7 ± 1.7 b	1.9 ± 1.9 b	0.0 ± 0.0 b	0.0 ± 0.0 a	4.2 ± 2.7 a

[a]Means followed by a common letter in the same column are not significantly different (Bonferroni *t*-test for mean separation.)

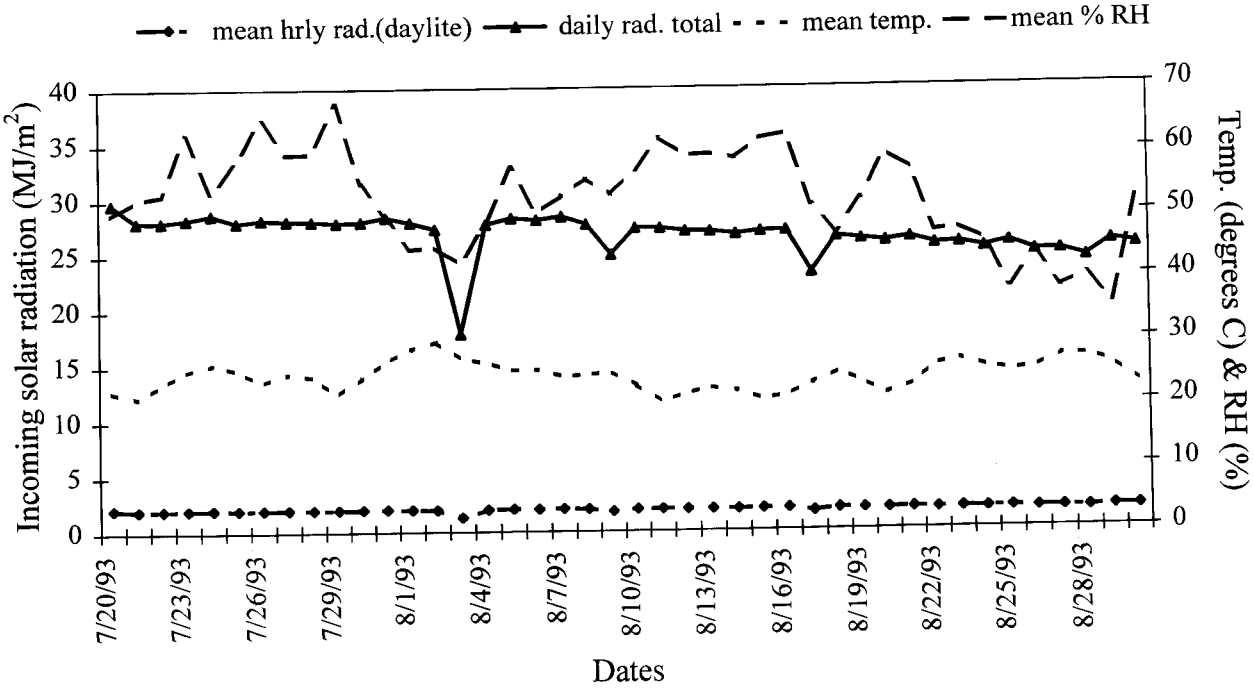

FIG. 3. Weather conditions during M2R persistence trials, Five Points, CA, 1993.

TABLE 3. Average Percentage Mortality of *Helicoverpa zea* Exposed to Cotton Leaves as Neonatal Larvae at Given Days Post-Application, Five Points, CA, 1995

Treatments	Percent mortality ± SEM[2]				
In vivo preparation:	Day 0	Day 3	Day 7	Day 10	Day 14
Alone	72.5 ± 11.1 a	42.5 ± 14.9 abc	12.5 ± 6.3 cd	20.0 ± 7.1 a	15.0 ± 8.7 a
+Blankophor	95.0 ± 5.0 a	40.0 ± 10.8 abc	32.5 ± 6.3 abcd	11.1 ± 4.5 a	22.5 ± 8.5 a
+Formulation 62191	100.0 ± 0.0 a	45.0 ± 10.4 abc	37.5 ± 13.8 abcd	26.4 ± 6.6 a	35.0 ± 15.0 a
+Blank. & Form.	92.5 ± 7.5 a	66.1 ± 11.2 ab	57.5 ± 12.5 ab	37.5 ± 15.5 a	25.0 ± 12.6 a
In vitro preparation:					
Alone	85.0 ± 6.5 a	32.5 ± 19.3 abc	20.0 ± 5.8 bcd	21.7 ± 4.6 a	10.0 ± 4.1 a
+Blankophor	90.0 ± 4.1 a	75.0 ± 21.8 a	45.0 ± 9.6 abc	26.9 ± 10.1 a	27.5 ± 11.1 a
+Formulation 62191	87.5 ± 7.5 a	85.0 ± 2.9 a	47.5 ± 10.3 abc	20.6 ± 8.9 a	17.5 ± 8.5 a
+Blank. & Form.	91.7 ± 8.3 a	77.5 ± 4.8 a	66.9 ± 9.2 a	32.2 ± 10.8 a	17.5 ± 7.5 a
Controls:					
Water blank	17.5 ± 6.3 b	2.5 ± 2.5 c	0.0 ± 0.0 d	5.3 ± 3.1 a	22.5 ± 13.2 a
Blankophor	7.5 ± 2.5 b	7.5.± 4.8 bc	2.5 ± 2.5 d	8.3 ± 2.8 a	12.5 ± 6.3 a
Formulation 62191	10.6 ± 4.6 b	2.8 ± 2.8 c	0.0 ± 0.0 d	5.0 ± 2.9 a	0.0 ± 0.0 a
Blank. & Form.	10.0 ± 4.1 b	2.5 ± 2.5 c	2.5 ± 2.5 d	13.6 ± 7.0 a	0.0 ± 0.0 a

[a]Means followed by a common letter in the same column are not signifantly different (Bonferroni *t*-test for mean separation).

of virus formulations to be used in the field.

ACKNOWLEDGMENT

We gratefully acknowledge the technical support of Freddie Cardenas, Shirley May and Vilay Lee. We are also grateful to M.R. McGuire of the USDA-ARS National Center for Agricultural Utilization Research for providing the flour/sucrose formulation; to the Keystone Aniline Corporation for providing Blankophor BBH; to biosys® for in vitro-produced occlusion bodies; and to Sandoz, Inc. for providing *H. zea* eggs.

Portions of the research were supported through a cooperative agreement with biosys®, now Thermo Trilogy.

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MANAGING THE ENTOMOPATHOGENIC FUNGUS *CONIDIOBOLUS THROMBOIDES*[1] IN RUSSIAN WHEAT APHID[2] CULTURES

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ABSTRACT

Six fungicides were evaluated in vitro for their efficacy in inhibiting vegetative growth and sporulation of the entomopathogenic fungus, *Conidiobolus thromboides* Drechsler, a contaminant in greenhouse cultures of the Russian wheat aphid, *Diuraphis noxia* (Kurdjumov). The fungicides copper oxychloride (Cupravit), mancozeb + oxadixyl (Sandofan), captab + metalaxyl (Apron C), bitertanol (Baycor), iprodione (Rovral Dip), and mancozeb (Sancozeb) at a rate of 0.1% active ingredient moderately to strongly inhibited *C. thromboides* (mean inhibition 81.1%). No true fungicidal activity was observed with any of the fungicides during the test period (7 d at 20°C). Amount of sporulation of the fungus varied among treatments, with no spores formed on the mancozeb + oxadixyl-treated medium, versus profuse sporulation on medium treated with bitertanol or iprodione. Because of strong inhibition of *C. thromboides* and relatively low cost, mancozeb at concentrations of 10.0, 2.0, 1.25, 0.5, 0.08, and 0.016% was further evaluated in vitro. The fungus growth cut-off point, midway between 1.25 and 2.0% mancozeb, was calculated, and a rate of 1.625% active ingredient per liter of water was used to treat greenhouses housing *C. thromboides*-infected Russian wheat aphid colonies. In addition, sanitary measures were followed diligently, which included fumigation of contaminated greenhouses with potassium permanganate + formaldehyde, construction of new rearing cages, and a change in the watering protocol. These curative and preventative measures were used successfully to decontaminate the Russian wheat aphid colonies.

INTRODUCTION

As an alternative to chemical insecticides, an integrated control program using resistant cultivars in combination with parasitoids, predators, and entomopathogenic fungi for the Russian wheat aphid, *Diuraphis noxia* (Kurdjumov) (Homoptera: Aphididae), is being developed at the Small Grain Institute of the South African Agricultural Research Council. Strains of the major aphid-pathogenic fungus (Humber 1991), *Conidiobolus thromboides* Drechsler (Zygomycetes: Entomophthorales), have been studied widely for potential control of aphids (Humber et al. 1977, Latgé and Papierok 1988). In this regard, *C. thromboides* is a beneficial organism.

Mention of a proprietary product does not constitute an endorsement or a recommendation for its use by the Small Grain Institute, the University of Natal, the USDA, or by the Texas A&M University System.

1 Zygomycetes: Entomophthorales.

2 Homoptera: Aphididae.

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Insect mass-rearing in the greenhouse and insectary often is challenged by both biotic and abiotic factors (King and Leppla 1984, Van Lenteren 1991). Insects reared in a greenhouse often are attacked by fungal pathogens because conditions such as high humidity, constant optimal temperatures, and artificial light promote disease development. Recently, a *C. thromboides* infection was detected in cultures of Russian wheat aphid maintained for use in the plant resistance and natural enemy programs at the Small Grain Institute. Despite scrupulous sanitation, reduction in humidity levels, and a regime of fluctuating temperatures, epizootics developed. The entire Russian wheat aphid stock cultures were destroyed by the fungus three different times. The continuation of several research programs aimed at developing new resistant wheat cultivars and the introduction of exotic parasitoids and predators required better management and/or elimination of fungal epizootics occurring in the greenhouse. The implementation of chemical control of the fungus was thus considered.

The objectives of the study were to (1) evaluate several chemical fungicides for their fungistatic/antibiotic (inhibition of germination of fungal spores/of vegetative growth) or fungicidal (kill of spores) activity on *C. thromboides* and (2) determine the concentration at which an effective, but inexpensive, fungicide, in combination with sanitary measures, should be applied for control of the fungus in the greenhouse.

MATERIALS AND METHODS

Details on the fungicides tested are given in Table 1. The isolate of *C. thromboides* used in the study originated from an infected Russian wheat aphid collected from a greenhouse at the Small Grain Institute. Cultures of *C. thromboides* were grown on wheat grain extract (WGE) (Srinivasan et al. 1964).

The fungicides at a rate of 0.1% active ingredient were tested for their effects on *C. thromboides*. The required quantity of each fungicide was added to 100 ml of sterilized WGE medium, cooled to 65°C in a water bath. WGE and fungicides were mixed on a magnetic stirrer for ≈5 min. Twenty-five ml of the mixtures then were poured into 9-cm diameter petri dishes. One fungal plug (4 × 4 × 2 mm) consisting only of mycelia was transferred to the center of each petri dish. Four replicated dishes of each treatment and control (nontreated WGE medium) were incubated at 20°C and photoperiod of 16:8 (L:D) h. Each treatment and control were incubated in isolation because fungicide volatiles could influence the fungal growth on other treatment or control dishes.

Vegetative (mycelial) radial growth was measured daily for 7 d. A millimetric ruler was used each day to measure along the same pre-marked radial line. Daily values were cumulated; the replicated values recorded for each treatment and the control were averaged (± SEM). The 7-d cumulative growth data were analyzed by one-way analysis of variance using SYSTAT software (Wilkinson et al. 1992), and means were separated using the Tukey Honestly Significant Difference (HSD) test. Percentage of difference at 7 d between the control and corrected amounts of fungal inhibition was calculated by using the equation: [(growth on nontreated medium - growth on fungicide-treated medium) / (growth on nontreated medium)] × 100.

The lid of each petri dish was inspected on day 7 for the presence of ballistospores (forcibly discharged entomophthoralean spores) as indicative of sporulation. Amount of sporulation was not quantified but estimated for intensity and classified as absent, sparse, dense, or profuse. Where no sporulation occurred after 7 d on fungicide-treated medium, the fungus was assessed for viability. Mycelial plugs (≈5 mm × 5 mm) were transferred from fungicide-treated medium to fresh, nontreated WGE medium (4 plugs were introduced into each of 3 replicated dishes). The dishes were incubated for 5 d at 20°C and photoperiod of 16:8 (L:D) h. The plugs then were examined for any sign of regrowth radially extending from each initial plug. The petri dish lid directly above each plug was checked for the presence of ballistospores. Regrowth and sporulation were indicative of a fungistatic rather than fungitoxic effect of a given fungicide on *C. thromboides*.

To identify fungal growth and sporulation cut-off points, one fungicide strongly inhibiting *C. thromboides* (Sancozeb; see Results) and widely available at low cost, was further tested in vitro at concentrations of 10.0, 2.0, 1.25, 0.5, 0.08, and 0.016% active ingredient (mancozeb) per 100 ml medium for its effects on the fungus. Methods and

TABLE 1. Details of the Fungicides Tested.

Common name (Trade name)	% active ingredient	Chemical name	Supplier
Copper oxychloride (Cupravit)	85	Basic cupric chloride	Bayer (Pty) Ltd. South Africa
Mancozeb + oxadixyl (Sandofan)	56 + 8	Manganese ethylenebisdithiocarbamate with zinc ion + 2-methoxy-N-(2-oxo-1,3-oxazolidin-3yl)acet-2',6'-xylidide	Sandoz Products Ltd. South Africa
Captab + metalaxyl (Apron C)	35 + 35	N-(trichloromethylthio)cyclohex-4-one-1,2-dicarboximide; 1,2,3,6-tetrahydro-N-(trichloromethylthio)phthalimide + Methyl N-(2-methoxyacetyl)-N-(2,6-xylyl)-DL-alininate	Ciba-Geigy (Pty) Ltd. South Africa
Bitertanol (Baycor)	30	β-([1,1'-biphenyl]-4-yloxy)-α-(1,1-dimethylethyl)-1H-1,2,4-triazole-1-ethanol	Bayer (Pty) Ltd. South Africa
Iprodione (Rovral Dip)	50	3-(3,5-Dichlorophenyl)-N-(1-methylethyl)2,4-dioxo-1-imidazolidinecarboxamide	Rhone-Poulenc Agrichem SA (Pty) Ltd. South Africa
Mancozeb (Sancozeb)	80	Manganese ethylenebisdithiocarbamate with zinc ion	Sanachem South Africa

analyses used were as described earlier.

Based on growth and sporulation cut-off points lying between 1.25 and 2.0% mancozeb (see Results), a 1.625% mancozeb solution (or 20 g Sancozeb) per liter of water was used, in combination with other curative and preventative measures, to decontaminate Russian wheat aphid rearing facilities.

Greenhouses contaminated with *C. thromboides* were washed and fumigated with potassium permanganate ($KMnO_4$) added to formaldehyde (4.3 liters formaldehyde + 1.8 kg $KMnO_4$ for treatment of 10,000 cubic feet). All cages were washed with the foaming detergent/sanitizer Asepto Z (a chlorinated alkaline with a concentration of 1.0-1.5%) [Klenzade SA (Pty) Ltd, South Africa]. Healthy aphids from the field were collected to start new colonies on a small scale before moving the aphids into the larger insectaries. As soon as Russian wheat aphids were introduced into the cages, a hand-held sprayer was used to spray Sancozeb at 20 g per liter of water onto the plants, aphids, and soil surface. At each aphid cycle (≈10 d) for 2 mo., the cages were washed again with Asepto Z, and the new plants, aphids, and soil were sprayed with Sancozeb. As a control, one greenhouse and the cages housing cultures of parasitoids were treated with all of the above except the fungicide treatment because of possible negative effects of the latter on the parasitoid wasps.

RESULTS AND DISCUSSION

The effects of fungicides at 0.1% rate of active ingredient on the development of *C. thromboides* on solid medium are shown in Fig. 1.

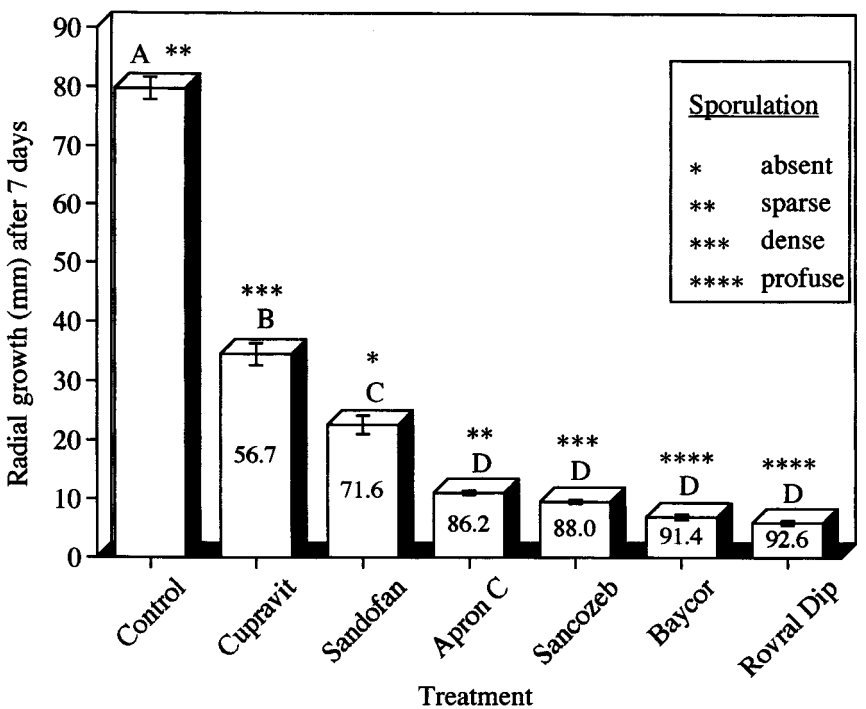

FIG. 1. Effect of 0.1% active ingredient of fungicides on radial growth (± SEM) and sporulation of *Conidiobolus thromboides* on solid medium. Letters above the bars represent separation of the means of radial growth (Tukey HSD test). Values inside the bars represent 7-d control-corrected percentages of inhibition of radial growth. Star symbols represent amount of sporulation.

All of the fungicides tested had some degree of fungistatis to *C. thromboides*, but no truly fungicidal activity was observed for any of the fungicides. There were significant differences in mycelial growth among the treatments ($F = 489.1$; df = 6, 21; $P < 0.001$).

Of the fungicides tested, both mancozeb + oxadixyl (Sandofan) and copper oxychloride (Cupravit) moderately inhibited *C. tromboides*, reducing mycelial growth by 71.6 and 56.7%, respectively. After 7 d of development, many spores were produced on the medium treated with copper oxychloride. No spores were produced on mancozeb + oxadixyl-treated medium. However, mycelial growth and sparse sporulation occurred after 5 d of subculturing *C. thromboides* on Sandofan-free medium. Captab + metalaxyl (Apron C), mancozeb (Sancozeb), bitertanol (Baycor), and iprodione (Rovral Dip) strongly inhibited growth of the fungus during the 7 d. The 7-d percentage of inhibition of vegetative growth by these four fungicides was >86%. Spore production at 7 d was sparse on the medium treated with captab + metalaxyl. However, as compared to the control, mancozeb, bitertanol, and iprodione enhanced the formation of spores.

Because of its strong inhibitory effect on *C. thromboides* (Fig. 1) and low costs , we further evaluated mancozeb at six different concentrations of active ingredient. The effect of these concentrations on fungal growth during 7 d is shown in Fig. 2.

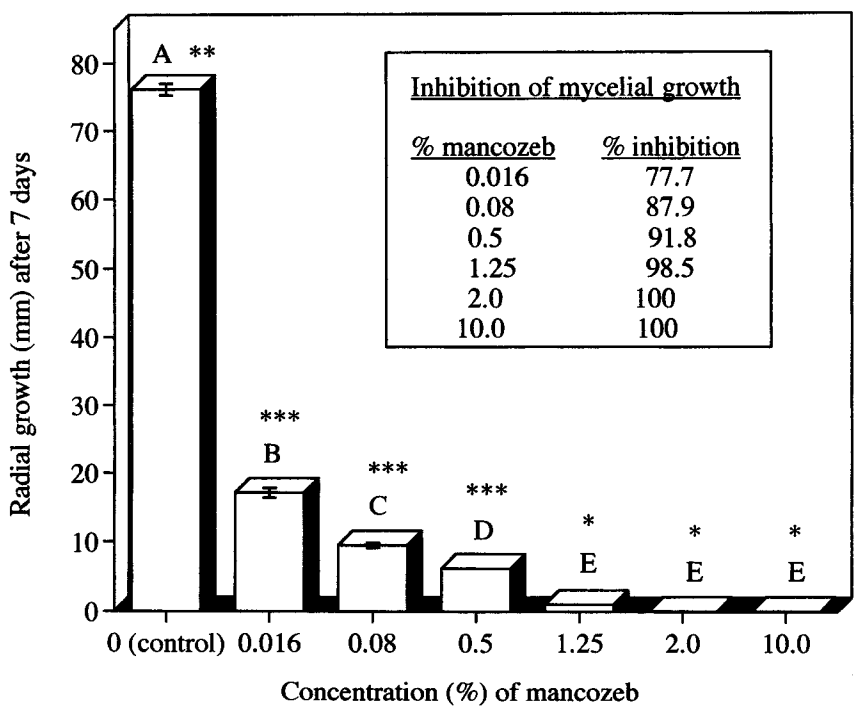

FIG. 2. Effect of different concentrations of mancozeb on radial growth (± SEM) and sporulation of *Conidiobolus thromboides* on solid medium. Letters above the bars represent separation of the means of radial growth (Tukey HSD test). Star symbols represent sporulation (* absent; ** sparse; *** dense).

There were significant differences in mycelial growth among the treatments ($F = 3,666$; df = 6, 21; $P < 0.001$). Mancozeb at 2.0 and 10.0% was fungicidal and completely inhibited growth and sporulation of *C. thromboides*. Mancozeb was moderately inhibitory at 0.016% and at 0.08 and 0.5% highly inhibitory of mycelial growth; however, sporulation was dense

at these three concentrations. Very limited mycelial growth and no sporulation occurred when mancozeb was used at a concentration of 1.25%. The fungus growth cut-off point, midway between 1.25 and 2.0% mancozeb, was calculated, and a rate of 1.625% mancozeb (20 g Sancozeb) per liter of water was used to treat fungus-contaminated greenhouses and rearing cages in the validation study.

No resurgence of the fungal problem occurred for 2 mo. after Sancozeb and sanitary measures were used to treat the *C. thromboides*-contaminated Russian wheat aphid cultures. Sancozeb had no acute or sublethal effects on Russian wheat aphid (data not shown). On the other hand, the fungal problem resurged in the greenhouse housing the parasitoid cultures when no Sancozeb was applied.

Our laboratory findings generally were consistent with previously published observations made in vitro. The effect in vitro of copper oxychloride and related copper-based inorganic compounds on entomopathogenic fungi is variable. For example, Soper et al. (1974) found copper hydroxide completely inhibited four species of Entomophthorales. Majchrowicz and Poprawski (1993) reported moderate-to-complete inhibitory action of copper oxychloride on *C. thromboides*, *Conidiobolus coronatus* (Costantin) Batko (Zygomycetes: Entomophthorales), and several species of entomopathogenic Hyphomycetes.

Fungicides such as bitertanol, based on triazoles, have been little studied with regard to their effects on entomopathogenic fungi. Hassan et al. (1991) reported that 0.0925% bitertanol was highly toxic to *Verticillium lecanii* (Zimmerman) Viégas, but harmless to *Beauveria bassiana* (Balsamo) Vuillemin (Hyphomycetes: Moniliales). Azoles were moderately harmful or harmful to the Hyphomycete *Beauveria brongniartii* (Saccardo) Petch (Coremans-Pelseneer et al. 1991, Coremans-Pelseneer 1994). Six species of hyphomycetous entomopathogenic fungi and *C. thromboides* usually were highly susceptible to the triazole triadimefon (Majchrowicz and Poprawski 1993).

In our study, the dithiocarbamate mancozeb (Sancozeb) at 0.1% strongly inhibited mycelial growth but not sporulation of *C. thromboides*. Most, if not all, of carbamic acid-derived fungicides such as iprodione (a dicarboximide) are slightly harmful or harmful to a wide range of entomopathogenic fungi (Nanne and Radcliffe 1971, Hassan et al. 1991, Majchrowicz and Poprawski 1993). Hall and Dunn (1959) showed that zineb at one-third the recommended rate exhibited various effects (from none to complete inhibition) on five entomophthoralean species pathogenic to the spotted alfalfa aphid, *Therioaphis maculata* (Buckton) (Homoptera: Aphididae). Majchrowicz and Mietkiewski (1976) reported the total incompatibility of mancozeb with entomophthoralean and hyphomycetous entomopathogenic fungi. Mancozeb had a fungicidal effect, and zineb and the combination of copper oxychloride + zineb greatly limited growth of entomopathogenic Hyphomycetes and Entomophthorales in laboratory tests (Machowicz-Stefaniak 1980, Majchrowicz and Poprawski 1993).

We found no published data on the effects of the combinations of mancozeb + oxadixyl, and captab (a dicarboximide) + metalaxyl on entomopathogenic fungi. The carbamates mancozeb and captab are discussed above, under Sancozeb. The oxadixyl (a phenylamide) component in Sandofan and the metalaxyl (an acylalanine) component in Apron C belong to the phenylamide group of fungicides. Metataxyl can adversely affect certain Entomophthorales (including *C. thromboides*) and Hyphomycetes, but also can enhance the activity of several other fungi (Majchrowicz and Poprawski 1993).

Entomopathogenic fungi usually are susceptible to fungicides (Roberts and Campbell 1977). Our data showed that the susceptibility of *C. thromboides* to fungicides varied between chemicals. Wilding and Brobyn (1980) suggested that under field conditions the action of fungicides on the infectivity of aphids by Entomophthorales might affect the germination of conidia. This hypothesis was supported by Delorme and Fritz (1981) for the foliar fungicides sulfur, maneb, and mancozeb. Indeed, these fungicides reacting with cell thiols inhibit the use of lipidic supplies and thus hinder spore germination (Fritz 1977). That there is so little known on the mode of action of fungicides on Entomophthorales and other Zygomycetes is because no members of these groups, except a few species of *Mucor*, are phytopathogens. No published supporting data could be found to suggest possible reasons for the variation in response of *C. thromboides* to the different fungicides. Such variations in sensitivity toward a particular fungicide in vitro were found by Olmert and Kenneth (1974) but not explained even among isolates of the same fungal species (*B. bassiana* and *V. lecanii*).

In addition to these cases where pesticides interfere with fungi, enhancement of activity of entomopathogenic and other fungi has been reported (Roberts and Campbell 1977, Majchrowicz and Poprawski 1993, Poprawski and Majchrowicz 1995). What may be occurring remains unexplained. Clearly, however, mancozeb (among other fungicides tested) inhibited elongation of vegetative hyphae but was not as effective against the elongation of conidiophores. We also may be seeing yet another aspect of the chemical differences between Zygomycetes and the 'higher' fungi (Ascomycetes, Deuteromycetes and Basidiomycetes), that some compounds that actively inhibit higher fungi either are ineffective or have modified activity among the Zygomycetes. An example of this is that nystatin has no apparent fungistatic activity against Zygomycetes, thus making it a suitable additive for a selective medium to suppress Hyphomycetes while allowing growth by the Entomophthorales.

We used Sancozeb in combination with other curative and preventative measures to decontaminate our Russian wheat aphid cultures. Despite fumigation, sanitation, and reduction in humidity levels, epizootics continued to develop in the control greenhouse. This indicated that the fungicide, not mere fumigation and sanitation, was responsible for control of *C. thromboides* in the validation study. We were able to resume mass-rearing Russian wheat aphid. The information presented here also can be used for other species of aphids.

ACKNOWLEDGMENT

We thank with appreciation Gary W. Elzen and Lawrence A. Lacey for their valuable suggestions in reviewing the manuscript.

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A NEW MECHANICAL METHOD FOR SAMPLING SELECTED BENEFICIAL AND PEST INSECTS ON CORN - THE CORN KISS

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ABSTRACT

A portable pneumatic insect collection device for aiding the sampling of resident insect populations on corn (*Zea mays* L.) plants was constructed by adding a modified outlet nozzle, a net support frame, and an insect collection net to a conventional engine-driven leaf blower. Insects on the leaf surfaces of individual plants being sampled with the device, designated the Corn KISS for Keep-It-Simple-Sampler, are dislodged by high speed air streams that carry them into the collection net. Field experiments indicated the efficacy of the Corn KISS for detecting the presence of corn rootworm adults on corn plants was approximately equal to that of a conventional visual plant inspection method and superior to that of a Berlese funnel detection method. Efficacies of the Corn KISS and Berlese funnel methods were not significantly different for detecting *Scymnus* spp. and other adult coccinelids, immature hemipters and chrysopids, and spiders; but the Berlese funnel method was significantly more effective than the Corn KISS for detecting both adult and immature *Orius* spp., immature coccinellids, and adult hemipters. In general, the effectiveness of the Corn KISS was equal or superior to the Berlese funnel method for field sampling of relatively large arthropods commonly found exposed on leaf surfaces; whereas, the Berlese funnel method was the more effective method for sampling smaller species that are usually found hidden in silks, leaf sheaths, and other protected areas on corn plants.

INTRODUCTION

Scientists with agricultural experiment stations in Kansas, Illinois, Indiana, Iowa, and Nebraska are currently conducting large scale research programs to evaluate a new adult-control strategy for areawide management of corn rootworms (*Diabrotica* spp.) (Calkins et al. 1997). The premise of the strategy is to kill adult beetles using semiochemical-based toxic baits before they oviposit the majority of their eggs, and thus, prevent economic infestations in corn (*Zea mays* L.) planted the following year. The center of corn rootworm areawide management research is the USDA-ARS National Grain Insects Research Laboratory (NGIRL) located at Brookings, SD. The USDA-ARS Areawide Pest Management Research Unit (APMRU) at College Station, TX, is supporting the research at NGIRL in several areas including development and evaluation of corn insect monitoring systems, and determination of detrimental effects of corn rootworm toxic baits on natural populations of beneficial insects. The APMRU research has been conducted primarily in corn production areas of Bell County, TX, that have historically had high infestation levels of Mexican corn rootworms (*Diabrotica virgifera zeae* Krysan and Smith). As a part of this research in 1997, the Corn Keep-It-Simple-Sampler (KISS) pneumatic insect collector described here was developed, and its performance evaluated in comparison to

that of a Berlese funnel method (Eastman 1980) and a visual plant inspection method for sampling populations of corn rootworm adults and other arthropods on corn plants.

MATERIALS AND METHODS

The Corn KISS (Fig. 1), which is a modified version of the row crop insect keep-it-simple sampler (KISS) described by Beerwinkle et al. (1997), was constructed by modifying a conventional engine-driven leaf blower (Echo, Model PB-210E) with addition of a sampling head that included a specially designed nozzle, an auxiliary handle, a plant guide, and a metal rod frame for supporting an insect collection net in front of the blower nozzle. The nozzle is designed to channel high speed air from the blower through three outlet orifices (center orifice $\approx$ 9.7 cm^2, side orifices $\approx$5.2 cm^2, Fig. 2) so that the air streams pass across the diamond shaped plant guide ($\approx$ 18-cm square) into the net (Fig. 3). The plant guide and net frame are constructed of 6.4-mm diameter steel rod in an integral unit that is attached at three points to the bottom of the nozzle (Fig. 2). The sampling head is attached to the blower with a single section of curved duct and is held in position with a rigid strut (6.4-mm steel rod) attached to the bottom of the blower housing.

In field use, the open side of the plant guide is fitted around the stalk at the base of a corn plant, and the sampling device is lifted as a unit to the top of the plant in a sweeping motion (Fig. 4). In this process, the plant guide channels the leaves and stalk of the corn plant into the high speed air streams passing between the nozzle outlets and the inlet of the net. The high speed air streams (center orifice $\approx$71.5 m/s, side orifices $\approx$ 60.4 m/s) dislodge insects from the corn plant surfaces and carry them into the collection net (30.5-cm dia. by 71.1-cm length, Cat. # 7212NA, BioQuip Products, Gardenia, CA.).

During the five-week period from 9 June to 10 July of the 1997 cropping season, the efficacy of the Corn KISS for detecting corn rootworm adults and other insects on field corn was compared to results obtained with a Berlese funnel method and a visual hand inspection method. Corn plants in nine fields located in Bell County were sampled weekly by the three methods. In each field on each sampling date, the Corn KISS was used to collect a composite sample from two plants in ten random locations. Each composite Corn KISS sample of collected insects and plant debris was placed in individual plastic bags and returned to the laboratory where insects were identified by visual inspection. On the corresponding sampling dates, ten samples, each comprised of two whole corn plants cut into manageable lengths and placed in large plastic garbage bags, were collected from each field and returned to the laboratory where they were placed in large Berlese funnels constructed with 38-L garbage cans (Fig. 5) for $\approx$ 48 hr. Heat generated by a 100-watt light bulb in the top of each Berlese funnel apparatus caused the plant material to dry and forced insects associated with the plant material to drop into receptacles containing an alcohol solution at the bottom of the funnel. Insects collected in the Berlese funnels were subsequently identified and counted by visual inspection. Similarly, on each sampling date, 40 individual plants at random locations in each field were sampled only for corn rootworm adults by visual whole-plant inspection.

Average numbers of rootworm adults detected per sampled corn plant on the same date in the same field by each of the three sampling methods were calculated, and the results obtained with the Corn KISS and Berlese funnel methods were each compared to the results obtained with the conventional visual sampling method by simple correlation analyses and linear models developed with regression analyses. In addition, the total numbers of corn rootworm adults and other insects of selected species collected by the Corn KISS and Berlese funnel methods, respectively, in the different fields on the same sampling dates were analyzed using ANOVA procedures to compare the results obtained by the two methods. Mean differences at significance levels of $P < 0.05$ were tested by the Least Significant Difference (LSD) method (Statgraphics,

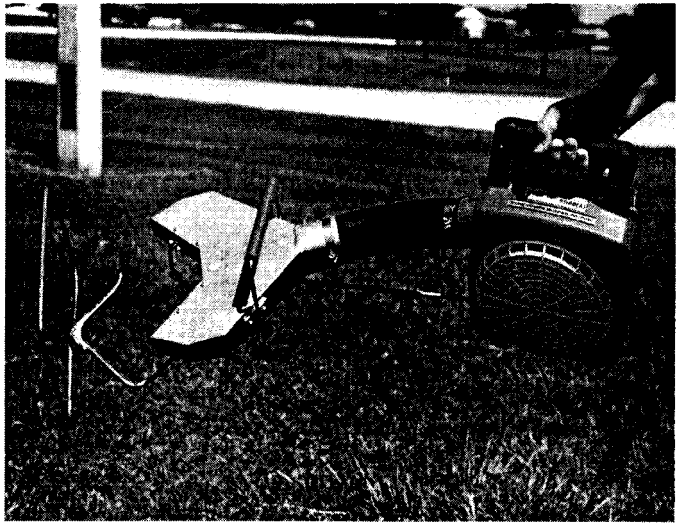

FIG. 1. Corn KISS constructed by modifying a leaf blower with the addition of a specially designed sampling head.

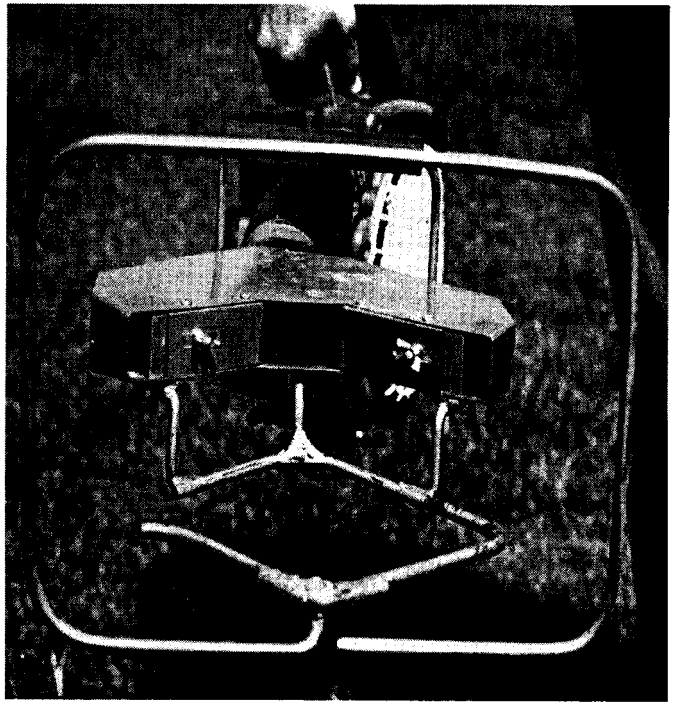

FIG. 2. Frontal view of Corn KISS outlet nozzle.

FIG. 3. Operator demonstrating operation of fully assembled Corn KISS sampler.

FIG. 4. Corn KISS in use sampling insect populations on individual corn plants.

Version 5, Statistical Graphics Corporation).

FIG. 5. Berlese funnels used to separate arthropods from plant debris.

RESULTS AND DISCUSSION

The efficacy of the Corn KISS method for detecting corn rootworm adults in field corn compared favorably with results obtained by visual plant inspection which is the conventional sampling method. Linear correlation between the paired sampling results obtained by the two methods over the course of the study was highly significant ($r = 0.80$; $P < 0.001$, Student's t-test; Fig. 6A), and the slope of the linear association model derived by regression analyses indicated a close relationship between the paired observations. In contrast, the correlation of the paired sampling results obtained for the Berlese funnel and visual sampling methods was not significant ($r = 0.24$, $P = 0.415$, Fig. 6B), indicating a relatively poor association between the paired results obtained by these two methods. These results indicated that the effectiveness of the Corn KISS method for sampling adult corn rootworm populations was approximately equal to that of the visual plant inspection method and superior to that of the Berlese funnel method.

The relative efficacies of the Corn KISS and Berlese funnel methods for detecting other arthropod species on corn plants are illustrated by the results of data analyses in Table 1. The efficacies of the two methods were not significantly different for detecting *Scymnus* spp. and other adult coccinelids, immature hemipters and chrysopids, and spiders; but the Berlese funnel method was significantly more effective than the Corn KISS for detecting both immature and adult *Orius* spp., immature coccinelids, and adult hemipters. These results indicate that the Corn KISS is the more effective method for sampling the more mobile, relatively large sized arthropods that are normally found exposed on plant surfaces; whereas, the Berlese funnel is the more effective method for sampling the smaller insects that are usually found hidden in silks, leaf sheaths, and other protected areas on corn plants.

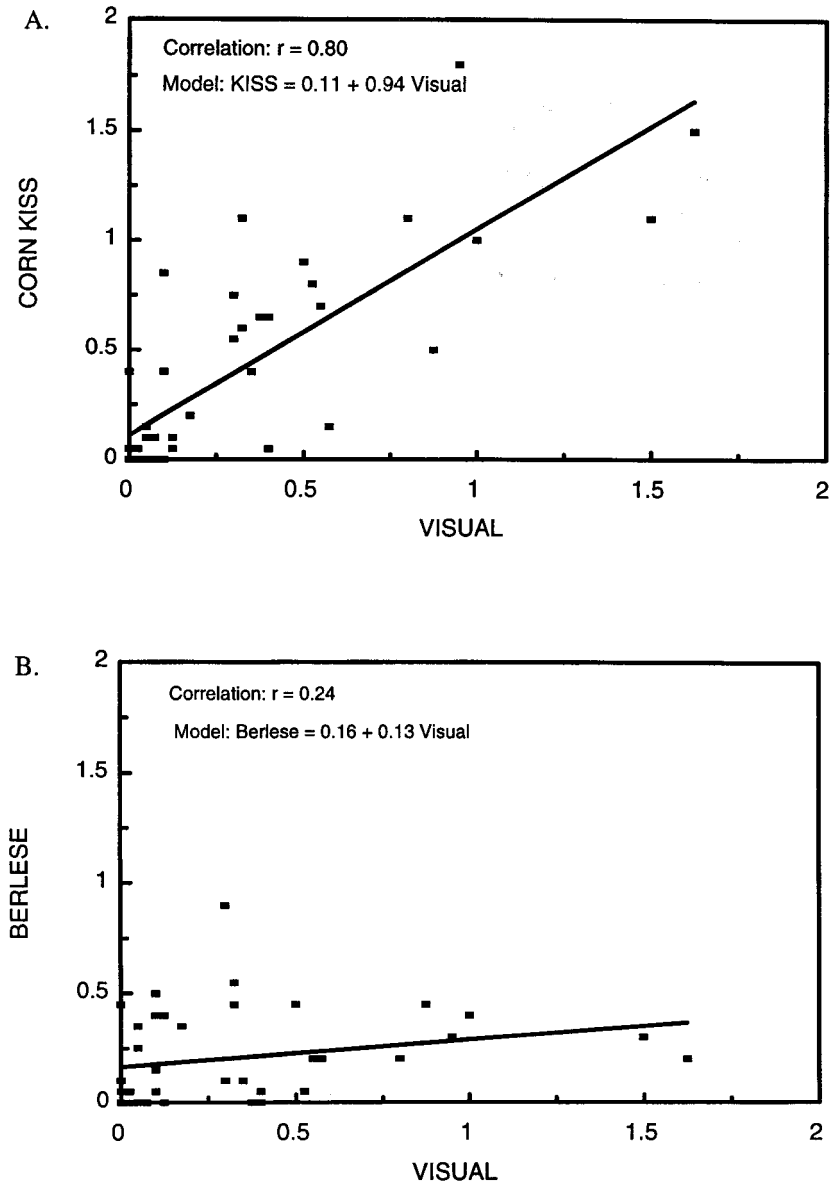

FIG. 6. Average numbers of corn rootworm adults detected per sampled corn plant in paired sampling comparisons between the Corn KISS (A.) and Berlese funnel methods, respectively, and the visual sampling method.

TABLE 1. Mean Numbers (± SE) of Selected Arthropods Collected per Field per Sampling Date by the Corn KISS and Berlese Funnel Sampling Methods, Respectively.[a]

Arthropods Collected	Corn KISS	Berlese Funnel
Corn rootworm spp.	6.9 ± 1.3 a[b]	3.7 ± 0.6 b
Scymnus spp.	3.1 ± 0.3 a	2.8 ± 0.4 a
Other coccinelids (adult)	0.6 ± 0.2 a	1.3 ± 0.6 a
(immature)	0.5 ± 0.1 a	2.1 ± 0.4 b
Orius spp. (adult)	1.1 ± 0.2 a	26.5 ± 2.5 b
(immature)	0.2 ± 0.1 a	38.5 ± 5.1 b
Other pred. hemipters (adult)	2.3 ± 0.3 a	8.7 ± 2.0 b
(immature)	0.8 ± 0,2 a	1.0 ± 0.3 a
Chrysopids (adult)	0.2 ± 0.1 a	0.0 ± 0.0 b
(immature)	0.5 ± 0.2 a	0.5 ± 0.1 a
Spiders	5.6 ± 0.5 a	6.2 ± 0.8 a

[a]A total of 20 plants in a each of nine fields was sampled by each method on each of five weekly sampling dates beginning when the corn plants were starting to tassel.
[b]Means in same row followed by different letters are significantly different (LSD test, $P \leq 0.05$)

Results of sampling comparison experiments demonstrated the Corn KISS portable pneumatic sampler to be an effective sampling aid for monitoring population trends of corn rootworm adults and other similar sized arthropods inhabiting corn plant leaf surfaces. Use of the device provides a method for fast, efficient sampling of large numbers of corn plants that minimizes human variation among samplers and provides specimens for positive identification of sampled arthropods. In addition, the Corn KISS is useful for collecting large numbers of corn rootworm adults at random locations in corn fields for purposes of determining colonization patterns and species identification, sex ratios, reproductive status, and other characteristics.

ACKNOWLEDGMENT

The authors acknowledge Charlie Jung, Steve Harp, Clint Mergele, and Bridget Killgore for their technical assistance.

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THE DIFFERENCES BETWEEN HORN FLY[1] DENSITIES ON CATTLE PASTURED IN WYOMING AND NEBRASKA AS POSSIBLY INFLUENCED BY ELEVATION

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ABSTRACT

Adult horn fly densities were determined at three elevations (800, 1800 and 2400 m) over two years using fly counts on cattle. In 1995, cattle at the 800 m elevation had the highest density of flies. In 1996, the greatest density of flies occurred on cattle at the 1800 m elevation. In both 1995 and 1996, the fewest flies were recorded on cattle at the 2400 m elevation, with densities below 90 flies per side. Based on published economic injury levels, control of horn flies at elevations above 2400 m may not be economically justifiable in Wyoming. Cattle grazed at elevations at or below 1800 m may not have horn fly populations large enough to merit control and therefore should be examined for fly densities before control measures are taken.

INTRODUCTION

The horn fly, *Haematobia irritans* (L.) (Diptera: Muscidae), is one of the most important pests of beef cattle in the United States (Bruce 1964). Its blood feeding habits and its occurrence in large populations cause decreased weight gains, feed efficiency and milk production (Byford et al. 1992).

Many researchers have attempted to determine the factors that influence levels of horn fly populations on cattle, including hair color (Ernst and Krafsur 1984, Schreiber and Campbell 1986), time of day (Schreiber and Campbell 1986), breed (Steelman et al. 1991, 1994) and age of cattle (Haufe 1986, Steelman et al 1993). These efforts have met with varying results. Studies investigating the relationship between elevation and horn fly density have not been reported in the literature.

Cattle production in the mountainous state of Wyoming occurs at a variety of elevations. If densities of horn flies are considerably lower at higher elevations, producers may be less likely to utilize prophylactic insecticide treatments, thereby reducing production costs and maintaining an insecticide susceptible population of horn flies. The objective of the study was to determine if differences existed in horn fly densities on cattle grazed at different elevations.

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MATERIALS AND METHODS

A study of adult horn fly densities on cattle was conducted during the summers of 1995 and 1996 at three locations at each of three elevations. The first elevation, 800 m (2,700 ft), was near North Platte, Nebraska. The second elevation, 1800 m (6,000 ft), was located near Cheyenne, Wyoming. The third elevation, 2400 m (8,000 ft), was near Albany, Wyoming in the Medicine Bow Mountains. Three locations which pastured heifers in 1995 were replaced in 1996 by locations with cow/calf herds.

Selection of cattle herds in this study was not based on animal breed or color. Ernst and Krafsur (1984) found no differences in horn fly densities due to host breed or color. In their study only 3.3% of the total variance in horn fly counts was attributable to the host animals, and 83% of the variance was attributed to the date of sampling. In this study, adult horn fly population densities on cattle were determined on three dates, in both years. Binoculars (7 x 50 mm) were used to count all visible horn flies on one side of up to 30 heifers or cows in each herd.

In each year, the first sampling was conducted at each location 10 to 14 days after adult horn flies were first observed on cattle. This resulted in staggered sampling dates during the summer. In 1995, adult horn flies were first sampled at the 800 m locations in mid June, at the 1800 m locations in early July and at the 2400 m locations in mid-late July. In 1996, adult horn flies were first sampled at the 800 and the 1800 m locations in the first week of July and at the 2400 m locations in the second week of July. In both years, the second and third sampling dates followed approximately one month after the previous sampling date.

The locations chosen for this study were considered to be fixed effects in all the analyses of variance because certain qualifications needed to be satisfied. These included: no insecticide use on the cattle, the cattle must be maintained at the same elevation for the duration of the study, and the animals must be grazed on unimproved native range pastures. The elevations used in this study were also considered to be fixed effects in the analyses of variance, as they were selected to represent specific zones at which cattle were pastured in Wyoming and Nebraska. A nested analysis of variance and a Tukey's multiple mean separation test between the three elevations at each sampling date were conducted on the horn fly data (GLM Procedure, SAS Institute Inc. 1992). In this analysis the variable, location, was nested within the elevation.

Because sufficient cow herds were not available in 1995, three herds of yearling heifers were used in that year's study. The variables, location and elevation, were considered to be fixed effects. A nested analyses of variance and a Tukey's multiple mean comparison using orthogonal contrasts was conducted to determine if a significant difference existed between adult horn fly densities on heifers and on cows sampled at an elevation and within a date (GLM Procedure, SAS Institute Inc. 1992). In this analysis the variable, location, was nested within the elevation.

RESULTS AND DISCUSSION

Monthly high temperatures and rainfall totals for 1995 and 1996 are presented for each elevation (Table 1). In both years, the highest average monthly maximum temperatures were recorded at 800 m followed by temperatures at 1800 m, with temperatures at 2400 m far lower. Season-long daily high and low temperatures for the summers of 1995 and 1996 at the three elevations are reported in Kaufman (1997).

The cow herds carried significantly more horn flies than did the heifer herds at every sampling date (Table 2). Based on these results, adult horn fly counts on heifers were eliminated from further analysis.

TABLE 1. Average High Temperatures and Total Rainfall for Three Elevations Sampled Over Two Years.

		Average High Temperature (°C)			Rainfall (cm)		
Year	Month	800 m[a]	1800 m[b]	2400 m[c]	800 m[a]	1800 m[b]	2400 m[c]
1995	Jun	25.8	24.0	18.8	8.86	10.11	5.16
	Jul	30.7	29.0	22.6	4.50	7.27	1.85
	Aug	33.4	31.0	22.8	1.40	2.45	1.09
	Sep[d]	---	27.0	21.4	---	7.97	5.18
1996	Jun	27.0	25.2	21.6	8.92	4.59	1.74
	Jul	28.1	27.5	24.4	15.77	7.95	3.44
	Aug	27.4	27.0	23.4	8.66	4.59	3.38
	Sep[d]	---	21.5	17.8	---	5.94	4.72

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[b]USDA-ARS High Plains Grasslands Research Station (Cheyenne, WY 82009).
[c]Centennial 1 N Station (National Climatic Data Center, Asheville, NC 28801). The Albany (2400 m) locations are approximately 8 miles from the Centennial weather station at the same elevation.
[d]September 1 - 15 reported.
--- no data collected.

In 1995, significantly more flies were found on cattle grazed at the 800 m elevation than on cattle at either the 1800 m or 2400 m elevations at the first sampling date (Table 3). No difference was found in horn fly densities on cattle between the 1800 m or the 2400 m elevations. No significant differences in horn fly densities were found at the 800 m and the 1800 m elevations at the second sampling date, but each had significantly greater horn fly densities than cattle at the 2400 m elevation. On the third sampling date, cattle at the 800 m elevation had significantly greater horn fly densities than cattle at the 1800 m elevation, which had significantly greater horn fly densities than cattle the 2400 m elevation.

Cattle at the 1800 m elevation were found to have significantly greater horn fly densities than cattle at either the 800 m or the 2400 m elevations at the first sampling date in 1996 (Table 3). However, no difference was found in horn fly densities on cattle between the 800 m or the 2400 m elevations. On the second sampling date, cattle grazed at the 800 m elevation had significantly greater horn fly densities than cattle at the 1800 m elevation, which had significantly greater horn fly densities than cattle grazed at the 2400 m elevation. No significant differences in horn fly densities were found between cattle grazed at the 800 m and the 1800 m elevations on the third sampling date, but each had a significantly greater fly densities than cattle at the 2400 m elevation.

In this study, factors other than the altitude at which cattle were grazed, undoubtedly influenced horn fly densities. Evaluation not only encompasses the height above the sea level, but also influences the biotic and abiotic factors that exist in such an ecosystem. These other factors include; latitude, longitude, climatological factors, vegetative influences, insect fauna of the dung pats, human alterations and others. The 800 m and 1800 m elevation locations are characterized by rolling plains and tablelands of moderate relief, and vegetation class is termed a shortgrass prairie (Bailey 1995). The 2400 m elevation locations are characterized by rugged glaciated mountains, with local relief of 900 to 2100 m, and striking vegetational zonation, controlled by a combination of altitude, latitude, direction of

TABLE 2. Comparison of 1995 Horn Fly Densities Between Yearling Heifers and Cows at the Same Elevation and Sampling Date.

Sampling Date[a]	Elevation (m)	Comparison[b]	N[c]	Mean No. Horn Flies	Mean Separation
1	800	Cow	20	74.35	a
		Heifer	59	41.11	b
		EMS = 765.60; F-value = 21.82			
	2400	Cow	55	38.80	a
		Heifer	30	13.53	b
		EMS = 765.60; F-value = 18.88			
2	800	Cow	30	162.67	a
		Heifer	60	71.61	b
		EMS = 3475.10; F-value = 47.71			
	2400	Cow	55	87.82	a
		Heifer	30	55.37	b
		EMS = 3475.10; F-value = 6.36			
3	800	Cow	30	139.00	a
		Heifer	60	64.57	b
		EMS = 1519.74; F-value = 72.91			
	2400	Cow	55	34.18	a
		Heifer	30	12.87	b
		EMS = 765.60; F-value = 6.50			

[a]800 m sampling date 1 = June 19, sampling date 2 = Aug. 1-4, sampling date 3 = Aug. 28-29; 2400 m sampling date 1 = July 20-25, sampling date 2 = Aug. 23, sampling date 3 = Sept. 13.

[b]Degrees of freedom = 1. Alpha = 0.05. 800 m cow herd size = 150 animals, heifer herd sizes = 72 and 48 animals; 2400 m cow herd sizes = 100 and 50 animals, heifer herd size = 200 animals.

[c]N = number of animals sampled.

TABLE 3. Adult Horn Fly Counts on Cattle Among Elevations and by Date for 1995 and 1996.

Sampling Date [a]	Elevation (m)[b]	1995 N[c]	1995 Mean No. Horn Flies[d]	1996 N[c]	1996 Mean No. Horn Flies[d]
1	800	20	74.35 a	90	51.61 b
	1800	88	32.71 b	60	85.68 a
	2400	55	38.80 b	79	47.18 b
1995: HSD = 13.72, DF = 157, n = 54, EMS = 908.16, F-value = 15.62, r = 3.346					
1996: HSD = 9.54, DF = 221, n = 76, EMS = 621.42, F-value = 47.21, r = 3.337					
2	800	30	162.67 a	90	108.80 b
	1800	90	154.74 a	90	126.19 a
	2400	55	87.81 b	79	72.53 c
1995: HSD = 30.68, DF = 169, n = 58, EMS = 4882.35, F-value = 18.4, r = 3.344					
1996: HSD = 14.55, DF = 250, n = 86, EMS = 1638.54, F-value = 42.85, r = 3.334					
3	800	30	139.00 a	60	135.60 a
	1800	90	87.84 b	90	155.17 a
	2400	55	34.18 c	88	36.78 b
1995: HSD = 20.15, DF = 169, n = 58, EMS = 2106.64, F-value = 53.50, r = 3.344					
1996: HSD = 19.66, DF = 230, n = 79, EMS = 2743.95, F-value = 126.17, r = 3.336					

[a]1995, 800 m cow herd size = 150 animals, heifer herd sizes = 72 and 48 animals; 1800 m cow herd sizes = 92, 100, and 800 animals, 2400 m cow herd sizes = 100 and 50 animals, heifer herd size = 200 animals; 1996, 800 m cow herd sizes = 150, 84, and 138 animals, 1800 m cow herd sizes = 92, 100, and 800 animals, 2400 m cow herd sizes = 50, 100 and 600 animals.

[b]1995, 800 m sampling date 1 = June 19, sampling date 2 = Aug. 1-4, sampling date 3 = Aug. 28-29; 1800 m sampling date 1 = July 5-6, sampling date 2 = Aug. 7, sampling date 3 = Sept. 8; 2400 m sampling date 1 = July 20-25, sampling date 2 = Aug. 23, sampling date 3 = Sept. 13; 1996, 800 m sampling date 1 = July 2-3, sampling date 2 = July 30-31, sampling date 3 = Aug. 13, 30; 1800 m sampling date 1 = July 5, sampling date 2 = Aug. 2, sampling date 3 = Aug. 28; 2400 m sampling date 1 = July 12, sampling date 2 = Aug. 9, sampling date 3 = Sept. 4.

[c]N = number of animals sampled.

[d]r = studentized 5% level for multiple range test, Alpha = 0.05.

prevailing winds and slope exposure. A more complete ecological description of the three areas sampled can be found in Bailey (1995).

Air temperatures in 1995 and 1996 at the 2400 m elevation did not surpass the maximum developmental threshold and activity temperature of 32.2 °C for horn flies reported by Bruce (1964). Temperatures at the 1800 m elevation exceeded the threshold 24 times in 1995 and 4 times in 1996, while temperatures at the 800 m elevation exceeded the threshold 40 times in 1995 and 7 times in 1996. These high temperatures at the 800 m elevation may be associated with the bimodal curve in Western Nebraska adult horn fly

populations reported by Campbell (1976), which was similar to those observed in Texas (Bruce 1964, Palmer and Bay 1984). Greenham (1972) and Palmer et al. (1981) found maximum temperatures in dung pats to be at least 10 °C higher than the maximum air temperature, while minimum temperatures in the pat were similar to minimum air temperatures. Horn fly larvae do not survive if subjected to more than 2 h daily of temperatures above 44 °C , a maximum of 34 °C air temperature (Palmer and Bay 1984) or 6 h at 42 °C (Palmer et al. 1981). Pupae do not survive if exposed to temperatures of 44 °C for 6 h (Palmer et al. 1981).

Adult horn fly populations at the 1800 m elevation were significantly greater than at the 800 m elevation on the second sampling date in 1995. The high temperatures observed in 1995 may have killed the horn fly larvae in Nebraska, thus slowing the growth rate of the adult population, resulting in a biomodal curve as reported by Campbell (1976). Temperature levels were not as high in 1996 as they were in 1995, which may account for the significantly larger horn fly levels observed at 800 m at the second sampling date in 1996.

At the second and third sampling date in both 1995 and 1996, significantly fewer horn flies were counted on cattle pastured at the 2400 m elevation compared to cattle grazing at the 800 m and the 1800 m elevations. These differences may reflect fewer season-long accumulated developmental degree days. Cool temperatures and other diapause-inducing signals also may have contributed to the reduced horn fly densities at the 2400 m elevation on the third sampling date. These observations suggest that temperature, as influenced by elevation, plays an important role in the size of horn fly populations. Bruce (1964) reported that the optimum conditions for adult horn fly activity are between the relatively narrow temperature limits of 26.7 and 32.2 °C. He also reported that at temperatures below 20 °C, the adult horn fly becomes sluggish and is inactive below 4.4 °C. Lysyk (1992) reported that nearly 100% of horn flies reared at less than 16 °C entered diapause, regardless of the photoperiod to which the parents were exposed.

Horn fly densities at the 2400 m elevation reached a maximum of 87 flies per side in 1995 and 73 flies per side in 1996. Densities at the 1800 m elevation reached a maximum of 155 flies per side in both 1995 and 1996, while densities at the 800 m elevation were 163 flies per side in 1995 and 136 flies per side in 1996. Haufe (1979), working in western Canada, suggested an economic injury level of ca. 250 horn flies per animal. Schreiber et al. (1987) concluded that an economic injury level for horn flies is at a density greater than 200 flies per animal. The horn fly counts in this study were conducted on only one side of the animal. If the numbers generated in this study are doubled, no control of horn flies would be needed for cattle maintained at elevations of 2400 m and above. Control of horn flies at the 1800 m and 2400 m elevations may be justified during the middle and/or end of the summer, if horn fly densities build to levels greater than 125 flies per side.

ACKNOWLEDGMENT

The authors thank D. W. Watson and D. A. Rutz for critical reviews of the manuscript. We thank M. A. Grubbs, N. R. Kumar, K. Davis for their help in the completion of this project.

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VARIATION IN BEET ARMYWORM SUSCEPTIBILITY AND PLANT RESISTANCE IN SELECTED BT COTTON VARIETIES

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ABSTRACT

The effects of plant nitrate levels, neonate diet, instar, and leaf developmental stage on expression of resistance to beet armyworm, *Spodoptera exigua*, (Hubner) (Lepidoptera: Noctuidae) were evaluated in three varieties of Bt cotton: Deltapine90B (DP90B), Deltapine35B (DP35B) and Deltapine33B (DP33B). High nitrogen levels were only associated with lower levels of beet armyworm mortality in one variety in one test. First-instar beet armyworm larvae fed DP90B from high nitrogen plots had 6% mortality, compared to 38% mortality for larvae fed DP90B from plots with only residual levels of nitrogen. Neonate feeding on artificial diet also reduced subsequent mortality on Bt cotton. Beet armyworms fed DP90B cotton leaves after 3-6 h on artificial diet had 7% mortality at 120 h compared to 38% mortality for beet armyworms fed DP90B throughout the test. Second instars are less susceptible than first instars, which are less susceptible than neonate larvae. Second-instar beet armyworm larvae had 0.1-4.0% mortality compared to 33-57% mortality of first-instar larvae fed DP33B. In another test, first instar larvae had only 7% mortality compared to 38% mortality for neonate larvae fed DP90B. Leaf developmental stage, approximately half grown vs. fully expanded, did not affect mortality. Variation in resistance was also found among varieties. DP90B produced lower levels of beet armyworm mortality than DP33B. While DP33B produced 33-57% mortality in first-instar larvae fed artificial diet as neonates, DP90B only produced significant mortality in larvae fed DP90B from egg hatch.

INTRODUCTION

Cotton plants, *Gossypium hirsutum* L., genetically modified to express endotoxin proteins from *Bacillus thuringiensis* Berliner var. *kurstaki* for control of lepidopteran pests were planted on extensive acreage for the first time in 1996. Available varieties have generally performed well but there were some notable exceptions. Multiple insecticide applications were made to Bt cotton varieties for bollworm, *Helicoverpa zea* (Boddie) (Lepidoptera Noctuidae), and other lepidopteran pests in seven southeastern states and the Brazos River bottom area in Texas (Hardee and Herzog 1997). In 1997, the southeastern portion of the Cotton Belt also experienced *H. zea* damage. Bollworm is approximately 10X less susceptible than tobacco budworm or pink bollworm (MacIntosh et al. 1990), and some damage was expected with high populations (Mahaffey et al. 1994). Field observations also suggested that lower levels of insect resistance might be

linked to highly stressed plants, high rates of nitrogen or highly vegetative plants (M. Bates, personal communication). Increased plant nitrate levels resulting from higher nitrogen rates have been associated with enhanced survival of insects (Schriber 1984). As the nitrogen content of the host plant increases, insect growth rates often improve (McNeil and Southwood 1978, Mattson 1980). Survival, reproduction and average population size also may increase with increased nitrogen (Onuf 1978, Prestidge 1982). However, enhanced nitrogen levels do not always improve larval growth rates (Auerbach and Strong 1981, Stark 1965). High rates of nitrogen also affect vegetative growth, and could indirectly affect resistance levels if vegetative plants have lower levels of expression.

Although all the Bt cotton varieties released are producing the same CryIa(c) protein, differences in performance should be expected due to differences in expression of resistance or differences in suitability of the host genotype that affect insect susceptibility to the toxin. These differences could be relatively stable, but could also vary, depending on specific developmental and environmental interactions with the host plant genotype.

The expression of resistance is not uncommonly affected by environment. Low light intensity and high relative humidity can reduce expression of resistance. (Tingey and Singh 1980). Temperature and soil fertility can also affect expression. Temperature, for example, has been correlated with allelochemical concentration and subsequent changes in feeding behavior of insects (Breen 1992). The host plant genotype can directly affect the level of allelochemical by affecting production. Host plant genotype can also affect relative levels of allelochemical indirectly. Rapid vegetative growth can reduce the concentration of allelochemical relative to plant biomass. The suitability of the host plant, determined by genotype, also can increase insects ability to tolerate toxins (Breen 1994).

Field performance problems, on the other hand, may be related to reductions in insect susceptibility. Diet and age (Firko and Hayes 1990, Shaver and Parrot 1970), for example, may decrease susceptibility of individual insects to toxins. Such effects have been apparently responsible for problems with field performance of Bt cotton infested with bollworm, *H. zea*. Eggs oviposited on flowers hatched into larvae that fed on bloom tags containing little CryIa(c) protein. After feeding on the bloom tag, the now older, less susceptible larvae were able to feed successfully and develop on plant parts that would have been toxic to younger larvae, thereby resulting in economic damage.

Bt cotton is highly toxic to tobacco budworm, *Heliothis virescens* (Fabricius) (Lepidoptera, Noctuidae), and pink bollworm, *Pectinophora gossypiella* (Saunders) (Lepidoptera, Gelichiidae) (Halcomb et al. 1996), and to a lesser degree, bollworm. Changes in expression of resistance may not be readily apparent in such insects. Beet armyworm, *Spodoptera exigua* (Hubner) (Lepidoptera, Noctuidae), was used for testing since it is less susceptible and thus more likely to be sensitive to changes in expression of resistance. Beet armyworm is not a primary target for Bt cotton; however, variation in insect susceptibility and plant resistance expression levels that are more evident in a less susceptible insect pest such as beet armyworm can provide insights into interactions that may also affect more susceptible insects, particularly bollworm.

The initial objective of this research was to evaluate the impact of high nitrogen rates on the expression of resistance in Bt cotton in the field. Three varieties of Bt cotton were included in the test to examine possible varietal differences in resistance, or in expression of resistance. The potential influence of beet armyworm susceptibility on performance of Bt cotton was also evaluated by examining the effect of diet and instar on mortality.

MATERIALS AND METHODS

The field test was conducted at the New Mexico State University Agricultural Science Center near Artesia, NM, on a Pima silt loam during 1997. Treatments included three varieties of Bt cotton and their recurrent parents, high and low nitrogen treatments, and a growth regulator treatment. The respective Bt and recurrent parent varieties were Deltapine33B (DP33B), Deltapine5415 (DP5415), Deltapine35B (DP35B), Deltapine5690 (DP5690), Deltapine90 (DP90), and Deltapine90B (DP90B).

Each nitrogen and growth regulator treatment was in a strip plot running through the length of the field in a completed randomized block design with six replicates. Varieties were placed in a split-plot across the main treatment strips. Each plot was 7.6 m long and 4.1 m wide (102-cm row spacing). Lorsban-15 was introduced in-furrow at planting on 9 May 1997. Conventional tillage practices for the area were followed.

Fertilizer was uniformly applied with ammonium phosphate to supply 25 kg P_2O_2 on 3 April 1997 before planting. The high nitrogen treatments were sidedressed with liquid 32-0-0 to supply 62.8 kg/ha nitrogen on 26 June and 28 kg/ha on 21 July. Mepiquat chloride (PIX®) was applied to the appropriate plots at 92 g/ha on 26 June 1997. The low nitrogen treatment plots received no additional nitrogen other than that supplied with monoammonium phosphate. The crop was watered according to GOSSYM-COMAX recommendations.

Cotton petioles from the youngest mature leaf were sampled weekly from each variety under the low and high nitrogen treatment beginning 30 June 1997. Petiole nitrate levels were determined using fresh sap for nitrate detection with a Cardy meter (Spectrum Technologies Inc; Plainfield, IL). Cardy meter values were confirmed by KCL extraction of dried petioles using an ion selective electrode.

Plots were spindle picked on 8 October and again on 22 October 1997. Yields were calculated by harvesting the center two rows of each plot. Cotton from each plot was then ginned to calculate lint weight of each plot using a 10-bladed sawtooth gin.

Plants for bioassays were selected from plots that had been confirmed as having appropriately high and low levels of nitrates. Leaves were bioassayed in the laboratory with lab-reared beet armyworm to determine differences in expression of resistance. Beet armyworms were reared on a standard wheat-germ casein diet. In all tests, one leaf was placed in a 237-ml paper cup with moistened filter paper. Leaves used were, unless otherwise noted, the first fully expanded leaf on the plant. Each cup was infested with 5-10 larvae /cup /treatment and each treatment was replicated a minimum of six times. Mortality was recorded at least every 48 h, until the test was terminated.

In Tests 1 and 2, the effect of high rates of nitrogen was tested using second-instar beet armyworm larvae. The first test included two Bt cotton varieties, DP33B and DP90B, and their respective recurrent parents, DP5415 and DP90B. In Test 2, DP35B and its recurrent parent were also tested. In Test 3, in addition to nitrogen, the effect of instar on beet armyworm susceptibility was examined by testing first- vs. second-instar larvae using one variety, DP33B. The effect of leaf development was also examined, by testing fully developed leaves vs. half-grown leaves. In the fourth test, the effect of diet and insect age on beet armyworm susceptibility were examined. Mortality of larvae feeding on Bt cotton from egg hatch was compared to those feeding on the recurrent parent and to those fed artificial diet prior to transfer to a DP90B leaf. Eggs were placed on leaves of DP90B, a Bt variety, and on leaves of the recurrent parent. After hatching, larvae were allowed to feed up to 6 h then transferred to fresh leaves of DP90B and

DP90. In the last treatment, larvae were transferred from artificial diet to DP90B leaves, as in previous tests. The remainder of the bioassay was similar to Tests 1-3.

Data were analyzed using SAS GLM (SAS Institute 1998). Beet armyworm mortality and lint yield per plot were correlated with the variables of interest: variety, Bt endotoxin, nitrogen, diet, and leaf size. Appropriate interactions were also tested for significance. Specific comparisons of means were tested with least square means and t tests.

RESULTS

In Test 1, mortality of beet armyworms was very low with 0.1-7% mortality of beet armyworms after 96 hours on the DP33B and DP90B. There were no significant differences in mortality due to variety ($P<0.46$), Bt endotoxin ($P<0.84$), or nitrogen ($P<0.84$) (Fig. 1).

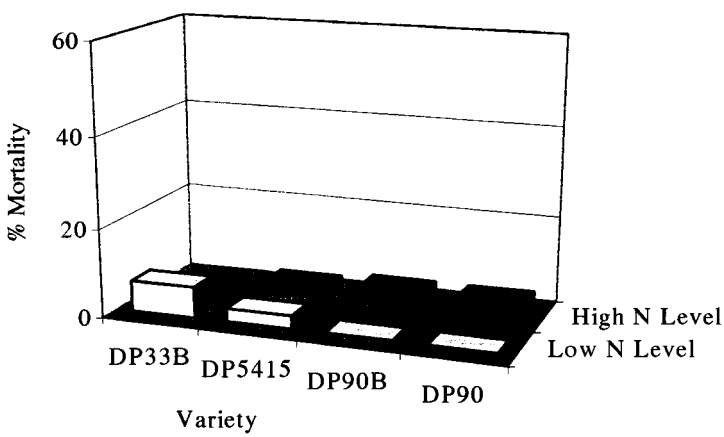

FIG 1. Effect of nitrogen levels on mortality of second-instar *S. exigua* fed Bt or recurrent parent cotton leaves.

As in the first test, in Test 2 there was no significant difference in mortality rates between nitrogen levels ($P<0.60$) of second-instar beet armyworm from 96 to 240 hours ($P<0.72$) after infestation (Table 1). There was significantly higher, albeit still low, mortality in the Bt varieties compared to the recurrent parents ($P<0.03$). The Bt varieties produced a mean 18.7% (0-62%) beet armyworm mortality compared to 6.2% (0.1-15%) mortality in the recurrent parents. Bt variety DP33B produced 27% mortality, significantly higher than DP35, with 6% mortality, or DP90B with 12% mortality ($P<0.03$).

TABLE 1. Effect of Nitrogen and Variety on Mortality of *S. exigua* after 96 Hours on Bt Varieties (DP33B, DP35B, DP90B) or their Recurrent Parents (DP5415, DP5690, DP90).

Nitrogen	Percent mortality of *S. exigua* after 96 hours feeding on					
	DP33B	DP5415	DP35B	DP5690	DP90B	DP90
Low	11.1	0.1	13.4	5.0	24.2	3.1
High	62.0	9.2	0.0	14.6	2.8	4.7

As a result of low mortality in the first two tests, possible differences in instar susceptibility larvae were evaluated in Test 3 by testing first-instar vs. second-instar beet armyworm using one variety, DP33B, and its recurrent parent. Leaf developmental stage and nitrogen effects were also tested. First-instar beet armyworm had significantly higher mortality than second instars, 42% and 1%, respectively, after 120 hours ($P<0.0008$) (Fig. 2). Leaf size and nitrate concentration had no effect on mortality ($P<0.69$, $P<0.63$). Beet armyworm first instars on fully developed leaves had 36% mortality vs. 48% mortality on half grown leaves. High nitrate leaves had a mean 45% mortality vs. 38% mortality in the low nitrate treatment.

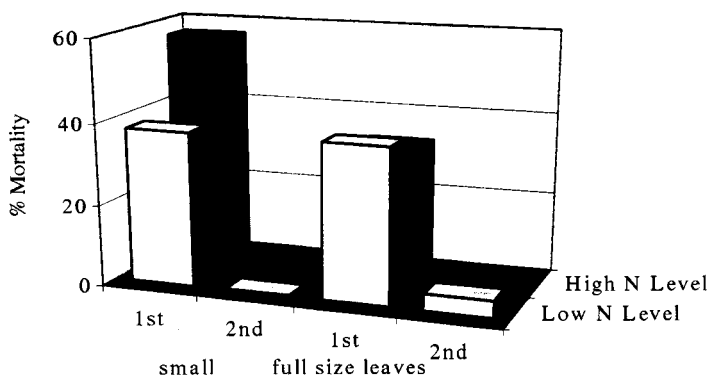

FIG 2. Effects of *S. exigua* instar, DP33B leaf size, and nitrogen on mortality.

In Test 4, beet armyworm neonate feeding on artificial diet for 3-6 h significantly increased survival after transfer to a Bt variety, DP90B ($P<0.004$) (Table 2). Mortality was also affected by a significant interaction between leaf nitrate concentration and neonate diet ($P<0.04$). Beet armyworm fed only DP90B had 6% mortality at high but 38% mortality at low nitrate concentrations, while those initially fed diet had 7% mortality at both high and low nitrate levels. Those fed DP90 alone also had similar mortality at low and high nitrate concentrations with 16 and 20% mortality, respectively.

TABLE 2. Effect of Neonate Diet on *S. exigua* susceptibility to Bt Cotton. Artificial Diet.

Nitrogen	Percent mortality of *S. exigua* fed initial / final diet		
	DP90B /DP90B	diet /DP90B	DP90/DP90
Low	38.5	7.1	16.3
High	6.0[a]	6.9	20.4

[a] Means within a column are significant at $P<0.004$

Yield was not significantly related to nitrogen treatment (F=1.0; df 1,109; $P<0.31$). Plots with one growth regulator application produced significantly higher yield (F=7.4; df 1,109; $P<0.0077$) with a mean yield 1064 kg/ha vs. 1005 kg/ha yield in plots without a growth regulator application. There was a significant interaction of Bt status by variety (F=36.4; df 2,109; $P<0.0001$). DP33B produced 951 kg/ha, a significantly 21% higher yield than its recurrent parent (t=4.1 $P<0.0001$) with 784 kg/ha. DP35B produced 1192 kg/ha, not significantly higher than the yield of its recurrent parent (t=1.9, $P<0.06$) with 1115 kg/ha. DP90B produced 1042 kg/ha, a significant decrease, and 7% less than the yield of its recurrent parent (t=2.1, $P<0.0395$) with 1124 kg/ha. Of the three Bt varieties, DP35B produced the highest yield followed by DP90B then DP33B (Table 3). Mean lint yields of DP33B, DP90B and DP35B were 951, 1192 and 1042 kg/ha, respectively.

TABLE 3. Mean Yield of Bt Cotton by Variety in Artesia, NM, 1997.

Variety	Bt	Yield (kg/ha)	Range	% Change [a]
DP33B	+	951	654-1192	+21[b]
DP5415	-	784	507-1052	
DP35B	+	1192	1027-1581	+7
DP5690	-	1115	909-1308	
DP90B	+	1042	745-1277	-7
DP90	-	1124	907-1371	

[a] Percent change compared to recurrent parent.
[b] Significant at $P<0.0001$

DISCUSSION

High plant nitrate levels may reduce insect resistance in Bt cotton. However, it is likely that nitrate levels will only reduce insect resistance under very particular conditions. High leaf nitrate concentrations did not reduce mortality of second-instar beet armyworm transferred from artificial diet to DP33B, DP35B, or DP90B or first-instar larvae transferred to DP33B. However in one test, in one variety, there was a dramatic difference between nitrogen treatments. Neonate larvae fed DP90B exclusively

had six-fold lower mortality in the high nitrogen treatment.

Very short exposure to a non-Bt diet can have a dramatic effect on the ability of beet armyworm to tolerate subsequent exposure. Beet armyworm neonate larvae fed artificial diet 6 h or less had, with 0-4% mortality, dramatically lower mortality compared to the 38% mortality of the control larvae fed only DP90B with low nitrogen levels.

Early feeding on non-Bt tissue for a very short period of time in the field is thought to affect the performance of Bt cotton. *H. zea* eggs deposited on flowers resulted in early instar larvae feeding on tissue with little to no CryIa(c) protein. Larvae can subsequently move to tissue with higher expression levels of the protein when they are older and less susceptible. Such examples illustrate the potential problems with using mixtures as a possible mechanism for managing resistance

Only neonate beet armyworm is consistently susceptible to Bt cotton. While second-instar beet armyworm had some significantly higher mortality on Bt varieties compared to their recurrent parent, the levels of mortality were very low. Differences in either baseline resistance or in expression of resistance will affect the level of control in neonate, first-, and possibly second-instar larvae. For example, first-instar beet armyworm were susceptible to DP33B but not DP90B. DP90B only produced significant mortality in neonate larvae, and then only with leaves at low nitrogen levels.

Variation in resistance among Bt varieties is a certainty. How much this will affect field performance remains unclear. DP33B had a higher level of insect resistance than DP90B. DP33B produced 27% mortality in second-instar larvae compared to 6% in DP90B. DP33B also produced 41% mortality in first-instar larvae while DP90B did not produce significant levels of mortality.

Such differences might not be evident in more susceptible insects such as pink bollworm and tobacco budworm; however, they may explain some of the problems with bollworm damage to Bt cotton. DP33B was the only variety that yielded, under low boll worm pressure, significantly more than its recurrent parent in the field. DP33B had yields 21% higher than its recurrent parent while DP90B yielded significantly less than its recurrent parent. Also, in a separate test, on a commercial farm, DP33B produced 13% higher yields than its recurrent parents while DP90B did not yield significantly more than its recurrent parent.

This lower level of resistance in DP90B may be a function of plant morphology. It has been suggested that highly vegetative, 'lush' plants may be associated with lower levels of performance in the field. DP90 is inherently a more vegetative variety. If so, management to minimize vegetative growth may increase the level of resistance of DP90B. On the other hand, highly vegetative plants are also known to attract higher numbers of bollworms, so at least part of the reason for lower field performance could be a function of higher populations in the DP90B.

The performance of Bt cotton can be influenced by a number of plant and insect interactions. The baseline level of resistance of a particular variety and the level of susceptibility of the insect pest will determine if such interactions actually affect performance in the field. A less susceptible insect, such as beet armyworm, is more likely to be affected by slight changes in expression of resistance or differences among varieties in baseline resistance. High nitrogen rates, for example, were associated with a reduction in resistance to beet armyworm, but only in one cultivar and only with neonate larvae. However, such effects will likely be additive with other interactions that affect expression of resistance.

ACKNOWLEDGMENT

This research was sponsored in part by funding from the New Mexico Agricultural Experiment Station and Cotton Incorporated. Technical assistance from Anna Flores, Lupe Carrasco and Manda Cattaneo is gratefully acknowledged.

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ELECTROANTENNOGRAM ACTIVITY AND ATTRACTION ASSAY OF *CERATITIS CAPITATA* TO AIRBORNE VOLATILES FROM PEACH AT THREE RIPENESS STAGES.

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ABSTRACT

Several components present in airborne volatiles produced by peaches at three stages of ripeness were identified. Some of these evolved from one ripeness stage to another. For example, ethanal (methyl phenyl) was detected in green fruits and became part of the ketone (metylphenyl) series of green ripe fruits; 3-ethyl-5-methyl benzyl alcohol detected in green ripe fruits became 4-isopropyl benzyl alcohol when these fruits ripened; 4-ethyl acetophenone appeared in all three stages. An increase in the concentration of esters was observed as ripeness developed. Electroantennogram studies using *Ceratitis capitata* (Wied.) males and females showed that the most active compounds were ethyl octanoate; naphthalene; 1,2,3,4-tetrahydronaphthalene; 4-ethyl acetophenone and ethyl ethyl benzoate. Males and females responded differently to 1,2,3,4¬tetrahydronaphthalene and 4-ethyl acetophenone. The entire airborne volatile fraction from peach gave a higher electroantennogram response than any of its individual components. These components gave a greater response when peaches were at the green-ripe stage. In all of the tests, female *C. capitata* individuals were more responsive than males. In attraction assays, female response was greater to those compounds that gave the highest signals in the electroantennogram studies with the exception of 4-ethyl acetophenone.

INTRODUCTION

The fruit fly, *Ceratitis capitata* (Wied.) is widely distributed in tropical and Mediterranean areas dedicated to fruit cultivation. Preferred host plants for the females are figs, peaches and apricots (Liquido et al. 1990). Control is currently achieved with the use phosphorated insecticides applied through air pulverization along with a food lure. The use of these toxic pesticides is rejected by many social sectors and has caused negative economic repercussions to producers. This situation justifies the growing interest in finding of new approaches for control of this pest.

Plant extracts and essential fruit oils have been described as attractants of fruit flies (Beroza and Green 1963, Fornasiero et al. 1969). The active ingredient has been attributed to α-copaene which elicits positive response in both sexes. Unfortunately this compound is found in small amounts in nature, and its chemical synthesis is complicated; therefore, its use for control purposes is not economically feasible (Jacobson et al. 1984, Teranishi et al. 1987, Warthen and McInnis 1989).

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Electroantennogram (EAG) studies of alcohols, esters, and saturated and non saturated aldehydes with chain lengths ranging from two to twelve carbon units that have been isolated from plants and vegetables have showed that those compounds with six carbon atoms elicit greater EAG responses in females flies than other compounds tested. (Guerin et al. 1983; Light et al. 1988, 1992). Additionally, volatile compounds emitted by some fruits have proven to be good attractants for fruit flies (Wong et al. 983, Nishida et al. 1985). In an earlier study (Hernandez et al. 1996), 26 compounds in fresh orange airborne volatiles were identified, and the activities of 13 of these compounds were evaluated by EAG using *C. capitata*. Three compounds elicited a greater response in females than in males. The objective of this study was to evaluate airborne volatiles from peaches (PAV) during three stage of ripeness by EAG on female and male *C. capitata* and by laboratory attraction assays with females of this same species.

MATERIALS AND METHODS

Peaches ('Katerine var.') were collected in Moncada (Valencia, Spain) during June and July 1992. Fruits were classified according to ripeness [i.e.,green, green-ripe (intermediate) and ripe]. Fruits were put into a sealed glass bottle through which air was circulated at 60 ml/min. The air at the outlet of the bottle was filtered by successive passage through 20% potassium permanganate, charcoal and Porapak Q. AV were collected on a stainless steel column (15 x 1cm) packed with 10mg Porapak Q (Jang et al. 1989). Fruits were changed every two days during a period of 15 days, except for the ripe fruit, which was changed daily. Volatiles that were trapped on Porapak Q were eluted by rinsing the resin with 20ml of dichloromethane followed by 20ml pentane in a soxhlet extractor for 12 h.

Airborne volatiles trapped on porapak Q were analyzed by GLC using a Perkin-Elmer Sigma 3B gas chromatograph system equipped with a flame ionization detector and HP 3396A recorder. The separation was performed on an HP Ultra 1 capillary column (crosslinked methyl silicon gum, 25m x 0.2mm x 0.33μm film thickness) that was initially equilibrated at 50°C, after which sample injection temperature was increased 4°C/min to 180°C and then by 10°C/min to 280°C; nitrogen was the carrier gas at a flow rate of 1.8 ml/min.

Fractions were analyzed by GLC-MS using a Hewlett Packard 5988A quadrupole with electronic impact at 70eV. In this system, an HP Ultra 2 capillary column (crosslinked 5% phenyl methyl silicon, 25m x 0.2 mm x 0.33 μm film thickness) was used. Chromatographic conditions were: initial temperature 50°C, followed by two temperature gradients of 4°C/min to 180°C and then 10°C/min to 280°C; helium was the carrier gas at a flow rate of 1.8 ml/min.

Identification was also verified by comparing Kovats index numbers, determined experimentally on the purified compounds, with those of standard compounds supplied by Aldrich Chemical Company Inc. (Steinheim, Germany), Sigma Products (Desenhofen, Germany), Fluka Chemie AG (Buchs, Switzerland), Alfa Products (Karlsruhe, Germany), Extrasynthese (Genay, France) and Merck-Schuchardt (Darmstadt, Germany) or those synthetized in our laboratory.

EAG activity assays were performed using a laboratory colony of *C. capitata* maintained at 26±1°C, 70±5% relative humidity and a 16L:8D photoperiod. Virgin adults, segregated according to sex, were fed a diet of sugar, hydrolyzed protein and water and used in the assays five to six days after emergence.

Each purified compound was assayed at six different dosages ranging from 10^{-2} to 10^{3} μg, whereas entire fractions were assayed at single doses of 2,000 μg. The methodology

used for EAG activity assay was that previously described by Hernandez et al. (1996). Dichloromethane (10 µl) was used as a control and 100 µg of hexanal as the standard. The response to each substance was converted to a value relative to that of hexanal according to the following formula:{[mV(test)-mV(control)] x 100}/[mV(hexanal)-mV(control)]. Each assay (one substance x six dosages) was repeated using five different male and female individuals. Air was passed for a 2 min interval between each test (Roelofs 1984, Light et al. 1988). The signal was recorded which a Tektronic TDS 310 oscilloscope coupled to an IBM PC-AT.

Attraction assays were performed according to Robacker and Hart (1984) using laboratory cages (30 x 30 x 30 cm). These cages were homogeneously illuminated using a 60 W lamp and maintained at a temperature of 26±2°C. One hundred virgin females were introduced to each cage 24 hours before the assay (adaptation period). Food was removed 30 min before the assay and water was continuously available during the experiment.

At the start of the assay, individual compounds were applied on 3 x 3cm filter papers placed in one extreme at the top of the cages. A second paper with the control substance was placed at the opposite extreme. The dosages to be assayed were determined for each substance based on data from the EAG assays (1,000 µg for all compounds except 100 µg for 4-ethylacetophenone). The number of flies that were attracted to the paper with the tested sample and control were counted every minute during a 15min period.

Statistical analysis was carried out using mixed analysis of variance (ANOVA) which accounted for the structure of the experiment (mixture of crossed and hierarchical factors) and also for the nature of factors (combination of fixed and random effects) (Kendall and Stuart 1976, Dixon 1992). In addition to simple effects, the applied statistical model also evaluated the following interactions: substance-sex, substance-concentration, concentration-sex, and substance-concentration-sex. Data from attraction assays were evaluated statistically using a student *t*- test.

RESULTS

Identified compounds in fresh peach airborne volatile at three ripeness stages are shown in Table 1. The most abundant compounds in green peaches were two isomers of ethyl acetophenone followed by the terpene 1-(10) aristolene. In green-ripe fruits, the most abundant compound was 1,2-diethylbenzene followed by 1,3 diethylbenzene and ethyl acetophenone. In ripe fruits, the most abundant compounds were the esters, ethyl octanoate and ethyl (Z)-4- decenoate. The only chemical we found in all three ripeness stages was 4-ethyl acetophenone; however, as the fruit ripened, the concentration of this substance decreased. Some of the compounds seemed to evolve from one ripeness stage to another. For example, ethanal (methyl phenyl) in the green fruits appeared to be transformed into the ketones series (methyl phenyl) in the green-ripe fruits, and 3-ethyl-5-methyl benzyl alcohol in green-ripe fruits appeared to become 4-isopropyl benzyl alcohol in ripe fruits. As a general trend, an increase in the concentration of esters was observed as ripeness advanced.

Fig.1 shows the responses obtained by EAG assays of the 12 identified compounds at six concentrations with both females and males of *C. capitata*. For many of the studied compounds, the response was greater as the dosage increased (1,000µg). One exception was 4-ethyl acetophenone which elicited a maximum response at a dosage of 100 µg. For some compounds, the antennae apparently became saturated before the highest dosage was reached. This occurred at 100 µg in the case of the 4-hydroxy-4-methyl-2-pentanone (females), 4-ethyl acetophenone (males), 1,3,5-triethylbenzene (males), ethyl-ethyl benzoate (both sexes),1,2, diethylbenzene (males) and γ-decalactone (males); at 10 µg for 4-isopropyl

benzyl alcohol (males) and γ-decalactone (females); and at 1μg for 1,4-diacetyl benzene (males) and 4-hydroxy-4-methyl-2-pentanone (males).

TABLE 1. Compounds Identified in Fresh Peach Airborne Volatiles.

SUBSTANCE	RT[a] (min)	Ripeness[b] stages	Area (%)	Kovats Index[c]	
				Lit.	Exp.
4-Hydroxy-4-methyl-2 pentanone	4.7	G	5.6		970
1,2-Diethyl benzene	6.2	GR	20.4	1010[I]	1023
1,3-Diethyl benzene	6.3	GR	10.8	1013[I]	1029
Ethyl styrene	6.4	GR	2.4		1033
(Methyl phenyl) ethanal	9.1	G	8.8		1114
1 (Methyl phenyl)-1-propanone	9.2	GR	2.5		1118
1,2,3,4-Tetrahydronaphthalene	10.0	GR	3.1	1140[I]	1144
1-(Methyl phenyl)-1-butanone	10.4	GR	1.0		1157
Naphthalene	10.9	GR	3.5	1157[II]	1162
Ethyl octanoate	11.0	R	38.5	1180[II]	1171
1,3,5-Triethyl benzene	11.7	GR	0.7	1163[I]	1191
4-isopropyl benzyl alcohol	12.1	R	0.6	1182[I]	1199
3-ethyl-5-methyl benzyl alcohol	12.6	GR	7.3		1219
Ethyl acetophenone	12.6	G	14.7		1210
Ethyl acetophenone	12.4	R	2.8		1210
4-Ethyl acetophenone	13.1	G	9.4	1242[I]	1220
4-Ethyl acetophenone	13.4	GR	10.2		1245
4-Ethyl acetophenone	13.0	R	1.2		1228
1,4-Diacetyl benzene	14.3	G	4.0	1214[I]	1230
2-Ethyl ethyl benzoate	14.4	GR	1.1	1294[I]	1272
Ethyl (Z)-4-decenoate	17.0	R	20.7	1361[II]	1345
1-(10)Aristolene	17.6	G	9.2		1370
Pyrazine	17.9	R	2.4		1372
γ-Decalactone	18.9	R	3.0	1422[I]	1408
$C_{17}H_{36}$	27.2	R	2.8		1672

[a] Retention time on an Ultra 1 analytical column 25 m x 0.2 mm x 0.33 μm film thickness (initial T 50°C; ΔT 4°C min^{-1} to 180°C and 10°C min^{-1} to 280°C. N_2 was the carrier gas at 1.8 mL min^{-1}). Flame ionization detector.

[b] G, Identified in green peaches. GR, Identified in green-ripe peaches. R, Identified in ripe peaches.

[c] Exp., Experimental; Lit., Literature; [(I)] Calculated with an authentic standard; [(II)] Takeoka et al., (1992).

The evaluated interaction between compound and concentration (P x C) indicated a difference in the effect of concentration depending on the chemical. The increase in the

response at higher concentrations was greatest for ethyl octanoate followed by ethyl-ethyl benzoate. The significant interaction between compound and sex (PxS) was due to the high response of naphthalene by males and 4-ethyl acetophenone and 1,2,3,4-tetrahydro naphthalene by females. There were no significant differences between male and female responses to the standard (7.8±0.8 mV).

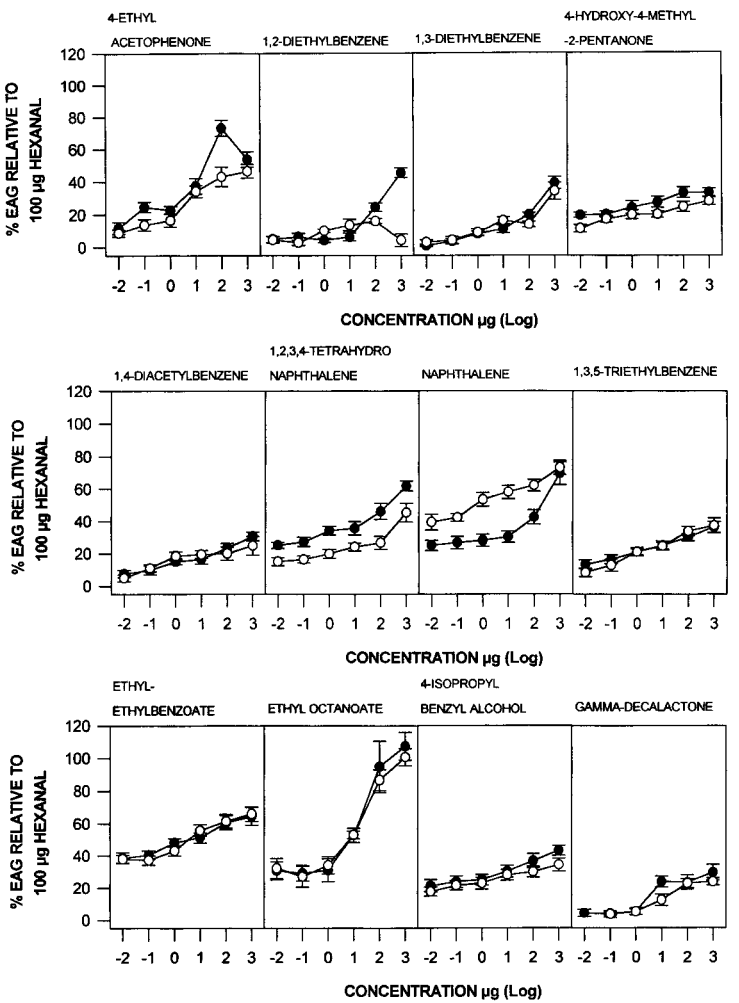

FIG. 1. Dose-response curves of EAGs for *C. capitata* females (●) and males (○) at serial dilutions (from 10^{-2} to 10^{-3} µg) of substances in fresh peach airborne volatiles. Vertical lines show the ± S. D. (n=5). The 100% response approximately corresponds to 8mV.

Table 2 summarizes the ANOVA results. The significance of the PxC and PxS interactions showed that differences in EAG responses among the different compounds depended as much on concentration as they did on sex of the fly.

TABLE 2. Analysis of Variance for EAG Data.

V.S.	S.S.	D.F.	M.S.	F-ratio
P_i	145 915.67	11	13 265.06	71.16[b]
C_j	98 257.68	5	19 651.54	413.28[b]
S_k	671.83	1	671.83	3.6
$F_{l(ij)}$	17 895.98	96	186.42	3.92[b]
$(P \times C)_{ij}$	35 928.64	55	653.25	13.74[b]
$(P \times S)_{ik}$	9 387.29	11	853.39	4.58[b]
$(C \times S)_{jk}$	991.07	5	198.21	4.17[b]
$(P \times C \times S)_{ijk}$	3 159.41	55	57.44	1.21
$(C \times F)_{jl(ik)}$	22 825.71	480	47.55	-
TOTAL	335 033.28	719		

[b] Significance level = 0,01

V.S., Variation source; S.S., Square sum; D.F., Degrees of freedom; M.S., Mean square; P, Product; C, Concentration; S, Sex; F, Fly

Fig. 2 shows the responses of males and females to the entire peach airborne volatile fraction obtained at the three ripeness stages. Females were more sensitive than males to the three extracts. The intermediate green-ripe stage elicited the highest EAG response.

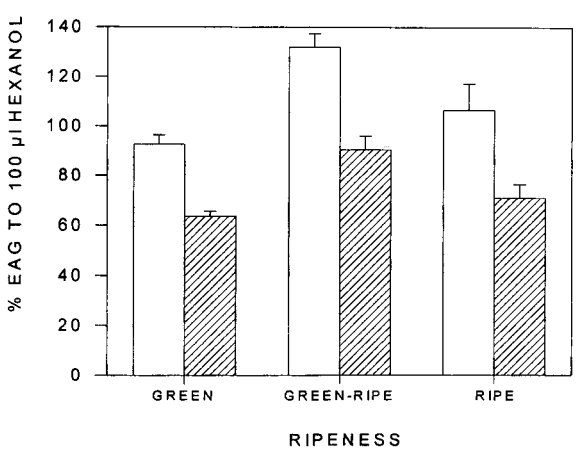

FIG.2. Mean EAG optimal responses of *C. capitata* females (▭) and males (▨) from 2,000 µg of entire peach airborne volatiles fraction obtained at three ripeness stages. mV.

Of the 12 compounds studied, only naphthalene, 1,2,3,4-tetrahydro naphthalene, ethyl-ethyl benzoate and ethyl octanoate were attractive in the assays (Fig. 3). For many of the chemicals tested, responses were very low initially but increased with time (6-10min). Ethyl octanoate was the most effective substance in attracting females. For these compounds, the attraction effect decreased after 12 min; however, three showed a light recovery after 14 minutes. The activity at the final point of this test was the same for these three compounds. Ethyl octonoate, in spite of showing an irregular behavior, maintained its attraction effect until the end of the assay period.

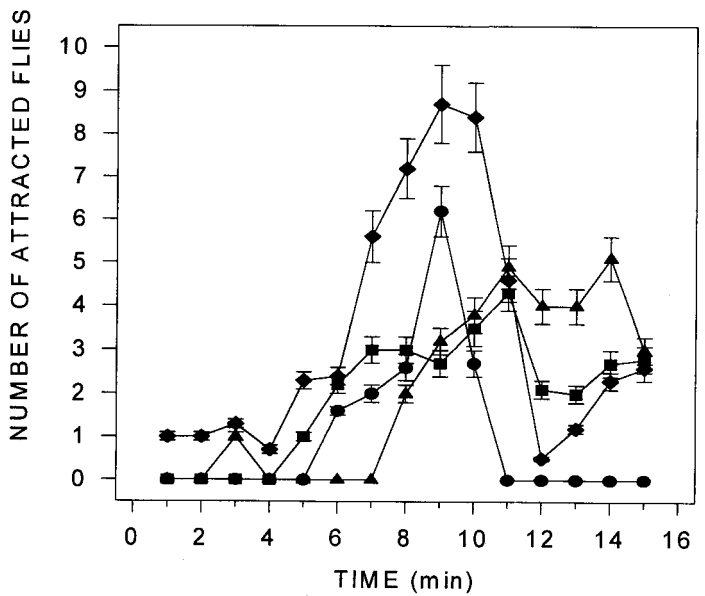

FIG. 3. Mean response of laboratory attraction assays using *C. capitata* females. Compounds tested were naphthalene (●), tetrahydronaphthalene (■), ethyl ethyl benzoate (▲), and ethyl ocatanoate (◆) each at a dosage of 1,000μg.

DISCUSSION

None of the five compounds (4-hydroxy-4-methyl-2-pentanone, methyl phenyl ethanal, ethyl acetophenone, 1(10)-aristolene, 1-4-diacetyl benzene) isolated in the first ripeness stage of peaches has been reported previously from this fruit; however, there are reports of these compounds in other vegetable products. For example, 4-hydroxy-4-methyl-2-pentanone has been identified in oil of *Osmanthus fragans* and *Chimonanthus praecox* L. (Knudsen et al. 1993), ethyl acetophenone in the oils of *Ophrus insectifera* L. and *O. speculum* Link. (Knudsen et al. 1993) and 1-(10) aristolene in oils of *Aristolochia longa* (De Pascual Teresa et al. 1983, Connolly and Hill 1991).

The compounds 1,2 diethyl benzene; 1,3 diethyl benzene; ethyl styrene; methyl phenyl propanone; 1,2,3,4 tetrahydronaphthalene; methyl-phenyl butanone; naphthalene; 1,3,5 triethyl benzene; 3 ethyl-5-methyl benzyl alcohol; 4-ethyl acetophenone; and 2-ethyl-ethyl benzoate were identified in the second ripeness stage. Spencer et al. (1978) and Sumitani et al. (1994) identified naphthalene and some of its derivatives and the 1,3,5-triethyl benzene in essential oil extracted from peaches. Ethyl styrene was described in *Rosa chinensis* and *Helianthus annuus* L. (Knudsen et al. 1993). Some compounds previously described in the essential oil from peach have structures similar to ones that we have identified in this study; this was the case for 1,2,3,4-tetrahydro-1,1,6-trimethylnaphthalene which was identified instead of 1,2,3,4-tetrahydronaphthalene (Kemp et al. 1971), benzyl alcohol appeared instead of 3-ethyl-5-methyl benzyl alcohol (Horvat et al 1990), ethyl benzoate instead of 2-ethyl-ethyl benzoate (Sevennats and Jennings 1966, Do et al. 1969) and acetophenone instead of ethyl acetophenone (Sumitani et al. 1994).

In ripe peaches, we identified eight compounds, four of which have been previously described. Ethyl octanoate and γ-decalactone have been reported from *Cuminum cyminum* and *Eucalyptus bakeri* (Ahmed and Vishwakarma 1988), and ethyl (Z)-4-decenoate in the essential oil of nectarines (Takeoka et al. 1992). Jang et al. (1989) have reported a compound, ethyl (E)-3-decenoate, similar to ethyl (Z)-4-decenoate, in emissions from males of *C. capitata*. The remaining four compounds are reported for the first time in peach airborne volatiles in this study. Females in the EAG tests responded stongly to 4-ethyl acetophenone and 1,2,3,4-tetrahydronaphthalene.

When the results of the EAG assays are compared to those in the attraction tests, the compounds that were more attractive to females (1,2,3,4-tetrahydronaphthalene, ethyl ethyl benzoate, naphthalene and ethyl octanoate) corresponded to those that were more effective in the EAG tests. Ethyl octanoate has a structure similar to (E)-3-ethyl octenoate, a compound that was identified as a pheromone emitted by males of *C. capitata* (Jang and Light 1996). In this study, naphthalene and its derivatives demonstrated good attraction qualities. Teranishi et al. (1987) also reported that some derivatives of naphthalene were attractive to this species.

In the EAG assays, we showed that the entire PAV fraction elicited higher responses than any of its individual compounds. The results obtained here also show that the intermediate ripeness stage elicited higher responses than the other two stages. Additionally, female response was stronger than that of males. In previous research, we found similar results for orange volatiles (Hernandez et al 1996). Therefore, we conclude that *C. capitatas* females select peaches that are in their intermediate ripe stage for oviposition since the antennae posses more receptors than do males for these volatiles (Levinson et al. 1987, Light et al. 1988).

ACKNOWLEDGMENT

The authors wish to thank P. Esteban at the Bioelectronics Department of the U.P.V. for developing the electroantennograph, Ph D. Gerardo Alvarez manilla for the critical review of this manuscript. Ph. S. Ballach at the Statistical Department of the UPV for assistance statistical managment as well as the Instituto Valenciano de Estudios e Investigación for its financial assistance.

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PERSPECTIVE

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A PROPOSED RATING SYSTEM FOR ESTIMATING THE WINTER SURVIVAL POTENTIAL OF BOLL WEEVILS [1]

D. R. Rummel, S. C. Carroll, and M. D. Arnold

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Early researchers recognized that the boll weevil survived cold, host-free periods in a state of comparative if not complete inactivity (Sanderson 1907, Hinds and Yothers 1909, Newell and Dougherty 1910, Hunter and Hinds 1904, Fenton and Dunnam 1927). However, Brazzel and Newsom (1959) in their landmark publication, "Diapause in *Anthonomus grandis* Boh." were the first to describe diapause in this insect. These authors stated that diapause in the boll weevil was characterized by cessation of gametogenesis, atrophy of gonads, increase in fat content, decrease in water content, and decrease in respiratory rate.

Brazzel and Newsom (1959) reported that they were able to separate boll weevils collected from the field during the late fall period into two distinct groups based upon the amount of fat accumulated. Some individuals had marked hypertrophy of the fat body and neither the digestive tract nor the tracheae could be seen through the dorsum after the removal of the elytra and hind wings. Others had accumulated little fat, and parts of the digestive tract and the tracheae were clearly visible after removal of the wings. Striking differences in the reproductive status of the two groups also were revealed when they were dissected. The lean weevils were reproductively developed while the gonads in fat individuals showed marked regression.

Descriptions of classic reproductive and diapausing male and female boll weevils were presented by Brazzel and Newsom (1959). Testes of lean, reproducing males were white, opaque at the center and translucent around the edges. They were distinctly lobed, fluffy in appearance, and ranged from 0.7 to 1.3 mm in diameter. Testes of fat, diapausing males were yellow in color, indistinctly lobed, filled with fat globules, dense in appearance, and ranged from 0.4 to 0.6 mm in diameter. Reproductive females were lean with ovaries ranging from 2.0 to 6.0 mm or more in length and all stages of oogenesis were evident. In contrast, ovaries of fat, diapausing females ranged from 1.2 to 2.0 mm in length and showed no evidence of oogenesis.

The work of Brazzel and Newsom (1959) demonstrated that only boll weevils in diapause were capable of surviving cold, host-free winters. Therefore, entomologists have long used the Brazzel-Newsom method of determining diapause in order to estimate the overwintering potential of this insect. Slosser et al. (1996) stated that a primary constraint to verifying diapause status with the Brazzel-Newsom method was that the dissection process was tedious thereby limiting sample size, and the insect had to be killed, precluding its use for further study. In addition, the method is rather subjective and different observers may sometimes reach different conclusions. However, long experience has shown that the Brazzel-Newsom method is quite accurate when practiced by an experienced observer.

Extensive development of the fat body and reduced reproductive organs are the basic criteria for identifying diapausing boll weevils (Brazzel and Newsom 1959). However, the actual role of large fat body reserves in the boll weevil diapause phenomenon is not entirely clear. It is generally considered that a feeding period of approximately 2 weeks is required for a boll weevil to accumulate adequate fat body reserves to survive the winter (J. R. Brazzel,

[1] Coleoptera: Curculionidae

personal communication). Long experience with feral boll weevils used in overwintering survival and spring emergence studies (Rummel and Carroll 1983, Carroll and Rummel 1985, Carroll et al. 1993) indicate that Brazzel's estimate of the prehibernation feeding requirement is quite reasonable. However, studies conducted by Mitchell and Taft (1966) indicated that boll weevils needed very little fat reserve to survive the winter. These authors suggested that the accumulation of large fat reserves by diapausing boll weevils was coincidental to the occurrence of diapause and occurred primarily because diapausing weevils continued to feed at a time when their rate of metabolism was considerably reduced.

Rankin et al. (1994) documented a direct relationship between the amount of fat body reserve and the propensity of adult boll weevils in the Lower Rio Grande Valley of Texas to engage in long-duration flight, but found no relationship between the size of the fat body and ovarian development. These authors suggested that large fat body reserves per se were unreliable as a criterion for diapause. This suggestion is supported by the fact that boll weevils exhibiting both oogenesis and hypertrophy of the fat body are sometimes found in field populations during the fall. One explanation of this phenomenon is that such individuals are in the process of absorbing the eggs and entering diapause. In studies conducted in central Texas, Walker (1967) reported that during the fall period there was apparently some reversion of diapausing (fat) female boll weevils to reproductive status. However, work by Palmer and Cate (1992) showed that even limited reproductive output by female boll weevils precluded winter survival. The findings of Palmer and Cate (1992) support the suggestion of Rankin et al. (1994) that accumulation of extensive fat reserves alone is not a reliable indicator of diapause in the boll weevil.

Mitchell and Taft (1966) noted that in studies of boll weevil diapause, it was common for researchers to classify individual weevils with atrophied reproductive organs as being in "intermediate" or "firm" diapause depending upon the amount of accumulated fat. Generally, very fat weevils meeting the classic characteristics of diapause as described by Brazzel and Newsom (1959) were considered as being in "firm" diapause while those with lesser amounts of fat were placed in the "intermediate" diapause category. However, Lloyd and Merkl (1961) placed some female boll weevils containing eggs in the intermediate diapause category if the ovaries were reduced in size and some fat was present. Walker and Brazzel (1959) stated that samples of adult boll weevils collected from cotton in the fall could be divided into three categories: a) reproductive individuals, b) weevils in the "intermediate" stages of diapause with little fat accumulation, and c) diapausing individuals with greater levels of accumulated fat. These authors stated that weevils which survived the winter would be individuals from category "c" that had fed sufficiently to be in "firm" diapause.

Mitchell and Taft (1966) questioned the proposition that only weevils in "firm" diapause were capable of surviving the winter. They pointed out that Brazzel and Newsom (1959) found that while diapausing boll weevils apparently used little of their fat reserves during the winter hibernation period, "intermediate fat" weevils were found in overwintering sites throughout the winter. Studies conducted by Mitchell and Taft (1966) indicated that a sizable percentage of boll weevils classified as being in "intermediate" diapause successfully survived the 1963-1964 winter in Mississippi. These authors concluded that the atrophied condition of the reproductive system was a better indicator of diapause in late season boll weevils than the amount of accumulated fat.

The objective of the present study was to develop a rating system which would allow reasonable estimates of the winter survival potential of diapausing boll weevils. This system should prove useful in evaluating boll weevil suppression or eradication programs.

Proposed Rating System. A proposed rating system for estimating the winter survival potential of diapausing boll weevils is shown in Table 1. This system is designed to allow an observer to estimate the winter survival potential of different groups of weevils which might be tested during the fall period. In this system, diapause is determined by the method described by Brazzel and Newsom (1959). However, the "intermediate" diapause category is not considered. The proposed rating system presents two basic categories of boll weevils; diapausing and reproductive. While fat content is not considered as the major determinate of the diapause condition, it is considered the major indicator of winter survivability for diapausing weevils. The amount of fat present in diapausing individuals is dependent upon the quality and quantity of food, and the feeding time available. Thus, when faced with a reduced food supply in the fall, especially when mostly large, hard bolls are available, many diapausing boll weevils may enter overwintering habitat with reduced body fat content.

Boll weevils which fully meet the characteristics described as "firm" diapause (Brazzel and Newsom 1959, Walker and Brazzel 1959) are considered as Category 1 diapausing individuals with the highest probability of winter survival. Individuals with intermediate fat content are placed in diapause Category 2 rather than being considered as being in "intermediate" diapause. Category 2 individuals should exhibit reduced winter survival potential and a trend toward earlier spring emergence compared to Category 1 weevils. Category 3 individuals are diapausing weevils with light to very light fat body content. These individuals have poor winter survival potential, with those that do survive emerging earlier than Category 1 and 2 individuals. Category 4 is reserved for reproductive weevils which are considered to have no chance of winter survival.

TABLE 1. A Proposed Rating System for Estimating the Winter Survival Potential of Boll Weevils.

Category 1. Specimen examined fully meets the classic characteristics of diapause as described by Brazzel and Newsom (1959). This type specimen has previously been described as "firm" diapause by some authors. Boll weevils in this category have a high to very high winter survival potential.

Category 2. Specimen examined meets the classic characteristics of diapause, except fat content is approximately 1/4 to 1/2 that found in Category 1 specimens. However, fat is firm and formed into well defined globules. Other than having less fat, this specimen looks like Category 1 individuals. Specimens in this category are considered as "intermediate" diapause by some authors. Category 2 individuals are expected to have good winter survival potential, but they will probably exhibit greater mortality under severe winter conditions than those in Category 1. Surviving weevils may emerge from winter habitat earlier than Category 1 weevils.

Category 3. Specimen exhibits atrophied reproductive system with no indication of reproductive activity. Body fat content is very light to light. Light fat body may be formed into well defined fat globules, but also may be soft, stringy and indistinctly formed. Category 3 individuals have very poor survival potential. Under mild winter conditions, some individuals will survive long periods but most will emerge well in advance of Category 1 and 2 weevils.

Category 4. Specimen is reproductive. Most individuals in this category are typical reproductive males and females as described by Brazzel and Newsom (1959). Some fat may be present, but fat content is light. Occasionally, intermediate types such as fat females containing eggs are found. However, these individuals also are considered as reproductive. Individuals in this category are assigned a zero winter survival rating.

Supporting Evidence. The study of Mitchell and Taft (1966) demonstrated that diapausing boll weevils with intermediate amounts of fat survived the winter in Mississippi as well as weevils collected from surface woods litter in mid- and late November. Therefore, the findings of these authors support the rejection of the term, "intermediate" diapause.

Slosser et al. (1996) used a cold bath device to study the survival of diapausing and nondiapausing boll weevils by subjecting them to freezing temperatures for different periods of time. In these studies, diapausing weevils were allowed to emerge from late season, field collected squares and maintained under diapause inducing conditions in the laboratory. These weevils were allowed to feed for several weeks before testing. Nondiapausing boll weevils used in the test were obtained from a laboratory colony which was inoculated with feral boll weevils annually. Prior to testing, samples were taken from all test cohorts and dissected to determine diapause and/or reproductive status. Test weevils were classified as Category 1, 2, 3 or 4 as shown in Table 1. Slosser et al. (1996) reported that the percentage of test weevils classified as Category 1 was similar to the percentages of weevils which survived exposure to -10° C for 6 hours. They suggested that this finding might mean that an exposure of 6 hours

to a temperature of -10° C discriminated against all diapausing boll weevils except those with a high fat content (i.e., those typically referred to as being in "firm" diapause). Although there was no actual experimental verification that it was, in fact, Category 1 boll weevils which survived this temperature regime, this finding does lend indirect support to the validity of the proposed rating system.

Studies conducted in 1992-1993 to determine the effects of various food sources on the winter survival and spring-summer emergence pattern of boll weevils (S.C. Carroll, D.R. Rummel, T. W. Fuchs, J. E. Slosser, L.T. Wilson, unpublished data) lend additional support for the proposed rating system. In these studies, boll weevils were reared from field collected squares, (Tom Green Co., TX) and held in the laboratory under diapause inducing conditions (Slosser et al. 1996). Weevils were divided into four groups with each group fed one of the following diets: 1) artificial diet (Sterling and Adkisson 1966), 2) field collected squares, 3) field collected small bolls (≤ 2.54 cm diameter), and 4) field collected large bolls (> 2.54 cm diameter). After 3 weeks of feeding, the test weevils were released into overwintering cages on 6 November, 1992. Prior to release, samples were randomly selected from each group and dissected to determine diapause/reproductive status. After dissection, weevils were classified as reproductive, or as diapause Category 1, 2 or 3 as shown in Table 1.

In a late season cohort in this test, weevils fed on large, field collected bolls had a noted reduction in body fat compared to the other feeding categories. In this group, 90.0% of the weevils were placed in Category 3, and the remaining 10.0% in Category 2. All of the weevils fed on artificial diet were classified as having maximum body fat and placed in Category 1. Eighty nine percent of the weevils fed on squares were placed in Category 1, and the remaining 11.0% in Category 2. Of the weevils fed on small bolls, 67.0, 22.0, 6.0, and 5.0% were placed in Categories 1, 2, 3, and 4 respectively.

The winter of 1992-1993 was quite mild and survival of all boll weevil test groups was high. However, the survival of weevils fed on large bolls (9.50%) was significantly less than that of weevils in the other three groups (artificial diet - 71.3%, squares - 50.0%, small bolls - 57.7%). Thus, significantly lower total winter survival was recorded for the group of weevils which had the lowest accumulation of body fat (90.0% of individuals in Category 3).

Differences in the spring-summer emergence patterns were evident among the various weevil test groups. Weevils fed large bolls exhibited the earliest emergence profile, while square fed weevils had the most delayed emergence pattern. Weevils fed small bolls and those fed artificial diet had a very similar emergence pattern which fell between the large boll and square fed groups. The group of weevils with the lowest body fat (large boll fed) reached the 25.0% emergence point on 25 March compared to 15 May for square fed weevils. The low fat weevil group reached the 50.0% and 75.0% emergence points on 26 April and 10 May, respectively. Square fed weevils reached the 50.0% emergence level on 15 May and the 75.0% level on 28 May.

Weevils fed artificial diet and those fed small bolls had very similar emergence patterns. While emergence of these two groups was considerably later than the group fed large bolls, emergence at the 25.0, 50.0, and 75.0% levels varied from 10 to 14 days earlier than weevils fed squares. All specimens examined from the group fed artificial diet were placed in Category 1 based on fat content. Why the emergence pattern of these weevils was not similar to those high fat weevils fed on squares is unknown. However, a clear emergence pattern was evident with the low fat weevil group. This group of weevils had a significantly lower winter survival rate, and they emerged from winter habitat significantly earlier than groups of weevils with higher fat content ratings.

Bodden (1997) compared the winter survival and spring-summer emergence pattern of boll weevils collected from pheromone traps on 17 November and released into overwintering cages on 18 November to that of weevils reared from field collected squares and fed fresh squares and small bolls for 2 weeks prior to release into cages. Prior to release into overwintering cages, samples of weevils from each group were dissected to determine diapause status. Dissected weevils were divided into three categories: 1) reproductive, 2) diapause, and 3) questionable. Weevils classified as questionable were those with no evidence of reproductive activity but with very light fat content. The November control group (fed weevils) had only 5.0% classified as questionable while weevils collected from traps had 25.0% in the questionable category. Diapause percentages were 65.0% and 55.0% for the November control group and the November trapped weevils, respectively. However, the

author noted that weevils from the November trapped group classified as diapausing had less fat than diapausing weevils from the November control group.

Bodden (1997) reported significant differences in the winter survival rate and the emergence profile of the two different groups of boll weevils. Only 1.0% of the November trapped weevils survived the winter compared to 6.0% for the November control group. Even more striking was the difference in the emergence pattern of the two weevil cohorts. The first boll weevils to emerge were from the November trapped cohort with 25.0% emergence by 1 March, 50.0% by 15 April, and 75.0% by 19 April. In contrast, weevils emerging from the November control cohort reached the 25.0% emergence point on 20 May, with 50.0 and 75.0% emergence occurring on 30 May and 6 June, respectively. Bodden (1997) concluded that the low survival rate and early emergence pattern of the November trapped cohort was likely due to the failure of these weevils to accumulate adequate fat reserves. While the weevils in the November control cohort were fed fresh squares and small bolls in the laboratory, weevils in the November trap cohort were dependent on foraging for food in commercial cotton fields. A series of light freezes beginning on 23 October had greatly depleted suitable weevil food by November.

While the proposed rating system was not used in the above cited study, comparisons can be made. Weevils classified as "questionable" by Bodden (1997) would fit Category 3 of the proposed rating system. Most of the trapped weevils which were classified as diapausing would likely have fit Category 2 because of the noted reduced body fat. The study of Bodden (1997) strongly indicates that diapausing weevils with reduced body fat have a propensity toward lower winter survival and early emergence.

A test similar to that reported by Bodden (1997) was initiated by the present authors in the fall of 1997 and completed during the summer of 1998. In this study, one boll weevil cohort was obtained by collecting boll weevil infested squares from the field during early October and allowing the adults to emerge and feed in the laboratory under diapause inducing conditions (Rummel and Carroll 1983). The adult weevils were fed for 3 weeks on fresh squares and small bolls obtained from a plot of cotton planted in late June. This cohort was designated as the control cohort. A second cohort was composed of newly captured boll weevils taken from pheromone traps on 30 October. Prior to release into overwintering cages on 31 October, samples were taken from each cohort and dissected to determine the diapause/reproductive status. Dissected weevils were classified according to the system shown in Table 1.

Classification of dissected weevils according to fat body content are shown in Table 2. Weevils from the control cohort which were fed fresh squares and small bolls were classified as 80.0% Category 1 and 8.0% Category 2. The remaining 12.0% of the weevils in this cohort were classified as reproductive. The majority of the weevils taken from traps (53.0%) had very light fat content and were placed in Category 3. Only 11.0% of the trapped weevils were placed in Category 1, and 25.0% were classified as Category 2 (Table 2). The remaining 11.0% of the trapped weevils were classified as reproductive.

The percentage of weevils from both cohorts which survived the winter also is shown in Table 2. The winter survival rate of the trapped cohort was 1.27% compared to 14.8% for the control cohort. The difference in the overwinter survival rate of the two cohorts was similar to that reported by Bodden (1997) in that survival of the control weevils was considerably greater than that of the trapped weevils.

The cumulative emergence distributions of the trapped and control weevils were compared using the Kolmogorov-Smirnov two sample test (Siegel 1956). Following a very mild winter, a highly significant ($P < 0.01$) difference in the spring-summer emergence profile was noted between the trapped and control cohort (Fig. 1). The 25.0% emergence point was reached by the trapped weevil cohort on 19 April, while the control cohort reached this point on 12 May. Trapped weevils reached the 50.0 and 75.0% emergence points on 14 and 28 May, respectively, while weevils from the control cohort reached these levels on 26 May and 5 June. Emergence data from the present study clearly demonstrated that the trapped cohort with a high percentage of low fat weevils had a lower winter survival rate and a significantly earlier emergence profile compared to the control cohort.

TABLE 2. Winter Survival Ratings and Overwinter Survival Rates of Late Season Boll Weevils Taken from Pheromone Traps and a Control Group. Lubbock Co., Texas 1997-98.[a]

	Survival ratings	
	Control weevils	Trapped weevils
Category 1	80.0%	11.0%
Category 2	8.0%	25.0%
Category 3	0.0%	53.0%
Category 4	12.0%	11.0%

Overwinter survival	
control weevils – 14.8%	trapped weevils – 1.27%

[a]Control weevils were fed fresh squares and small bolls for three weeks prior to release into overwintering cages. Both groups were released into overwintering cages on 31 October 1997.

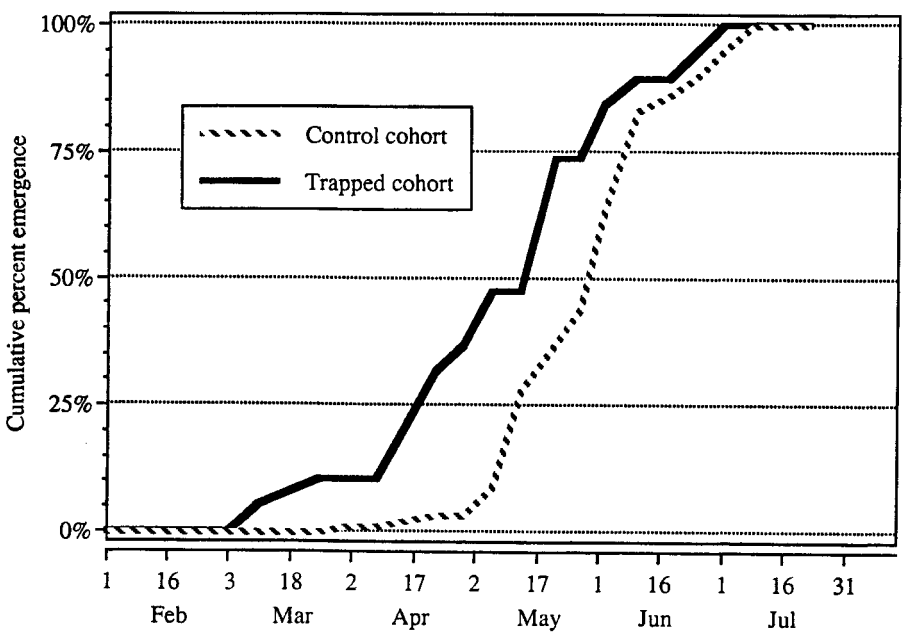

FIG. 1. Spring and summer emergence profiles of diapausing boll weevils taken from pheromone traps, and a control group of diapausing weevils. Control weevils were fed for 3 weeks prior to release into overwintering cages; trapped weevils were not fed and were released 24 hours after capture. All weevils placed in overwintering cages on 31 October. Emergence profiles of control and trapped cohorts are significantly different ($P<0.01$) according to the Kolmogorov-Smirnov two sample test. Lubbock Co., Texas, 1998.

We do not present direct experimental evidence that diapausing boll weevils with reduced fat are indeed those which exhibit low winter survival and early spring emergence. For example, one could argue that the early emerging weevils shown in Fig. 1 are the survivors of the small percentage of Category 1 individuals from the trapped cohort (Table 2). However, if this were true, the emergence profile of the trapped cohort and the control cohort would be similar, not significantly different. Thus, the consistent early emergence pattern noted in tests with high percentages of low fat weevils lends support to the proposed rating system.

Based upon data reviewed and presented in the present report we suggest that the terms "firm" and "intermediate" diapause are inappropriate and should not be used. Rather, weevils exhibiting diapause characteristics as defined by Brazzel and Newsom (1959) should be considered as diapausing and then categorized according to the amount of fat reserve. While fat reserve alone is not a reliable indicator of diapause, the amount of stored fat does appear to influence percentage of winter survival and the spring-summer emergence pattern of diapausing boll weevils. In instances where boll weevil cohorts were composed mainly of Category 3 and some Category 2 individuals, winter survival was greatly reduced and the emergence pattern considerably earlier than cohorts composed of Category 1 weevils. Mitchell and Taft (1966) did not report on the severity of the winter in their test. However, other studies reviewed in this report were conducted under mild winter conditions and overall boll weevil survival was high. Under more severe winter conditions, the survival rate of weevils with low fat reserves would probably be greatly reduced. In fact, few if any Category 3 individuals would be expected to survive a severe winter. We suggest that in cotton producing areas with cold, host-free winters, the proposed rating system can be useful for estimating the overwintering survival potential of designated late season boll weevil cohorts.

ACKNOWLEGEMENT

This study was funded by the Boll Weevil Steering Committee of Plains Cotton Growers, Inc., Lubbock, Texas. The support of the committee is greatly appreciated. Additional support was provided by Texas Agricultural Experiment Station project H-6991.

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MINUTES OF THE 1999 ANNUAL MEETING OF THE EXECUTIVE COMMITTEE OF THE SOUTHWESTERN ENTOMOLOGICAL SOCIETY

The Executive Committee met at 6:00 p.m., February 23, 1999, at the Hilton Hotel in Las Cruces, New Mexico, during the Annual Meeting of the Southwestern Branch of the Entomological Society of America. Present were President Jerry Michels, Editor Darrell Bay, Secretary-Treasurer Allen Knutson and President-Elect James Webster. The Secretary-Treasurer's and Editor's reports were reviewed and discussed. Upon review of compensation for the secretary to the Journal and for the Society, a motion was made and passed to increase compensation to the secretary for the Journal from $1920 to $2,000 and for the Society from $900 to $1,000. Darrell Bay and Pete Teel served on a committee appointed by Past President Coppedge to consider a student scholarship supported by the Society. The major concern was the lack of Society funds for a scholarship. Figures compiled by Allen Knutson showed that during the past ten years, 1989-1998, the amount of funds carried over to the next year ranged from $11,000 to $16,600. Memberships and subscriptions have been relatively constant during this time, ranging from 345-405 and 98-116 per year, respectively. A significant increase in membership dues, subscriptions or page charges would be necessary to generate additional funds to support an annual scholarship. The committee concluded none of these options was desirable at this time and unless other funds were identified, a scholarship could not be provided. The committee then discussed electronic publishing of the Journal. Allen Knutson distributed copies of The Future of Scientific Journals: Free Access or Pay Per View by Thomas J. Walker (American Entomologist, Fall 1998). This paper details how and why the Florida Entomologist became a free on-line publication. (Also see http://csssrvr.entnem.ufl.edu/~walker/fewww/aedraft.htm for this and other related articles). Revenue from page charges and electronic reprints supports this publication, and income from page charges has increased as authors seek greater exposure available through free on-line access. Darrell Bay noted that electronic manuscripts are not accepted at this time. Also, John Jackman has established a site for the Society on the Texas A&M Entomology Department web site (http://swentsoc.tamu.edu/intex.html). The possibility of the Entomological Society of America hosting the Society's web site was also mentioned. The committee agreed to ask John Jackman to chair a committee to develop a feasibility study and budget for maintaining the Society's web site and for making the Journal accessible on the World Wide Web. Allen Knutson provided the 152 ballots for President-Elect and the ballots were tallied. There being no further business, the meeting was adjourned at 6:50 p.m.

MINUTES OF THE 1999 ANNUAL MEETING OF THE SOUTHWESTERN ENTOMOLOGICAL SOCIETY

The 23rd Annual Meeting of the Society was called to order by President Jerry Michels at 7:00 p.m. following the Executive Committee meeting. Minutes of the 1998 annual meeting, as published in the June issue of the Southwestern Entomologist, were distributed. The Secretary-Treasurer's report and Editor's report for 1998 were distributed, reviewed and approved. President Michels discussed the Society's web site and the Executive Committee's decision to ask John Jackman to chair a committee to explore electronic publication of the Journal. Members interested in this initiative were invited to read the American Entomologist article cited above and contact the Society's officers with their suggestions. President Michels noted that the Society would again award a one year's membership to the first place winners in the Student Poster and Student Paper Competition. As was later announced, the winners were Chad Smith and Carlos Bogran, both of Texas A&M University. Greg Cronholm, Society Archivist, reported that he had deposited material relating to the history of the Society with the University Archivist at TAMU and asked that anyone with material relating to the history of the Society to please contact him. Darrell Bay noted that Volume 25 of the Journal is planned for the year 2000, and suggested it would be an appropriate time to

recognize W. F. Chamberlain for the instrumental role he played in organizing the Southwestern Entomological Society. A motion was made and passed to send a letter of appreciation and plaque honoring Dr. Chamberlain's contributions to the formation of the Society. President Michels called for the Nominating Committee's report. Jeff Slosser, Chair, reported that Roy Parker and John Burd were nominated for President-Elect. He reported that 152 ballots had been received and tallied and that Roy Parker was elected President-Elect. President Michels then thanked the officers of the Society for their assistance and passed the gravel to the incoming President, James Webster. President Webster then presented J. Michels a plaque in recognition his service to the Society. There being no other business, the meeting was then adjourned.

1998 SECRETARY-TREASURER'S REPORT

Balance on hand as of January 31, 1998		$16,620.94
Income: February 1, 1998-January 31, 1999		
Memberships	$ 2,400.00	
Subscriptions	1,840.00	
Page Charges	19,325.89	
Back Issues, Misc.	28.79	
Royalties	105.60	
Total Income		$23,700.28
Expenses: February 1, 1998-January 31, 1999		
Journal:		
Printing	$16,529.54	
Secretary	1,920.00	
Admail Mailing Service	813.35	
Postage	1,381.50	
Editor Fee (1998,1999)	4,000.00	
Society:		
Secretary	900.00	
Supplies	120.47	
Postage, Invoices	507.01	
Bank Charges	23.45	
Secretary-Treasurer Fee	1,500.00	
Total Expenses		$ 27,695.32
Balance on hand January 31, 1999		$ 12,625.90

As of January 31, 1999, there were 366 paid members and 103 institutional subscribers in the Southwestern Entomological Society. There were 15 unpaid page charges (primarily for the December, 1998 issue) totaling $3,047.00.

Respectfully submitted,
Allen Knutson
Secretary-Treasurer

AUDIT COMMITTEE REPORT

I examined the 1998 financial records of the Southwestern Entomological Society as prepared by Secretary-Treasurer Allen Knutson and found the records of income and expenses to be in order.

Respectfully submitted,
Roy Parker

EDITOR'S REPORT

There were 44 manuscripts, totaling 384 pages, published in the four regular issues of Volume 23 of the Southwestern Entomologist during 1998 compared to 46 manuscripts and 500 pages in Volume 22 during 1997. There were no supplements published during the year.

A total of 55 manuscripts were received for consideration for publication, compared to 59 in 1997. A number of these are still in the review process; however, three have been rejected. In addition, five manuscripts originally submitted in 1997 were also rejected during 1998. The total of eight manuscripts rejected represents a decrease of one from the nine rejected during 1997.

Editor's Financial Report

Date	Description	Receipts/Expenditures	Balance
01/01/98	Balance Forward		$74.29
02/11/98	Airborne Express: Vol. 23(1)	5.00	69.29
02/19/98	From Treasurer: $200.00		269.29
04/30/98	Airborne Express: Vol. 23(2)	5.00	264.29
06/06/98	Stamps	29.25	235.04
06/09/98	Stamps	55.00	180.04
07/22/98	Airborne Express: Vol. 23(3)	5.00	175.04
10/08/98	Airborne Express: Vol. 23(4)	5.00	170.04

Cash Summary

Balance Forward 01/01/96	74.29	
Receipts	200.00	
Expenditures	104.25	
	$170.04	Ending Balance

Respectfully submitted,
Darrell E. Bay, Editor

A NEW GALL MIDGE (DIPTERA: CECIDOMYIIDAE) PEST OF LIVE OAK IN TEXAS

Raymond J. Gagné[1] and Edward G. Riley[2]

ABSTRACT

A gall midge (Diptera: Cecidomyiidae) new to science, described and named here as *Arnoldiola atra* Gagné, forms a previously unreported rosette gall on buds of live oak, *Quercus virginiana* Mill, in southeastern Texas. The galls form unsightly masses on specimen trees. Adults of both sexes, pupae and larvae of the gall midge are described. The origin of the new species is unknown. It is placed tentatively in *Arnoldiola*, a genus heretofore unreported for the Americas.

INTRODUCTION

A previously unreported gall on twigs of live oak (*Quercus virginiana* Mill) was recently found in Harris and Austin counties in Texas. The prominent galls were first noticed in 1997 and again in 1998 in ornamental plantings. They spoiled the appearance of specimen trees, especially when the galls occurred in large numbers. The gall (Fig. 1), about 1 cm in diameter, is an aggregate of a large number of narrow, elongate rosettes made up of scalelike leaves.

The gall is formed by a new species of gall midge, described here in the genus *Arnoldiola*, that flies during several weeks in spring. Several larvae of the gall maker live among the leaf scales making up each individual rosette. Full-grown larvae are white and about 3 mm long. Larvae feed until fall when they spin a silken cocoon among the leaf scales of the gall (Fig. 3) where they overwinter. Pupation occurs there in early spring and, shortly afterwards, the black adults, about 2 mm long, emerge from the cocoons in April and May, leaving their pupal exuviae hanging from the galls (Fig. 2). At least 60 adults were reared from one sample of four galls from one 20 cm-long twig.

A search of the literature of oak galls of the Old World and North America uncovered no gall identical to this one. The most similar gall is figured in Houard (1940), the single best guide to North American oak galls. This is the "leafy oak gall," also on *Q. virginiana*, but made by the cynipid wasp *Andricus quercusfoliatus* Ashmead. Further, the bracts of that gall, however, are very narrow and enclose at their base a single closed cell in which the cynipid lives. The galls made by the new species are multiple, wide-bracted rosettes that do not have larval cells at their bases. The most similar oak gall in

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Europe is made on *Quercus coccifera* L. and *Quercus* ilex L. by another gall midge, *Blastodiplosis cocciferae* Tavares, but it belongs to a different supertribe than *Arnoldiola* and its rosette gall is made up of a single series of scalelike leaves (Tavares 1901, Houard 1908).

The new gall midge forms a gall that is quite different from other North American cecidomyiid galls on oak, most of which are leaf rolls or simple leaf blisters (Gagné 1989). We consider the galls to be a new introduction into Texas as well as the United States because it seems unlikely they would not have been noticed previously. The fact that no hymenopterous parasitoids, usually present in great numbers on plant-feeding cecidomyiids, were reared from the galls is further evidence that the gall midge is new to the area. The range of live oak is almost completely in southeastern United States, from SE Virginia to Florida, west along the Gulf Coast, and into West Central Texas. The tree occurs also in a few localites in northeastern Mexico and western Cuba. Possibly the gallmaker occurs in those outlying areas or on another oak.

MATERIALS AND METHODS

The first collection of the galls was made by J.R. Rivers who found them on 18-XI-1997 on live oak in Houston, Harris Co., Texas and sent them to one of us (EGR). The next collection submitted was found also on live oak by B. Batchelor on I-22-1998 in Bellville, Austin Co., Texas. The most recent was again from Houston and collected by F. von Schenk on live oak. All adults studied were reared in spring of 1998 from this latest collection. Larvae were taken from the Houston galls found in 1997 and also from the most recent Houston collection. Larvae and adults were killed and stored in 70% ethyl alcohol. Specimens for microscopic study were mounted on glass slides in Canada balsam, using the method outlined in Gagné (1989, 1994a). The study specimens are deposited in the U.S. National Museum of Natural History, Washington, DC (USNM) and Texas A&M University Department of Entomology Collection. Tthe senior author (RJG) was responsible for the taxonomic portion of this study. In the species description anatomical terminology of the adult stage follows McAlpine et al. (1981) and that of the larval stage follows Gagné (1989).

Arnoldiola atra Gagné, new species

Adult: Color black. Wing length: male, 1.7-1.8 mm (n=6); female 1.8-2.0 mm (n=6).

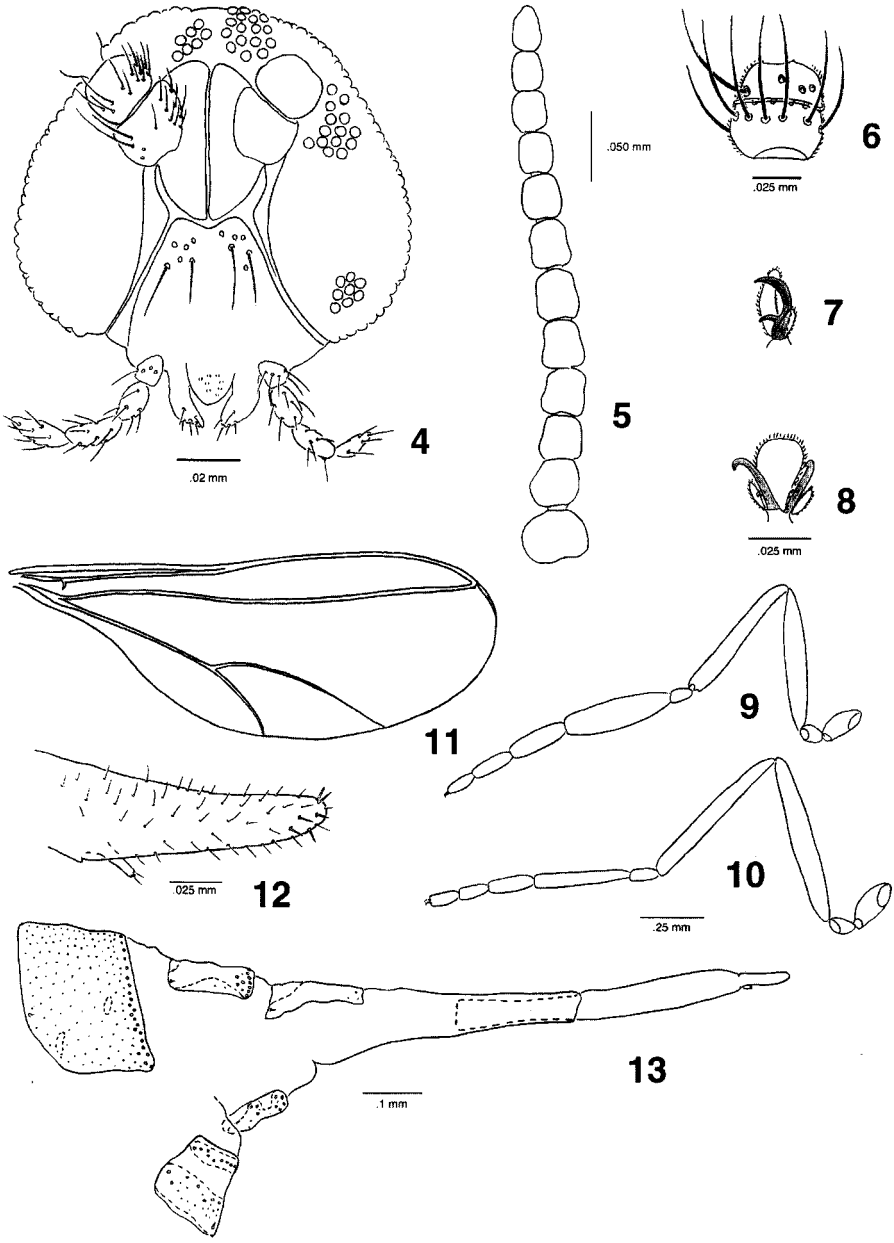
Head (Figs. 4-6): Eyes nearly contiguous at vertex, separated at midline by 1-2 eye facet diameter, the slightly interrupted eyebridge about 6 facets long; facets circular, closely approximated except farther apart dorsolaterally, separated there by up to 1/2 facet diameter; lower half of eye convex. Vertex of occiput broadly rounded. Male antenna gynecoid, without the long necks usually associated with male, and otherwise similar to that of female: scape with scales ventrolaterally and ventromesally, naked dorsally; pedicel with surrounding setae; flagellum with 10-13 (avg. 11, n=12), small flagellomeres, usually girdled with 1 circumfilum at midlength and at least a partial one apically, the two united by one connective strand. Frons with 6-20 setae per side, 0 scales, convex anteriorly.

FIGS. 1-3. Galls of *Arnoldiola atra* from live oak. 1, Several galls. 2, Two galls with pupal exuviae and adult of gall maker. 3, Gall with some leaf scales removed to show cocoon of gall maker.

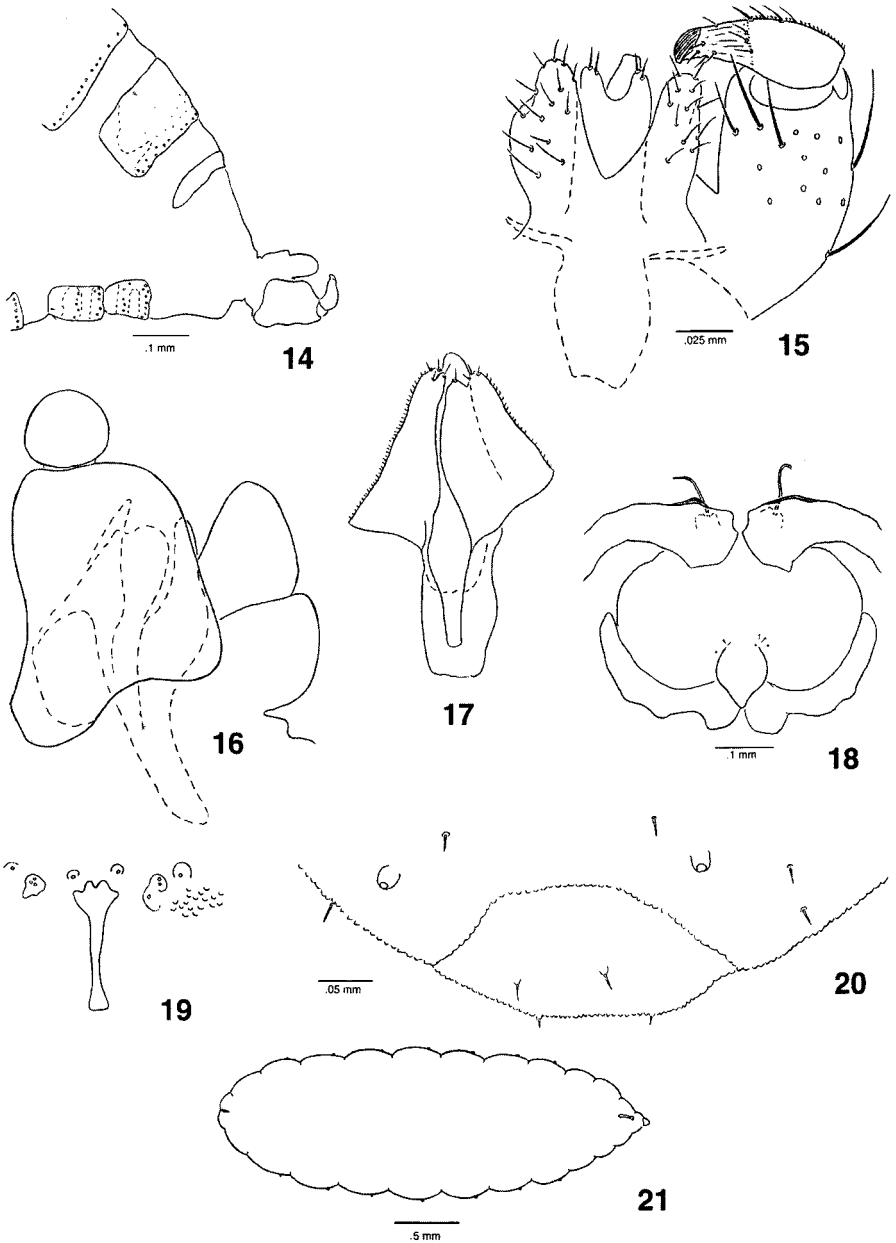
1

2

3



FIGS. 4-13, *Arnoldiola atra*. 4, Male head (dorsal; only scape and pedicel of antennae shown). 5, Male pedicel and flagellum. 6, Male third flagellum (ventral). 7, Tarsal claws, empodium, and pulvilli (dorsal). 8, Same (lateral). 9, Male leg. 10, Female leg. 11, Wing. 12, Female abdomen from sixth segment to fused cerci (lateral). 13, Detail of female cerci and hypoproct (lateral).



FIGS. 14-21, *Arnoldiola atra*. 14, Male abdomen from end of sixth segment to genitalia (lateral). 15, Male genitalia (dorsal; one gonopod not shown). 16, Male genitalia (lateral; diagrammatic). 17, Male claspettes and aedeagus (dorsal). 18, Pupal head. 19, Larval spatula and associated papillae (ventral). 20, Larval eighth and terminal abdominal segments (dorsal). 21, Larva (ventral).

Labellum hemispherical, with 8-10 setae. Palpus 4 segmented, the first segment shortest, the remaining segments of approximately equal size.

Thorax: Scutum with 2 lateral and 2 dorsocentral broad rows of mixed setae and scales. Scutellum with a group of setae and scales on each side. Mesanepisternum mostly covered with scales. Mesepimeron mostly covered with setae. Wing (Fig. 11) with R_5 joining C noticeably anterior to wing apex, slightly bowed away at midlength from leading edge of wing. Male tarsi (Fig. 9) of all legs inflated, noticeably thicker than those of female (Fig. 10). Tarsal claws (Figs. 7-8) toothed, empodia slightly longer than claws, pulvilli about 1/2 length of claws.

Male abdomen (Figs. 14-17): First through sixth tergites entire, rectangular, although not always evenly sclerotized, with single, uninterrupted, posterior row of setae, covered elsewhere with scales, and with anterior pair of trichoid sensilla; seventh tergite as for the sixth except for many fewer scales; eighth tergite reduced to short, wide sclerotized band with pair of trichoid sensilla the only vestiture.
Sternites about 2 times wider than long, each secondarily subdivided into two parts, the posterior portion with setae, the anterior, larger portion covered with setae and scales and pair of closely adjacent trichoid sensilla. Genitalia: cercus rounded apically, with several setae dorsally; hypoproct with two lobes, each with pair of distal seta; claspette elongate, broad, with two distal lobes; gonocoxite broader in lateral view than in dorsoventral view, with anteroventral convexity; gonostylus bulbous and setulose at base, tapering to short, cylindrical, carinate shaft and ending with distal tooth.

Female abdomen (Figs. 12-13): First through sixth tergites and sternites generally as for male; seventh and eighth tergites much narrower, more or less rectangular but not completely sclerotized laterally and anteriorly, the seventh with posterior group of setae, few scales, and anterior pair of trichoid sensilla, the eighth with a few posterior scales and pair of trichoid sensilla; distal half of ovipositor elongate; cerci fused, cylindrical, slightly bilaterally flattened, with widely scattered, short setae, with several equally long but thickened setae posterolaterally; hypoproct narrow, elongate.

Pupa (Fig. 18): Antennal base weakly prominent. Face smooth, clypeus with 2-3 papillae at each side near base, 1-2 on each side with short seta. Cephalic setae and prothoracic spiracle elongate. Abdomen evenly covered dorsally with short spicules.

Third instar larva (Figs. 18-21): White; length, 2.5-2.6 mm; integument rugose; spatula (Fig. 19) clove shaped; lateral papillae usually only 3, occasionally 2 or 4, on each side of ventral midline of thoracic segments; hind papillae without seta on prothorax, with setae on other segments; terminal papillae (Fig. 20) 4, 2 each side of midline, 1 of each pair with seta slightly longer than other.

Holotype: Male, ex aggregate bud gall, *Quercus virginiana*, Houston, Texas, collected 2-IV-1998, F. van Schenck, deposited in the National Museum of Natural History (USNM), Washington, DC. Other material examined (divided between USNM and Department of Entomology Collection, Texas A & M University College Station, TX): 5 ♂♂, 8 ♀♀, 10 pupal exuviae, larva, all on slides and with same data as holotype; 10 ♂♂, 10 ♀♀, pinned, same data as holotype; 5 larvae on slides, 50 in alcohol, Harris Co., Texas, collected 18-XI-1997, J. Rivers.

Etymology: The specific name, *atra*, is an adjective meaning black, with reference to the completely black adults.

REMARKS

Unlike most gall midges in which the antenna is much longer in males than in females, the antennae of both sexes of this species are equally short. Males of the new species have greatly enlarged tarsi, an unusual sexual difference in gall midges. The new

species is tentatively placed in *Arnoldiola*, a genus previously known only from Europe, because it shares some characters with that genus. These characters are: the reduced number of antennal flagellomeres, to 10-13 in the genus; and the seventh and eighth female tergites both similarly reduced in size and quadrate, the eighth not strongly divided mesally to form two separate sclerites as in *Dasineura* and relatives. The other known species of *Arnoldiola* are all from Europe and eight of the nine described species whose biology is known are from various simple or complex galls on buds and leaves of oak (Skuhravá 1986). Larvae of the type species, *Arnoldiola quercus* Binnie from Europe, live gregariously in aborted buds of *Quercus robur* L., but no modified leaves are associated with their damage.

The following combination of characters will separate the new species of *Arnoldiola* from other Nearctic Cecidomyiinae: R_5 is only slightly shorter than the wing; the antenna has a variable number of 10-13 flagellomeres that have no necks and each with at most 2 circumfilar strands in both sexes; the palpus is 4 segmented; the tarsal claws are curved beyond midlength and have large teeth; and the male and female tarsi are dissimilar in size.

No other *Arnoldiola* species is known to have such an extreme sexual difference in the size of the tarsi. Males of *Arnoldiola quercus* Binnie from Sweden (E. Sylvén in litt.) have a tendency to shorter, only slightly wider tarsi than females, but the legs of two species seen in the course of this study, *Arnoldiola cerris* Kollar and an undescribed species from Israel, do not show sexually dimorphic tarsi.

ACKNOWLEDGMENT

We thank Nit Malikul for making the slide preparations; Cathy Anderson and Lucrezia Rodriguez for computer assistance in preparing the plates; E. Sylvén for suggesting *Arnoldiola* as a home for the new species; and, for their comments on drafts of the manuscript: Keith M. Harris, International Institute of Entomology, CAB, London, UK; Thomas J. Henry and Allen L. Norrbom, Systematic Entomology Laboratory, PSI, ARS, USDA, Washington, DC; and Edvard Sylvén, Swedish Museum of Natural History, Stockholm.

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EFFECTS OF SOME NEW FORMULATIONS OF *BACILLUS THURINGIENSIS* FOR MANAGEMENT OF CABBAGE LOOPER AND DIAMONDBACK MOTH ON CABBAGE IN SOUTH TEXAS

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ABSTRACT

Seven experimental formulations of *Bacillus thuringiensis* Berliner were evaluated in the laboratory and field for controlling cabbage looper, *Trichoplusia ni* (Hübner), and diamondback moth, *Plutella xylostella* (L.), on cabbage in 1997-1998. Dipel DF, a commercial formulation of *B. thuringiensis*, and SpinTor (spinosad, a fermentation by-product based compound derived from a naturally occurring soil actinomyces bacterium, *Saccharopolyspora spinosa*, were used for comparison in some experiments. All formulations were highly toxic to third-instar *T. ni* larvae under laboratory conditions. Percentage mortalities to third instars of *T. ni* were 20-43% at 24 h, 51-84% at 48 h, 81-97% at 72 h and >98% at 96 h. LC_{50} values for ABG-6405, Troy-Bt and Dipel DF were 46.6-67.9 mg/l, the lowest, followed by ABG-6406 and ABG-6405D; whereas, LC_{50} values for SS-6696A and ABG-6475 were almost 15-fold higher than that for Dipel DF. Under field conditions, effectiveness of these *B. thuringiensis* formulations varied greatly. When pest pressure was high, all formulations were more effective to *P. xylostella* than to *T. ni*; whereas, no differences occurred when pressure was low. Five weekly applications of *B. thuringiensis* formulations significantly reduce larval populations of both pest species. However, in two of the three field trials, application of *B. thuringiensis* alone did not reduce the number of larvae below the economic threshold (1 larva/3 plants), and the market value of the plants was significantly reduced.

INTRODUCTION

Cole crops, including cabbage, broccoli, and cauliflowers, are one of the major vegetable types in Texas with 5,300 ha and 4,250 ha planted in 1996 and 1997 and total values of over $44 and $38 million, respectively (Anonymous 1998). Cabbage looper, *Trichoplusia ni* (Hübner), and diamondback moth, *Plutella xylostella* (L.), are the two most destructive pests of cabbage and other cole crops in Texas (Cartwright et al. 1987). Control of these pests is becoming increasingly difficult due to resistance to many common synthetic insecticides and the imposed damage restrictions on fresh market vegetables. Management recommendations for these lepidopterous pests in cole crops were based on either a low

economic threshold (1 larva/3 plants) (Cartwright et al. 1987) or scheduled weekly sprays. Growers often relied on prophylactic applications of broad spectrum insecticides with over 10 applications in a single season (J. Anciso, personal communication). Because of frequent applications of insecticides, control failures to *P. xylostella* have been reported in south Texas since 1986 (Magaro and Edelson 1987) and were particularly bad during 1989 and 1990.

The current array of commercially available microbial insecticides, including *Bacillus thuringiensis* Berliner, have demonstrated their potential as effective and environmentally safe alternatives to synthetic chemical insecticides for lepidopterous pests (Wilding 1986). Currently, over 20 commercial formulations of the toxins produced by *B. thuringiensis* play an important role in integrated pest management programs of lepidopterous pests on vegetables and other field crops because of their high toxicity to many lepidopterous pests and low toxicity to non-target organisms including beneficial insects. *B. thuringiensis* products have been used in south Texas for decades. Most reported data indicated that *B. thuringiensis* reduced populations of *T. ni* and *P. xylostella*, but overall control has not been adequate under high population pressures (Edelson et al. 1993; Sparks and Anciso, unpublished data). Edelson et al. (1993) found that applications of *B. thuringiensis* (Dipel 2X) together with fatty acid soap and release of *Chrysoperla carnea* Stephen were equally effective against the lepidopterous complex on broccoli compared with applications of methamidophos and endosulfan, two synthetic organic insecticides, but required more applications for effective control.

The objectives of this study were to determine the efficacies of some new formulations of *B. thuringiensis* for management of *T. ni* and *P. xylostella*, the two most important lepidopterous pests on cabbage and other cole crops in south Texas. Reported here are results of both laboratory bioassays and field trials of seven *B. thuringiensis* formulations conducted during 1997 and 1998.

MATERIALS AND METHODS

A total of seven new formulations of *Bacillus thuringiensis* var *kurstaki* Berliner (Bt) were tested in field and laboratory experiments. Among the seven formulations, four (ABG-6405, ABG-6405D, ABG-6475 and ABG-6492) were supplied by Abbott Laboratories (North Chicago, IL), one (Troy-Bt) by Troy BioSciences (Phoenix, AZ), and two (SS-6696A and SS-6712) by the Next Wave, Samsung (Seoul, Korea). Dipel DF (Abbott Laboratory, North Chicago, IL), a commercial formulation, was used as an industry standard for comparison, and water-treated or untreated plants as controls. In some trials, SpinTor 2SC (spinosad, a fermentation by-product based compound derived from a naturally occurring soil actinomyces bacterium, *Saccharopolyspora spinosa* by Dow AgroSciences, Indianapolis, IN) was also used for comparison.

Laboratory Bioassays. Only *T. ni* was used in this experiment. First instar *T. ni* (7 d from egg) fed on the multiple species diet (Southland Products, Lake Village, AR) were obtained from Entopath, Inc. (Easton, PA). The larvae were maintained in a growth chamber at 20 ± 2 °C until they grew to third instar. Cabbage plants, about 30-40 cm high with 8-10 leaves, were placed on a bench in a greenhouse for treatment. To evaluate efficacy over time (24, 48, 72 and 96 h), plants were treated with the field rate (1.5 g/liter or 0.5 lb/30 gal water). To determine the relative toxicity (LC_{50} and LC_{90}) of these formulations, a series of concentrations were used to produce a range from very high to low percentage mortalities of third instars. In both experiments, water was used as a control. The plants were first sprayed to run-off with the dilutions or water. After air-drying for 3 h, the treated plants

were moved to an insectary with a photoperiod of 14:10 (D:L) h at 25 ± 2 °C and 50-55% relative humidity (RH). Cabbage leaves were removed from plants and leaf disks (10-12 cm in diam) were cut and placed in a plastic petri dish (2 cm deep x 15 cm diam). Small holes (20-25, 2 mm in diam) were drilled on the petri dish lids for ventilation. Four pieces of paper tissue were placed at the bottom of the petri dish, and a few drops of water were added daily for moisture. Ten third-instar larvae were introduced to each petri dish. Dead and live larvae were checked at 24, 48, 72, and 96 h. Leaf disks were replaced every 48 h. *T. ni* larval mortalities for 72 h were used for calculating LC_{50}, LC_{90} and related parameters.

Field Trials. All field trials were conducted at the Texas Agricultural Research and Extension Center Research Farm, Texas A&M University at Weslaco. Cabbage was established in double-row on 1-m beds and 25-cm apart. Each treatment plot consisted of two rows of cabbage plants (9.14 m long). Plots were separated with sorghum wind breaks between beds and a 1.5-m alleyway. All treatments were arranged in a randomized complete block design with four replications. A standard production protocol developed by the Texas Agricultural Research and Extension Center was followed. Fertilization consisted of 112 kg/ha of N-32 at planting, and the herbicide Prefar 4E was used at 14 liter/ha at planting.

All formulations were applied using a tractor-mounted sprayer with three ceramic hollow cone nozzles (TX-6 red) per row (one over the plants, one on each side of the row on drops directed into the plant) at 689.5 kPa (7.03 kg/cm^2) at a delivery rate of 374 liter/ha with a traveling speed of 4.83 km/h. Spray began when mean numbers of larvae per plant reached the economic threshold of one larva per three plants, and thereafter, the plants were sprayed once per week for five or more times depending on the trial.

Larval sampling was initiated four weeks after planting in each test, and conducted once per week after the first application. The numbers of small and large larvae per plant were counted a few hours before each application by checking both the upper and lower surfaces of every leaf for 10 randomly selected plants from each plot.

At termination, a damage/quality evaluation was made on 10 randomly selected plants from each plot based on the following six categories (Greene et al. 1969): 0 - no apparent damage; 1-minor feeding damage on wrapper outer leaves or 1% leaf area eaten; 2 - minor-moderate feeding damage, or 2-5% leaf area eaten; 3 - moderate damage, or 6-10% leaf area eaten, but no head damage; 4 - moderate-heavy damage on wrapper and outer leaves with minor damage on head, or 11-30% leaf area eaten; and 5 - heavy damage on wrapper and head, or >30% leaf area eaten.

Fall 1997. Two new experimental formulations, ABG-6405 and ABG-6492, were tested in this trial. Dipel DF was used for comparison, and untreated plants for control. The materials were applied five times at weekly interval on 23, 30 October and 6, 14, and 25 November. Damage was evaluated at termination on 1 December.

Spring 1998. Four formulations, SS-6696A, SS-6712, Troy-Bt, and ABG-6405, were used in this trial. Dipel DF was used for comparison, and untreated plants for control. The materials were applied on 17 March as threshold level exceeded 0.3 larvae per plant, and once per week for four more times on 24 March, and 1, 8, and 15 April. Damage was evaluated on 20 April. During the season, Aphistar (Rohm and Haas Company, Independence Hall West, Philadelphia, PA), an insect growth regulator that is selectively effective only against aphids, was applied at 0.132 kg (AI)/ha on 25 March to suppress turnip aphid [*Lipaphis erysimi* (Kaltenbach)]. The plants were also sprayed twice with the fungicide, Bravo-750, at 2.34 liter/ha to control diseases.

Fall 1998. Three formulations, ABG-6405, ABG-6405D, and ABG-6475, were used in this trial. Dipel DF and SpinTor were used for comparison, and untreated plants for control. The applications were initiated on 1 October when larval population of *T. ni* and *P.*

xylostella reached the economic threshold, and four more times on 8, 15, 22 and 29 October, respectively. Sampling began four weeks after the planting. Numbers of small and large larvae of both pest species per plant were counted by checking both the upper and lower surfaces of every leaf on each plant, and 10 plants per plot were examined. A total of 440 plants were examined per week. Damage was evaluated on 5 November.

For the laboratory bioassay data, percentage mortality of larvae was transformed to the arcsine square root [arcsine (percent mortality/100)$^{1/2}$] before analysis to stabilize error variance (Gomez and Gomez 1984, Steel and Torrie 1980), and mean percentage mortality was analyzed using analysis of variance (ANOVA) and was separated using the least significant difference test (LSD) following a significant F-test at $P = 0.05$. Although all tests of significance were based on the transformed data, we report the untransformed percentage mortality (percentage mean ± SE). LC_{50} and LC_{90} with related parameters [90% fiducial limits (FL), slope, and SE] for the larvae of *T. ni* in laboratory bioassays were analyzed using POLO (LeOra Software 1994), and the lack of overlap of the 90% FL was used as the criterion to separate the toxicity among the formulations. For field data, numbers of larvae per plant were subjected to ANOVA, and means were separated using LSD following a significant *F*-test at $P = 0.05$ (SAS Institute 1996).

RESULTS

Laboratory Bioassays. All seven formulations caused significant mortality of third instar *T. ni* under laboratory condition 3-4 d after treatments compared with the water treated control (Fig. 1). Efficacies among the seven formulations and time (h) after treatment were significantly different (formulations: $F = 145.59$; df = 3, 600; $P = 0.0001$; h: $F = 347.25$; df = 3, 600; $P = 0.0001$), as were the interactions of formulation and time (h) ($F = 7.43$; df = 21, 600; $P = 0.0001$). Without including the water control in the analyses, efficacies of the seven formulations at 24 h and 96 h were not significantly different (for 24 h: $F = 0.74$; df = 6, 68; $P = 0.6194$; for 96 h: $F = 0.74$; df = 6, 68; $P = 0.6194$). However, efficacies among the formulations at 48 and 72 h were significantly different (For 48 h: $F = 3.39$; df = 6, 68; $P = 0.0056$; for 72 h: $F = 0.2.87$; df = 6, 68; $P = 0.0148$). The efficacies of ABG-6475 were significantly lower than these of the other formulations. These results indicated that most larvae did not die within 24 h; only 50-85% died within 48 h, and almost all larvae died by 72 and 96 h after treatment.

Probit analysis of the concentration-mortality response of third instar *T. ni* to the seven formulations indicated that the LC_{50} and LC_{90} values varied greatly (Table 1). Two new formulations, ABG-6405 and Troy-Bt, and the standard, Dipel DF, provided the lowest LC_{50} and LC_{90} values, followed by ABG-6406 and ABG-6405D. The highest LC_{50} and LC_{90} values were found in ABG-6475 and SS-6696A. The LC_{50} (899.9 mg/l) and LC_{90} (3647.5 mg/l) values of ABG-6475 were over 15- and 20-fold higher than those of Dipel DF (58.1 and 169.3, respectively).

Field Trials. Fall 1997. T. ni populations were high during this season. Although plants treated with all three *B. thuringiensis* formulations had significantly fewer larvae than those on the untreated plants (Table 2), the numbers of larvae on the treated plants exceeded the economic threshold, beginning with the second week after the first application. *P. xylostella* populations were low throughout the entire season. All three formulations significantly reduced the larval population (Table 1) with no significant difference among them. The numbers of larvae on most treated plants was below the economic threshold.

Spring 1998. All *B. thuringiensis* formulations significantly reduced the larval population 1 - 2 weeks after the first application, and the population was suppressed

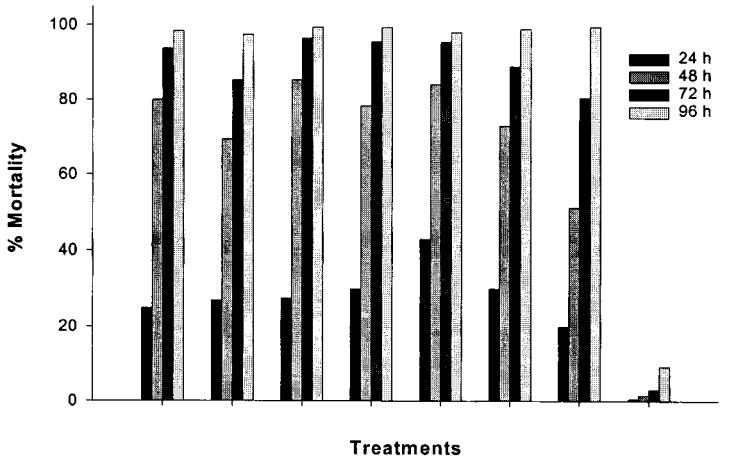

FIG. 1. Efficacies of seven *Bacillus thuringiensis* formulations at 1.5 g/liter against third instar *Trichoplusia ni* over 24, 48, 72 and 96 h under laboratory condition

TABLE 1. Toxicity (LC_{50} and LC_{90}, mg/liter) of Seven *Bacillus thuringiensis* Formulations to Third-Instar Larvae of *Trichoplusia ni* on Cabbage in the Laboratory 72 h after Treatment.

Formulation	LC_{50} (90% FL)	LC_{90} (90% FL)	Slope ± SE
ABG-6405	52.8 (44.4-62.1)	204.1 (158.8-289.2)	2.18 ± 0.23
ABG-6406	79.8 (93.8-271.0)	371.2 (288.7-513.9)	1.92 ± 0.18
ABG-6405D	91.4 (77.3-107.1)	352.8 (284.0-464.4)	2.19 ± 0.21
ABG-6475	899.9 (736.6-1084.1)	3647.5 (2870-4948)	2.11 ± 0.21
Dipel DF	58.1 (50.1-67.9)	169.3 (130.0-257.8)	2.76 ± 0.34
SS-6696A	261.9 (214.2-326.2)	1406.3 (987.8-2288.0)	1.76 ± 0.19
Troy-Bt	54.5 (46.4-63.5)	164.5 (131.1-226.4)	2.67 ± 0.27

throughout the season (Table 3). However, these materials at the tested rates did not maintain the larval population below the economic threshold (1 larva/3 plants). Among the formulations, Dipel DF at the three tested rates provided the best control of *P. xylostella*, and number of larvae on the treated plants was below or close to the economic threshold. The larval population of cabbage looper at the beginning was relatively low (below the economic threshold). Numbers of larvae increased gradually on the untreated plants, and exceeded the economic threshold 2-3 weeks after the first application. However, plants treated with *B. thuringiensis* had significantly fewer larvae than those in the untreated control. The

TABLE 2. Effects of Some New *B. thuringiensis* Formulations on *Plutella xylostella* and *Trichoplusia ni* on Cabbage in South Texas (Weslaco, Fall 1997).

Treatment	Rate (kg/ha)	10/16[b]	10/21	Mean number of larvae per plant[a] 10/28	11/3	11/11	11/17	11/24	12/1	Plant damage
					T. ni					
ABG-6405	0.56	0.05a	0.30a	0.60b	2.95bc	3.65ab	2.80ab	2.45b	1.05b	3.5b
ABG-6405	1.12	0.10a	0.40a	1.45ab	3.70ab	3.15b	2.10b	1.30bc	0.55b	2.3b
ABG-6492	1.33	0.05a	0.20a	1.00b	2.15c	2.40b	1.55b	1.55bc	0.55b	2.3b
Dipel DF	0.56	0.05a	0.35a	2.30a	2.90bc	2.50b	2.80ab	1.00c	1.30b	2.8b
Dipel DF	1.12	0.05a	0.20a	1.65ab	1.90c	3.40b	2.40b	1.40bc	1.35b	2.0b
Untreated	-	0.00a	0.25a	2.53a	4.50a	5.25a	3.90a	5.85a	4.20a	5.0a
					P. xylostella					
ABG-6405	0.56	0.05a	0.30a	0.90b	0.60b	0.60b	0.20b	0.10b	0.00b	3.5b
ABG-6405	1.12	0.10a	0.55a	1.25b	0.65b	0.10b	0.20b	0.10b	0.00b	2.3b
ABG-6492	1.33	0.10a	0.85a	1.25b	0.10b	0.15b	0.35b	0.10b	0.00b	2.3b
Dipel DF	0.56	0.15a	0.70a	2.45a	0.55b	0.10b	0.00b	0.00b	0.00b	2.8b
Dipel DF	1.12	0.25a	0.90a	0.80b	0.15b	0.20b	0.10b	0.05b	0.00b	2.0b
Untreated	-	0.45a	0.75a	2.58a	2.60a	2.55a	2.75a	1.15a	0.95a	5.0a

[a] Means followed by the same letter(s) do not differ significantly ($P = 0.05$, LSD [SAS Institute 1996]).

[b] Pre-treatment samples.

treatments Dipel DF, Troy-Bt and SS-6712 maintained effectiveness for the entire season The number of larvae in each treatment remained below the economic threshold. All *B. thuringiensis* formulations showed a trend toward better control with the higher rates.

Fall 1998. *P. xylostella* and *T. ni* populations were slightly lower than normal due to the heavy and frequent rains during the period of the experiment in October (Table 4). All *B. thuringiensis* formulations performed equally well against the two pest species. Low pest density resulted in only minor differences compared to the untreated plants. However, even with low pest densities, foliage damage was significantly different among the treatments, with the higher rate of ABG-6405 providing the best protection, and the untreated control with the highest damage rating.

DISCUSSION

For decades, *B. thuringiensis* has been widely used for management of various pest species in Lepidoptera, Diptera and Coleoptera on various crop systems, and has gained well-earned popularity because of its distinct advantages over other pesticides: negligible hazards to humans; short waiting period from time of application to time of harvest; no waiting period from time of application before re-entering the field; not harmful to beneficial or non-target insects, birds or other animals that may feed on the dead insects; no phytotoxicity to crops; and not considered harmful to the environment. Results in this study also indicated that new formulations and commercial products are highly toxic to *T. ni* larvae from a laboratory colony on cabbage under laboratory condition. However, under field conditions, particularly when pest population pressure was high, most tested formulations,

TABLE 3. Effects of Some New *B. thuringiensis* Formulations on *Trichoplusia ni* and *Plutella xylostella* on Cabbage in South Texas (Weslaco, Spring 1998).

Treatment	Rate kg/ha	Mean number of larvae per plant ± SE[a]							Plant damage	
		March 16[b]	March 23	March 30	April 6	April 14	April 17	April 20	Head	Outside
					T. ni					
SS-6696A	0.56	0.1a	0.2a	1.0a	1.1ab	1.6ab	1.2a	1.6ab	2.22b	2.65bc
SS-6696A	1.12	0.1a	0.2a	0.4a	0.9a-d	0.7def	1.0a	1.3abc	1.95bc	2.32de
SS-6696A	1.33	0.0a	0.2a	0.6a	0.6cde	0.7c-f	1.0a	1.7a	1.75cd	2.40cd
SS-6712	0.56	0.0a	0.1a	1.0a	1.2a	1.2bcd	0.9ab	0.8cd	2.05bc	2.78b
SS-6712	1.12	0.0a	0.1a	0.4a	0.7b-e	0.9cde	0.7bc	0.3de	1.45def	1.92fg
SS-6712	1.33	0.1a	0.1a	0.8a	0.5de	0.5ef	0.3cd	0.7cde	1.30ef	2.28de
Troy-Bt	0.28	0.1a	0.1a	0.5a	0.7b-e	0.6ef	-	0.4de	1.43def	2.05ef
Troy-Bt	0.56	0.1a	0.3a	0.6a	0.5cde	0.6ef	-	0.3de	1.08fg	1.83fgh
ABG-6405	2.24	0.0a	0.2a	0.1a	0.3e	0.5ef	-	1.1bc	1.33ef	1.83fgh
Dipel DF	0.56	0.1a	0.1a	0.4a	1.5cde	0.5ef	0.3cd	0.5de	1.48de	1.83fgh
Dipel DF	1.12	0.0a	0.2a	0.4a	0.7b-e	0.9cde	0.4bcd	0.5de	1.20efg	1.68gh
Dipel DF	1.33	0.0a	0.1a	0.3a	0.3e	0.3f	0.0d	0.2e	0.85g	1.60h
Untreated		0.1a	0.3a	0.6a	1.0abc	1.8a	1.2a	1.7a	3.25a	4.18a
					P. xylostella					
SS-6696A	0.56	0.3a	0.5bcd	1.4b	0.5bc	1.8b	1.8b	3.5b	2.22b	2.65bc
SS-6696A	1.12	0.5a	0.5bcd	0.6bcd	0.2bc	1.0bcd	0.8b	2.1b-e	1.95bc	2.32de
SS-6696A	1.33	0.7a	0.8ab	0.7bcd	0.2bc	0.7bcd	1.0b	2.2bc	1.75cd	2.40cd
SS-6712	0.56	0.5a	0.7abc	1.0bcd	0.7b	1.5bc	0.4b	2.1bcd	2.05bc	2.78b
SS-6712	1.12	0.6a	0.5bcd	0.6bcd	0.2c	0.5bcd	0.9b	1.0c-f	1.45def	1.92fg
SS-6712	1.33	0.5a	0.5bcd	1.2bc	0.2c	0.5bcd	0.5b	0.9c-f	1.30ef	2.28de
Troy-Bt	0.28	0.5a	0.3d	0.5cd	0.2bc	0.5bcd	-	0.7c-f	1.43def	2.05ef
Troy-Bt	0.56	0.9a	0.2d	0.6bcd	0.3bc	0.1d	-	0.6c-f	1.08fg	1.83fgh
ABG-6405	2.24	0.7a	0.3d	0.2d	0.1c	0.1d	-	1.0c-f	1.33ef	1.83fgh
Dipel DF	0.56	0.6a	0.3cd	0.5cd	0.1c	0.2cd	0.2b	0.3c-f	1.48de	1.83fgh
Dipel DF	1.12	0.7a	0.3cd	0.5cd	0.1c	0.5bcd	0.1b	0.2f	1.20efg	1.68gh
Dipel DF	1.33	0.5a	0.3d	0.3d	0.1c	0.0d	0.0b	0.3ef	0.85g	1.60h
Untreated		0.7a	1.0a	2.4a	2.7a	7.1a	9.9a	10.0a	3.25a	4.18a

[a] Means in the same column followed by different letters differ significantly at $P = 0.05$ (LSD, SAS Institute 1996).

[b] Pre-treatment samples.

applied at weekly intervals, did not reduce the populations to levels below the economic thresholds or to levels sufficient for meeting industrial standards, although the populations of both *T. ni* and *P. xylostella* were significantly reduced. Damaged plants are not

TABLE 4. Effects of Some New Formulations of *B. thuringiensis* on *Trichoplusia ni* and *Plutella xylostella* on Cabbage in South Texas (Weslaco, Fall 1998).

Material	Rate kg/ha	Mean number of larvae per plant ± SE[a] Oct. 1	Oct. 8	Oct 15	Oct. 22	Oct. 29	Nov. 5	Plant damage
				T. ni				
ABG-6405	0.5	0.8a	1.2a	0.4b	0.8b	1.3b	1.0a	0.9b
ABG-6405	1.0	0.8a	1.4a	0.2b	0.7b	0.8b	0.3b	0.1d
ABG-6405D	1.2	0.5a	1.3a	0.3b	0.8b	1.3b	0.6b	0.6bc
ABG-6405D	2.0	1.0a	1.8a	0.2b	0.6b	1.0b	0.5b	0.7bc
ABG-6475	2 qts	1.2a	1.6a	0.2b	0.9b	0.9b	0.3b	0.9b
SpinTor	3 oz	0.8a	0.6a	0.1b	0.2b	0.8b	0.0c	0.3cd
Untreated		0.8a	1.3a	0.6a	1.4a	2.0a	1.2a	1.9a
				P. xylostella				
ABG-6405	0.5b	0.4a	0.3ab	0.0a	0.1bc	0.1b	0.0b	0.9b
ABG-6405	1.12	0.4a	0.2b	0.0a	0.0c	0.1b	0.0b	0.1d
ABG-6405D	1.33	0.2a	0.4ab	0.0a	0.2bc	0.1b	0.0b	0.6bc
ABG-6405D	2.24	0.4a	0.4ab	0.0a	0.1bc	0.0b	0.0b	0.7bc
ABG-6475	2.12[b]	0.3a	0.3b	0.1a	0.2ab	0.0b	0.0b	0.9b
SpinTor	0.045[c]	0.1a	0.6a	0.1a	0.0c	0.1b	0.0b	0.3cd
Untreated		0.1a	0.2b	0.1a	0.4a	0.1a	0.0a	1.9a

[a] Mean number of larvae per plant (± SE) in the same column followed by different letters differ significantly at P = 0.05 (LSD, SAS Institute 1996).
[b] liter/ha.
[c] kg (AI)/ha

marketable or the market value will be greatly reduced if the damage levels are greater than 2.0 or 5% leaf surface was removed.

Loss of residual activity of *B. thuringiensis* products under field conditions due to various environmental conditions has long been a source of discussion and concern among pest management decision makers (Ignoffo et al. 1977, Leong et al. 1980). *B. thuringiensis* toxins must be in the feeding zone of the target pests which exposes them to adverse environmental conditions (Gillespie et al. 1994, Mulla 1990). Exposure to solar radiation (sunlight) and wash-off by rain will degrade and inactivate the spores and crystals, and reduce the effectiveness of the activity of *B. thuringiensis* products (Leong et al. 1980, Akiba 1991, McGuire et al. 1996, Tamez-Guerra et al. 1996). High leaf temperature and vapor pressure deficit also affect *B. thuringiensis* endospore viability and pathogenicity (Leong et al. 1980). Although all manufacturers have indicated that they have used the most advanced technology in their formulations (e.g., encapsulation, ultraviolet protectants, sticker or surfactants) (Dunkle and Shasha 1988, 1989; Gelernter 1990; Tamez Guerra et al. 1998), the effectiveness of these tested formulations to *T. ni* and *P. xylostella* was far better under laboratory conditions than under field conditions. These differences clearly indicated that the quality of these *B. thuringiensis* products needs significant improvement for wider scale acceptance by growers and consultants making pest management decision.

T. ni and *P. xylostella* and most other lepidopterous larvae feed from the underside of the leaf surface. Therefore, *B. thuringiensis* products should be applied to all plant surfaces, including the underside of the leaf surface where most larvae are located. This may be best accomplished using adequate spray volumes (at least 370 l/ha) and higher pressure (at least 0.70 kPa) (T.-X. Liu, unpublished data). In addition, application to the undersides of the leaf surfaces will prolong *B. thuringiensis* activity by avoiding exposure to sunlight and rain.

Most *B. thuringiensis* products have better activity against young larvae rather than old or mature larvae. This means that the initial *B. thuringiensis* application should be made immediately before or just after the insect eggs have hatched and the larvae are still young. The best way to apply *B. thuringiensis* products at the proper time is to monitor plants for eggs. Pheromone traps are useful to determine when adult moths are in the area and when egg laying is likely to occur.

Optimization of *B. thuringiensis* products for management of lepidopterous pests on cabbage and other vegetable crops include better spray coverage, integrated with pest resistant varieties, alternating with other insecticides, and formulation quality that improve persistence under field conditions. Recently, Meade and Hare (1995) reported that a combination of *B. thuringiensis* applications and insect resistant varieties significantly reduced the feeding damage of lepidopterous complex on celery in California.

Although resistance of *P. xylostella* to some commercial *B. thuringiensis* products on cabbage has been observed in the laboratory (Tabashnik et al. 1997) and under field conditions in Florida (G. Leibee, University of Florida, personal communication), little additional resistance has been documented for *T. ni* to date. *B. thuringiensis* products will be recommended as alternatives to replace some synthetic chemical insecticides for control of the lepidopterous complex on cabbage and vegetables. However, *B. thuringiensis* used alone may not be the best strategy for managing the lepidopterous pests on cabbage and other crops. Rotation with other biorational insecticides and insecticides with different modes of action and chemistry is strongly recommended to prolong effectiveness and delay the development of resistance by lepidopterous pests on cole crops (Jaques 1988). I suggest that all *B. thuringiensis* formulations should be used alone only when pest pressure is low when greater damage can be tolerated. Otherwise, they have to be used more frequently with better spray coverage, at higher rates, or mixed with some more effective and expensive insecticides to reduce cost. Implementation of the Food Quality Protection Act of 1996 will likely limit the application of some organic chemical insecticides in the near future. With the developments of new toxins and formulation technology, *B. thuringiensis* products will be more effective, receive more consideration as a synthetic chemical insecticide alternative, and certainly play an even more important role in the integrated management programs of lepidopterous pests on cole crops and other vegetables.

ACKNOWLEDGMENT

I thank A. N. Sparks, Jr. (Texas Agricultural Extension Service, Weslaco) and V. French (Texas A&M University - Kingsville Citrus Center at Weslaco) for review of the manuscript; and L. Larson and A. M. Adair (Abbott Laboratories, IL), B. Brown (Troy BioSciences, Phoenix, AZ), S. H. Sik (NextWave, Samsung Corporation, Seoul, Korea) for providing testing materials and partial financial support. I also thank B. Yue, J. Martinez, M De Leon and J. Martinez, Jr. for technical assistance, and L. Arndt (Dow AgroSciences, Indianapolis, IN) for supplying the cabbage looper larvae for the greenhouse and laboratory bioassays. This manuscript has been approved for publication by the Director of Texas

Agricultural Research and Extension Center, Texas A&M University System at Weslaco, and the Head of the Department of Entomology, Texas A&M University, College Station.

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PRODUCTION OF MONOCLONAL ANTIBODIES SPECIFIC FOR HYPODERMIN C, A COLLAGENASE OF THE COMMON CATTLE GRUB, *HYPODERMA LINEATUM*[1]

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ABSTRACT

Murine hybridomas were constructed that secreted monoclonal antibodies specific for hypodermin C (HyC), a collagenase produced by first instar larvae of the common cattle grub, *Hypoderma lineatum* (Villers). Monoclonal antibodies produced by the hybridoma cultures, HLl-Cl, HLl-C2, HL1-C3 and HLl-C4 were each of subclass IgG1.

INTRODUCTION

The common cattle grub, *Hypoderma lineatum* (Villers), and the northern cattle grub, *Hypoderma bovis* (L.), are obligate parasites of cattle (Scholl 1993). The host serves as the sole source of nutrition since adult flies do not feed. Economic impact occurs from meat and hide damage, as well as from injury or production loss resulting from attempts to escape the host-seeking adult flies (Kunz 1987). Cattle grubs are host-specific, therefore, control of the pests in livestock effectively removes the breeding population. However, use of most chemical pesticides on lactating dairy cattle is severely restricted, making area-wide population control of cattle grubs difficult to achieve.

In an effort to develop new control technologies, we chose to study augmentation of natural host resistance to subsequent infestation through vaccination. *H. lineatum* proteases designated hypodermins A, B, and C, were chosen as candidate immunogens based on skin reactions of previously exposed vs. naive animals, antibody response to natural infestations, preliminary vaccine trials using natural materials, and immunological crossreactivity between the two major species, *H. lineatum* and *H. bovis* (Pruett et al. 1987, 1988, 1990; Schwinghammer et al. 1988). Recombinant DNA technology provides an alternate method for production of large quantities of immunogenic material for use in vaccine trials but presents new problems with regard to production, purification, and immunoreactivity of the recombinant antigens.

Monoclonal antibodies (mAbs) could be used to provide a method for monitoring the immunogen during production as well as to purify immunogenic protein by immunoaffinity

[1] Names are necessary to report factually on available data; however, the USDA neither guarantees nor warrants the standard of the product, and the use of the name by USDA implies no approval of the product to the exclusion of others that may also be suitable.

chromatography. Such antibodies would also be useful probes for dissection of protein structure and location of tissue-fixed antigens. This report describes the construction of hybridomas which secrete mAbs specific for hypodermin C (HyC), the major collagenase of *H. lineatum*.

MATERIALS AND METHODS

First instar larvae of *H. lineatum* were collected from esophagi of slaughtered cattle, frozen in liquid nitrogen, and ground in 10 mM tris-HCl, pH 7.4 at 4°C in a Tenbroeck tissue homogenizer to prepare crude extract (HL1) as described by Pruett et al. (1988).

Immunization of 6- to 12-week-old BALB/c mice (Charles River Laboratories, Wilmington, MA) was as described previously (Temeyer and Pruett 1998), except that immunizations used crude HL1 extract instead of purified protein and booster immunizations were given at 3-wk intervals. Antibody development was monitored in mouse serum using a kinetic-ELISA (Pruett et al. 1987) and hybridoma construction was as described previously (Temeyer and Pruett 1998) except that immune splenocytes and myeloma cells were fused at a 4:1 ratio. Hybridoma culture supernatant was screened for production of antibody specific for *H. lineatum* antigens using a microtiter format ELISA test with HL1 bound to microtiter plates (4 μg HL1 crude antigen ml^{-1} incubated overnight at 30°C in 50 mM Tris-HCl, 300 mM KCl, 2 mM Na_2EDTA, pH 8.0 [Mathews et al. 1984]) which had been pretreated with 0.5% glutaraldehyde. Wells which scored positive for production of *Hypoderma*-specific antibody were repeatedly subcloned as described previously (Temeyer and Pruett 1998).

Monoclonal antibody was prepared from hybridoma culture supernatants or ascites fluid, isotyped and characterized by Western blotting as described previously (Temeyer and Pruett 1998) except that nondenaturing electrophoresis was in 7% polyacrylamide gels (7% PAGE) and denaturing gels were 10% polyacrylamide (10% SDS-PAGE). Proteins were transferred from polyacrylamide gels to nitrocellulose (BA-85, Schleicher & Schuell, Keene, NH) or Zeta-Probe (Bio-Rad Laboratories, Richmond, CA) membranes by electroblotting and incubated for 1 hr in 10% nonfat dry milk to block non-specific protein binding sites. Western blots were shaken at room temperature for 2 hr with mAb (hybridoma supernatant) diluted 1:25 in antibody dilution buffer (ADB2; 2% nonfat dry milk containing 1% [w/v] Tween-80 and 0.01% [v/v] antifoam A [Sigma Chemical, St. Louis, MO]). Goat anti-mouse IgG-HRP conjugate (BioRad) was diluted 1:1000 in ADB2 and shaken with the blot for 2 hr at room temperature. Color development with o-dianisidine was for 10 min (Temeyer et al. 1986).

Cells were collected from cell culture or ascites fluid by centrifugation, rinsed and resuspended at ca. 4-8 X 10^6 cells ml^{-1} in serum-free DMEM (Temeyer and Pruett 1998). An equal volume of fetal bovine serum was mixed with the cell suspension. To this volume, an equal volume of 30% dimethylsulfoxide (DMSO, ATCC, Rockville, MD) was slowly added while mixing gently to provide a final concentration of 1-2 X 10^6 cells ml^{-1} in DMEM containing 15% DMSO and 25% fetal calf serum. The cell suspension was aliquoted into cryopreservation vials and cooled stepwise to 4°C, -20°C, and -70°C (allowing for a cooling rate of 1°C min^{-1} at each step) before transfer to storage in liquid nitrogen.

RESULTS AND DISCUSSION

Mice immunized with HL1 crude extract in Freund's adjuvant did not respond uniformly as measured by kinetic ELISA. We chose spleens from high responding mice in an attempt to maximize the presence of antibody-producing cells available for fusion. We succeeded in obtaining hybridomas secreting antibody specific for HyC using splenocytes from animals that

had received three immunization injections at 3-wk intervals, with harvest of splenocytes for fusion 3-4 days after the final booster injection. It was noted during the subcloning stage, that the hybridomas appeared to be dependent on the presence of feeder cells, and were not easily adapted to grow in their absence. Supplementation of the growth medium with hybridoma growth factor (Janssen Biochemica, Beerse, Belgium) as specified by the manufacturer appeared to relieve the requirement for feeder cells.

Hybridoma supernatants which scored positive by ELISA test using HL1 crude antigen and negative by a control ELISA using antigen from *Bacillus thuringiensis* (Temeyer et al. 1986) were tested for antibody specificity by immunoblotting (Pruett et al. 1990). As seen in

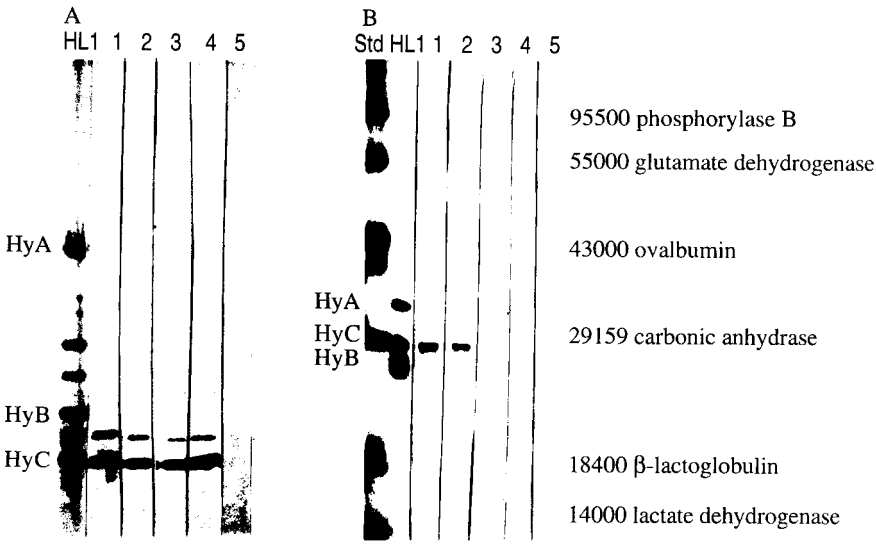

FIG. 1. Binding of monoclonal antibodies to hypodermin C. Soluble proteins from first instar larvae of *H. lineatum* were separated by polyacrylamide gel electrophoresis (PAGE). Hypodermins A, B, and C are identified in the margins for lane HL1 gel photos. Lanes 1, 2, 3 and 4 show Western blots of gel samples as shown in lane HL1 demonstrating binding specificity of monoclonal antibody of HL1-C1 (lane 1), HL1-C2 (lane 2), HL1-C3 (lane 3), and HL1-C4 (lane 4) to native (panel A) or denatured (panel B) *H. lineatum* larval proteins. Lane 5 was a negative control incubated with mAb specific for *Bacillus thuringiensis* crystal protein. (A) Nondenaturing-PAGE and Western blots. (B) SDS-PAGE and Western blots. Lane Std contains protein standards (prestained SDS standards, kit #823; Diversified Biotech, Boston MA) as indicated.

Fig. 1A (lane HL1), relatively few bands constitute the bulk of soluble proteins from *H. lineatum*. Four hybridoma cell lines were isolated which secreted mAb specific for HyC.

Hybridomas HL1-C1 (isolation #1D2A-F1), HL1-C2 (isolation #1D2B-E9), HL1-C3 (isolation #74C1-E12), and HL1-C4 (isolation #234C1-F4) each produced antibody that was determined to be IgG1 by double immunodiffusion and bound both the native and SDS-denatured forms of HyC on immunoblots (Fig. 1). Immunoaffinity columns exhibited the same specificity for HyC as shown on immunoblots. Ascites fluid was collected and found to contain high titer antibody at 500-4000 times that found in cell culture supernatant. IgG precipitation from ascites fluid by ammonium sulfate yielded 0.9-1.2 mg ml^{-1}.

In conclusion, we have demonstrated the construction, isolation, specificity, and production of mAbs specific for HyC of *H. lineatum*. These mAbs were demonstrated to bind both native and denatured forms of HyC and were specific for HyC even under blotting conditions of high primary and secondary antibody concentration (Fig. 1). The mAbs were observed to bind to a band which ran just below the hypodermin A band on nondenaturing PAGE, but this is believed to represent a minor isoform of hypodermin C as evidenced by a single band on SDS-PAGE. These hybridoma cell lines and their mAbs present new and useful biochemical tools for monitoring, quantification, and purification of HyC and related recombinant proteins.

ACKNOWLEDGMENT

The authors thank M. Haufler, B. Burkett, and A. Barwick for excellent technical assistance and thank Dr. V. Howard (Department of Biology, Bismark State College, Bismark, ND) for construction of the immunoaffinity columns.

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OVERWINTERING HABITAT INFLUENCE ON BOLL WEEVIL (COLEOPTERA: CURCULIONIDAE) TRAP CAPTURES IN NEW MEXICO

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ABSTRACT

During the mid 1990's the cotton boll weevil, *Anthonomus grandis grandis* Boheman, became established in much of New Mexico. A study was conducted in 1997 and 1998 to evaluate the effect of various overwintering habitats on trap captures in two areas of New Mexico. Urban habitats produced high overwintering trap captures in both Lea County in the High Plains Test and in Eddy County in a desert river valley. Urban habitats produced the highest spring trap captures in Lea County in 1997 and Eddy County in 1998. Sand shinnery oak produced the greatest trap captures in Lea Co. in 1998 with 142 boll weevils /trap compared to 66 in urban habitats. However, the highest number of late captures were in urban habitats, with 83% of the seasonal total in captured after 23 May. Sand shinnery oak trap captures were over a longer period and only 22% of the total were collected after 23 May. Hostable squares were initially available 20-27 June both years and locations. Trap captures in urban habitats peaked the end of May-early June in the High Plains site in 1997 and 1998. However, captures in urban habitats in the desert valley in 1998 peaked earlier, on 9 May. Within urban habitats, the highest overwintering trap captures were in densely vegetated areas such as weedy borders. There was no evidence of high numbers overwintering in buildings. Desiccation in less protected desert habitats may provide high levels of control, but favorable urban overwintering habitats and proximity of cotton fields to those habitats are major factors in developing economic populations of boll weevil in New Mexico.

INTRODUCTION

The cotton boll weevil, *Anthonomus grandis grandis* Boheman, entered the United States in 1892 and rapidly became established in most of the Cotton Belt. Establishment in the West, however, was delayed. Although boll weevil became a key pest in the Southeast within 30 years of its initial entry into south Texas, there were no persistent economic infestations in the West for over 70 years (Bottrell et. al. 1972a). In 1953, boll weevil became an economic pest of cotton in the Presidio Valley in the Trans-Pecos region of Texas (Robertson 1957). Several years previously, boll weevil infestations were detected immediately west of the Pecos River, near Imperial, Texas, approximately 60 miles south of the New Mexico border (Robertson et al. 1966). Incipient infestations were detected near El Paso, Texas, in 1960, 1962 and 1963 (Fye and Parencia 1972). Despite lack of an eradication program, these infestations did not persist.

Detection of boll weevil in Arizona was complicated by the presence of the thurberia weevil, *Anthonomus grandis thurberia* Pierce, a subspecies of boll weevil. But it seems

clear that economic infestations in cultivated cotton in the 1960's originated with boll weevils from northern Mexico (Fry and Parencia 1972). Subsequent damage in cultivated Arizona cotton from 1982 until it was eradicated in 1990, demonstrated that boll weevil could become established and attain pest status in a desert environment.

In the 1960's, populations were overwintering on the Texas High Plains, a semi-arid area of Northwestern Texas that extends into the eastern border of New Mexico (Bottrell et al. 1972a). For the next 30 years, limited overwintering habitat, diapause control programs, and cold winters kept boll weevil populations low enough to limit expansion (Rummel et al. 1975). But in the 1990's, a series of warm winters and discontinuation of the fall diapause control program led to larger populations in the High and Rolling Plains, and expansion into New Mexico and the Trans-Pecos Region of Texas. Boll weevil is now established in most cotton-growing counties in New Mexico and a number of counties in the Trans- Pecos Region of Texas.

The quality and availability of favorable overwintering habitat is the primary factor affecting weevil survival when boll weevil populations are low (Rummel and Adkisson 1970). The best habitats are those where large amounts of deciduous leaf litter accumulate. Sand shinnery oak, *Quercus havardii* Rydb., provides a very favorable habitat for boll weevil overwintering survival in the High Plains of Texas. (Rummel and Adkisson 1970, Bottrell et al. 1972b). Dense mesquite, *Prosopis glandulosa* Torr, is a somewhat less favorable habitat, but can be important during mild winters due to its prevalence (Brown and Phillips 1989, Bottrell et al. 1972b). Most grasses have not been considered favorable overwintering habitat (Boring 1972). However, one Conservation Reserve Program (CRP) grass, lovegrass, *Eragrostis curvula* (Schrader), produced survival similar to shinnery oak habitat during two relatively mild winters in the Rolling Plains of Texas (Brown and Phillips 1989).

Boll weevil survival in overwintering habitats determines, in part, the level of infestation in nearby fields the following spring, but the timing of emergence is also critical. Early or suicidal emergence will not affect a cotton crop. Boll weevils cannot reproduce in any crop but cotton, and they cannot reproduce in cotton until flower buds are of a size to support full development. These are generally at least 4-mm diameter and are referred to as 'hostable' squares. The 'effective emergence', emergence shortly before hostable squares are available, most accurately predicts subsequent damage (Fenton and Dunham 1927, Rummel and Carroll 1983).

Timing of emergence can vary considerably between years (Stone et al. 1990) and habitats. Emergence was delayed in shinnery oak motts compared to less dense shinnery brush, and was related to lower spring temperatures in litter (Slosser et al. 1984). Emergence of boll weevils in the Rolling Plains in Texas also ended earlier in mesquite litter compared to broadleaf litter (Slosser and Fuchs 1991). Differences in emergence curves have been determined using both pheromone traps and large emergence cages infested with boll weevils. Carroll and Rummel (1985) compared both techniques and found that the emergence profiles were similar.

Most cotton in New Mexico is grown in arid regions in the Chihuahuan desert where average rainfall ranges from 22 cm /year in the Mesilla Valley by the Rio Grande River to 31 cm/year in the Pecos River Valley. In these river valleys, there is little leaf litter habitat and no CRP grasses. The primary natural habitat is dominated by mesquite, creosote, *Larrea tridentata* (DC.) Coville, and tarbush, *Flourensia cernua* DC. Weedy borders and lots are dominated by kochia, *Kochia scoparia* (L.) Schrad., and Russian thistle, *Salsola kali* L. Approximately 10,000 acres of cotton are grown in Lea County on the eastern border of New Mexico, which is geographically within the High Plains. Potential favorable overwintering sites in this area, as in Texas, include shinnery oak, CRP grasses, and mesquite. Rainfall averages 36 cm/yr, 80% of which falls in the six-month

period between May through October.

The climate in the desert valleys is characterized by high temperatures during the summer and moderate temperatures with light freezes during the winter. Relative humidity is low during the day, generally 20-30% and rises to at least 70-80% at night.

The identification of the most favorable boll weevil overwintering habitats is of particular importance in eradication and suppression programs. When such areas can be identified, scarce resources can be allocated more efficiently, with monitoring and control efforts concentrated in the areas at highest risk. Identifying the most favorable habitats also gives producers the opportunity to modify the most problematic habitats (Slosser et al. 1984), avoid planting near such habitats, or otherwise manage their crop with the knowledge that such fields are more susceptible.

The objective of this study was to determine which habitats produce the highest trap captures of overwintered boll weevils in New Mexico, and to determine if there could be differences in emergence curves of overwintered boll weevils suggested by trap captures in the various habitats.

MATERIALS AND METHODS

Population Monitoring with Pheromone Traps. In 1996 through 1998, pheromone traps (Technical Precision Plastics, Mebane, NC) were used to monitor boll weevil populations in the Pecos valley of New Mexico. At least one trap was placed in each cotton field in the valley. Trapping rates varied over the years, but there were generally 400-700 traps with a trapping rate of 1:5 to 1:40/acre. Traps were monitored from late April to November with traps checked weekly or biweekly. Ten-mg lures (Hercon Environmental Corp., Emingsville, PA) were replaced biweekly.

Habitat sampling. In 1997 and 1998, traps were placed in overwintering habitats in Lea County on the High Plains, and in Eddy County in the Pecos Valley. Boll weevil traps were placed in five to seven different habitats in each county, each year. Traps were placed in habitats in February with 30 traps per habitat, with the exception of the urban habitat which had 20-30 traps. Lures were changed every two weeks. Traps were checked biweekly in 1997 and weekly in 1998. Traps were removed in early July, shortly after all cotton had flower buds (squares).

Habitats sampled in the Pecos Valley in 1997 were urban densely vegetated backyards, grassland, mesquite, borders between fields of cotton and alfalfa, Medicago *sativa* L., and salt cedar, *Tamarix ramosissima* Ledeb. Habitats tested in Lea County in 1997 and 1998 were sand shinnery oak, *Quercus havardii* (Rydberg), urban densely vegetated backyards in Hobbs and Lovington, cotton field borders, grassland, and CRP grasses. Habitats tested in the Pecos Valley in 1998 were located in two areas, one urban, and one rural. In the urban location, traps were placed in three habitats throughout Carlsbad, NM, in a 7 square-mile area. The rural habitats were in an area beginning 10 miles south of Carlsbad in a 14 square-mile area, 30 miles north of the Texas border. All traps were checked weekly with lures changed every two weeks.

The urban area was divided into three habitats. The first habitat had structures but sparse vegetation. Traps were placed in areas with few weeds or dense vegetation and where the closest building had wood roof shingles. The second habitat had high levels of vegetation, either weedy borders or urban weedy lots. The third habitat was sparse vegetation without structures nearby. These traps were placed on golf courses. It was considered a control habitat. Rural habitats included grassland, salt cedar, borders between cotton and alfalfa fields, and mesquite rangeland.

No choice survival and emergence tests. Boll weevils were also tested for overwintering survival and emergence curves of multiple cohorts. Techniques were

similar to Carroll et al. (1993). Boll weevils were reared from infested squares collected from August to October. Adults were collected as they emerged, then fed clean squares and small bolls for 3 weeks before being placed in a rangeland habitat near Artesia, NM. In the emergence test, four cohort groups were placed in the field in field-emergence cages with four cages per cohort and approximately 200 boll weevils per cage. Due to the lack of preferred overwintering habitat (i.e., deep leaf litter) artificial insulation was added by covering the bare ground with either 72 or 12.0 cm of alfalfa hay. In the survival test, adults were placed in open cylinders on the ground with 25 boll weevils per cylinder and covered with hay. Forty-eight cylinders were placed in the field, eight cylinders for each date when they would be removed and evaluated for survival. Temperature/relative humidity dataloggers (Onset Corp., Borne, MA) were placed at ground level for each treatment, and two inches below the surface in the 12.0-cm treatment with temperature and relative humidity recorded hourly.

RESULTS AND DISCUSSION

By the early summer of 1996, overwintered populations of boll weevils were detected with pheromone traps in the Pecos Valley. Initially, the highest numbers of weevils were detected in the easternmost cotton growing areas near the small villages of Hagerman and Malaga. However, with time, greater populations became established in more urban areas.

Boll weevil captures in the small village of Malaga (population 350) were twice as high as those in Carlsbad (population 25,000), 15 miles north, in the fall of 1996 but only half as high the following spring (Fig. 1). From September to December 1996, an average of 17.8 boll weevils per trap were captured in Malaga (n=32 traps) compared to 8.2 in Carlsbad (n=94 traps). The following spring, 0.63 were collected per trap in Carlsbad (n=118 traps) compared to 0.32 in Malaga (n=84 traps) for three weeks prior to production of hostable squares.

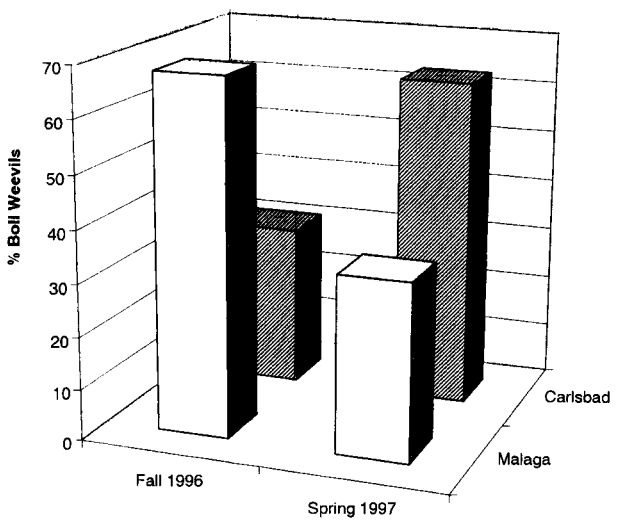

FIG. 1. Percentage of total overwintered boll weevil captures in an urban (Carlsbad) and nearby rural (Malaga) area of south Eddy County NM, 1996-1997.

Lea Co 1997. The greatest number of boll weevils were captured in urban habitats with a seasonal average of 48 boll weevils per trap over the season (Table 1). CRP and sand shinnery oak habitats followed, both with 23 boll weevils per trap. The lowest numbers were collected at cotton borders and range grassland with 14 and 13 boll weevils per trap, respectively. Emergence curves for the best habitats, urban, CRP and shinnery oak, were similar with peak emergence the two weeks prior to 9 June (Fig. 2). After that date, captures declined precipitously. Peak captures in the cotton borders and grassland were about a month earlier, with peak emergence the two weeks prior to 11 May. The majority of boll weevils, 84%, emerged between 11 May and 9 June. Hostable squares were available 20 June .

TABLE 1. Seasonal Average and Percent of Total Boll Weevils Captured in Pheromone Traps in Various Overwintering Habitats in Lea County, NM, 1997 and 1998.

Habitat	Mean[a] 1997	% of total 1997	Mean[a] 1998	% of total 1998
Urban	48a	40	66b	24
Sand shinnery oak	23b	19	142a	51
CRP	23b	19	49b	17
Grassland	13c	11	11c	4
Cotton Border	14c	12	12c	4

[a]Means followed by similar letters are not significantly different by DMRT ($P<0.05$)

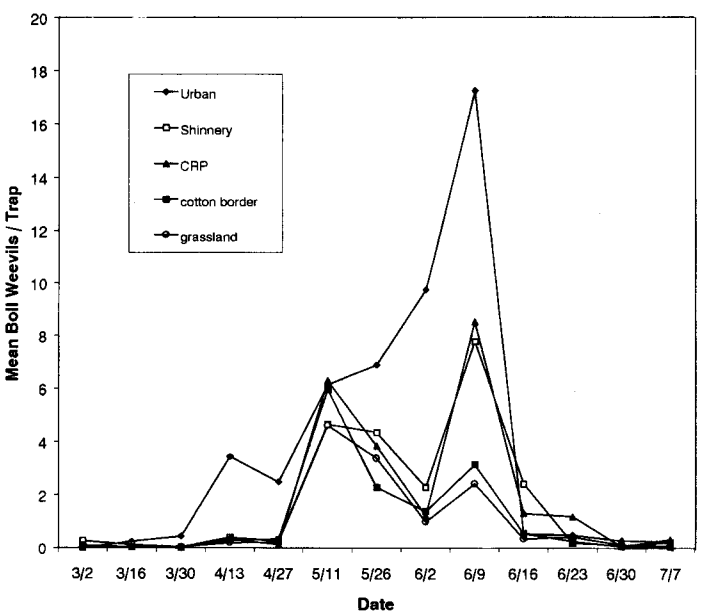

FIG. 2. Overwintered boll weevils captured in habitats in Lea County, NM, 1997.

Eddy Co. 1997. Boll weevil populations in Eddy County were still low in the spring of 1997. Only 115 boll weevils were collected from 140 traps up to 3 July. The low numbers made comparison of habitats impossible. However, pooled data from all habitats indicated peak captures were from 6 May to 2 June, with 54% of the total collected during that time period. This peak period was similar to that in Lea County the same year with the highest captures from 27 April to 9 June.

Lea Co. 1998. During 1998, the greatest number of overwintered weevils was captured in shinnery oak habitat, with a seasonal total of 142 boll weevils per trap or 51% of the total collected in all traps (Table 1). Traps in urban habitats had a seasonal total of 66 boll weevils per trap, 24% of the total collected, and significantly fewer than sand shinnery oak. CRP traps collected a seasonal total of 49 boll weevils/trap, significantly less than in shinnery oak but more than in grassland and cotton border traps. Grassland and cotton field border traps captured the fewest boll weevils with seasonal totals of 11 and 12 boll weevils per trap, respectively (Table 1). Although total captures were higher in shinnery oak in 1998, the highest number of late captures was in urban habitats. After 23 May, traps in urban habitats collected 83% of the season total for that habitat compared to 42% in CRP grasses and only 22% in shinnery oak (Fig. 3).

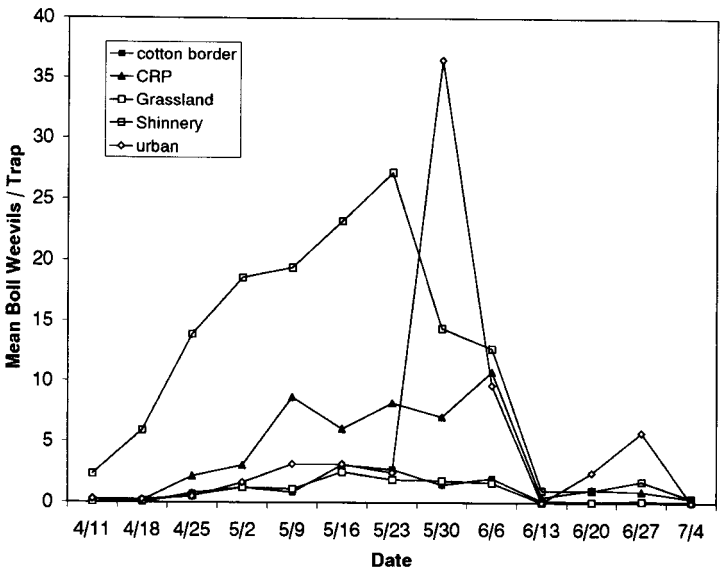

FIG 3. Overwintered boll weevil captures in habitats in Lea Co. NM 1998.

1998 Eddy Co. Urban Habitat.: Traps located in densely vegetated areas captured more boll weevils than traps in both the sparse vegetation/high structure habitat and sparse vegetation/low structure habitat (Table 2). Dense vegetation traps captured 15.7 boll weevils per trap compared to 2.8 boll weevils in the sparce vegetation/high structure habitat and 1.4 in the sparce vegetation/low structure habitat. Peak capture was between 26 April and 9 May, with a total of 8.2 boll weevils per trap, or 52% of the total captured in the dense vegetation habitat (Fig 4). During the same period, traps in the sparse vegetation/high structure and low structure habitats captured 0.97 and 0.91 weevils/trap or 35% and 65% of the total captured in those habitats, respectively.

TABLE 2. Overwintered Boll Weevil Captures in Rural and Urban Habitats in Eddy County NM in 1998.

Habitat	Total/trap[a]	% of total
	Urban	
High vegetation	15.7a	68
Low vegetation/low structure	4.7b	20
Low vegetation/high structure	2.8b	12
	Rural	
Salt cedar	3.7a	37
Mesquite	2.2ab	22
Grassland	3.1a	31
Cotton /alfalfa field borders	1.0b	10

[a]Means followed by similar letters are not significantly different ($P<0.05$) by DMRT

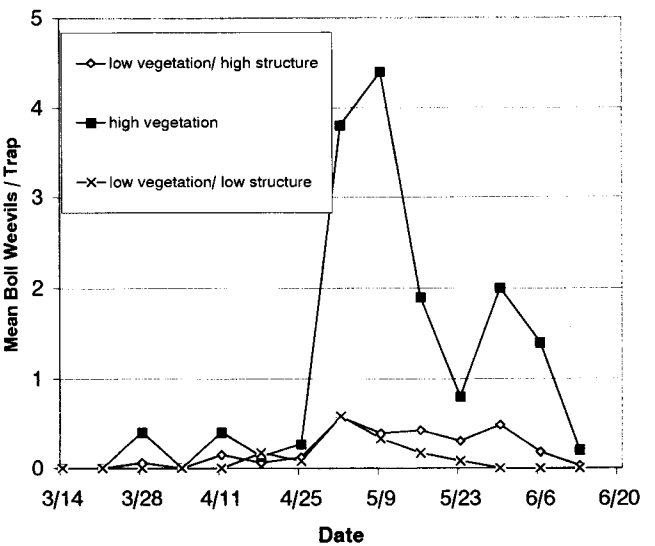

FIG 4. Overwintered boll weevil captures in urban habitats in Eddy Co. 1998.

1998 Eddy Co. rural habitat. Boll weevil captures were significantly higher in salt cedar and in grasslands compared to cotton/alfalfa field borders (Fig 5). Salt cedar traps captured a total of 3.7 boll weevils/trap by 13 June compared to 3.1 in grassland traps and only 1.0 in borders between alfalfa and cotton. Traps located in mesquite areas collected 2.2 weevils/trap. Peak capture was similar to that in the urban habitats with peak captures 26 April to 9 May in salt cedar and grassland habitats. (Fig. 5).

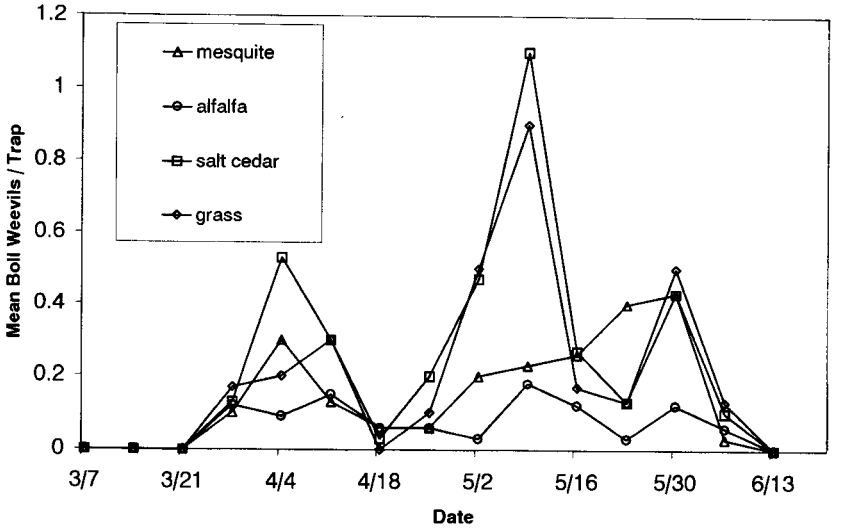

FIG 5. Overwintered boll weevil captures from rural habitats in Eddy Co. 1998.

Survival test. With insulation, boll weevils did successfully survive the coldest part of the winter in December and early January. On 6 January, 63% of boll weevils sampled were alive. Of those surviving weevils, 48% were alive two weeks later after being held at 25°C with only water. Data collected after 6 January were compromised by a fungal infection in the weevils. However, during the winter of 1997-1998, the coldest period was prior to 6 January. Any further mortality would be due to some factor other than low temperatures.

Temperatures at a depth of 7.2-12.0 of insulation were not low enough to cause any mortality. Temperatures at a depth of 4.8 cm of insulation were not low enough to cause high mortality. The lowest extended period of low temperatures was -2.5°C for 12 hours at 4.8 and 7.2 cm depth (Table 3) which Slosser et al. (1996) found would not result in high mortality. Temperatures at a depth of 7.2 cm or more of insulation were less likely to produce mortality than 4.8 cm. In laboratory tests, Slosser et al. (1996) recorded only 5% mortality at -5°C for 8 hours and 29% mortality at -7.5°C for 12 hours. However, even very short periods at 10°C did result in relatively high mortality, with 48% mortality in boll weevils exposed to -10°C for only 1 hour. Temperatures at 4.8 cm were -0° C for 1 hour and below -7.5 and -5.0°C for 6 and 9 hours, respectively. The lowest temperature at 7.2 cm was only -2.5 ° C for 12 hours.

TABLE 3. Temperatures below alfalfa hay insulation from 4 December to 6 January, 1998 in Artesia, NM.

Depth (cm)	Low °C	No. continuous hours below °C			
		-2.5	-5.0	-7.5	-10
0	-20.5	70	22	15	4
4.8	-16.3	12	9	6	1
7.2	-4.1	12	0	0	0
12.0	-2.3	0	0	0	0

One of the greatest barriers to boll weevil establishment on the High Plains was the scarcity of suitable overwintering habitat (Rummel and Adkisson 1970). The lack of suitable overwintering habitat will be even more important in the desert valleys of New Mexico and Texas since prolonged winter temperatures below -4.9°C, which are responsible for much mortality in the High and Rolling Plains, are rare.

The preferred overwintering habitat, leaf litter, is not common in eastern New Mexico on the edge of the High Plains, and is much more rare in the desert valleys. The data presented indicate that the best overwintering habitat in the desert valleys of New Mexico and Texas are urban habitats. Although there has been much speculation that boll weevils are overwintering in buildings, particularly under wood shingle roofs, these data do not suggest that this is one of the better overwintering habitats. High levels of vegetation, both in urban lots and borders, produced the highest number of boll weevils captures. This difference may have been due to higher numbers of boll weevils selecting such habitats in addition to, or rather than, higher survival rates.

In monitoring and eradication programs, the influence of urban habitats on boll weevil establishment has become evident. In both the Pecos and Mesilla Valleys, the initial infestations were not in urban areas. But the present 'hotspots,' in both valleys, are in cities. The finding that the highest numbers were in highly vegetative areas in weeds is encouraging. If significant numbers were overwintering in buildings, control would be difficult. Weeds, particularly those in borders by the field, are manageable.

These data also suggest that desiccation during long, dry springs may be one of the most important sources of mortality in the desert valleys of New Mexico and Texas. Although, in comparison to other areas, every spring in these desert valleys is dry, the spring of 1998 was particularly dry and hot. Boll weevil captures that spring were earlier than in 1997 and lower than expected. Early captures in 1998 may have been due to early emergence but also could have been a result of very high mortality in overwintering habitat after 9 May 1998 or both. The primary stress deserts impose on insects is desiccation from high temperatures, radiation, and low humidity. The data presented suggest that the most favorable overwintering habitats may provide protection not only from low winter temperatures, but also from exposure to high temperatures and radiation that could reduce survival, particularly in late spring. Equally, or perhaps more important, respiring plants could provide a microclimate with higher relative humidity than inert habitats. Bottrell (1972b) noted that unexpectedly low overwintering survival in one habitat was likely due to drier conditions during a droughty year in the Rolling Plains. In the desert valleys of New Mexico and Texas, the highest climatic control may be a combination of high temperatures, high radiation, and low relative humidity producing high suicidal emergence and direct mortality from desiccation in exposed habitats. Plants that protect boll weevils from direct sunlight, and provide insulation from extreme temperatures could provide the most favorable overwintering habitat. Such a habitat will likely also produce later emergence, more closely timed to availability of hostable squares. Bunch grasses, particularly those in irrigation ditches, under trees or in shaded urban areas could provide such a habitat and will be examined further.

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SNAKEWEED FLOWER HEAD WEEVIL: A FINICKY CONSUMER OF WOODY SNAKEWEED?

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ABSTRACT

Research was conducted to determine whether the snakeweed flower head weevil, *Anthonomus tenuis* Fall, showed either feeding differences or differential larval survival between broom snakeweed, *Gutierrezia sarothrae* (Pursh) Shinners, and threadleaf snakeweed, G. *microcephala* (DC) Gray. Results indicate that while adult weevils feed on both species, larvae are found only in the seed heads of broom snakeweed.

INTRODUCTION

Broom snakeweed, *Gutierrezia sarothrae* (Pursh) Shinners (Asteraceae: Astereae), and the closely related threadleaf snakeweed, *G. microcephala* (DC) Gray, are aggressive, potentially toxic to livestock, low-growing perennial half shrubs (Dollahite and Anthony 1957, Dwyer and DeGarmo 1970, Ueckert 1979, McDaniel et al. 1981, Smith et al. 1991.) Because of these undesirable characteristics, the native woody snakeweeds generally are considered to be economically important; control is necessary on some rangelands. Various herbicide, burning, and biological control methods have been used to control snakeweed in New Mexico, although the results after herbicide or burning treatments are often unsatisfactory (Wangberg 1982, Dwyer 1967). In both cases, seeds in the soil may provide a rapid resurgence of snakeweed. Of these methods, only biological control appears to have a chance of controlling the seed production and viability through agents that attack the buds, flowers and seeds directly.

Out of seven species of Curculionidae and two species of Apionidae found in the flower and seed heads (Foster et al. 1981) of both woody species of snakeweed, only *Anthonomus tenuis* appears to actually develop in the capitula as larvae (Richman and Thompson 1999). *Anthonomus tenuis* was extremely abundant on woody snakeweeds in this study, especially in Socorro County, although it was observed early on that the beetles were much less common on threadleaf snakeweed than on broom snakeweed. Unfortunately there are no published life history studies on this weevil to our knowledge, although Burke (1968) described the pupa and noted that it was found in the flower heads of both "*Xanthocephalum*" (= *Gutierrezia*) *sarothrae* and "*Chrysothamnus*" (= *Ericameria*) *nauseous* (Pallas ex Pursh) Britt (Roalson and Allred 1995).

Experiments were thus devised to determine if *Anthonomus tenuis* weevils discriminate between the two species of woody snakeweed as adults and whether this extends to selection of oviposition sites. In *G. sarothrae* the flowering heads or capitula are small, yellow and average seven total florets, including both tube and ray flowers; some capitula may contain as

few as two tube and two ray flowers. In *G. microcephala*, the capitula are reduced usually to one tube and one ray flower (Loomis 1983). Average mature plants of *G. sarothrae* usually produce as much as 10,000 seeds in a year (McDaniel et al. 1984).

MATERIALS AND METHODS

Several experiments were devised in 1994 and 1995 to test feeding preference of adults of *Anthonomus tenuis*. At the same time field collections of capitula from both woody snakeweed species in several New Mexico counties also were conducted to determine actual prevalence of the weevil larvae. As the sexes are difficult to separate, the proportions of each sex were unknown.

A two-choice olfactometer was constructed of a T-shaped response chamber connected to two glass jars (with removable lids) by clear plastic tubes (Tygon R-3603, 7.0 mm in diameter and 30 cm in length). Air was blown over snakeweed clippings in the glass chambers by an electric pump (Second Nature Whisper7 700A aquarium pump). The air stream passed from the pump through plastic tubing to the jars, then through the response chamber and exhausted out of the device.

Each test was replicated at least ten times, with test materials alternated in consecutive replications. Response chambers, plastic tubes and the two glass chambers were cleaned with ethanol and then air-dried for about 5 minutes after each run. All experiments were conducted at about 36°C and 30-40% relative humidity, between 0900 and 1600 hr MDT during the fall of 1994 and between 1000 and 1800 hr MDT during July-September of 1995. Eight, 40-watt fluorescent lamps positioned 1.8 m above the olfactometer provided illumination.

Single and double choice olfactometer experiments ran for 30 or 60 minutes. In these tests five weevils (August 1994) or ten weevils (1995) were given a choice between *G. sarothrae* (five replicates) and *G. microcephala* (five replicates) in one chamber and nothing in the other chamber. The second test was a choice between *G. sarothrae* in one chamber and *G. microcephala* in the other (ten replicates), again with five weevils per replication. Infested capitula collected in the field from Torrance and Lincoln counties, New Mexico, were held in the laboratory for adult emergence. The adult weevils were not aged or sexed for the feeding preference experiments. Responses were counted as positive if a weevil moved past the t-tube into a chamber tube or plant chamber. Responses were counted as negative if a weevil remained in the entry tube.

Petri dish experiments also were conducted during the summer of 1994 in which one 4.0-cm long stem of both species of snakeweed was placed in a 60 mm divided Petri dish lined with #2 filter paper. One ml of distilled water was added to the dish to maintain humidity, and five to ten adult weevils (not aged or sexed) were placed in the dish. In the fall of 1994 and the fall of 1995, the piece of filter paper was placed in the bottom of a 15 or 12.5-cm plastic Petri dish. A stem of *G. sarothrae* was placed on top of the filter paper on the far-left side and a stem of *G. microcephala* on the far right side. The weevils were placed in the center of the Petri dish and the cover replaced. At the end of one hour, the cover of the Petri dish was removed and weevils on each stem were counted and recorded. In one set of experiments in the summer of 1994 the number of live weevils was counted on each side daily and plant stems were changed as needed for 11 days. All the weevils had moved to *G. sarothrae* by that time, and so the experiment was concluded.

In a second series of experiments, weevils were collected from the Leyendecker Plant Science Research Center snakeweed plots in Doña Ana County, NM, during 1995. In the laboratory, the weevils were fed plant stems that consisted of approximately 50% *G. sarothrae* and 50% *G. microcephala*. The plant stems were inserted into a water soaked floral block placed in a 10-ml plastic cup. The cup with the stems was set on a Petri dish bottom, weevils

were placed on the stems and everything was enclosed inside an inverted 355-ml clear plastic cup. The bottom of the 355-ml cup was removed and covered with insect netting to allow air exchange for the plant stems and weevils. The weevils were removed from their regular food source and held in 10-ml plastic containers without food for 24 hours before each experiment. Non-flowering plant stems were used in the first series of experiments (Petri #1, Double Choice #1 and Single Choice #1). Flowering plant stems were used in the second series of experiments (Petri #2, Double Choice #2 and Single Choice #2). The plant stems for 1995 Petri #1 experiment came from labeled plants on the Jornada Experimental Range snakeweed plots at South Well, Doña Ana Co., New Mexico. As plants from this site were in poor condition, stems from the Leyendecker snakeweed plots were used for the remaining experiments.

A cage experiment was also conducted in which stems of *G. sarothrae* and *G. microcephala* were clipped (10-12 cm long) from the Leyendecker snakeweed plots and caged with ten weevils. A total of five to ten stems of one species were provided in each cage. The number of live weevils on both plants was counted daily and stems were changed when necessary to keep the material fresh.

Collections were made at 12 sites in 10 counties in western New Mexico in July 1994 and at 21 sites in 12 counties in the eastern half of New Mexico during August 1994. In each case, the plants were identified by examining the capitula, whether in bloom or in bud. Flower heads were collected and the number of larvae in 50 flower heads was recorded. Parasitoids found were also collected.

In 1995 snakeweed flower capitula were collected from each of five marked *G. sarothrae* and *G. microcephala* at the Jornada (South Well) site and from each of five marked Jornada *G. microcephala*, five marked Arizona *G. microcephala*, five marked Jornada *G. sarothrae* and five marked Lovington (Lea County) *G. sarothrae*. Twenty capitula were dissected per plant, for a total of 100 capitula per site. The dissections were conducted during November 1995. All larvae of *A. tenuis* were counted and recorded.

RESULTS AND DISCUSSION

Weevils in the 1994 olfactometer bioassays showed a definite preference for snakeweeds over blanks and for *G. sarothrae* over *G. microcephala*, with 94% of the weevils choosing one or the other species of snakeweed (data pooled) over the blank (4.70± 0.48 for plants, 0.2 ± 0.42 for blank, Chi-Square with 1 DF 41.3265 $P < 0.0001$.) Most (84%) of the weevils in olfactometer tests offered a choice between the two species of snakeweed tended to move toward the glass container which held *G. sarothrae* stems (4.1 0± 0.74 for *G. sarothrae*, 0.90± 0.74 for G. microcephala, Chi-Square with 1 DF 20.4800 $P < 0.0001$). Petri dish and cage experiments with weevils resulted in nearly all weevils moving to *G. sarothrae*. Overall survival of adult weevils in the cage study was higher for *G. sarothrae*, with 50% alive after 5 days, than for *G. microcephala*, with 50% alive after 2 days.

Attempts to repeat these experiments (olfactometer and Petri dish) in 1994-1995 resulted in no significant preference being shown in any of the experiments. The two Petri dish double choice experiments (Double Choice #1 and #2) were typical, showing no statistically significant results with 5.50±1.72 weevils attracted to *G. sarothrae* and 4.40±1.71 weevils attracted to *G. microcephala* (Chi-Square with 1 DF 1.2222 $P = 0.2689$, n = 10, N = 99) and 4.50±1.90 weevils attracted to *G. sarothrae* and 4.40±1.71 weevils attracted to *G. microcephala* (Chi-Square with 1 DF 0.0112 $P = 0.9156$, n = 10, N =89, all tests starting with 100 weevils, n = number of weevils in each replication, N= total live weevils recovered in all replications.) All of these weevils had been collected in Doña Ana County, New Mexico,

whereas in the earlier experiments the material came from Lincoln and Torrance counties. In an attempt to use weevils that had not been exposed to *G. microcephala* in mixed populations, as had those from Leyendecker Plant Science Research Center site or the Jornada site, weevils were brought from the Socorro County, New Mexico, site where only *G. sarothrae* grew. These experiments also resulted in data clearly showing no preference, with 4.60±1.17 weevils attracted to Socorro *G. sarothrae* and 4.80±1.40 weevils attracted to Doña Ana *G. sarothrae* (Chi-Square with 1 DF 0.0426 $P = 0.8366$, n = 10, N= 94) in the first experiment. In the second experiment, a mean of 4.40±1.51 weevils were attracted to Socorro *G. sarothrae* and 4.00±1.16 weevils were attracted to Doña Ana *G. microcephala* (Chi-Square with 1 DF 0.1905 $P = 0.6625$, n = 10, N = 84, all tests started with 100 weevils; n = average number of weevils in each replication; N= total live weevils recovered in all replications.) The discrepancy between the early olfactometer tests and the later tests with the same equipment is puzzling. The shorter time (30 minutes as opposed to one hour) involved in the later olfactometer tests could have been a factor, but further Petri dish experiments over the one-hour period used in the earlier tests also showed no preference. It is possible that the first tests involved a large number of females close to oviposition and that this influenced the results, resulting in a higher number moving to *G. sarothrae* stems.

Data from field collected material confirmed the view that *Anthonomus tenuis* either prefers *G. sarothrae* for oviposition, or does not survive on *G. microcephala* as a larva. Indeed, no larvae of *A. tenuis* were found in the capitula of *G. microcephala* from 11 sites, while 149 larvae were collected in the capitula of *G. sarothrae* from ten sites in August 1994. Results from the dissection of the 500 capitula (300 *G. sarothrae* and 200 *G. microcephala*) from the Jornada and Leyendecker sites tends to confirm this, as again no larvae were ever found in a capitulum of *G. microcephala.* In addition, sweep samples collected in 1989-1993 by Richman from *G. sarothrae* seemed to have higher numbers of adult *A. tenuis* than those collected from *G. microcephala.* Unfortunately, only a few samples were taken from *G. microcephala* during this period, but the general impression was that when large numbers of weevils were collected, the plant involved was always *G. sarothrae.* It thus seems possible that *A. tenuis* prefers *G. sarothrae* for oviposition, but may feed on either species as an adult if the preferred host is not available or if oviposition is not occurring. In the case of the November tests with the Socorro weevils, oviposition may have already occurred and adults were probably not discriminating. A similar situation could be occurring with the Leyendecker and Jornada weevils, only in this case it may be that the weevils were tested before oviposition or missed a relatively narrow oviposition period in August. We did not test preference by sex and thus another possibility is that males may never discriminate between the two species and that the weevils used were disproportionately one sex or the other.

Parasitoids recovered from the 1994 study include only one that attacks *Anthonomus tenuis.* This was the eulophid *Tetrasticus* sp. Two other parasitoids were also recovered, but these seem to be attacking a cecidomyiid fly that was occasionally found in the capitula. These were the pteromalid *Zatyopis* sp. and the eurytomid *Eurytomis* sp. No data were collected on the effect of *Tetrasticus* on *A. tenuis* populations.

The following conclusions are warranted. 1) *Anthonomus tenuis* adults will feed on *G. sarothrae* and *G. microcephala* although they may show a preference toward *G. sarothrae* at some time of the year. 2) *Anthonomus tenuis* larvae are found in the seed heads of *G. sarothrae*, but not on *G. microcephala.* 3) At least one species of eulophid parasitoid (*Tetrasticus* sp.) attacks *A. tenuis* larvae. The question now arises as to the reason for the prevalence of *A. tenuis* larvae in the capitula of *G. sarothrae*, when adults do not seem to discriminate in the laboratory between the species of snakeweed for at least some of the year. At least in part, we believe the answer is related to the number of seeds and the size of the capitula. *Anthonomus tenuis*, despite its small size, may need more food as a larva than *G.*

microcephala provides. Selection may cause *G. microcephala* to have fewer seeds because it then avoids seed predation by *A. tenuis* larvae. If this is so, then under some circumstances (and assuming no counter selection for large capitula with more numerous seeds), *G. microcephala* may invade and replace *G. sarothrae*. This in fact may be happening, as *G. microcephala* was found at several sites during our 1994 eastern New Mexico sampling trip where *G. sarothrae* was expected. In addition, *G. microcephala* seems to be more numerous on the Jornada than it was a few years ago. Unfortunately, these observations are just anecdotal, but future researchers should examine their populations of snakeweed carefully for this alteration in populations.

ACKNOWLEDGMENT

We would like to thank the various ranchers who allowed us to sample snakeweed on their properties, especially those associated with the Youngblood Ranch near San Antonio. We would also like to thank Eric Grissell of the Systematic Entomology Laboratory, United States Department of Agriculture in Beltsville, Maryland, for identifying the parasitoids; Gloria Maese, Senior Analyst for the Agricultural Biometric Service, New Mexico State University, provided the Chi Square test results for those data that allowed statistical testing. David Thompson of the Department of Entomology, Plant Pathology and Weed Science, New Mexico State University, and Carol Sutherland, New Mexico Cooperative Extension/New Mexico Department of Agriculture, both reviewed the manuscript and offered a number of useful suggestions. This project was supported in part by an USDA CSREES special grant and the Agricultural Experiment Station, New Mexico State University, Las Cruces.

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LETHAL AND SUBLETHAL EFFECTS OF SELECTED INSECTICIDES[1] ON *GEOCORIS PUNCTIPES*[2]

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ABSTRACT

Adults of the big-eyed bug, *Geocoris punctipes* (Say), from a laboratory culture, were exposed to selected insecticides and insect growth regulators (IGR's) using a foliar insecticide residue bioassay. Methoxyfenozide, tebufenozide, and spinosad were not toxic to male and female *G. punctipes* at the recommended rates, while chlorfenapyr produced only partial mortality. At recommended rates, tebufenozide and chlorfenapyr significantly reduced the fecundity of *G. punctipes* compared with a control. Most of the chemicals tested had no effect on host egg consumption of *G. punctipes*, whereas a recommended rate of chlorfenapyr increased egg consumption. Tebufenozide, methoxyfenozide, and chlorfenapyr appear very good for inclusion in IPM in cotton while chlorfenapyr seems fairly good.

INTRODUCTION

The concept of integrated pest management (IPM) was developed as a consequence of the perceived incompatibility of pesticides and biological control. Integrated pest management in cotton production recommends the preservation of beneficial insects for control of various insect pests. Emphasis on IPM is especially important in early season cotton, when beneficials are capable of maintaining some pests below economic thresholds. Chemical insecticidal sprays become necessary, however, as the growing season progresses and numbers of pest insects increase and plant fruiting structures become more susceptible to attack (Frisbie et al. 1989).

Primary pest release and resurgence and increases in populations of secondary pests, may occur as a result of the selective destruction of beneficial arthropods by chemical pesticides. Even the newly developed biorational pesticides, which are based on natural products and are more host- or pest-specific, can have profound side effects (Croft 1990). Pest release and resurgence have been widely reported as a consequence of pesticide use or over-use (Michelbacher et al. 1946, Doutt 1948, DeBach and Bartlett 1951, Lingren and Ridgway 1967, Flint and van den Bosch 1981).

Cotton IPM is highly complex and relies on many factors, including the selectivity of pesticides. Information on the toxicities of various cotton insecticides to key beneficial species is therefore important in selection of compounds that will minimize mortality of these species.

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

[2]Hemiptera: Lygaeidae

The literature is replete with studies which document the toxic effects of pesticides on beneficial arthropods in cotton. For example, the effects of four new compounds [spinosad (Tracer®), fipronil (Regent®), chlorfenapyr (Pirate®), and imidacloprid (Provado®)] at field rates on populations of beneficials have been determined on cotton beneficials (Pietrantonio and Benedict 1997, Sparks et al. 1997, England et al. 1997, Peterson et al. 1996, Murray and Lloyd 1997). Elzen et al. (1998) also reported the toxic effects of insecticides to the cotton beneficials *Geocoris punctipes* (Say), *Orius insidiosus* (Say), *Hippodamia convergens* Guerin-Meneville, and *Chrysoperla carnea* Stephens. Several other studies document the toxic effect of cotton insecticides on beneficials (Pape and Crowder 1981, Yokoyama and Pritchard 1984, Butler and Las 1983, Scott et al. 1983, Rajakulendran and Plapp 1982, Plapp and Vinson 1977, Plapp and Bull 1978). However, pesticides often can have more subtle effects on beneficial arthropods, including sublethal effects (for review see Elzen 1989, Jepson 1989, Croft 1990).

The big-eyed bug, *G. punctipes*, is an important predator of several economic pests of cotton (Sterling et al. 1989). The objective of this study was to document some of the lethal and sublethal effects of selected, newer chemicals with novel modes of action on *G. punctipes* using a foliar insecticide residue bioassay which mimics field application of formulated chemicals. The chemicals tested were chlorfenapyr, an insecticidal pyrrole with a mode of action related to the uncoupling of oxidative phosphorylation (Kuhn et al. 1993); tebufenozide and methoxy-fenozide, IGR's that mimic the action of ecdysone (Walton et al. 1995); and spinosad, which causes persistent activation of nicotinic acetylcholine receptors and prolongation of acetylcholine responses (Salgado et al. 1997).

MATERIALS AND METHODS

The *G. punctipes* culture was originally collected from cotton near Weslaco, TX, in September 1996. Adult *G. punctipes* were maintained on green beans as an ovipositional substrate and were provided *Helicoverpa zea* (Boddie) eggs as food and moistened cotton wicks as a water source. Adults were maintained in 14.5 cm x 2.5 cm ventilated plastic petri dishes, with 40-60 insects per dish. All colonies were held at 26 ± 2°C, 55-60% RH, and a 14L:10D photoperiod. Adults were less than one week old when treated.

Formulated insecticides tested were spinosad [Tracer 4 suspension concentrate (SC); Dow Agrosciences, Indianopolis, IN], chlorfenapyr [Pirate 3 (SC); American Cyanamid, Parsippany, NJ], methoxyfenozide [Intrepid 80 wettable powder (WP); Rohm and Haas, Philadelphia, PA], and tebufenozide [Confirm 2 flowable (F); Rohm and Haas, Philadelphia, PA].

Cotton plants, *Gossypium hirsutum* L. ('Sure-Grow 125'), grown in 11-cm diameter plastic pots in a greenhouse, were treated with insecticides using a laboratory spray chamber (DeVries Mfg., Hollandale, MN) (Elzen et al. 1998). Air was exhausted from the chamber through a built-in exhaust fan, and the chamber and spray system were washed with water between treatments. The sprayer was calibrated to deliver 56 liters per hectare using one TX-4 nozzle at 1.7kg/cm² and 4.8km/h.

Rates of formulated insecticides applied to cotton plants were selected by referring to an appropriate control guide (Norman and Sparks 1997) or from manufacturer's recommendations (in the case of non-registered materials). These rates are shown in Tables 1 and 2. Treatments were mixed with water using analytical methods and precision laboratory glassware. In the case of chlorfenapyr, treatment was applied at the recommended rate and at 30% of the recommended rate. One leaf from each randomly sprayed cotton plant was excised 30 min after treatment and placed in a 14cm x 2.5cm ventilated petri dish, with water-moistened filter paper placed on the bottom of each dish. *Geocoris punctipes* adults were sexed with the aid of a stereo microscope. Five adult males and five adult females were

aspirated into each dish and six dishes were replicated for each treatment. *Helicoverpa zea* eggs were supplied as food and moistened cotton wicks served as a water source. Adults exposed to each compound were held for 96 h at 26 ± 2° C, 55-60% RH, and a 14L:10D photoperiod. Data were taken at 96-h after exposure; mortality was assessed by failure of movement when prodded by a probe. Control mortality was never greater than 10%; data were corrected for control mortality using Abbott's (1925) formula. Percentage mortalities were arcsine transformed and analyzed by analysis of variance; means were separated by least significant difference [$P \leq 0.05$ (SAS Institute 1988)].

Cotton plants were randomly sprayed as outlined above with formulated insecticides, leaves were excised and exposed to *G. punctipes* as previously outlined. Five adult females were placed in the petri dishes containing the sprayed leaves and six dishes were replicated for each treatment. Six 4-cm diameter gauze-faced pads were spread apart exposing both inner surfaces. Both inner pieces were placed in the holding cages as oviposition substrates. Preliminary data had shown that *G. punctipes* in laboratory culture readily lays eggs on these gauze pads. The number of eggs on each surface was counted every 24 h for a total time of 96 h.

Cotton plants were sprayed with formulated insecticides and leaves were excised and exposed to *G. punctipes* as above. Five adult males and five adult females were aspirated into the petri dishes containing the sprayed leaves. Six dishes were replicated per treatment. Newly laid, white *H. zea* eggs were frozen at -5°C for 24 h. Exactly 23 mg of *H. zea* eggs were put in a 45 x 45 mm polystyrene weigh boat and one weigh boat was placed in each petri dish. Egg consumption was determined every 24 h for a total period of 96 h. Weight of the eggs consumed was determined at the end of each 24 h period and the total weight consumed within that period was calculated by subtracting the 24 h weight from the initial weight. At the conclusion of each 24 h period, 22.8 mg of fresh eggs were supplied to the petri dishes. Egg consumption was expressed as a percentage of the original amount of eggs supplied for a total of 96 h.

RESULTS AND DISCUSSION

Male and female *G. punctipes* were very tolerant of most of the insecticides tested, especially methoxyfenozide, tebufenozide, and spinosad (Table 1). Methoxyfenozide and tebufenozide are growth regulators which interfere with normal growth and molting in insects; therefore, they were predicted to have low toxicity to adult insects. When ingested, these insecticides mimic insect molting hormone, causing larvae to undergo a premature lethal molt, leading to death within 3 to 7 days (Walton et al. 1995).

Spinosad treatment resulted in no toxicity to *G. punctipes* males or females in the current tests (Table 1). Elzen et al. (1998) previously found that spinosad, at the same rate reported herein, produced no toxicity of *G. punctipes* in spray chamber bioassays carried out for 72 h. These data are consistent with the study of Murray and Lloyd (1997) who reported that spinosad was not disruptive to predator populations in Australian cotton and suggested that the product has an important role in integrated management programs. Further, Hendrix et al. (1997) reported that spinosad was softer on beneficials than chlorfenapyr, deltamethrin (Decis), *lambda*-cyhalothrin (Karate), or acephate (Orthene), and Pietrantonio and Benedict (1997) rated spinosad as harmless (causing <25% mortality) to *Cotesia plutellae* (Kurdjumov) in laboratory studies.

Chlorfenapyr, at 30% of the recommended and at the recommended rate, was significantly more toxic to males than the other chemicals tested, causing 34.5 and 55.3 % mortality, respectively (Table 1). Chlorfenapyr also was significantly more toxic to females at a full rate than the other chemicals tested except methoxyfenozide (Table 1). Wiley et al. (1995)

TABLE 1. Toxicity of Selected Insecticides to *G. punctipes* Adults in a Foliar Insecticide Residue Bioassay.

Treatment	Kg (AI)/ha	% Mortality ± SE[a] Males	Females
Chlorfenapyr	0.12	34.5 ± 17.0b	10.0 ± 5.1a
Chlorfenapyr	0.39	55.2 ± 18.7b	23.9 ± 11.5b
Methoxyfenozide	0.28	0.0 ± 0.0a	10.8 ± 10.8ab
Tebufenozide	0.28	0.0 ± 0.0a	0.0 ± 0.0a
Spinosad	0.099	0.0 ± 0.0a	0.0 ± 0.0a
		F = 8.60	F = 2.90
		df = 4, 20	df = 4, 20

[a]Values within a column followed by the same letter are not significantly different ($P \geq 0.05$; least significant difference [SAS Institute 1988]).

stated that chlorfenapyr has low to moderate impact on beneficials. Elzen et al. (1998), using a spray chamber bioassay that terminated at 72 h, found that chlorfenapyr, at the same rate used in the current study, was intermediate in toxicity to *G. punctipes*, causing 66.7 % mortality in a mixed population of males and females.

Tebufenozide and chlorfenapyr at the recommended rate significantly reduced fecundity of *G. punctipes* compared with the control treatment. Interestingly, the 30% of recommended rate of chlorfenapyr significantly increased the fecundity of *G. punctipes* compared with a control (Table 2).

TABLE 2. Effect of Selected Insecticides Applied in a Foliar Insecticide Residue Bioassay on *G. punctpies* Adults.

Treatment	Kg (AI)/ha	Eggs/female ± SE[a] Fecundity	% *H. zea* Consumption
Chlorfenapyr	0.12	3.3 ± 0.3d	5.1 ± 0.41ab
Chlorfenapyr	0.39	1.5 ± 0.2a	7.2 ± 0.6c
Methoxyfenozide	0.28	3.1 ± 0.2cd	6.3 ± 0.5bc
Tebufenozide	0.28	0.9 ± 0.1a	6.1 ± 0.5bc
Spinosad	0.099	2.3 ± 0.2b	4.8 ± 0.3a
Control	---	2.5 ± 0.1bc	5.4 ± 0.3ab
		F = 12.59	F = 4.04
		df = 5, 114	df = 5, 114

[a]Values in a column followed by the same letter are not significantly different ($P \geq 0.05$; least significant difference [SAS Institute 1988]).

Most of the treatments had no negative effect on egg consumption. However, the recommended rate of chlorfenapyr significantly increased the egg consumption by *G. punctipes* compared with the control (Table 2).

Pesticides have been found to both increase and decrease fecundity in natural enemies, and many sublethal effects are multi-faceted (Croft 1990). The stimulatory effects of pesticidies are reviewed in Luckey (1968). Some of these results are difficult to interpret, which underscores the complexity of the sublethal effects of pesticides.

Most of the insecticides tested (i.e., tebufenozide, methoxyfenozide, and spinosad) are biodegradable (Salgado et al. 1997, Walton et al. 1995). Tebufenozide is specific to a wide

variety of pestiferous lepidopterous larvae and laboratory data indicate that it has no effect on Coleoptera, Heteroptera, or Orthoptera (Smagghe and Degheele 1994). Spinodad is highly effective against lepidopterous pests of cotton at low application rates and is safe to many beneficial arthropods (Peterson et al. 1996). Chlorfenapyr is a broad spectrum insecticide-miticide (Wiley et al. 1995).

Unlike most insecticides which are very toxic to beneficials (or equally toxic to pests and beneficials), we have presented here examples of insecticides which are less toxic to beneficials. Therefore, they should fit very well into IPM progarms. These results suggest that these chemicals may serve as the basis for future IPM programs. However, care should be taken when making assumptions based on laboratory toxicity data (Stark et al. 1995).

ACKNOWLEDGMENT

We thank Martin Avila and Sergio Maldonado (USDA, ARS, Weslaco, TX) for assistance in the research, and F. W. Plapp, Jr. (formerly, Dept. of Entomology, Texas A&M University, College Station, TX) for comments on an earlier copy of the manuscript.

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BEMISIA ARGENTIFOLII (HOMOPTERA: ALEYRODIDAE): FACTORS AFFECTING ADULT AND NYMPH HONEYDEW PRODUCTION

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ABSTRACT

Laboratory studies were conducted to determine the quantity and quality of the major honeydew sugars produced by *Bemisia argentifolii* Bellows and Perring (= *B. tabaci* strain B) (SPW) adults and nymphs. The effects of temperature, light intensity, and SPW adult density on honeydew production were also determined. In the field, we studied the effects of insecticides on SPW populations and on honeydew production of adults and nymphs collected in treated and untreated cotton plots. In the laboratory, adult females lived longer than males, produced more and larger honeydew drops, and, in most cases, larger amounts of the measured honeydew sugars compared with males. Both male and female adults produced more honeydew at 26.7±1°C and 32.2±1°C compared with 21.1±1°C. Under our conditions neither light intensity (30 or 450 μmol/second/m^2, respectively), nor adult density affected honeydew production. SPW development from egg hatch to adult emergence averaged 12.2 days at 26.7±1°C. Honeydew production began the first day of nymphal life and peaked on day 3 following emergence. First and second nymphal instars produced more drops than third and fourth nymphal instars. However, honeydew drop size was larger for third and fourth instars compared with first and second instars. The third and fourth instars produced more trehalulose compared with the earlier instars. Adults produced more honeydew than nymphs. Trehalulose as a percentage of all sugars measured in the study was greater for adults compared to nymphs. Melezitose, as a percentage of all measured sugars, was greater for nymphs compared with adults.

INTRODUCTION

Bemisia argentifolii Bellows and Perring (= *B. tabaci* (Gennadius) (Strain B) (SPW) was first reported in Florida about 1986 (Costa and Brown 1990). SPW has since been reported from many parts of the world where it is a major pest of vegetables, other cultivated food and ornamental crops, and cotton (Debarro 1995). Pest status is associated with direct feeding damage and reduced crop yields, virus transmission, plant physiological disorders, and contamination of crops with excreted honeydew that serves as a substrate for several fungus species. Fungal growth on honeydew on the surface of edible produce and ornamentals is aesthetically objectionable and reduces economic value. Fungal growth on foliage can reduce photosynthesis and on cotton lint causes discoloration. SPW is a phloem feeder and synthesizes a number of sugars, polyols, and other compounds from predominantly sucrose-laden phloem sap (Hendrix and Salvucci

1998). The sugar components of honeydew excretions are major factors in the sticky cotton problem. Honeydew deposits on cotton lint adhere to the working surfaces of cotton processing equipment resulting in reduced ginning rates and stoppages of lint processing machinery at the textile mill (Hector and Hodkinson 1989). The two most abundant sugars found in SPW honeydew are trehalulose and melezitose (Tarzcynski et al. 1992, Hendrix et al. 1992). These sugars have been shown to increase on cotton lint in the field in conjunction with increasing SPW populations and to be correlated with elevated cotton lint stickiness (Henneberry et al. 1995, 1996, 1998a, 1998b). Other honeydew sugars cause sticky cotton (Miller et al. 1994), but some of these are also plant physiological sugars and cannot be directly identified with SPW when extracted from honeydew contaminated lint.

The importance of the sticky cotton problem to the textile industry prompted us to investigate factors affecting honeydew production. An equally important reason for conducting the research was the entomological interest in increasing our knowledge of SPW that might lead to better insight into nutrition, physiology, and biochemical pathways that could provide unique manipulations of the biology of the insect leading to population suppression methods. The objectives of our laboratory studies were to determine (1) the amounts and distribution of honeydew production by adult males, females and nymphs over their life-spans, (2) the relative size of male and female adults honeydew drops, and (3) the effects of light intensity, population density, and temperature on honeydew production. In the field, we collected adults and nymphs from insecticide-treated and untreated cotton plots to determine the effect of several insecticides commonly used for SPW control on honeydew production.

METHODS AND MATERIALS

Laboratory. Adult. SPW used in all studies were reared in the greenhouse on cotton plants. Newly emerged adult males and females were obtained by removing all existing adults at 0800 h from the plant(s). The adult free plants were placed in 0.45 m wide x 0.61 m high muslin-covered cages. Adults that emerged on the plants thereafter until 1300 h were collected, sexed and held separately. We determined adult honeydew production over their life spans by placing two males or two females, in each of five, 3.8 cm diameter x 0.85 cm high leaf-clip cages on intact cotton leaves on plants in the two to four-leaf stage of development. Adults were collected from each cage with an aspirator every three or four days and transferred to new leaf-clip cages on new plants to avoid egg hatch in the female infested cages. For these and all others tests, numbers of honeydew drops or amounts of individual sugars produced are reported for the number of surviving insects on each sample date. We determined the effect of disturbance during transfer to new cages on honeydew production by placing two males in each of five leaf-clip cages. These males were not transferred to new cages, but remained in the same cages until death. For all treatments, males transferred, females transferred, and males not transferred to new cages, cotton plants with leaf-caged SPW were placed in 26.7°C temperature-controlled chambers in a randomized complete block design under 14:10 light:dark (L:D) conditions. Temperatures in all studies varied ±1°C from the control settings. The experiment was replicated five times and repeated on each of three occasions.

Honeydew Drop Counts, Collection and Sugar Identification. For all studies, each leaf cage had a snap-on, removable polyethylene cage bottom. Cage bottoms were removed on each day that adults were transferred to new cages. Numbers of honeydew drops on leaf-cage bottoms were counted. Honeydew, on each collection day, was washed from the leaf-cage bottoms with 3ml of warm deionized water and frozen.

Frozen honeydew samples were lypholized and reconstituted in 60-100 µl of deionized water. The identity and amounts of trehalulose, melezitose, glucose, fructose and sucrose in the samples were determined using the high performance liquid chromatography (HPLC) methods of Hendrix and Wei (1994) and sampled sugars quantified by comparison with peak areas of known sugar standards.

Honeydew Drop Size. The relative sizes of adult honeydew drops were determined by confining 30 individual adult males or adult females in leaf-clip cages on cotton leaves for ≈ 2 h. The plastic cage bottoms were detached and all drops counted and diameters measured using a calibrated ocular micrometer. The honeydew drops were assumed to be spherical in shape and volumes calibrated using the formula, $\mu l = 4.189r^3$ (Anonymous 1964).

Effects of Light Intensity. The effects of light intensity on honeydew production were determined by confining two males or two females in each of five leaf cages on cotton plants in constant temperature chambers at 26.7° C and 14:10 L:D conditions. Light intensity in one of the chambers was 450 (27750 lux) and in the second chamber 30 (1420 lux) µmols/second/m^2. Light intensities were measured with a Li-Cor LI-250 photometer (Li-Cor Inc., Environmental Division, Lincoln, Nebraska). Adult transfer schedules to new leaf cages on new plants were as described above but for 14 days. The experiment was conducted in a five replicate randomized complete block design and repeated on two occasions.

Effects of Whitefly Density. Honeydew production of adults at different population densities was determined by confining 1, 5, 10, 15 or 20 males or females, respectively, in leaf cages. Plants with adults in leaf cages were placed in a constant temperature box at 26.7°C and 14:10 L:D conditions for four days. Numbers of honeydew drops were not counted because with high numbers of SPW, honeydew drops on the plastic cage bottoms often overlapped or coalesced. The experiment was replicated six times using a randomized complete block design.

Temperature. We determined the effect of temperature on adult honeydew production by confining two males or two females in leaf cages on each of five cotton plants. Plants with adults in leaf cages were placed in constant temperature boxes at 21.1, 26.7, or 32.2°C. Each controlled temperature cabinet was under 14:10 L:D conditions. Adults were transferred to new cages and on new plants every 3 or 4 days for 14 days. The experiment was conducted in a five replicate complete block design and repeated twice.

Nymphs. Honeydew production by SPW nymphs was determined in 26.7°C controlled temperature cabinets under 14:10 L:D conditions. Eight adult pairs were placed in each of 15 leaf cages on cotton plants for 2 h. Adults were removed and each caged leaf examined for eggs. Plants with eggs were placed in the constant temperature chambers and examined each day for egg hatch. When eggs began to hatch, two to six, first-instar nymphs were left on each leaf, all other eggs and nymphs were removed. On the first day of nymph emergence and each day thereafter until adults emerged, numbers of honeydew drops were counted and subsequently collected for HPLC analysis, as described for adults.

Field. Effect of Insecticides on Adult and Nymph Populations. The experiment was conducted at the University of Arizona, Maricopa Agricultural Experiment Station, Maricopa, AZ. Cotton seed was planted 8 April 1997. Individual plots were 12 rows wide with 1 m row spacing, and rows were 15.3 m long. Treatments, in a four-replicate randomized complete block design, were, one application, in each case, of buprofezin [Applaud®, 405C, AgrEvo Corp., NC, 0.28 kg (AI/ha)], pyriproxyfen [Knack®, Valent, 0.48 kg (AI/ha)], endosulfan [Thiodan®, FMC Corporation, PA, 2.2 Kg (AI/ha)], or a mixture of fenpropathrin and acephate (Danitol®, Orthene®), Valent Corp., CA 0.56 Kg

(AI/ha) plus 0.56 Kg (AI/ha), respectively. Applications were made on 1 August with a four row, rear-mounted boom mounted on a Hi-boy tractor. There were seven hollow-cone nozzles per row that delivered 193 liters per hectare under 80 psi. Untreated plots were controls. SPW adults on the fifth mainstem node leaves were counted weekly on 15 plants in each plot from 22 July to 2 September using the leaf-turn method described by Naranjo and Flint (1995). Immature life stages were counted weekly on 3.88cm^2 leaf disks taken from the fifth mainstem nodes of 15 plants from each plot (Naranjo and Flint 1994).

Effect of Insecticides on Honeydew Production. Adults. The effect of insecticide applications on adult honeydew production was determined by collecting adults from each plot using a modified battery-operated Dust Buster (Black and Decker® Car Vac™, Hunt Valley, MD) vacuum cleaner at weekly intervals beginning on 22 July and ending on 2 September. Collected adults were transported from the field to the laboratory in styrofoam, cooled boxes. Fifteen to 20 adults were aspirated from field collection containers from each plot and introduced into 7.62 cm-diameter, ventilated, plastic petri dishes containing leaves of rooted cotton seedlings. A vertical groove was cut into the side of each petri dish to accommodate cotton-wrapped seedling stems. Seedling roots were placed in 50-ml cylindrical vials containing water. After 48 h, petri dish bottoms were collected and honeydew washed from the dishes in 3 ml of deionized water.

Nymphs. The effect of insecticide treatments on nymph honeydew production was determined by collecting two, fifth mainstem node leaves with petioles intact from each plot. Individual leaves were examined under a microscope to determine the presence of living nymphs and record their numbers. All adults were removed. Leaves with nymphs were placed in the petri dishes described, leaf petiole ends were cut-off at an angle and placed in cylindrical vials containing 10 ml of water. Honeydew in the petri dish bottoms was collected as described for adults. Based on the location of nymphs on the undersides of leaves and the dorsal position of the anal opening (van Lenteren and Noldus 1990), we expected negligible or no honeydew accumulation on the leave substrates.

Honeydew collections on each sampling date from both adults and nymphs was frozen, lypholized and HPLC analyzed to determine amounts of individual sugar components as previously described.

Statistical Analysis. All data were analyzed using analysis of variance (ANOVA) (MSTAT-C 1988), a factorial treatment arrangement was applied when appropriate, and means separated contingent on a significant F test using the method of least significant differences at $P \leq 0.05$. Relative sizes and volumes of adult male and female honeydew drops were compared using Students "t" tests.

RESULTS

Laboratory. Male SPW transferred to new leaf cages every 3 or 4 days over their life span lived 22.9 ± 3.2 (S.E.) days compared with 23.3 ± 2.0 days for males not transferred to new leaf cages (Table 1). Females transferred to new leaf cages every 3 or 4 days lived 29.7 ± 3.3 days. The mean total numbers of honeydew drops per male for those transferred or not transferred to new cages were 594.3 ± 85.2 and 769.3 ± 184.4, respectively, compared with 1916.9 ± 359.3 honeydew drops for females. Honeydew of females contained significantly more glucose, fructose, trehalulose and total measured honeydew sugars than males (Table 1). Although larger amounts of melezitose were produced by females compared with males, and larger amounts of sucrose by males compared with females, the differences were not statistically significant. Numbers of

TABLE 1. Mean (± S. E.) Longevity (Days), Numbers of Honeydew Drops and Total Micrograms of Honeydew Sugars per SPW Adult During Their Life Span at 26.7° C.

Treatment[a]	Longevity (days)	Total honeydew drops (no./adult)	Sugars (µg/adult)					
			Glucose	Fructose	Trehalulose	Sucrose	Melezitose	Totals
Males								
Transferred	22.9 ± 3.2 a	594.3 ± 85.2 b	1.81 ± 0.52 b	2.09 ± 0.66 b	3.18 ± 1.08 b	10.62 ± 3.93 a	1.36 ± 0.42 a	19.05 ± 5.64 b
Not transferred	23.3 ± 2.0 a	769.3 ± 184.4 b	1.30 ± 0.43 b	0.88 ± 0.30 b	1.43 ± 0.47 b	09.24 ± 4.19 a	0.73 ± 0.28 a	13.58 ± 5.22 b
Females								
Transferred	29.7 ± 3.3 a	1916.9 ± 359.3 a	3.98 ± 0.88 a	6.97 ± 2.08 a	48.04 ± 14.23a	07.26 ± 2.13 a	2.37 ± 0.84 a	68.63 ± 17.72 a
F, df 2,14	2.08	7.27	7.29	6.87	10.88	0.37	3.05	9.44

[a] Means of 8 replications, 2 adults/replications. Means in a column not followed by the same letter are significantly different. Method of least significant differences $P \leq 0.05$.

honeydew drops for males and females increased to a peak on days 4 to 7 following emergence, decreased through days 8 to 14 and increased on days 15 to 17 following emergence (Fig. 1A). Drops per female increased thereafter to a second peak on days 29 to 31 and decreased thereafter. Male honeydew drop excretion was more variable during the period of 18 to 38 days following emergence. Females produced more trehalulose than males on all sampling dates (Fig. 1B). Amounts of melezitose, glucose, and fructose in honeydew were low and variable for both males and females (Fig. 1C, 2A, 2B, 2C). On several sampling dates (< 1 to 3, 11 to 14, 18 to 21, and 22 to 24) males produced more sucrose than females but variability was high and differences were not statistically significant.

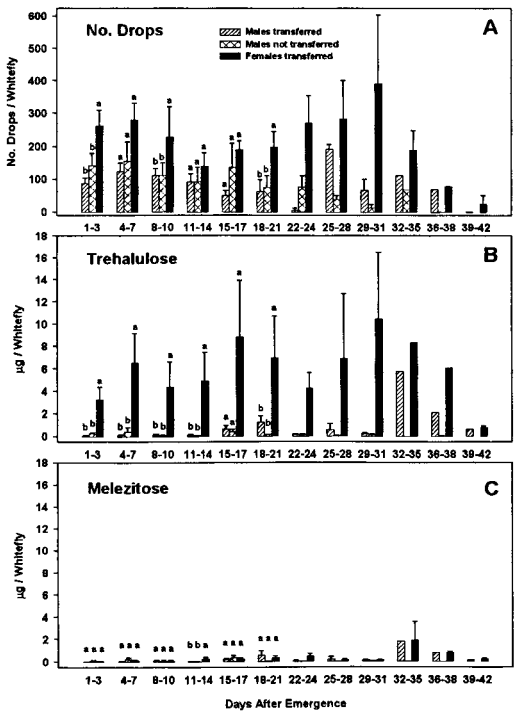

FIG. 1. Mean (± S.E.) numbers of honeydew drops (A) and micrograms of trehalulose (B) and melezitose (C) produced per adult male and female SPW on days following emergence. Columns not labeled with the same letter are significantly different. Method of least significant differences. $P \leq 0.05$. Data after 18-21 days following emergence, due to male or female mortality and loss of replication are presented as means ± S.E.

Honeydew Droplet Size. The average diameters of 533 honeydew drops produced by 30 males and 539 honeydew drops produced by 30 females were 0.061 and 0.095 mm, respectively (t = 7.58, df = 58, $P \leq 0.01$). Assuming spherical honeydew drop proportions, the calculated average volume of honeydew drops was $6.84 \pm 1.06 \times 10^{-5}$ µl for males and $25.60 \pm 2.68 \times 10^{-5}$ µl for females ($t = 6.512$, df = 58, $P \leq 0.01$).

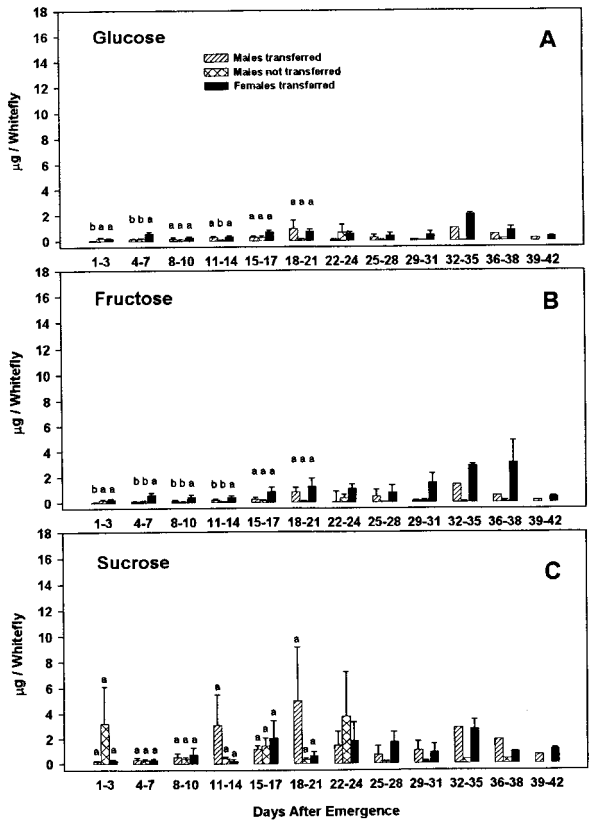

FIG. 2. Mean (± S.E.) numbers of micrograms of glucose (A), fructose (B) and sucrose (C) produced per adult male and female SPW on days following emergence. Columns not labeled with the same letter are significantly different. Method of least significant differences. $P \leq 0.05$. Data after 18-21 days following emergence, due to male or female mortality and loss of replication are presented as means ± S.E. where possible.

Effects of Light Intensity. There were no significant differences for numbers of honeydew drops or amounts of individual or total honeydew sugars measured when SPW male or female adults fed on cotton leaves under low (30 μmol/s/m^2) versus high light intensity (450 μmol/s/m^2) conditions (Table 2). There were also no statistically significant interactions for any category of male or female data collected from plants at low or high light intensities. Thus, only the overall means for light intensity and SPW sex are tabulated. Females produced more honeydew drops and, over the entire test period, larger amounts of all sugars compared with males under both low and high light intensity conditions. On individual sampling dates, variability was high for sucrose and melezitose. Although larger amounts of sucrose were produced by females compared with males, the differences were not statistically significant, except for the total amount of sucrose produced over the entire test period. Similar results occurred for melezitose on the first sampling date, but significantly more melezitose was produced by females compared with males on all other sampling dates.

Adult Density. Whitefly adult density in leaf cages had no effect on the amounts of any of the measured honeydew sugars produced per male and female over the 4-day test period (Table 3). Female honeydew contained significantly more of each honeydew sugar compared with males.

Effects of Temperature. More honeydew drops were produced by SPW at 26.7°C compared with 21.1 and 32.2°C (Table 4). Mean numbers of drops produced at 26.7°C were significantly higher than numbers produced at 21.1°C but not 32.2°C. Over all temperatures, females produced larger numbers of drops than with males. Temperature had no effect on the quality of sugars produced, but females produced larger amounts of glucose, fructose and trehalulose than males. No detectable sucrose and very little melezitose were produced by either adult males or females at any of the experimental temperatures.

Nymph Development and Honeydew Production. The average number of days from egg hatch to adult emergence for 42 nymphs at 26.7°C (14:10L:D) was 12.20 ± 0.19 days. Honeydew production began the first day of nymphal life (Fig. 3A). The peak number of drops occurred on day 3 following egg hatch. Numbers of drops decreased thereafter to day 10 following egg hatch, and increased slightly thereafter for the remainder of the nymphal development cycle. Trehalulose found in nymphal honeydew, in general, increased with nymphal age except for large amounts found on days 5 and 10, and a decrease in amounts on day 14 (Fig. 3B). Generally, lesser amounts of melezitose, glucose, and fructose except for melezitose on day 2 and fructose on day 14 were found on most days of nymphal development (Fig. 3C; 4A, B). Amounts of sucrose produced were highly variable (Fig. 4C). The size of honeydew drops (visual observation) and total honeydew production increased with increasing nymphal instar (Table 5). First- and second-instar nymphs produced significantly more honeydew drops than third- and fourth-instar nymphs (Table 5). Fourth-instar nymphs produced larger amounts of glucose and fructose than first- and second-instar nymphs and larger amounts of trehalulose and totals of all sugars measured when compared with all other instars.

Field. Effect of Insecticides on SPW Populations and Honeydew Production. The single application of each individual chemical or chemical mixture was made on 1 August. Numbers of adults, nymphs and eggs were significantly reduced in all insecticide-treated plots (except for Buprofezin) on 6 August. Numbers of adults were also significantly lower in fenpropathrin-acephate, endosulfan and pyriproxyfen, buprofezin, and fenpropathrin-acephate treated plots on 12 and 19 August, respectively, and all insecticide-treated plots on 2 September compared with the untreated plots. Results were more variable for nymphs and eggs. Significantly fewer nymphs and eggs occurred in all insecticide-treated plots on 6 August and for nymphs only in

pyriproxyfen-treated plots on 26 August. Higher numbers of eggs occurred in pyriproxyfen-treated plots compared with all other insecticide-treated plots on 19 August. This may have occurred because of the sterilizing effect of pyriproxyfen on SPW adults resulting in an accumulation of that life stage in the treated plots. A 0.3 cm rain on 3 August may have contributed to some reduction of adult population in all plots, but did not affect numbers of nymphs or eggs. These results are not tabulated in this paper. However, orthogonal comparisons for all insecticides compared with untreated plots are shown in Fig. 5.

Honeydew Production. Adults. There were no significant differences on any sampling date between the amounts (μg/adult) of each individual measured adult honeydew sugar for any insecticide treatment when compared with untreated controls, (F range = 0.20 to 1.29; df 4, 19 for all comparisons; $P \leq 0.05$) (Fig. 6). Individual insecticide treatment data for sugars are not graphed, however the orthogonal comparisons (also no significant differences F range = 0.02 to 0.95; df 1, 15; $P \leq 0.05$) for the averages of all insecticide treatments compared with the averages for untreated controls are shown in Fig. 6. Honeydew collected from adults in the insecticide-treated and untreated fields was highest in trehalulose followed by sucrose, fructose, melezitose, and glucose. Amounts (μg/adult) of all the measured sugars in honeydew from field-collected adults were low in July and early August, increased in mid to late August and decreased in September.

Nymphs. Similar to the results with adults there were no significant differences in the amounts of sugars found in honeydew collected from nymphs in insecticide-treated and untreated fields (Fig. 7). However, trehalulose and melezitose were found in the greatest amounts followed by fructose, sucrose, and glucose.

DISCUSSION

There is little published information comparing the quantity or quality of honeydew produced by SPW male or female adults and nymphs. Several authors have reported on the quality of sugars found in honeydew produced by SPW feeding on cotton but no distinction was made for male or females. For adults, Tarzcynski et al. (1992) collected honeydew for 48 h from mixed sex adult populations of SPW and reported that sucrose (10.1%), glucose (5.3%), fructose (11.7%), trehalulose (43.1%), and oligosaccharides (29.5%) were the major honeydew carbohydrates. Similarly, Hendrix et al. (1992) collected honeydew for 48 h from mixed adult and nymph populations and found that the honeydew contained 18.9% monosaccharides, 16% sucrose, 43.8% trehalulose, 16.8% melezitose, and approximately 3% of a novel trisaccharide (bemisiose) (Hendrix and Wei 1994) as well as several other oligosaccharides. Our results also show that trehalulose was the largest component of the five major sugars found in SPW honeydew (glucose, fructose, sucrose, trehalulose, and melezitose). Davidson et al. (1994) reported similar trehalulose results for SPW adults feeding on sucrose solution through artificial membrane. We further show that females produce more honeydew and more trehalulose and total sugars than males. Honeydew drops of females, on average, were also larger in size compared with those of males. The difference reported in this paper are relative since water loss was not considered and sphere droplet dimensions were assumed. Hendrix and Salvucci (1998) and others have suggested that the larger saccharides (trehalulose, melezitose, and others) may play a significant role in honeydew physical and chemical characteristics and in osmotic regulation of insect body fluids. The implication of this regarding the differences in sugars excreted by males and females is unknown. However, differences in amounts of sugars produced may be partially explained by differences in the sizes of males and fe-

TABLE 2. Mean ± S. E. Numbers of Honeydew Drops and Micrograms of Honeydew Sugars Produced by SPW Males and Females Feeding on Cotton Leaves Under Low and High Light Intensity Conditions at 26.7 ° C.

Treatment	Days After Emergence[a]				Total
	< 1-3	4-7	8-10	11-14	
			Honeydew Drops (No.)		
Light Intensity[b]					
450	294.80 ± 65.00 a	253.20 ± 51.60 a	272.20 ± 87.90 a	266.80 ± 71.30 a	1086.90 ± 248.60 a
30	268.90 ± 65.40 a	186.40 ± 50.70 a	234.90 ± 103.70 a	348.30 ± 129.00 a	1038.40 ± 288.60 a
F[c]	0.11	1.55	0.13	0.39	0.04
Males	149.90 ± 27.90 b	101.80 ± 18.60 b	35.80 ± 10.70 b	116.20 ± 11.40 b	403.50 ± 46.00 b
Females	413.80 ± 61.20 a	337.80 ± 45.10 a	471.30 ± 88.90 a	498.90 ± 117.50 b	1721.80 ± 215.50 a
F	11.16	19.37	18.51	8.67	26.11
			Glucose		
Light Intensity					
450	0.23 ± 0.07 a	1.02 ± 0.77 a	0.18 ± 0.03 a	3.09 ± 1.18 a	4.58 ± 1.63 a
30	0.37 ± 0.15 a	0.37 ± 0.20 a	0.25 ± 0.10 a	4.95 ± 2.42 a	5.94 ± 2.65 a
F	0.79	0.86	0.74	0.70	0.41
Males	0.13 ± 0.02 b	0.87 ± 0.02 a	0.08 ± 0.02 b	0.54 ± 0.22 b	0.83 ± 0.21 b
Females	0.48 ± 0.15 a	1.30 ± 0.75 a	0.35 ± 0.09 a	7.50 ± 2.17 a	9.62 ± 2.34 a
F	4.75	2.94	11.45	9.77	15.60

Fructose

Light Intensity					
450	0.51 ± 0.77 a	0.03 ± 0.01 a	0.36 ± 0.09 b	3.09 ± 1.39 a	5.77 ± 2.17 a
30	1.32 ± 0.72 a	1.74 ± 1.22 a	0.83 ± 0.27 a	3.91 ± 1.22 a	7.79 ± 2.48 a
F	1.46	0.00	21.66	0.49	1.71
Males	0.14 ± 0.02 b	0.07 ± 0.02 b	0.09 ± 0.04 b	0.66 ± 0.36 b	0.96 ± 0.33 b
Females	1.69 ± 0.67 a	3.50 ± 1.70 a	1.08 ± 0.19 a	6.34 ± 1.24 a	12.59 ± 1.85 a
F	5.29	5.37	96.64	23.55	56.86

Trehalulose

Light Intensity					
450	6.50 ± 2.52 a	9.26 ± 6.34 a	3.11 ± 1.21 a	46.27 ± 16.93 a	65.15 ± 22.87 a
30	9.39 ± 3.56 a	7.77 ± 3.07 a	4.75 ± 1.67 a	38.67 ± 14.66 a	60.57 ± 19.92 a
F	1.06	0.06	3.07	0.58	0.25
Males	0.27 ± 0.06 b	0.13 ± 0.06 b	0.02 ± 0.01 b	2.20 ± 1.21 b	2.61 ± 1.18 b
Females	15.62 ± 2.52 a	16.90 ± 5.84 a	7.88 ± 1.00 a	82.74 ± 11.89 a	123.10 ± 10.58 a
F	30.04	8.18	69.85	65.40	172.56

			Sucrose		
Light Intensity					
450	0.90 ± 0.39 a	0.73 ± 0.48 a	0.28 ± 0.07 a	1.39 ± 0.59 a	3.31 ± 0.82 a
30	0.93 ± 0.51 a	0.26 ± 0.10 a	0.28 ± 0.13 a	0.84 ± 0.34 a	2.31 ± 0.59 a
F	0.01	1.10	0.0	1.04	1.74
Males	0.47 ± 0.20 a	0.12 ± 0.03 a	0.17 ± 0.06 a	0.83 ± 0.35 a	1.58 ± 0.30 b
Females	1.37 ± 0.57 a	0.87 ± 0.47 a	0.39 ± 0.13 a	1.40 ± 0.59 a	4.03 ± 0.81 a
F	1.59	2.81	2.50	1.09	10.42
			Melezitose		
Light Intensity					
450	0.12 ± 010 a	0.19 ± 0.14 a	0.03 ± 0.01 b	6.14 ± 4.83 a	6.46 ± 4.98 a
30	0.20 ± 0.11 a	0.31 ± 0.18 a	0.14 ± 0.05 a	25.20 ± 17.11 a	25.84 ± 17.10 a
F	0.29	0.56	19.80	1.23	1.28
Males	0.11 ± 0.10 a	0.00 ± 0.00 b	0.00 ± 0.00 b	0.87 ± 0.53 b	0.98 ± 0.52 b
Females	0.21 ± 0.11 a	0.48 ± 0.20 a	0.17 ± 0.05 a	30.47 ± 16.95 a	31.32 ± 16.93 a
F	0.47	7.64	41.29	2.77	3.15

[a] Means of 5 replications, 2 adults of each sex per replication, means in a column not followed by the same letter are significantly different, method of least significant differences $P \leq 0.05$.

[b] µmol/second/m^2

[c] df = 1,12, in each case, for light intensity and whitefly sex.

TABLE 3. Effects of Adult Density on the Mean (± S.E.) Micrograms of Honeydew Sugars Produced per SPW Adult in 4-Day Feeding Periods on Cotton.

Adult Density/ Cage	Sugar[a]					
	Glucose	Fructose	Trehalulose	Sucrose	Melezitose	Total
1	0.19 ± 0.05a	0.30 ± 0.08 c	0.20 ± 0.07 a	0.00 ± 0.00 a	0.13 ± 0.08 a	0.82 ± 0.19 a
5	0.22 ± 0.06 a	0.28 ± 0.05 a	0.11 ± 0.04 a	0.09 ± 0.04 a	0.13 ± 0.04 a	0.80 ± 0.20 a
10	0.01 ± 0.03 a	0.19 ± 0.05 a	0.23 ± 0.08 a	0.04 ± 0.02 a	0.09 ± 0.04 a	0.71 ± 0.15 a
15	0.05 ± 0.01 a	0.08 ± 0.03 a	0.07 ± 0.03 a	0.01 ± 0.01 a	0.14 ± 0.08 a	0.35 ± 0.12 a
20	0.16 ± 0.06 a	0.17 ± 0.07 a	0.10 ± 0.04 a	0.02 ± 0.01 a	0.08 ± 0.04 a	0.53 ± 0.21 a
F =[b]	2.33	2.39	1.84	4.98	0.22	1.77
Males	0.10 ± 0.20 b	0.12 ± 0.03 b	0.09 ± 0.03 b	0.01 ± 0.01 b	0.06 ± 0.02 b	0.39 ± 0.08 b
Females	0.22 ± 0.04 a	0.27 ± 0.05 a	0.19 ± 0.04 a	0.05 ± 0.02 a	0.17 ± 0.05 a	0.90 ± 0.14 a
1 F =[c]	10.20	10.61	4.40	4.98	5.28	14.62

[a] Means of 6 replications in a column not followed by the same letter are significantly different $P \leq 0.05$ method of least significant differences.
[b] df = 4, 45
[c] df = 1, 45

TABLE 4. Effects of Temperature on Mean ± S.E. Numbers of Honeydew Drops and Micrograms of Honeydew Sugars Produced by SPW Adults.

	Days After Emergence				
Treatment[a]	< 1-3	4-7	8-10	11-14	Total
			Honeydew Drops (No).		
Temperature (°C)					
22.1	44.0 ± 16.1 b	81.4 ± 25.4 b	72.5 ± 19.9 b	88.9 ± 27.4 b	286.7 ± 40.1 b
26.7	178.3 ± 34.8 a	177.8 ± 34.8 a	179.8 ± 42.1 a	186.0 ± 28.3 a	721.9 ± 84.4 a
32.2	89.0 ± 15.7 b	135.0 ± 32.0 ab	178.2 ± 29.4 a	222.7 ± 44.0 a	625.0 ± 55.7 a
F[b]	9.04	4.74	5.43	4.41	21.34
Males	64.0 ± 20.4 b	105.2 ± 24.9 b	110.0 ± 23.4 b	117.0 ± 21.8 b	396.3 ± 53.2 b
Females	143.6 ± 21.4 a	157.6 ± 26.2 a	176.9 ± 29.6 a	214.7 ± 33.2 a	692.8 ± 57.5 a
F	9.18	4.18	4.81	6.59	26.96
			Glucose		
Males	0.01 ± 0.01 b	0.00 ± 0.00 b	0.00 ± 0.04 b	0.03 ± 0.028 b	0.05 ± 0.04 b
Females	0.10 ± 0.04 a	0.15 ± 0.08 a	0.37 ± 0.10 a	0.42 ± 0.119 a	1.05 ± 0.27 a
F	8.53	3.77	8.54	11.99	20.09

			Fructose		
Males	0.02 ± 0.01 b	0.00 ± 0.00 a	0.01 ± 0.00 b	0.02 ± 0.01 b	0.04 ± 0.03 b
Females	0.09 ± 0.04 a	0.14 ± 0.08 a	0.26 ± 0.08 a	0.36 ± 0.11 a	0.85 ± 0.24 a
F	4.63	NS	4.65	10.16	17.96
			Trehalulose		
Males	0.07 ± 0.06 b	0.00 ± 0.00 b	0.01 ± 0.01 b	0.000 ± 0.00 b	0.08 ± 0.05 b
Females	0.52 ± 0.05 a	1.45 ± 0.43 a	2.11 ± 0.76 a	2.260 ± 0.81 a	7.73 ± 2.20 a
F	13.14	11.76	13.14	10.11	18.29
			Melezitose		
Males	0.01 ± 0.01 a	0.00 ± 0.00 a	0.13 ± 0.12 a	- -	0.14 ± 0.14 a
Females	0.25 ± 0.01 a	0.02 ± 0.01 a	0.09 ± 0.04 a	- -	0.13 ± 0.06 a
F	NS	NS	NS	- -	NS

[a] Means of 8 replications, 2 adults of each sex for each replicate. Means in a column not followed by the same letter are significantly different. Method of least significant differences $P \leq 0.05$.

[b] df for temperature comparisons = 2,40; df for all male – female comparisons = 1,40.

TABLE 5. Mean ± S.E. Numbers of Honeydew Drops and Micrograms of Individual Honeydew Sugars Produced per SPW Nymphal Stage During Development.

Nymphal Instar	Honeydew Drops (no.)	µg Honeydew sugars produced/nymph[a]					Totals
		Glucose	Fructose	Trehalulose	Sucrose	Melezitose	
First	185.3 ± 42 a	0.11 ± 0.02 bc	0.10 ± 0.02 c	0.12 ± 0.03 b	0.50 ± 0.16 a	0.20 ± 0.06 a	1.04 ± 0.18 b
Second	144.5 ± 21 a	0.06 ± 0.02 c	0.14 ± 0.03 bc	0.36 ± 0.13 b	0.14 ± 0.03 a	0.10 ± 0.04 a	0.78 ± 0.16 b
Third	71.2 ± 11 b	0.16 ± 0.03 ab	0.30 ± 0.05 ab	0.43 ± 0.10 b	0.26 ± 0.07 a	0.12 ± 0.02 a	1.26 ± 0.14 b
Fourth	47.4 ± 14 b	0.23 ± 0.07 a	0.35 ± 0.14 a	0.84 ± 0.22 a	0.52 ± 0.25 a	0.32 ± 0.11 a	2.26 ± 0.59 a
F[b]	7.98	5.06	3.54	6.62	1.47	2.47	4.09

[a] Means of 15 replications, 2-6 nymphs per replication for a total of 52 nymphs. Means in a column not followed by the same letter are significantly different. Method of least significant differences $P \leq 0.05$.
[b] df for honeydew drops = 3, 18; df for sugars, in each case; = 3, 42.

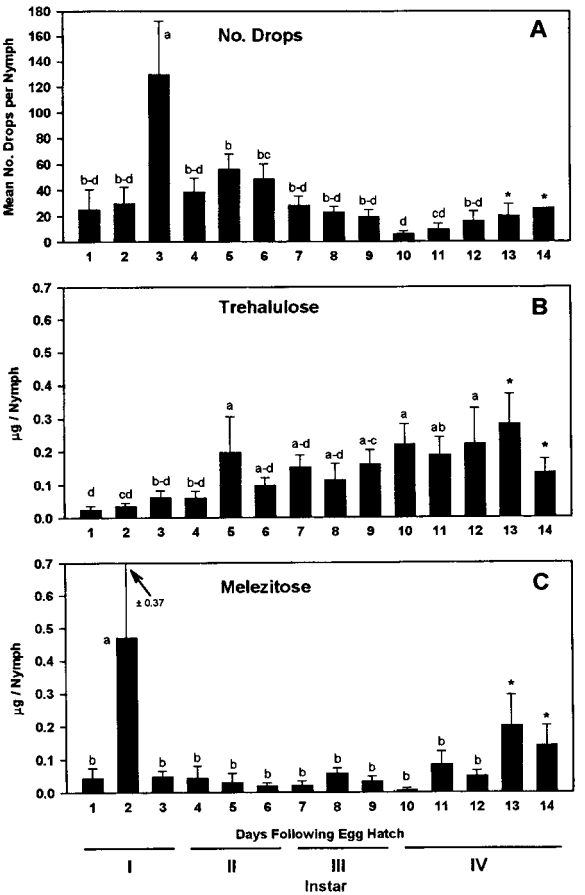

FIG. 3. Mean (± S.E.) numbers of honeydew drops and micrograms of honeydew sugars produced per SPW nymph on each of 14 days following emergence. Columns not labeled with the letter are significantly different. $P \leq 0.05$. Method of least significant differences. * average time for nymph development was 12.2 days. Data presented are for 11 and 3 nymphs for days 13 and 14, respectively and are means ± S.E. only.

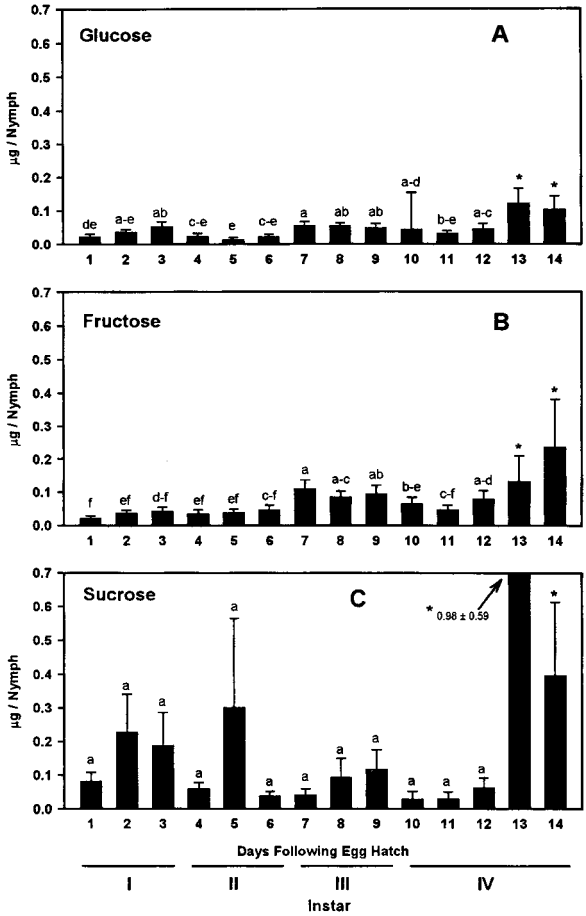

FIG. 4. Mean (± S.E.) numbers of micrograms of honeydew sugars produced per SPW nymph on each of 14 days following emergence. Columns in the same graph not labeled with the same letter are significantly different. $P \leq 0.05$. Method of least significant differences. * average time for nymph development was 12.2 days. Data presented are for 11 and 3 nymphs for days 13 and 14, respectively and are means ± S.E. only.

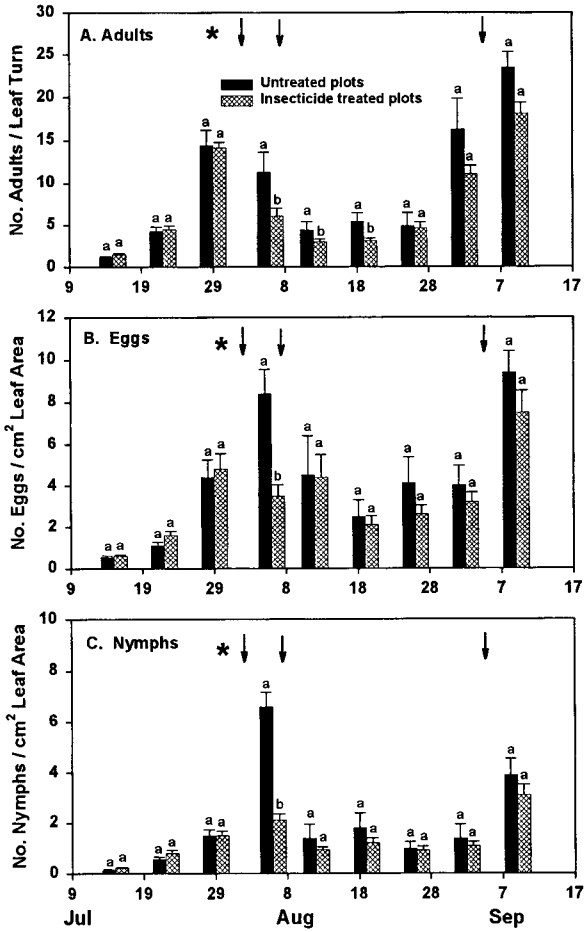

FIG. 5. Orthogonal comparisons for mean (± S.E.) numbers of SPW adults per leaf turn and eggs and nymphs per cm^2 of cotton leaf disk from untreated and all insecticide treated plots. Means of 4 replications not followed by the same letter are significantly different. $P \leq 0.05$. Method of least significant differences. * = insecticide treatment dates. Arrows = rainfalls.

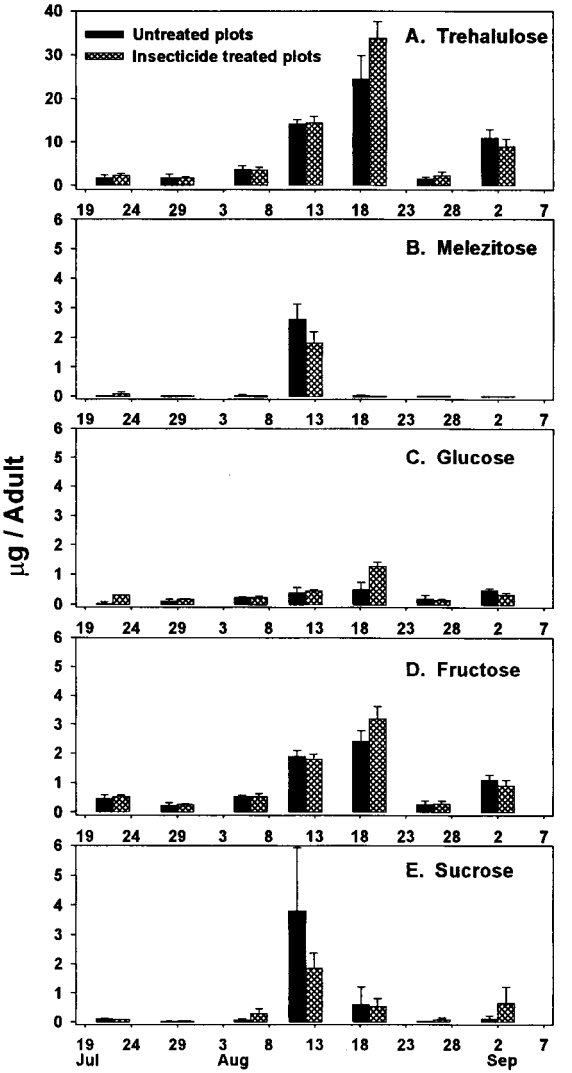

FIG. 6. Orthogonal comparisons for mean (± S.E.) micrograms of sugars in honeydew produced in the laboratory per SPW adult collected from insecticide-treated and untreated cotton fields. No statistically significant differences on any sampling date.

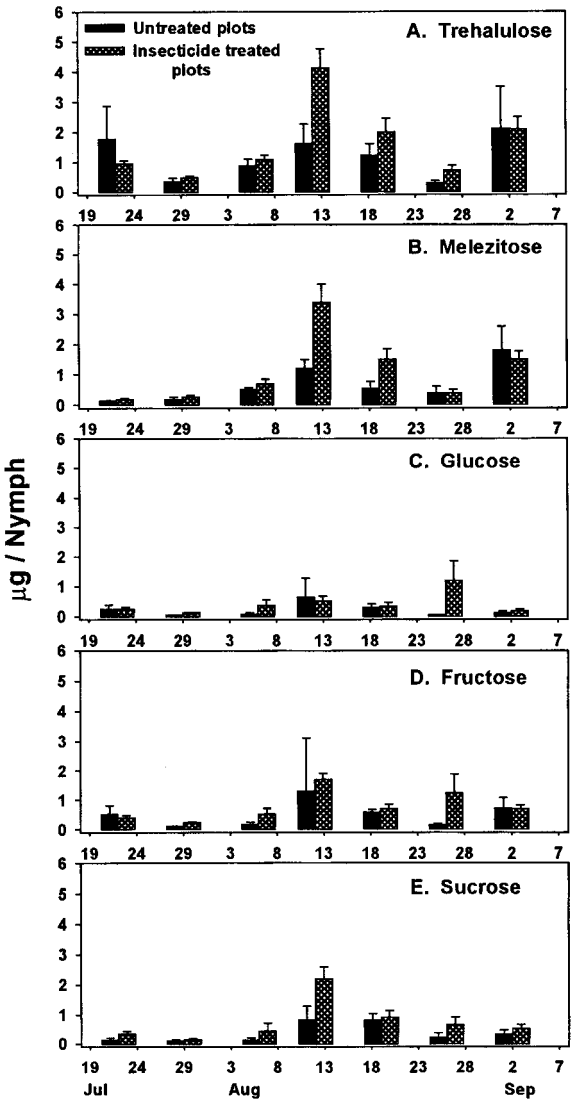

FIG. 7. Orthogonal comparisons for mean (± S.E.) micrograms of sugars in honeydew produced in the laboratory per SPW nymph collected from insecticide-treated and untreated cotton fields. No statistically significant differences on any sampling date.

males. Females weigh nearly 2.5x (51 μg) more than males (21 μg) (Isaacs et al. 1998). The carbohydrate aspects of the SPW cotton host interaction is complex and may significantly reflect on biological and ecological adaptations of the insect. Salvucci et al. (1997) proposed that trehalulose is synthesized for excretion by SPW feeding on cotton when carbon input from sucrose is in excess of metabolic needs. Isaacs et al. (1998) found greater carbohydrate concentrations in phloem sap from water-stressed melons *Cucumis melo*, compared to phloem sap from non-water stressed melons and also that SPW feeding on the higher carbohydrate-content phloem sap produced significantly more trehalulose than when feeding on lower carbohydrate-content phloem sap. In all cases, no comparisons for males and females were made. The authors speculated on biological advantages that may be afforded to SPW by having the ability to control internal osmotic haemolymph relationships in diverse plant-ecological systems. An understanding of this interaction and knowledge of the total carbohydrate composition of honeydew may help to develop potential approaches to SPW control and or chemical (or enzymatic) methods to reduce cotton lint stickiness in harvested cottons (Hendrix et al. 1993, 1996) by designing chemistry and/or enzymes specifically interactive with known honeydew sugars.

In one report of studies on honeydew produced by SPW nymphs feeding on cotton, Blua and Toscano (1994) found that during 24-h feeding periods, fewer honeydew drops were produced while feeding on high nitrogen fertilized seedlings compared with nymphs feeding on low nitrogen fertilized seedlings. They also reported that nymphs feeding on plants supplied high nitrogen initiated honeydew production earlier (2 days) than nymphs feeding on plants fertilized with low or medium amounts of nitrogen. Costa et al. (1999) (16 h and 24 h feeding periods) reported that second- and third-instar SPW nymphs had higher percentages of sucrose, trehalulose, and melezitose, and lower percentages of fructose and glucose compared to first- and fourth-instar nymphs. They also found a clear pattern of honeydew drop production consisting of periods of drop production followed by periods of no drop production. Blua and Toscano (1994) reported that nymphs did not initiate honeydew production for 3 to 5 days following emergence. Our results differed in that nymphs began producing honeydew drops on the day of emergence. Costa et al. (1999) observed honeydew drop production in some cases before the first molt but results were variable. The reason for the differences remain unclear but may be related to the methods of honeydew drop collection. In each case, Blua and Toscano (1994) and Costa et al. (1999) monitored honeydew drop production on water sensitive paper strips placed beneath the nymphs. Our results were obtained by counting drops collected on plastic leaf cage bottoms. Small drops of honeydew produced by first-instar nymphs on water sensitive paper may not in all cases produce a visible distinctive blue spot as is the case for larger honeydew drops produced by older nymphs. Similar to our results, the production of larger honeydew drops by older nymphs were reported by Blua and Toscano (1994) and Costa et al. (1999). Our results showing more but smaller honeydew drops produced by early instars are also in accord with the results of Costa et al. (1999). This has also been reported in other whitefly species. Lei et al. (1996) reported that *Trialeurodes vaporariorum* (Westwood) nymphal numbers of honeydew drops decreased with older nymphs but the volume of honeydew increased and was explained by the increased size of the drops produced by older nymphs.

Our field results suggest that several commonly used insecticides for control of SPW on cotton reduced numbers of adult and nymphs, in some cases, for 18 and 5 days, following application but there were no measurable effects on honeydew production of adults or nymph collected from the plots. These results suggest that effectiveness of insecticides for reducing cotton lint stickiness (Henneberry et al. 1995, 1996) is a result

of reduced populations as opposed to any direct effect on honeydew production. Additional studies need to be conducted to verify these results. Only one application of each chemical or chemical mixture was applied. Rainfall occurred on days 3 and 6 following the applications and may have resulted in reduced residual insecticide activity. Additionally, adult SPW disperse readily from plant to plant and between small plots. In the case of our study with adults, some of our collected SPW may have entered our plots from untreated cotton on other sources. This shortcoming can be resolved by collecting honeydew in the field from SPW infested leaves intact on plants. However, it will be difficult to sample adults or nymphs only under field conditions.

Overall, adult SPW produced more honeydew than nymphs. Adult females produced more honeydew than males and female honeydew was higher in trehalulose than male honeydew. The relative differences between honeydew production for males and females and between amounts adults produced compared with nymphs were consistent. However, honeydew production by adult and nymph individuals was subject to large degrees of variation. For example, in the laboratory production of trehalulose ranged from >1 to 3 μg for adult males and 7-123 μg for adult females. Nymphs (all stages) produced < 1 to 2.3 μg of trehalulose. For adults and nymphs collected in the field, amounts of trehalulose produced ranged from 1.6 to 34.0 μg for adults and < 1 to 4 μg for nymphs. The reasons for the large variations remain unknown, however a partial explanation may be inferred from the work of Isaacs et al. (1999). The authors found that trehalulose produced by SPW more than doubled when feeding on water stressed compared with non-water stressed melons. Thus changes in plant physiology may be reflected in SPW feeding biology. Increasing our knowledge in these areas of pest-host plant interaction has much potential for practical application. For example, water management in cotton culture is a farmer-adopted method of reducing cotton plant stress and SPW populations (Flint et al. 1996).

ACKNOWLEDGMENTS

The authors appreciate the support of Cotton Incorporated through Cooperative Agreement 5344-21220-009-07R that partially defrayed the costs of these studies.

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TEMPORAL DISTRIBUTIONS OF *CONOTRACHELUS* AND *ANTHONOMUS* WEEVILS (COLEOPTERA: CURCULIONIDAE) AND *MALPIGHIA GLABRA* FRUIT YIELDS IN EL TRAPICHE, MEXICO

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ABSTRACT

The temporal and spatial distributions of *Conotrachelus* spp., primarily *C. eburneus* Champion, in an acerola, *Malpighia glabra* (L.), grove in El Trapiche, Mexico, were determined from 1995 to 1998, and compared with populations of *Anthonomus sisyphus* Clark, *A. caracasius* (Faust), and *A. alboscutellatus* Champion from 1997 to 1998. The temporal distributions of *M. glabra* fruits were also determined from 1995 to 1998, and spatial distributions among blocks within the grove were determined from May to October 1998. *Conotrachelus* were found in highest numbers from June to November in all 4 years, coinciding with the rainy season. *Anthonomus* spp. were also most abundant from June to November in 1998. *A. sisyphus* and *A. caracasius* populations were distinctly bimodal, with peaks in July and November, after the second and third major fruit harvests. Few weevils of any species were present during the first major fruit harvest. Correlations of temporal abundance from 1 October 1997 to 11 December 1998 (176 dates) between *Conotrachelus* and *A. sisyphus* and *A. caracasius* were >0.500. *Conotrachelus* and *Anthonomus* were most abundant 3-4 weeks after peak fruiting times, and there were no correlations between temporal distributions of any weevil species and fruit yields. Spatial distributions among blocks within the *M. glabra* grove for *Conotrachelus* were always random in 1995 and 1996. Distributions for *Anthonomus* species were random most of the year, but *A. caracasius* and *A. sisyphus* distributions were slightly aggregated in May. In contrast, fruit distributions among blocks from May to October 1998 (after the peak fruiting period) were more aggregated. Because the temporal distributions of *Conotrachelus* and *Anthonomus* weevils were correlated (except with *A. alboscutellatus*), control measures against these weevils need to be conducted simultaneously or fairly close in time. Weevil distributions within the homogenous *M. glabra* grove were generally random, suggesting a relatively small portion of such groves need to be sampled to generate representative population estimates, regardless of fruit aggregation. Factors that influence even slight aggregation tendencies of *Anthonomus* weevils may be helpful in devising control methods.

INTRODUCTION

The acerola or Barbados cherry, *Malpighia glabra* (L.) [Malpighiaceae], is a shrubby tree found in the subtropical regions of the New World (Vivaldi 1979) that is noted for the high

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vitamin C content of its fruits (Asenjo and Moscoso 1950). Stems, leaves, flowers, and fruits of *M. glabra* are fed upon by more than 10 insect species in western Mexico, of which five are curculionid weevils (Yee 1999). *Anthonomus sisyphus* Clark, *A. unipustulatus* Champion, and at least one *Conotrachelus* sp., *C. eburneus* Champion, attack fruits and two species, *A. caracasius* (Faust) and *A. alboscutellatus* Champion, attack flower buds of *M. glabra*. These weevils can cause significant losses in fruit production and greatly reduce the aesthetic value of the fruits.

While data are available for *Anthonomus* populations on *M. glabra* in western Mexico (Yee 1999), there are no such data for *Conotrachelus* spp., including the major species on *M. glabra, C. eburneus*. Aside from its formal description, virtually nothing is known about this species, other than that it is found in Mexico (Champion 1904). The only other description (Fiedler 1940) with this name was a misidentification, and this homonym was renamed *Conotrachelus homonymus* Blackwelder (Blackwelder 1947). The genus *Conotrachelus* is confined to the New World and has 1,060 species (Papp 1979).

Both *Anthonomus* and *Conotrachelus* larvae feed on *M. glabra* fruits, but the general ecology of *Conotrachelus* is different from that of *Anthonomus* on *M. glabra*. *C. eburneus* develops in fruits, but unlike *A. sisyphus*, which pupate in fruits (Yee 1999), *C. eburneus* leaves the fruit to pupate in the ground (Yee, personal observations). The plum curculio, *C. nenuphar* (Herbst), also leaves fruits to pupate in the soil (e.g., Smith 1957), and this characteristic is true of other economically important *Conotrachelus* that attack tree nuts (Metcalf and Metcalf 1993). *C. nenuphar* is mostly crepuscular or nocturnal (Racette et al. 1991), whereas *A. sisyphus* and *A. caracasius* have been observed mating and feeding on green *M. glabra* fruits and flowers, respectively, during the day (Yee, personal observations). Differences in ecology between *Anthonomus* and *Conotrachelus* on *M. glabra* may extend to the relationship between their population trends and fruit production. The population trends of *C. eburneus* need to be determined if an overall weevil management program on *M. glabra* is to be successful. The ecological differences between the two genera suggest that different control strategies for *Conotrachelus* and *Anthonomus* on *M. glabra* are needed.

In this study, the temporal distributions of *Conotrachelus* from 1995 to 1998 were documented, and data for 1998 were compared with those for three *Anthonomus* species on *M. glabra*. Relationships between weevil abundance and fruit yields were also determined for all weevil species. Spatial distributions of *Conotrachelus* and *Anthonomus* spp. within a *M. glabra* grove were also described. Results are discussed in terms of weevil ecology and management.

MATERIALS AND METHODS

The study site was Rancho Santa Clara Maria, a *M. glabra* farm located 600 m above sea level in El Trapiche (19° 16' N, 103° 40' W) in the state of Colima, Mexico. The rainy season begins in June and lasts through October, with the dry season beginning in November and lasting until May. Temperatures range from ≈25 to 35°C. Yearly pluvial precipitation is ≈1,000 mm. Approximately 40,000 *M. glabra* trees of approximately six varieties of the 'B-series' were obtained from Puerto Rico and transplanted in El Trapiche on loam soil as small leafless trees in 1991. Trees were drip irrigated for 2 to 3 h daily during the dry months. Trees were planted in 29 blocks, each block typically arranged in 40 rows, 37 trees per row, in a grove occupying 55 hectares. Trees within blocks were spaced 3.3-4 m apart; blocks were separated by 3.3 m. Diflubenzuron (Dimilin) applications were made every 15 to 20 days to control weevils beginning on 20 March 1995 and continuing through 1998.

The first sampling began on 17 November 1994 and continued to 11 December 1998. A cloth sweep net (38 cm diameter) was used to knock weevils off trees. Thirty-seven to 50 trees per block were sampled by beating from underneath the trees twice, once on each side. Twenty-one (1994 and 1995), 21 or 14 (1996), or 13 (1997 and 1998) blocks were sampled daily, 3 or 4 days a week, or 3 days a week, respectively, during daylight (0800-1200 h). The sampling unit was number of weevils per 100 beats per block. Trees within blocks were sampled haphazardly. Weevils were identified in the field and released.

Fruits were found on trees 8 months of the year. Green, unripe, or slightly red fruits were picked. Fruits were picked before they ripened, as much as possible. During the peak periods of fruit production, all fruits were picked daily by teams of up to 400 people and placed into crates. Full crates held an average of 18.5 kg of fresh fruit. From 1995 to 1998, daily fruit yields from all 29 blocks were combined, but fruit yields within each of the 29 blocks were kept separate beginning 25 May 1998. Fruit yields were recorded up to 27 November 1998.

For graphical presentation of *Conotrachelus* spp. populations among years, 141 days per year were matched as closely as possible. Correlations among numbers (n = 136 or 140 days because of a few unmatchable or missing dates) of *Conotrachelus* spp. in 1995, 1996, 1997, and 1998 were calculated. Correlations among numbers of *Conotrachelus* and fruit yields from 1995 to 1998 and of *Anthonomus* spp. from 1 October 1997 to 27 November 1998 were also calculated.

The spatial dispersion of weevils was analyzed using Green's (1966) coefficient, according to the formula:

$$C_x = \frac{[s^2/m]-1}{\sum x - 1}$$

where s^2 = variance, m = mean, and $\sum x$ = total numbers of weevils collected on the sample date. According to Green, when $C_x = 0$, populations are randomly distributed, whereas when $C_x = 1$ or approaches it, the populations are more aggregated.

From 25 May to 10 October 1998, the spatial dispersion of fruits among the 13 blocks that were sampled for weevils was analyzed. These blocks were approximately the same area (each ≈1.6 hectares, total area ≈21 hectares). The sampling unit per block was the number of full crates of fruit.

RESULTS

Conotrachelus from 1994 to 1996 were not identified to species. In 1997 and 1998, only specimens identified as *C. eburneus* were counted. The mottled white patterns on the elytra easily distinguished it from two other species of *Conotrachelus* on *M. glabra*; 71% belonged to this species in a representative sample (n = 17) from 22 July to 23 September 1997. It is likely that most weevils collected from 1994 to 1996 were also this species.

Conotrachelus spp. were found at >5 per 100 beats during November 1994, but numbers dropped to <1 by late December (Fig. 1). From 1995 to 1998, highest numbers of *Conotrachelus* spp. or *C. eburneus* adults were found on *M. glabra* trees over a 5-month period from mid June through the end of November (Figs. 2A, 2B, 2C, 2D), coinciding with the rainy season. Adults were found during periods when there were few or no fruits, from late June until late August or early September. Abundance was greatest in July and November, but no well defined bimodal population trend was seen. Almost no adults were found during the first harvest of the year. Population trends were slightly different among years; they were most strongly correlated between 1995 and 1996 (r = 0.783), followed by 1996 and 1998 (r = 0.450), and were weaker among other years (Table 1).

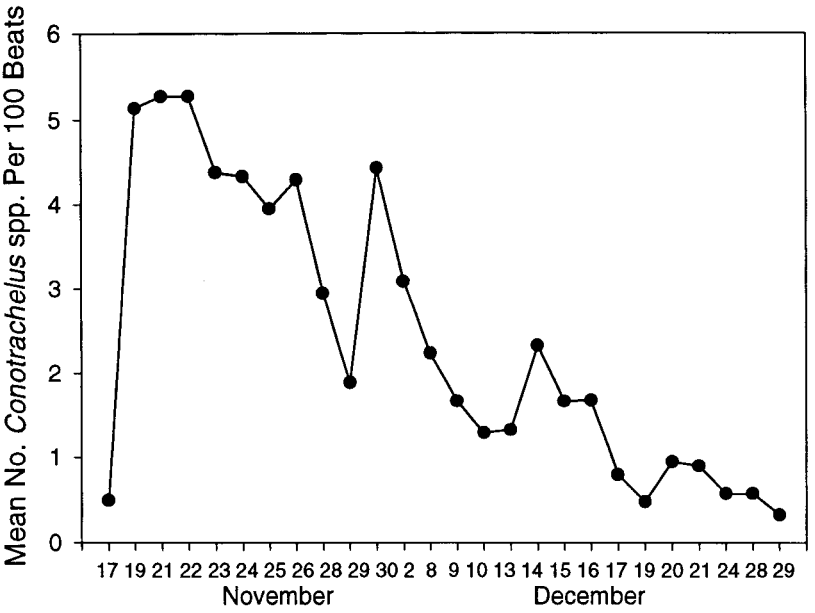

FIG. 1. Mean numbers of *Conotrachelus* spp. per 100 beats from *Malpighia glabra* trees collected in 1994 in El Trapiche, Mexico.

Unlike *Conotrachelus* spp., which showed no clear population peaks separated by periods of absence or near absence, *M. glabra* showed three or four distinct (protracted) fruiting periods from 1995 to 1998. In general, these periods occurred between March to April, May to June, and September to November. Fruit yields increased from 1995 to 1997, but yields in 1998 were similar to those in 1996. In contrast, *Conotrachelus* spp. numbers were highest in 1994, at least during November (Fig. 1), decreased slightly in 1995 (Fig. 2A), declined in 1996 (Fig. 2B), and declined further in 1997 (Fig. 2C). Numbers in 1998 (Fig. 2D) were similar to those in 1997.

Populations of two other weevils, *A. sisyphus* and *A. caracasius* (Figs. 3A and 3B, respectively), like those of *C. eburneus,* were high from June through November 1998. However, unlike those of *C. eburneus*, their population trends were distinctly bimodal, peaking in July and November, each time ≈3-4 weeks after peak fruiting times in late 1997 and in 1998. High populations of *A. caracasius* and *A. sisyphus* were present when fruits were nearly absent during August. *A. alboscutellatus* populations showed a distinct peak in November 1997 (Fig. 3C), but no clear pattern emerged afterward. The population trend of *C. eburneus* in 1998 was most similar to those of *A. sisyphus* and *A. caracasius*, and least similar to that of *A. alboscutellatus* (Table 1). Temporal distributions of weevils were not correlated with those of *M. glabra* fruit yields (Table 1). Like *C. eburneus*, the three *Anthonomus* species were nearly absent during the first fruit harvest of 1998.

Assuming the beat net collection method caused the different species to drop equally from trees, the abundance of adult weevils on *M. glabra* from 1997 to 1998 during daylight hours ranked as follows: *A. caracasius*>*A. sisyphus*>*A. alboscutellatus*> *C. eburneus. A. unipustulatus* was rarest of all and was not included in the analyses.

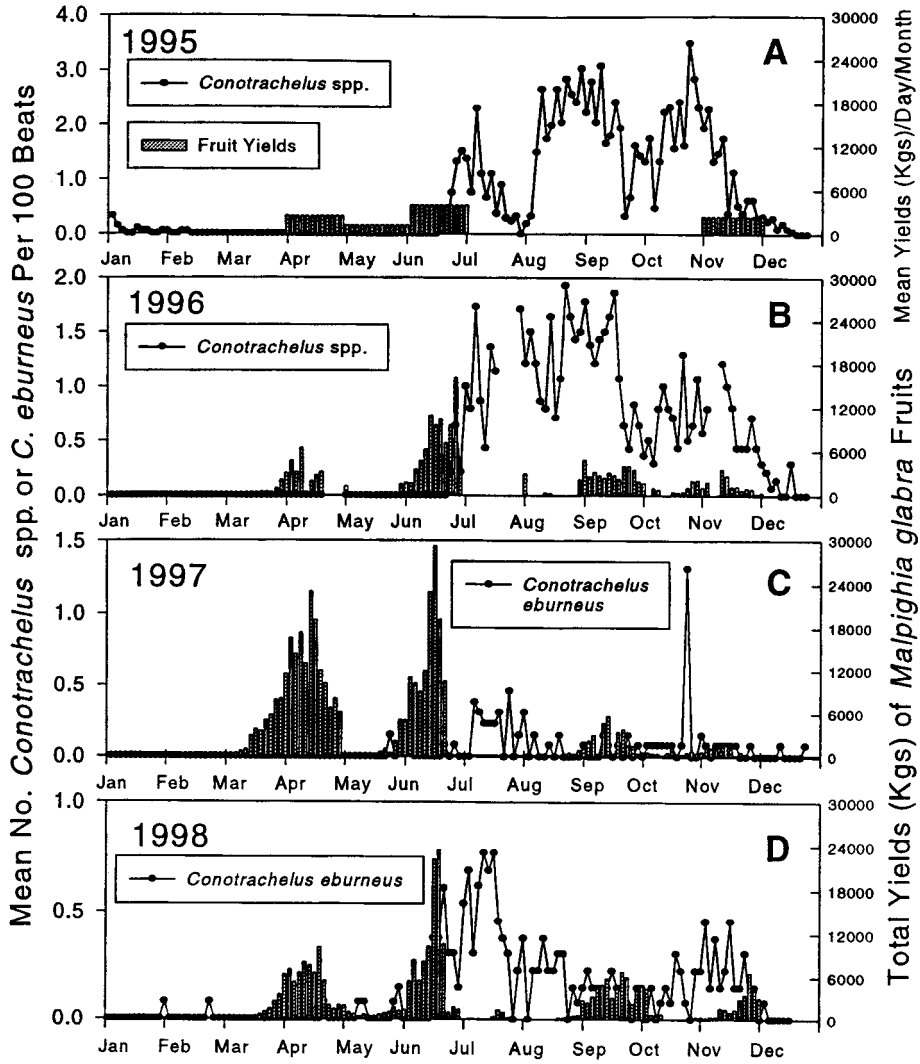

FIG. 2. Mean numbers of *Conotrachelus* per 100 beats from *Malpighia glabra* trees collected in (A) 1995, (B) 1996, (C) 1997, and (D) 1998 in El Trapiche, Mexico. Mean daily fruit yields/month (monthly total/13 sample days per month) are shown in 1995; yields in 1998 were recorded up to 27 November 1998.

Conotrachelus spp. were distributed randomly among 13-21 blocks in 1994, 1995, and 1996 (C_x values were <0.10, except in 2 cases). *C. eburneus* numbers among blocks in 1997 and 1998 were too low for calculations. *Anthonomus sisyphus*, *A. caracasius*, and *A.*

TABLE 1. Correlations Among Years in Daily *Conotrachelus* (all *C. eburneus* in 1997 and 1998) Numbers from 1995 to 1998 and Correlations among Weevil Species and *Malpighia glabra* Fruit Yields from 1997 to 1998 in El Trapiche, Mexico.

Correlations of *Conotrachelus* populations among 4 years (136 or 140 dates per year)				
		1995	1996	1997
	1996	0.783	---	---
	1997	0.297	0.212	---
	1998	0.329	0.458	0.290
	Correlations among weevil species (176 dates between 1 October 1997 to 11 December 1998)			
	Conotrachelus	*A. sisyphus*	*A. caracasius*	*A. alboscutellatus*
A. sisyphus	0.502	---	---	---
A. caracasius	0.537	0.580	---	---
A. alboscutellatus	-0.057	0.150	0.342	---
	Correlations between different weevil populations and fruit yields			
1995[a]	-0.210	---	---	---
1996[b]	0.035	---	---	---
1997[c]	-0.108	---	---	---
1997-1998[d]	0.048	-0.132	0.111	-0.176

[a] n = 141 sample dates, using mean yields/day/month.
[b] n = 137 sample dates.
[c] n = 141 sample dates.
[d] n = 170 sample dates between 1 October 1997 and 27 November 1998.

alboscutellatus were also distributed randomly among 13 blocks during most times of the year, as C_x values approached 0 in most cases (Figs. 4A, 4B, 4C). In no case (when $n \geq 4$) did C_x values for *Anthonomus* weevils reach 0.4. However, values for *A. sisyphus* and *A. caracasius* were higher than for *C. eburneus*. In contrast to weevil distributions, *M. glabra* fruit distributions were more aggregated, reaching 1.0 in a few cases (Fig. 4). C_x values were high in late June and July, when overall fruit production was relatively low (Fig. 3). Daily picking insured that most fruits were picked as soon as they reached the green stage.

For *A. sisyphus*, evidence for weak aggregations was detected from November to December, April and May, and late July 1998, coinciding with periods during the end of or after major harvests (Fig. 3A). For *A. caracasius*, evidence of a weak aggregation was detected only between April to May 1998 ($C_x \approx 0.20$). On 13, 18, 22, and 25 May, one of the 13 blocks accounted for 35% (n = 26), 51% (n = 72), 57% (n = 69), and 50% (n = 78), respectively, of the total weevils. Fruit yields from this block (3,737 kg) between 25 May and 4 June 1998 exceeded those of the other 12 blocks (mean of 965 kg).

On 13 and 14 October 1998, 28 randomly chosen trees were beaten ten times each. The C_x values (among trees) obtained for *C. eburneus* was -0.037 (mean per tree $\pm$ s^2, 0.11 $\pm$ 0.10); *A. sisyphus* 0.136 (0.14 $\pm$ 0.20); and *A. caracasius* 0.101 (0.21 $\pm$ 0.32).

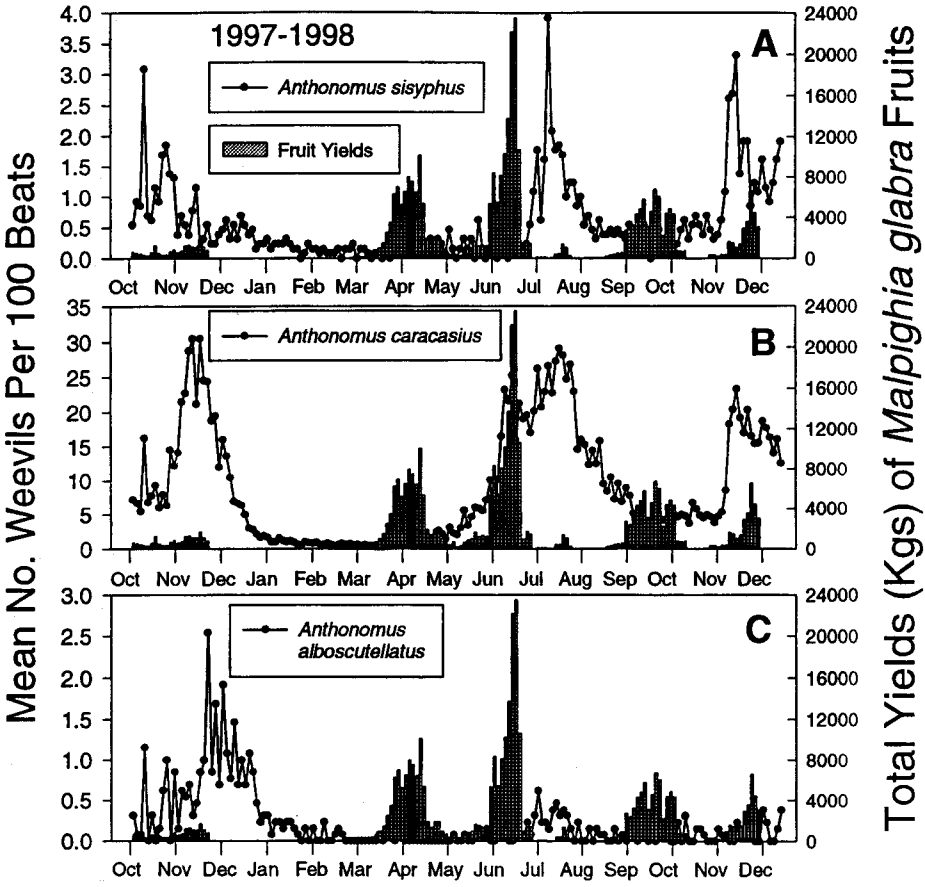

FIG. 3. Mean numbers of (A) *Anthonomus sisyphus*, (B) *Anthonomus caracasius*, and (C) *Anthonomus alboscutellatus* per 100 beats from *Malpighia glabra* trees collected 1 October 1997 to 11 December 1998 in El Trapiche, Mexico. Fruit yields were recorded up to 27 November 1998.

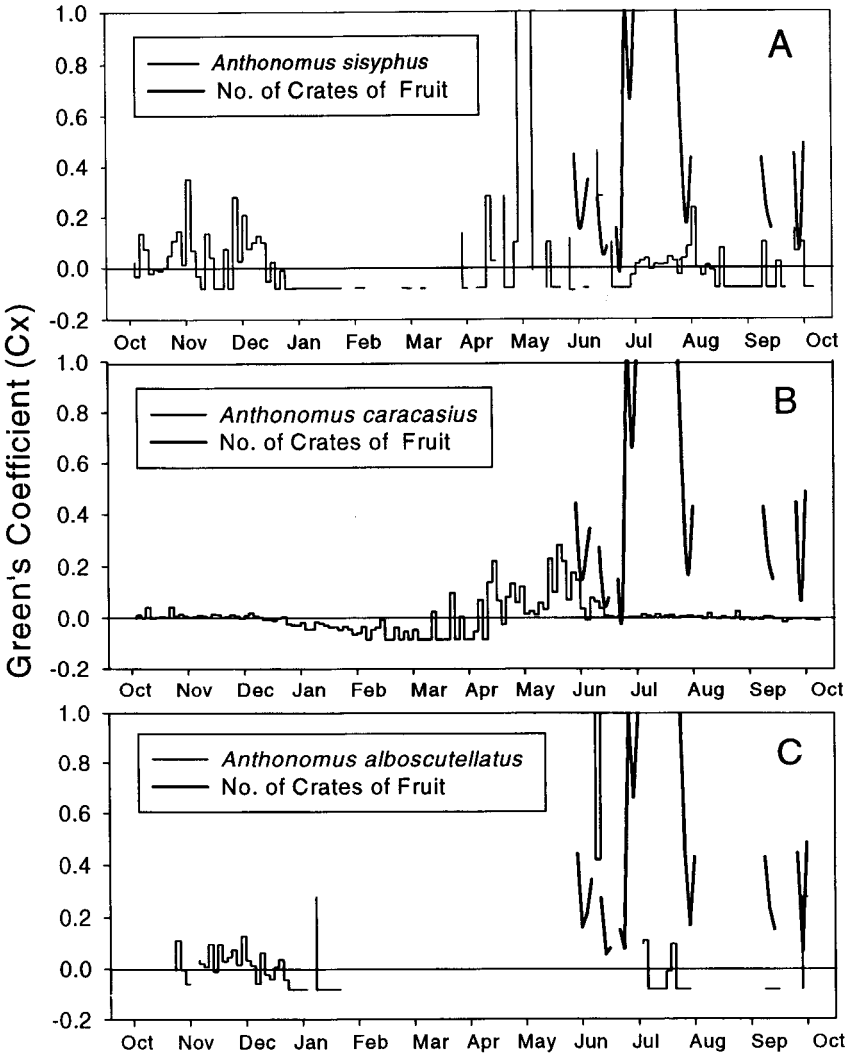

FIG. 4. Green's coefficients for (A) *Anthonomus sisyphus*, (B) *Anthonomus caracasius*, and (C) *Anthonomus alboscutellatus* among 13 blocks related to Green's coefficients for *Malpighia glabra* yields from October 1997 to October 1998. Numbers of *A. alboscutellatus* were too low between February and June to generate reliable coefficients.

DISCUSSION

Several key ecological characteristics of *Conotrachelus* and *Anthonomus* weevils on *M. glabra* differ, but their population trends were similar in that they were out of phase with

that of *M. glabra* fruit production. For all weevil species (except *A. alboscutellatus*) in 1998, abundance was greatest in July after the second major harvest of the year, suggesting that emergence from the ground (*Conotrachelus*), flower buds (*A. caracasius*), and fruits (*A. sisyphus*) peaked during this time because of the high abundance of breeding sites during or after the harvest. High numbers of ripened fruit that failed to be picked or that naturally dropped from trees were major sources of adult *A. sisyphus* (Yee 1999) and probably *C. eburneus* (depending on duration of the pupal stage). The consistent increase in numbers of *Conotrachelus* (1995-1998) and *A. sisyphus* and *A. caracasius* (1997-1998) after peak *M. glabra* production during the second and third major harvests of the year reveals the existence of a predictable host-pest cycle. The same pattern for *Anthonomus* was seen from 1995 to 1997, even though *A. sisyphus* and *A. caracasius* were grouped into one category during those years (Yee 1999).

The scarcity of fruits during July and August 1998 suggests that adult *C. eburneus* and *A. sisyphus* survived this period by feeding on other plant parts or else found the few fruits present. No observations on *C. eburneus* survival exist, but 18 adult *A. sisyphus* that emerged from *M. glabra* fruits on 25 September 1997 in El Petacal, Jalisco, Mexico (≈40 km from El Trapiche), survived only 2.3 ± 1.8 d (excluding one that survived >8 d) at ≈27-30 °C inside 15 cm^3 vials containing water-moistened paper tissue. Four *A. sisyphus* that emerged from fruit on 26 September 1998 survived only 4 d, and of 14 that emerged 29 September 1998, eight died in a mean of 3.8 ± 1.0 d; the other six survived >7 d. These observations suggest adult weevils cannot survive the flower- or fruit-free period long on stored reserves but need an outside source of nutrition. *A. caracasius*, which attacks flowers buds, may feed on other plant parts. *A. caracasius* were observed attacking the young growth buds in September 1998, even though numerous flower buds were present (Yee, personal observations). The maintenance of breeding weevil populations may be enhanced and made more complicated because blooming and fruiting by *M. glabra* were asynchronous, resulting in at least some flowers and fruits always being present 8 months of the year.

Conotrachelus eburneus, *A. sisyphus*, *A. caracasius*, and *A. alboscutellatus* populations should have been more aggregated during July and August 1998 if the weevils were searching to oviposit on the relatively few trees that had abundant flowers and fruits. However, for *A. caracasius*, this apparent aggregation tendency (at the block spatial scale) occurred during April and May. The prolonged period during which the C_x was >0.10 (six out of seven dates over a 14-d period) suggests this phenomenon was biologically significant and not just a statistical artifact, and may have been related to high abundance of flowering within one block. It is unclear why this did not occur during July and August 1998. The daily picking that was employed within the grove may have obscured patterns of food or oviposition site searching behavior by the weevils. After trees were stripped of fruit, the weevils apparently did not disperse immediately to blocks that had trees with fruit, resulting in the discrepancy between their spatial distributions and that of fruits. The homogeneity of the trees and their arrangement within the grove may have played a larger role in explaining weevil distributions than the heterogeneity in fruit distributions.

Several management practices can be implemented based on the temporal distributions of weevils: (1) treatment for *C. eburneus* and *Anthonomus* weevils need to begin in June and May, respectively; (2) prompt removal of fruits from the first and second harvests may reduce weevil emergence; and (3) because weevil populations were highest during the wet season (June to November), fungal pathogens such as *Beauveria bassiana* [Balsamo] Vuillemin and *Metarhizium anisoplae* may be effective; two *B. bassiana*-infected *A. sisyphus* were collected on 13 and 14 October 1998 in El Trapiche. *C. eburneus* may be especially affected because this species pupates in the soil. Because numbers of all weevil species were low from January

through May (*Conotrachelus*, 1995 to 1998; *Anthonomus*, 1998), early blooming varieties of *M. glabra* that produce most of their fruit during these months should be less impacted by weevils. These varieties can be identified and potentially selected for their early fruiting times. Fruits collected in March 1997 had almost no weevil damage, whereas those in September 1997 were heavily damaged (Yee 1999).

Weevils were randomly distributed most of the year (1998) within the homogenous *M. glabra* grove of 21 hectares, suggesting that a relatively small portion (blocks) of such groves needs to be sampled to generate representative population estimates. The random distributions suggest an entire grove can be treated based on such estimates, regardless of the heavier concentration of fruits among certain blocks. In cases where some weevil aggregation is detected ($C_x \approx 0.20$), the relevant blocks may need an extra insecticide treatment. Determining factors that affect even slight aggregation tendencies might prove useful in an overall weevil control program.

ACKNOWLEDGMENT

Charles O'Brien (Florida A & M University) confirmed identification of the *Conotrachelus eburneus* weevils and Ramon Guitierrez, Rosario Naranjo, and Luis Bayardo (Nutrilite, S. de R.L. de C.V., Rancho Santa Clara Maria) assisted in field collections.

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POPULATION DYNAMICS OF THE VARIEGATED LEAFHOPPER, *ERYTHRONEURA VARIABILIS*[1] BEAMER AND ITS EGG PARASITOIDS IN NORTHWESTERN MEXICO

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ABSTRACT

During the summer and fall of 1995 and 1996, two vineyards were sampled for egg parasitoids of the variegated leafhopper, *Erythroneura variabilis* Beamer. The vineyards sampled were "Las Calabazas," located 20 km east, and the vineyard at the Agricultural Experiment Station, 22 km west of Caborca, Sonora. Two egg parasitic wasps were detected and identified: *Anagrus epos* Girault (Hymenoptera:Mymaridae) and a species of Trichogrammatidae, *Ittysella lagunera* Pinto and Viggiani, which had not been collected before in this area. An overall apparent parasitism rate of 58.4% was observed in the "Las Calabazas" vineyard, versus 56.5% at the Agricultural Experiment Station for both species. In both vineyards the parasitism index rate reached 100% by the end of the season. *Anagrus epos* was the most abundant parasite, representing 78% of the total of specimens collected from the "Las Calabazas Vineyard" and 70% at the Agriculture Experiment Station.

INTRODUCTION

The variegated leafhopper, *Erythroneura variabilis* Beamer, has been an economically important pest of vineyards since grapes were first planted in Northwestern Mexico in the area of Caborca, Sonora, in 1967. This leafhopper is present in vineyards from late April through the middle of November, where it feeds on grape leaves by puncturing the leaf cell and sucking the sap (Guerra 1993). This causes premature defoliations causing extemporaneous regrowth of the vines, especially after rains, thereby affecting the following season's production. *E. variabilis* has a wide range of alternate hosts where it overwinters as an adult in a state of reproductive diapause (Barnes 1982). In this area they overwinter on grasses, alfalfa fields, and winter weeds along ditches and at the edges of vineyards. In the Caborca, Sonora, area, the variegated leafhopper is more abundant from June through October and requires chemical control before, and especially after harvest (Guerra 1993).

In Europe, grape leafhopper populations are parasitized by a minute mymarid wasp, *Anagrus atomus* (L.), (MacGill 1934). *Anagrus epos* Girault and *Anagrus armatus* Girault are reported to be effective biological control agents of grape infesting leafhoppers in Canada and the United States (Armstrong 1936, Doutt and Nakata 1965, McKenzie and Beirne 1972, Williams 1984).

Kido et al. (1983) and Barnes (1982) reported *Anagrus epos* Girault to be one of the most important egg parasitoids of the western grape leafhopper, *Erythroneura elengantula*

[1] Homoptera: Cicadellidae

Osborn, in the San Joaquin Valley of California. A near relative, the variegated leafhopper, *E. variabilis* Beamer, is also parasitized, but at a much lower rate (Settle et al. 1986). Six species of *Anagrus* are known from North America (Gordh and Dunbar 1977).

Attempts to regulate the variegated leafhopper populations by biological control have never been initiated nor have surveys been conducted on its egg parasitoids in northwestern Mexico. The purpose of this investigation was to determine the population dynamics of the variegated leafhopper and its egg parasitoids in Caborca, Sonora, Mexico, to obtain a better understanding of the effectiveness of native egg parasitoids in regulating population densities of *Erythroneura variabilis* Beamer and in the implementation of biological control as part of an integrated pest management strategy for vineyards in the future.

MATERIALS AND METHODS

Research was conducted during the summer and fall of 1995 and 1996 in two pesticide-free vineyards, one 20 km east of Caborca, known as "Las Calabazas," and the other 22 km west of Caborca, at the Agricultural Experiment Station (A.E.S.). The vineyard east of Caborca was 85 hectares and the one west of Caborca was 76 hectares, both planted in 1988 with the "Thompson Seedless" cultivar. The results of the 1996 season are presented here, as in 1995 the vineyard at "Las Calabazas" did not receive adequate irrigation due to problems with the well and the leafhopper population being low so that few parasitoids were collected.

Weekly samples of 20 vines were cut from the lower canopy of the plants in each vineyard. The lower canopy vines were chosen because they showed more vigorous growth and produced most of the mature leaves where adult females laid their eggs. The vines also were cut from the shaded part of the vineyard, as these are preferred by females for oviposition (Jensen and Flaherty 1982). Each vine had approximately 15 to 20 leaves. The cut end of each vine was placed in a 100-ml plastic bottle filled with water and placed in an 11.3- liter circular paper carton (50cm long x 17cm wide) with a lid. Each carton had a glass vial (7.5ml) partially inserted and secured into the carton lid to allow light to enter and attract emerging parasitoids and newly emerged leafhopper nymphs. The carton was held at 24° C.

Each carton was examined approximately on the third and seventh day after date of collection; leafhopper nymphs and parasitoids were completely removed from the vials and the numbers of parasitoids and leafhoppers per sample were recorded. Seven days after collection, the majority of the leaves on the vine had wilted. By this time there were no signs of emerging leafhopper nymphs or parasitoids; therefore, all nymphs and parasites were removed. A true parasitism rate could not be determined since leafhopper eggs were not counted, and some eggs could have died of natural causes or parasites may have not emerged; therefore, a parasitism index or an "apparent parasitism" was determined. The parasitism index was determined by dividing the total number of parasitoids by the sum of the total number of parasites and leafhopper nymphs obtained each day that cartons were examined. Data is presented at approximately three and seven day intervals.

Population density trends of the leafhopper nymphs also were monitored by sampling 500 leaves at random (50 leaves from each of 10 center rows) from each of the two vineyards approximately every third and seventh day. The economic threshold for grapes range from 10 to 15 nymphs per leaf for table grapes and 15 to 20 nymphs per leaf for grapes destined for raisins or wine.

The relative population densities of the variegated leafhopper nymphs, as indexed by the leaf samples taken in the fields, together with the parasites emerging from the cartons, were recorded as nymphs per leaf.

RESULTS AND DISCUSSION

Two species of egg parasites were found: *Anagrus epos* Girault (Hymenoptera:Mymaridae) and *Ittysella lagunera* Pinto and Viggiani (Hymenoptera:Trichogrammatidae). *I. lagunera* previously had been recorded only in the Torreon, Coahuila, area in north central Mexico (Pinto and Viggiani 1987) and had not been reported in this area.

A. epos is about 0.5mm in length. The antennae are longer than the body, having 13 filiform segments in males and are clubbed with nine segments in females. The wings are long and narrowed with fringed edges. By comparison, *I. lagunera* has red eyes and is more robust than *Anagrus epos*, with six-segmented antennae. The wings are wide and round with a long fringe on the forewings.

In the "Las Calabazas" vineyard, the population of both parasitoids increased gradually beginning in July but showed a slight decrease during August and was highest from September through October, at which time it began to decrease until the end of the season in early November. On the other hand, the nymph population of the leafhopper was highest during July, falling in early September and remaining low throughout the remainder of the season. The numbers of nymphs per leaf ranged from 1 to 21, averaging 7.2 nymphs per leaf for the season.

When the population of leafhoppers was high (10-15 or more nymphs per leaf), the emergence of both parasitoids was also high; however, from August through early November, populations started decreasing, and the parasitoids showed an increase from the end of August through the end of October. This increase in parasitoid emergence reflected an increment on the percentage parasitism index registered at the end of the season (Fig. 1).

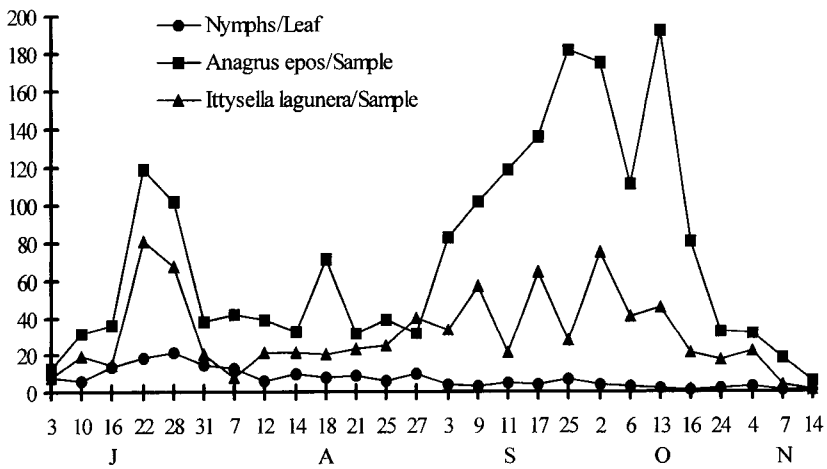

FIG. 1. Population Dynamics of the Variegated Leafhopper and Its Egg Parasitoids in the "Las Calabazas" Vineyard. Caborca, Sonora, Mexico.

Ittysella lagunera showed an erratic fluctuation from July through late September when it began to increase; however, it remained less abundant than *A. epos,* which was more

numerous from September through the end of October. Both species decreased by the end of the season (Fig. 1).

The emergence of parasitoids was high in July when the nymph population was also high; however, as the nymph population decreased, the emergence of the parasitoids also decreased, due mostly to lower egg ovipositions. During September, October, and the middle of November, when nymph per leaf infestations were at their lowest, the emergence of parasitoids increased and remained high until early November. This is also reflected in the apparent percentage parasitism registered for both species on leafhopper eggs which at the end of the season reached an apparent 100% parasitism (Fig.2).

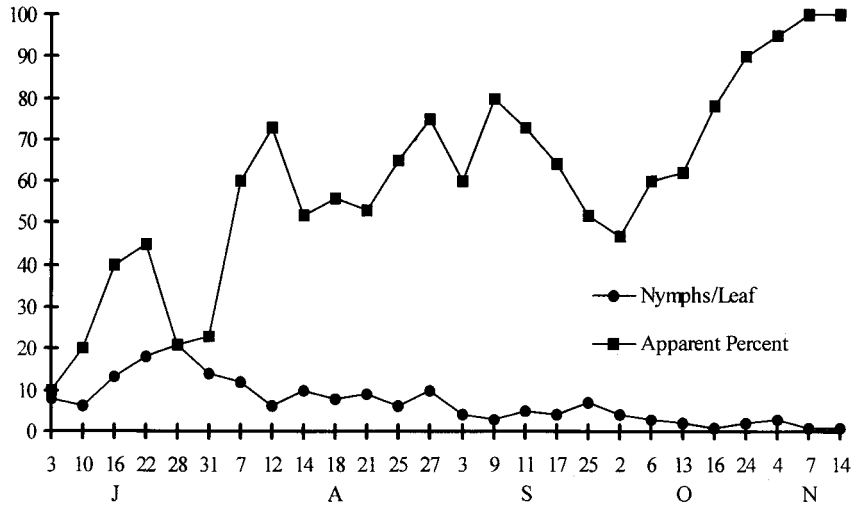

FIG. 2. Apparent Percentage Parasitism in Relation to the Population Dynamics of the Variegated Leafhopper in the "Las Calabazas" Vineyard. Caborca, Sonora, Mexico.

When fewer leafhopper nymphs were emerging, the apparent parasitism was high and actually reached 100%, but this was due to the decrease in leafhopper egg oviposition at the end of the season, as few adults were observed at this time. However, the overall average parasitization rate, as obtained by the parasitism index, registered 58.4% for the season, which is a good indication that parasitization by these wasps has a beneficial effect as biological control agents of the leafhopper populations in this area.

Leafhopper nymph populations in the Agricultural Experiment Station vineyard were more abundant than those at "Las Calabazas." Numbers of nymphs per leaf ranged from 1 to 17, averaging 8.8 nymphs for the season; however, the parasitoid population was less abundant. Population trends, for both leafhoppers and egg parasitoids, were similar to those observed in the "Las Calabazas" vineyard.

The leafhopper population was relatively high from July through August, fell rapidly in September, and decreased considerably during October and the first two weeks of November. The population of *A. epos* was always greater throughout the season in both vineyards. The same trend was apparent with *I. lagunera*, which was less abundant throughout the season in both vineyards. However, with both species, especially *Anagrus epos*, the population increased and became more abundant from the middle of September through the end of October (Fig. 3).

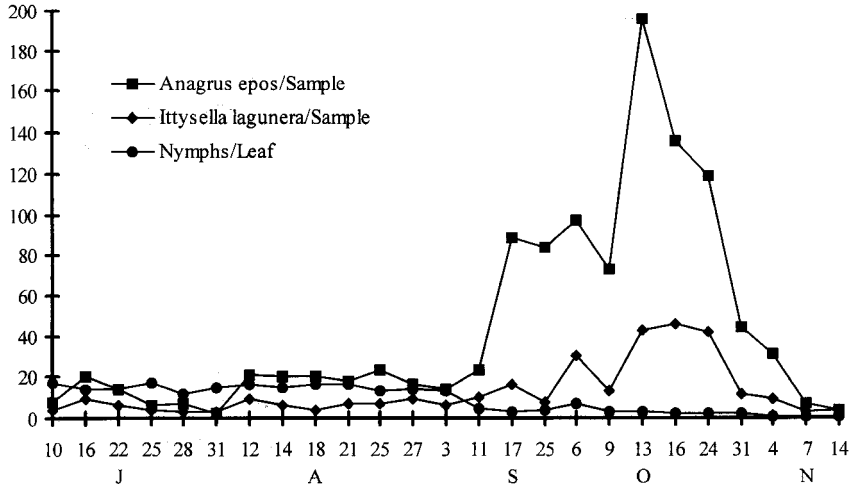

FIG. 3. Population Dynamics of the Variegated Leafhopper and Its Egg Parasitoids in the Agricultural Experiment Station. Caborca, Sonora, Mexico.

When the infestation of leafhopper nymphs was high (15-18 nymphs per leaf), due to increased in oviposition by adults early in the season, parasite emergence was also high and the parasitism index also increased as indicated in Fig. 4. There was an increase in emergence of parasites at the end of the season, which also was reflected in a greater apparent percentage parasitism at the end of the season (Fig. 4).

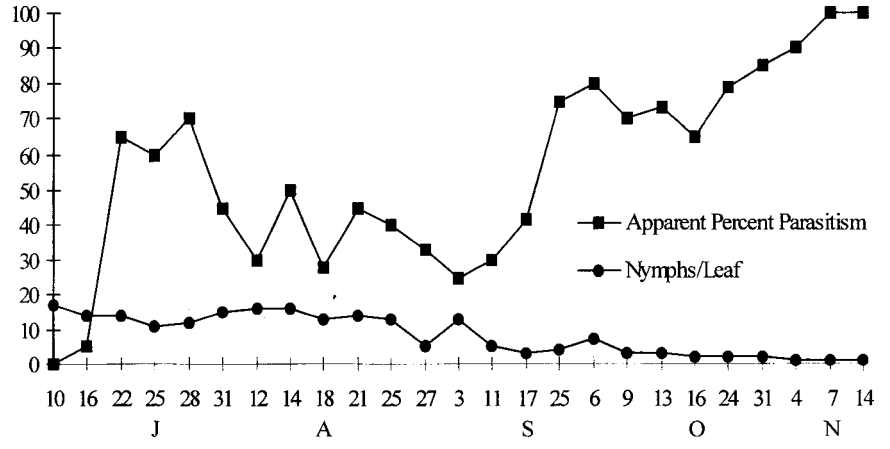

FIG. 4. Apparent Percentage Parasitism in Relation to the Population Dynamics of the Variegated Leafhopper in the Agricultural Experiment Station. Caborca, Sonora, Mexico

The combined apparent percentage parasitization of both parasites is somewhat erratic during July through August, but becomes more stable from September through November as the parasite population increased. The average overall apparent parasitism rate for the season in this vineyard was 56.5%.

This data indicates that, at the beginning of the oviposition period by leafhopper females, there were few parasites; however, egg hatch then was more abundant and parasite populations increased, leading to superficially high apparent percentage parasitism which actually reached 100% in both vineyards. The parasitism index was unrealistically high when leafhopper nymphs were low (as at the end of the season), but it was also high when leafhopper nymphs were high (as at the beginning of the season).

Anagrus epos accounted for 70 and 78% of the total specimens collected from the "Las Calabazas" and the Agricultural Experiment Station vineyards, respectively.

It is evident that these egg parasitoids play an important role in limiting the populations of the variegated leafhopper in this area; however, more research is needed to determine when parasitism can be more effective as part of an integrated pest management program.

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CHEMICAL CONTROL OF THE VARIEGATED LEAFHOPPER, *ERYTHRONEURA VARIABILIS*[1] BEAMER IN RELATION TO HEAT UNITS IN NORTHWESTERN MEXICO

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Caborca, Sonora

ABSTRACT

Research was conducted in 1996 to evaluate the effectiveness of a single insecticide application for the control of the first, second and third generations of the variegated leafhopper, *Erythroneura variabilis* Beamer, based on 2,500, 3,000 and 3,500 Accumulated Heat Units (AHU). Insecticide application at 2,500 AHU was effective in reducing the nymphal infestations, resulting in 8.5 nymphs per leaf compared to 16.9 nymphs per leaf for the untreated control plot, a reduction of approximately 50%. The adult population measured 26.8 adults per 25 sweep net samples for the treated plot and 60 adults per 25 sweep net samples on the untreated control plot, a reduction of 56%. The control of both adults and nymphs was continued for approximately 62 days from the date of application. Insecticide application at 3,000 AHU reduced the adult population by 63% and the nymphal infestation by 62% for approximately 45 days from the date of application, while the application at 3,500 AHU reduced the adult population by 63% and the nymph infestations by 77% but only for approximately 15 days from the date of application.

INTRODUCTION

The variegated leafhopper, *Erythroneura variabilis* Beamer, is the most important pest of vineyards in Caborca, Sonora, in northwestern Mexico. Both adult and nymphs feed on leaves by puncturing the leaf and sucking the sap. This causes premature defoliation and permits the vines to regrow extemporaneously, especially after the late summer rains. This weakens the vines and affects production potential for the next season. Furthermore, excessive fruit spotting of table grapes result from the insect`s excrement, leading to further economic loss. High adult populations of leafhoppers annoy pickers during harvest, and nymphs sometimes pierce the exposed skin of pickers, causing them to leave some vines unharvested. Grape growers usually do not apply insecticides before harvest to control the variegated leafhopper in order to avoid insecticide residual problems because most of the table grapes are exported to the United States, Japan, Canada and Europe. Harvest of table grapes begins in late May and ends by the middle of June; the remainder of the grapes, which are used for wine, juice and raisins, are harvested by the middle of June through the middle of August. Infestations of the variegated leafhopper are so great by August that damage is extensive, and several applications are needed to bring them under control. A single application usually has an effect on variegated leafhopper populations for approximately 15 to 20 days when

[1](Homoptera:Cicadellidae)

infestations are mild, while in severe infestation, the effect is shortened to 12-15 days. In some cases, up to four applications are needed to bring the leafhoppers under control.

It is difficult to determine when to initiate chemical control of this pest based on adult counts because the population of adult leafhoppers is very mobile and is active from April through the end of October. Furthermore, population increases vary from year to year in such a manner that infestations sometimes occur late in the season or one to two weeks earlier from what is expected, due mostly to weather conditions.

Accumulated heat units (AHU)have been used to predict the initiation and timing of chemical control measures for other insects. Research has shown that insects as well as plants respond in their development according to the number of heat units accumulated during the season (Newman 1971, Huber 1981) or to degree days accumulated (Herbert et al. 1988, Pruess 1983, Wilson and Barnett 1983, Shaffer 1983, Welch et al 1978).

In two previous years of research, Guerra (1992 and 1993) determined the periods of major incidence of adult and nymph populations of the variegated leafhopper in grapes based on AHU as described by Huber (1979). It was established, from the data obtained during these years, that adult and nymph populations reached their highest levels at approximately 3,000 AHU.

This research was conducted to determine the most appropriate time, based on AHU, at which chemical control could be initiated in order to have the greatest impact on the adult and nymph leafhopper populations.

MATERIALS AND METHODS

During the season, a commercial vineyard planted with the "Thompson Seedless" cultivar was divided into four plots, approximately one acre in size. Plots consisted of 12 rows of vines, each 100 meters in length, with 4 meters between rows. The experimental design was completely randomized with six repetitions, which consisted of the sampling dates. A statistical test on the comparison of two independent samples as described by Snedecor and Cochran (1974)was used. The average of 10 samples of 25 sweep net samples for adults, taken with a standard entomological net were used for each date sampled. Samples were taken from each of the 10 center rows from the treated and untreated plots. In addition, the average of 10 samples of 50 leaves each were taken completely at random from 10 center rows from each treated and untreated plot to determine the density of the leafhopper nymphs for each date sampled. Each treated plot was compared with the untreated control plot; no comparisons were made among the treated plots. Beginning on 1 January, the number of heat units accumulated were recorded. Heat units were determined by using the computerized heat unit tables developed by Huber (1979). From these tables, heat units are obtained by the interception between high and low temperatures for each day. Although, Huber's tables were developed for the cotton pink bollworm, *Pectinophora gossypiella* Saunders, the high and low temperature thresholds were the same as those for the development of the variegated leafhopper in grapes in this area.

A single insecticide application with carbaryl (Sevin 80) at 1.6 kg AI per hectare was applied with a vineyard insecticide ground sprayer, delivering 1,000 liters per hectare. Carbaryl was used because it can be applied up to one day before harvest. The equipment sprayed two rows at a time. All the applications were done between 08:00 and 11:00 hours. The first application was initiated at 2,500 AHU (28 May), the second at 3,000 AHU (18 June), and the third at 3,500 AHU (8 July), independent of the degree of infestation or stage of development of the crop.

After each application, weekly samples of adults and nymphs were taken until 4 August, at which time a general insecticide application was given to the whole vineyard and the

experiment was terminated. Populations of adults and nymphs of the variegated leafhopper began to increase, and extensive damage to the foliage was evident. Infestations of the grape leaf skeletonizer, *Harrisina brillians* Barnes and McDunnough, were also present at that time, especially in the control untreated plot.

RESULTS AND DISCUSSIONS

The incidence of adult and nymph populations throughout the duration of the experiment was continuous and reached its first peak by the middle of June; a second peak was reached by the end of June and a third and final peak was evident from the middle to the end of July. During the first week of July, both adults and nymphs showed a slight decline in infestation, but a noticeable increase was evident by the end of the month (Fig.1).

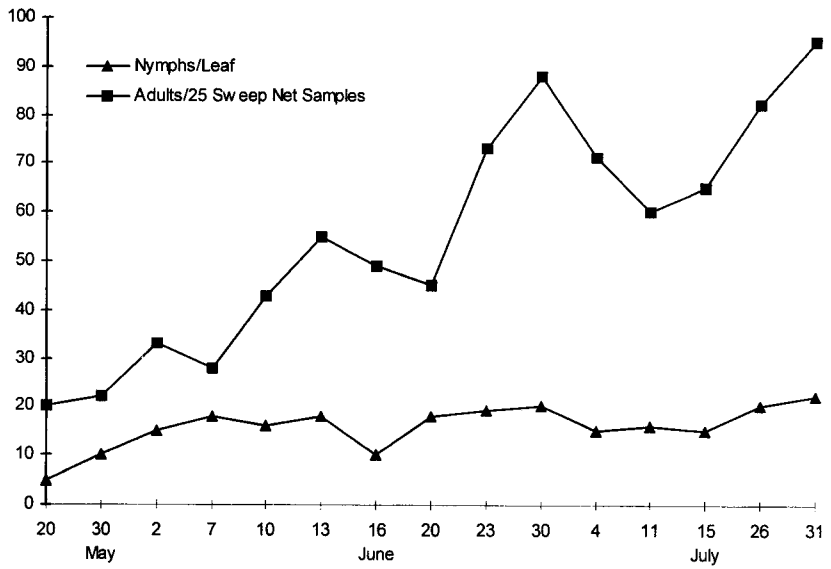

FIG. 1. Population Dynamics of the Variegated Leafhopper in an Untreated Vineyard. Caborca, Sonora, Mexico 1996

The established economic threshold for variegated leafhopper nymphs in this area is an average of 5 to 8 nymphs per leaf for table grapes and 10 to 15 for grapes destined for wine, juice or raisins. There is no economic threshold for adults. The number of nymphs per leaf registered in all the plots before the insecticide applications were above the established economic threshold.

The insecticide application initiated at 2,500 AHU had an impact on the adult and nymph populations, as the infestation on the treated plot was reduced. Statistical analysis of two independent samples indicated a significant difference in both the adult and nymph populations. The treated plot yielded an average of 26.8 adults per 25 sweep net samples, while the untreated control plot resulted in 61 adults per 25 sweep net samples throughout 62 days from the date of application. The nymphal infestations in the treated plot were also affected and indicated a general average of 8.5 nymphs per leaf, while the untreated plot had an average of 16.9 nymphs per leaf throughout 62 days from the date of application. The

infestation on the treated plot was slightly above the economic threshold established for this area for table grapes, but below for grapes destined for wine, juice and raisins. The infestation on the untreated plot was above the economic threshold (Table 1).

TABLE 1. Effectiveness of a Single Insecticide Application on the Infestations of the Variegated Leafhopper at 2,500 Accumulated Heat Units.

Treatments	Adults/25 Sweep Net Samples[a]	Nymphs/Leaf[a]
Untreated Control Plot	61.0*	16.9*
Treated Plot	26.8	8.5

[a]Test of Two Independent Samples (*Significant Differences,P=0.05)

In this case, a single insecticide application reduced the adult population by 56% and the nymphal infestation of the first, second, and part of the third generations by an average of 50% in relation to the untreated control plot during 62 days from the date of application.

The application at 3,000 AHU also reduced the adult population, yielding an average of 31.6 adults per 25 sweep net samples, while the untreated control plot indicated an average of 84.5 adults per 25 sweep net samples. The infestation of nymphs per leaf on the treated plot was 8.1, just slightly above the economic threshold for table grapes, but below that for grapes destined for wine, juice and raisin. The untreated control plot registered 21.2 nymphs per leaf which was considerably above the economic threshold for both table and industrial grapes (Table 2). The statistical analysis indicated a significant difference between the treated and the untreated plot for adults and nymphs. In this case, the adult population was reduced by 63% and the nymphal infestation by an average of 62% for approximately 45 days from date of application.

TABLE 2.Effectiveness of a Single Insecticide Application on the Infestations of the Variegated Leafhopper at 3,000 Accumulated Heat Units.

Treatments	Adults/25 Sweep Net Samples[a]	Nymphs/Leaf[a]
Untreated Control Pot	84.5*	21.1*
Treated Plot	31.6	8.1

[a]Test of Two Independent Samples (*Significant Differences, P=0.05)

At the initiation of the insecticide application at 3,500 AHU on 8 July, the adult population was very high and damage on foliage by the nymphal infestation was quite evident, as was that by the grape leaf skeletonizer, *Harrisina brillians* Barnes and McDunnough. The population of adult leafhoppers in the treated plot continued to increase indicating a general average of 32.3 adults per 25 sweep net samples, while the untreated control plot had a general average of 87.8 adults per 25 sweep net samples. This application reduced the adult population

by 63% but only for a short time. Nymphal infestation also was reduced, registering a general average of 3.6 nymphs per leaf, which is below the economic threshold, while the untreated control plot registered a general average of 15.4 nymphs per leaf, slightly above the economic threshold for table grapes and grapes used for wine, juice or raisin (Table 3). The statistically analysis indicated a significant difference between the adult and nymph infestations in the treated and untreated plot.

TABLE 3.Effectiveness of a Single Insecticide Application on the Infestations of the Variegated Leafhopper at 3,500 Accumulated Heat Units.

Treatments	Adults/25 Sweep Net Samples[a]	Nymph/Leaf[a]
Untreated Control Plot	87.8	15.4
Treated Plot	32.3	3.6

[a]Test of Two Independent Samples (*Significant Differences, P=0.05)

The nymphal and adult infestation was reduced on the treated plot but remained high on the untreated control plot throughout the course of this research. The nymph infestation was reduced by 77% in relation to the untreated control plot for approximately 15 days.

The data show that a single insecticide application at 2,500 AHU, which coincided with the development of the first nymphal generation, affected the population of adults and nymphs since these yeilded a marked reduction in relation to the untreated control plot. The adult female leafhoppers were affected since fewer eggs were deposited and the population of first generation nymphs was maintained at lower levels than those observed in the untreated plot.

The insecticide application at 3,000 AHU, which coincided with the initiation of the second generation of adults, affected this generation only. However, sufficient egg laying had been initiated, since the population of nymphs registered a substantial increase approximately 30 days after the application. The application at 3,500 AHU, which was initiated when the second generation was at its highest abundance, only affected that generation of adults and nymphs for approximately 15 days. It is evident that the economic threshold in general was below that established for table grapes and grapes destined for wine, juice or raisin in this area on the treated plots while it was above that on the untreated control plot.

The use of accumulated heat units can be incorporated in an integrated pest management program for the control of the variegated leafhopper, because a single insecticide application, timed when the populations of adult and nymph leafhoppers are increasing, as indicated by the amount of heat units accumulated, can substantially reduce the incidence of the first and second generations of this pest for approximately 62 days.

In conclusion, it was established that the infestations of variegated leafhopper nymphs, which merit initiation of chemical control occurs at approximately 2,500 AHU. If growers are alerted to apply insecticides at that time, based on the number of accumulated heat units, the population of adult leafhoppers and nymph infestations could be reduced substantially throughout the area, and there would be little need for additional applications.

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TOXICOLOGICAL ASSESSMENT OF *BEAUVERIA BASSIANA* AGAINST MEXICAN BEAN BEETLE (COLEOPTERA:COCCINELLIDAE)[1]

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ABSTRACT

Spore toxicity of ten *Beauveria bassiana* strains, grown on SDA medium at 27± 2^{0}C, and 80% RH, were evaluated against 3-day old larvae of *Epilachna varivestis* (Mulsant). Bioassays, using a dipped bean leaf assay, at ten doses between 1.23 and 1.27x10^7 spores/ml were performed to evaluate toxicological activity. Treatments were incubated at 25± 2^{0}C, 60% RH, and 12:12 (L:D) per 72 h. All strains of *B. bassiana* showed toxicity to *E. varivestis*. A strain isolated from *Diabrotica* sp. showed the highest toxicity with 96.6% mortality, while isolates from *Geraeus senilis, Diabrotica balteata, Hippodamia convergens*, and *Hypothenemus hampei* resulted in mortalities of 93, 90, 90, and 86.6%, respectively; strains isolated from Curculionidae showed mortalities of 86.5 and 80%. The lowest mortality was observed for strains isolated from *Sphenarium* sp. at 76.6%, *Oebalus mexicanus* at 70%, and *Cydia pomonella* at 66%. The isolates from coleopterous insects showed higher toxicity than isolates from other orders. The time required for emergence of fungus from infected insects was 24 hours. *Diabrotica* sp., *G. senilis,* and *D. balteata* strains sporulated from over 90% of infected insects, while the strain from *C. pomonella* sporulated from only 67% of those infected.

INTRODUCTION

In Mexico, the Mexican bean beetle (MBB), *Epilachna varivestis* Mulsant (Coleoptera:Coccinellidae), is a pest that defoliates soybean plants. Adults perforate the leaves while larvae feed mainly from the back side of the leaves without eating the leaf veins (Carrillo et al. 1990). This insect is broadly distributed in Mexico, but occurs mainly in regions of moderate climate such as Mexico State, Puebla, Tlaxcala, Hidalgo, Morelos, Guerrero, Michoacan, Queretaro, Guanajuato, Jalisco, San Luis Potosi, Aguacalientes, Zacatecas, Durango, and Chihuahua, where beans are cultivated (Sifuentes 1981).

Natural control of MBB by the parasitc wasps *Pediobius foveolatus* (García et al. 1993), *Aplomyiopsis epilachnae* (Aldrich) (Tenorio and Carrillo 1987) and *Perilus bioculatus* (Fabricius) (García and Piedra 1994) is sporadic in Mexico. Control of this pest using entomopathogen fungi such as *Beauveria bassiana* (Bals) has not been studied in depth.

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B. bassiana infects almost 700 species of insects, including important agricultural pests such as *Phyllophaga* sp., *Hypothenemus hampei, Geraus senilis, Diabrotica* sp., *Macrodactylus* sp., *Anthonomus grandis*, *Leptinotarsa* sp., *Chalcodermus aeneus,* and *Cerotoma arcuata* (SAGAR 1995). Other susceptible insects to *B. bassiana* are *Ostrinia nubilalis*, *Cydia pomonella*, *Popillia japonica,* and *Pieris brassicae* (Tanada and Kaya 1993).

In Mexico, reports have been published about isolates with pathogenicity for insects of economical importance such as *G. senilis*., *Phyllophaga* sp., *Diabrotica* sp., *D. balteata*., and *H. hampei* (SAGAR 1995). For *E. varivestis*, basic information regarding toxicity of *B. bassiana* is not available. Consecuently, this study was conducted to evaluate the toxicity of ten isolates of *B. bassiana* against *E. varivestis* and to detect the sporulation time of the fungi.

MATERIALS AND METHODS

Ten isolates of *B. bassiana* were obtained from the fungus collection of the National Center of Stock of Biological Control of the Agricultural Secretary in Colima, Mexico (CNRCB-SAGAR). Information regarding identification code, host, and origin of the strains is given in Table 1.

TABLE 1. Isolates of *B. bassiana* from Fungus Collection of the CNRCB-SAGAR

Identification Code	Host	Crop	Origin
BbP1	*Geraeus senilis*	Sorghum	Guanajuato, Mex
BbP7	Curculionidae	-	U.S.A
BbPM	*Cydia pomonella*	Apple	Chihuahua, Mex
BbUAT1	*Sphenarium* sp.	Corn	Tlaxcala, Mex
BbP5	Curculionidae	Corn	Colima, Mex
BbD2	*Diabrotica* sp.	Corn	Jalisco, Mex
Bb5	*Diabrotica balteata*	Corn	Colima, Mex
BbH1	*Hippodamia convergens*	Greenhouse	Guanajuato, Mex
Bb2B	*Hypothenemus hampei*	Coffee tree	Colombia
BbCH	*Oebalus mexicanus*	Sorghum	Guanajuato, Mex

Strains were propagated in culture medium SDA (840), 40 g dextrose, 10 g peptone, 15 g agar, 40 mg tetraciclina, and 0.2% yeast extract in 1,000 ml of sterile water (De la Rosa et al. 1998). Culture medium was poured into slant tubes under sterile conditions. The medium was inoculated with the strains and incubated at 27± 2°C and 80% RH to induce growth and sporulation. After 15 days of incubation, conidia were obtained by scrapping the fungal mass from the surface of the agar and subsequent dissolution in 5ml of sterile water and 0.25 ml of INEX dispersant. Spore concentration for each strain was determined with a Neubauer chamber (Cantwell 1970, Goetel 1984) and then stored at 5°C.

First-instar larvae of *E. varivestis* (3-days old) were obtained from the permanent culture kept at 25± 2°C, 60 ± 5% RH, and 12:12 (L:D) in the Entomology Laboratory of the Interdisciplinary Center for Research and Regional Development-Unit at Durango (CIIDIR-IPN).

Mortality of first-instar larvae of MBB caused by *B. bassiana* strains was assessed by a random experimental design. Each strain was tested against ten larvae and replicated three times. Three ml of each *B. bassiana* strain were applied to 1 cm^2 of bean leaf by immersion in a petri dish containing a suspension of each conidia strain. After the leaves were dried, ten larvae were placed on top of the treated leaves for each isolate; a control sample that contained only sterile water and dispersant was included with each replication. Cages were

incubated at conditions previously indicated. After 72 hours, dead insects were counted for each treatment. Mortality data was analyzed by ANOVA in a Statistics Graphics ver. 5.0 computer program, and means were separated using Duncan Nmn-Kls test. Dead insects were placed in a humidity chamber (RH 90 ± 5%) at 20°C to induce mycosis development. Emergence time and percentage of the fungus were recorded based upon the dead insects for each treatment.

RESULTS AND DISCUSSION

All ten isolates of *B. bassiana* were toxic to first-instar larvae of *E. varivestis* at concentrations between 1.23 and $1.27x10^7$ spores/ml. Average mortality ranged from 66 to 96.6% (Table 2).

TABLE 2. Mortality of First-instar Larvae of *Epilachna varivestis* Exposed to Ten Isolates of *Beauveria bassiana.*

Identification Code		Order of host	Concentration (10^7) Spores/ml	Average % Mortality [a]
BbD2	*Diabrotica* sp.	(Coleoptera)	1.23	96.6 $(2.95)_a$
BbP1	*G. senilis*	(Coleoptera)	1.24	93.3 $(3.10)_{ab}$
Bb5	*D. balteata*	(Coleoptera)	1.26	90.0 $(3.18)_{ab}$
BbH1	*H. convergens*	(Coleoptera)	1.24	90.0 $(3.25)_{ab}$
Bb2B	*H. hampei*	(Coleoptera)	1.25	86.6 $(3.30)_{ab}$
BbP5	Curculionidae	(Coleoptera)	1.26	86.5 $(3.34)_c$
BbP7	Curculionidae	(Coleoptera)	1.27	80.0 $(3.36)_c$
BbUAT1	*Sphenarium* sp.	(Orthoptera)	1.26	76.6 $(3.38)_d$
BbCH	*O. mexicanus*	(Hemiptera)	1.24	70.0 $(3.40)_e$
BbPM	*C. pomonella*	(Lepidoptera)	1.26	66.0 $(3.42)_f$

[a] Average of 3 replications. Standar error is in parentheses . Oneway anova: F ratio 9 and 0.001 probability. Means followed by the same letter are not significantly different Duncan test (P 0.05).

The isolate that resulted in the highest *E. varivestis* mortality was from the *Diabrotica* sp., followed by isolates from *G. senilis*, *D. balteata*, *H. convergens*, *H. hampei*, and Curculionidae, respectively. Isolates from *Sphenarium* sp., *O. mexicanus*, and *C. pomonella* resulted in a lower mortality. These results indicate that the isolates from a coleopterous host were more toxic to MBB than those from other orders. This observation agrees with Roberts (1989) who established that toxicity differences could be explained by biochemical diversity of the fungal strains with respect to their hosts. This diversity is evidenced by chemical and mechanical interactions between the fungal strain and the cuticle of the insect (Hajek and Leger 1994). This association between host and pathogen assumes that fungal isolates from coleopterous insects share biochemical and enzymatic characteristics that make possible infection and mortality in this group more rapidly than those isolates from insects from insects of other orders.

The mycelia of *B. bassiana* emerged from the intersegments of *E. varivestis* within 24 hours of exposure. Roberts (1989) reported similar findings. In this study, the larvae excreted a whitish liquid followed by the emergence of mycelia from the buccal and anal regions after 24 hours. After 48 hours, the mycelia changed to a creamy yellow, and at 72 hours the mycelia changed again to white and completely covered the body of the insect (Alves 1986). The highest percentages of sporulation of *B. bassiana* from the dead insects were observed for isolates from *Diabrotica* sp., *G. senilis*, and *D. balteata*, each with 90%.

Others strains such as those from *H. convergens*, *H. hampei*, and Curculionidae had less sporulation, with 70 and 90%. The lowest percentage (67%) was recorded for the strain from *C. pomonella*. The isolates of *B. bassiana* from coleopterous hosts sporulated faster from MBB larvae than fungal isolates from other orders. This study indicates a relationship between mortality and sporulation and confirms similar findings by other researchers (Hajek and Leger 1994).

In conclusion, all strains of *B. bassiana* showed toxicity to *E. varivestis* at concentrations from 1.23 to $1.27x10^7$ spores/ml in a leaf dip bioassay. The fungal isolates from *Diabrotica* sp., *G. senilis*, and *D. balteata* gave the highest percentages of mortality and sporulation on *E. varivestis*. This study provides information that will allow us to choose isolates with greater toxicity against MBB for later studies to determine production characteristics of the fungus.

ACKNOWLEDGMENT

This research is part of a grant entitled "Bioinsecticides against Bean's Pests" that is carried out under the auspices of the Regional Investigation System "Francisco Villa" dependent of the National Council of Science and Technology (SIVILLA-CONACYT). I am grateful to Robert W. Behle for the critical review of the manuscript.

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BEAN SEED MAGGOT, *DELIA FLORILEGA* (ANTHOMYIIDAE, DIPTERA): A POTENTIAL NEW PEST ON COLE CROPS IN THE LOWER RIO GRANDE VALLEY OF TEXAS

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In October 1997, over 20 acres of Chinese cabbage (also called Napa cabbage), *Brassica sinensis* L., near Edinburg in Hidalgo County in the Lower Rio Grande Valley (LRGV) of Texas, was infested by larvae of a dipterous fly. The larvae were found within the cabbage heads with little or no external damage. We found six larvae in a single head. The infested cabbages were shredded before harvest because they were not marketable.

Larvae and pupae were collected from damaged plants prior to shredding, and adults were reared out in about 7 d. Specimens were sent to E. G. Riley at the Insect Collection, Department of Entomology, Texas A&M University at College Station for identification. The fly was identified as *Delia florilega* (Zetterstedt) (Diptera: Anthomyiidae), formerly known as *Hylemya florilega* Zetterstedt. In literature, this species has been misidentified as *D. floralis* (Fallén) and *D. trichodactyla* (Rondani) (Brooks 1951).

Incidently, one of the authors (T.-X.L) found some similar larvae and damage by the larvae from heads of Chinese cabbage purchased from two local grocery stores in Weslaco, TX. The identity of the larvae was confirmed by comparison with the larvae collected from the cabbage field. After communication with the managers of the two local stores, it was determined that the Chinese cabbage shipment came from the same source in Salinas, CA. Although no confirmation exists of this pest in California, its introduction into Texas may have occurred by way of the local grocery stores.

The morphological characters and life cycles of *D. florilega* and the seedcorn maggot, *D. platura* (Meigen), can be easily confused because they are very similar in life stages and host range. Adults can be distinguished only by using adult male characters (Huckett 1924, Miles 1948, Brooks 1951, Eckenrode and Kim 1988).

The adult of *D. florilega* is a small fly. The body is grey with dark legs. Adults of *D. florilega* can be separated using the following morphological characteristics: mesotibia and metatibia in females (Kim and Eckenrode 1984), and length of the bristles on mid metatarsus in males (Brooks 1951). In female *D. florilega*, mesotibia has 4 or fewer bristles (1 on anterodorsal, 1 on posterodorsal and 2 on posteroventral surfaces [Fig. 1A]), metatibia lacks preapical setula, and a rather indistinct middorsal stripe is present on the abdomen (Ageyeva 1968); however, the color patterns may change with age. In adult female *D. platura*, mesotibia has 5 or more bristles (1-2 on anterodorsal, 2 on posterodorsal, and 2 on posteroventral surfaces [Fig. 1B]), and each metatibia has preapical setula. Kim and Eckenrode (1984) reported that the accuracy for the number of bristles on mesotibia was 90.2%, while the accuracy for the single preapical setula on the metatibia was 79.3% (Brooks

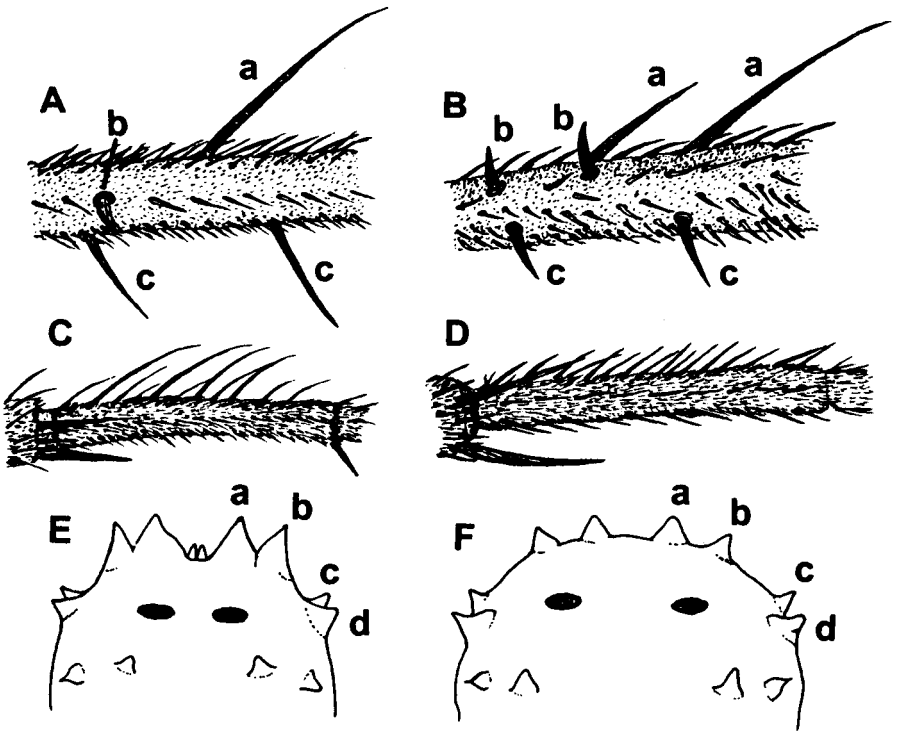

Fig. 1. Major morphological characters used for identification of *D. florilega*, with comparison to *D. platura*. Notes: We consulted the photos of Kim and Eckenrode (1983, 1984) for A-D; and J. C. Thompson (In F. Stehr. 1991. Immature Insects, vol. 2, p. 856) for E & F.

A. Mesotibia of *D. florilega* female, a. 1 anterodorsal bristle, b. 1 posterodorsal bristle, and c. 2 posteroventral bristles. **B.** Mesotibia of *D. platura* female, a. 2 anterodorsal bristles, b. 2 posterodorsal bristles, and c. 2 posteroventral bristles. **C.** Mid metatarsus of *D. florilega*, showing dorsal bristles at least twice as long as tarsal width. **D.** Mid metatarsus of *D. platura*, showing dorsal bristles less than tarsal width. **E.** Posterior end of *D. florilega* larva, showing tubercles a and b long, contiguous basally with a broader than b. **F.** Posterior end of *D. platura* larva, showing tubercles a and b short, separate basally, and subequal in breadth.

1951). In male *D. florilega*, the dorsally directed bristles on the middle part of metatarsus are at least twice as long as the tarsal width (Fig. 1C), whereas in male *D. platura*, the bristles are less than the tarsal width (Fig. 1D).

Maggots (larvae) of *D. florilega* are white to yellowish white, 5-8 mm long. The body is blunt or obliquely truncated at the posterior end, and pointed at the anterior end. It has a pair of black mouth hooks that curve downward for rasping. The posterior end has 6 posterior tubercles on each side, tubercles A and B, long and contiguous basally with A distinctly broader than B (Fig. 1E). This is the major character which separates this species from other similar species, *D. platura* (Fig. 1F). Eggs are white and tiny, 1-2 mm long. Pupae are red-brown, about 5 mm long.

The biology of *D. florilega* has been extensively studied by Eckenrode and his former students in Cornell University Agricultural Experiment Station at Geneva, NY (Eckenrode and Chapman 1971; Kim and Eckenrode 1983, 1984, 1987; Throne and Eckenrode 1986; Eckenrode and Kim 1988). *D. florilega* is extremely polyphagous, feeding on seedling corn, roots of cruciferous plants, including turnip, Brussels sprouts, and cabbage that have already been damaged by other Diptera larvae. They may also attack the cotyledons and shoots of beans, peas, and other vegetables (Brooks 1951, Miles 1950). It is mainly distributed in the holarctic region, and is possibly of Eurasian origin. In North America, it has been found from New Mexico in the southwest to New York in the east, and from Alaska to eastern Canada.

In the LRGV, the larvae bore inside the head of Chinese cabbage, and feed on leaves. This is the first record for this species feeding in the head of Chinese cabbage. Plants with multiple larvae and damage are not marketable; therefore, the infestation and damage in a large area could be serious.

The economic importance of this pest in Texas is currently unknown, and certainly needs to be determined. General recommendations for managing seed maggots include manipulating planting date; avoiding over fertilization with manure because maggots prefer moist, organically rich soil in which to lay their eggs; disk or rototill weeds at least 2 weeks before planting; and plowing under crop debris immediately after harvest. These management suggestions are targeted at preventing damage to sprouting seeds, seedlings, and roots. If damage to cabbage heads becomes a persistent problem, additional management strategies will be needed.

Voucher specimen are deposited in the Insect Collection, Texas Agricultural Research and Extension Center at Weslaco, and the Insect Collection (#980028), Department of Entomology, Texas A&M University, College Station.

We greatly appreciate E. G. Riley (Insect Collection, Department of Entomology, Texas A&M University, College Station) for identification of the insect, and to C. J. Eckenrode (New York Agricultural Experiment Station, Cornell University, Geneva) for providing references. We also would like to thank C. J. Eckenrode, T. H. Kim (Department of Agricultural Biology, Chonbuk National University, Chonju, Korea), and J. Jackman (Texas A&M University at College Station) for reviewing the manuscript. This paper was approved for publication by the Center Director of Texas Agricultural Research and Extension Center at Weslaco, and the Head of the Department of Entomology, Texas A&M University, College Station.

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USING INFRARED TRANSDUCERS TO SENSE GREENBUG (HOMOPTERA: APHIDIDAE) INFESTATIONS IN WINTER WHEAT

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ABSTRACT

Winter wheat was grown under four regimes (normally-watered, and not infested, normally-watered and infested, water-stressed but not infested, and infested and water-stressed) to determine if infrared transducers (IRTs) could be used sense greenbug infestations and distinguish them from water stress. The IRTs were able to sense temperature differences among plants of the different treatments, and when the IRT data were converted to differences from ambient temperatures, significant differences were found among the treatments. When sampled at 0900 hours, plants undergoing water stress and infested with greenbugs were always significantly warmer ($p = 0.05$) than normally-watered, noninfested plants. Temperatures of plants either water stressed or infested by greenbugs were between these extremes and were not statistically distinguishable from each other. However, on several occasions, they could be distinguished from nonstressed plants and those stressed by both factors. Analysis of greenbug abundance and temperature differences between normally-watered and water-stressed plants resulted in two significantly-different regressions that were nearly parallel, indicating that, within natural parameters, there may be a constant ratio between damage by greenbug and water stress in wheat. In conclusion, IRTs were found not to be a tool that can be used alone to distinguish greenbug infestations in winter wheat. Unless certain water stress conditions are met (absence or uniform), there is no practical way to differentiate greenbug infestations from water stress in winter wheat.

INTRODUCTION

As new and improved remote-sensing equipment and mapping systems are developed and used in production agriculture, site-specific management practices will become paramount (Schepers and Francis 1998). The ability to accurately sense and map infestations of pests (arthropods, plant pathogens and weeds) has been used in many management systems in the past. Zwiggelaar (1998) reviewed the current state of crop/weed discrimination technology, and Nilsson (1995) reviewed the use of remote sensing for crop disease detection and identification. Use of remote sensing to detect damage by insects on crops has not been studied adequately (Moran et al. 1997); however, several important studies have used indirect methods to determine amounts of damage caused by insects. These studies ranged from determining defoliation of hemlock forests by the hemlock woolly adelgid, *Adelges tsugae* Annand (Royal and Lathrop 1997) to using remote sensing to map vegetation to determine suitable habitats for tsetse, *Glossina* sp., in

Southern Africa (Robinson et al. 1997). Riley (1989) reviewed remote sensing uses in entomology, but the review concentrated primarily on optical and radar-related methods.

One method of remotely sensing biological stresses in plants is through the use of precision infrared transducers (IRTs). IRTs are used to detect differences in surface temperatures of plants rather than reflectance or energy units (Hatfield and Pinter 1993). IRTs are typically are calibrated to operate in the 8-14 or 10.5-12.5 μm bandwidths to eliminate problems with reflected solar radiation and signal attenuation caused by water vapor. This distinction lessens problems with distance between the IRT and the surface temperature being measured. In the past, IRTs have been used to determine frost and optimal time of planting in crops (Stewart et al. 1978), water potentials of plants (Erhler et al. 1978), predicting grain yields in wheat (Idso et al. 1977), and irrigation scheduling (Garden et al. 1981). Hatfield and Pinter (1993) stated that using IRTs detect biological stress is promising as long as the stress alters water flow in the soil-plant-atmosphere continuum and there is enough energy in the atmosphere to evaporate water from the leaves.

Damage by greenbugs, *Schizaphis graminum* (Rondani), to wheat, *Triticum aestivum* L., manifests itself in early stages as a chlorosis of the wheat tissue. In wheat fields, damage by greenbug is noticeable as yellowed plants in circular patches that expand over time if the pest is not controlled (Boring and Patrick 1990). Because the greenbug feeds in the phloem and directly affects water flow in a plant, its presence and amount of damage to wheat could theoretically be detected by IRTs. If IRTs can reliably sense damage caused by greenbugs, it may be possible to remotely determine greenbug infestations in the field through a combination of the damage they cause and the circular patterns they produce. This remote sensing could be accomplished through analyses of data collected by IRTs mounted on center-pivot irrigation systems, through an aerial "fly-over" with IRT-equipped aircraft, or possibly tethered balloons fitted with IRTs.

The ability to find greenbug infestations in the field could allow spot treatments of insecticides rather than whole-field applications of insecticides. These spot treatments could be made by using variable rate spray systems mounted on center-pivot irrigation systems, ground rigs, or possibly aircraft. Spot treating has the benefits of reducing the cost and amount of pesticides applied. In addition, this technique would allow biological control agents (ladybeetles, lacewings and parasitic wasps) to work in concert with chemical applications because the whole field would not be sprayed with chemicals. Therefore, natural enemies at the outer edge of a patch of greenbugs could control greenbugs that remain after a precision application of insecticides.

We developed three hypotheses regarding to the use of IRTs for remote sensing of greenbugs in winter wheat: 1) because greenbugs interfere with wheat's vascular system, there would be a temperature differential between infested and noninfested plants; 2) this differential would become more evident over time as the greenbug infestation grew and the infested wheat was increasingly damaged; and 3) the combination of yellowed wheat and a geometric pattern (i. e., greenbug "circles") could permit greenbug infestations to be distinguished from other wheat stress components that cause similar temperature differentials from nondamaged wheat. This experiment tested the first two of these three hypotheses.

In 1997 the Texas State Legislature approved an initiative to research and develop precision agriculture in the Texas High Plains. As part of this initiative, an experiment was conducted in the greenhouse to determine if greenbug infestations in winter wheat could be remotely sensed with IRTs. In addition, winter wheat often undergoes periods of water stress during the growing season when greenbugs are present. Because water stress may result in temperature differences that are similar to those caused by damage by greenbugs, and greenbugs tend to increase more rapidly in water-stressed wheat (Boring and Patrick 1990), we included water stress as a treatment in the experiment.

MATERIALS AND METHODS

TAM 105 winter wheat was grown in a peat-perlite soil medium in 16, 0.62 x 0.62 m wooden flats. Five days before infestation, all flats were sprayed with chlorpyrifos (Lorsban® 4E) at a rate of 68 g AI/ha to remove any insects or mites. When the wheat plants were ≈15 cm tall, eight flats were infested with biotype E greenbugs from a stock laboratory colony. Approximately 100 greenbugs (mixed adults and nymphs) were placed in the center of each flat. Wheat in four of the greenbug-infested flats was watered normally (3.6liters/flat/watering) at ≈7 d intervals. Plants in the remaining four flats of greenbug-infested wheat were moderately water-stressed, receiving 50% of the water given to the normally-watered plants. The same water regimes were imposed on the control of eight flats of noninfested wheat, and four flats receiving normal amounts of water and four receiving only half as much water. Water was applied shortly after greenbugs on the plants were sampled.

The flats were arranged in an alternating design (three columns and six rows) on benches in the greenhouse so that each treatment occurred at least once in each column and row. This arrangement was chosen to minimize effects of shading from greenhouse structural components. All noninfested wheat was sprayed weekly with chlorpyrifos at a rate of 68 g AI/ha to prevent greenbug infestations. There were four replications of each treatment.

A trolley system (Burk et al. 1999) was constructed in the greenhouse to carry four precision IRTs (Apogee Instruments, Logan, UT), four type-T copper-constantan thermocouples, and a Campbell Scientific 21X(L) data recorder (Logan, UT). The IRTs and thermocouples were interfaced to the 21X(L) with a Campbell Scientific AM25T multiplexer. The IRTs were placed to record the canopy temperature of the wheat, while the thermocouples were placed next to their respective IRT to record ambient air temperature at each sampling site. The trolley was programmed to continuously monitor the wheat flats, taking a reading from the center of the flats approximately once each hour. At the start of a cycle, the 21X(L) read the IRT and thermocouple values recorded from the flats in row one, 10 times at 10-second intervals. These values were averaged and stored in the 21X(L)'s onboard memory. The 21X(L) then moved the system to the next row of flats, paused for 60 seconds to allow any movement in the system to stop, and began a new set of readings as in row one. This recording continued until the system reached row six. After the readings were taken in row six, the system moved back to row one and began again. At the end of each 24-hr period, the data stored in the 21X(L) were automatically downloaded to a Campbell Scientific SM192 data storage module. Data stored in the SM192's were transferred at weekly intervals to a computer.

The experiment was conducted for 63 d beginning on day of the year (DOY) 256. The wheat was sampled on DOY 256 as a baseline before greenbug and water stress treatments were applied. Greenbug abundance and plant water parameters were sampled four other times (on DOY 270, 277, 286 and 291). By DOY 291, greenbugs were causing noticeable damage to the infested wheat. Greenbugs then were removed by using chlorpyrifos at the above-stated rate, and plants of the water-stressed treatments were given a normal amount of water to determine if the wheat returned to a status equivalent to the nontreated control when the stresses were removed. The final sampling occurred on DOY 319 when the final water parameter measurements were taken. To reduce variations in leaf water parameters at different times of the day, sampling was done as close to 0900 hours as possible, and usually concluded by 1100. At each sampling date, data collected were greenbug abundance on plants in infested flats and leaf water and osmotic potential of plants in all flats.

Greenbug abundance data were collected by randomly selecting five plants per treatment per replication and counting all greenbugs on each plant, for a total of 20 plants per treatment. At that time, one tiller from each plant used for assessing greenbug abundance was tagged for use in measuring leaf water parameters.

Leaf water potential (ψ_w) and osmotic potential (ψ_π) were measured using model 76-1A leaf cutter thermocouple psychrometers (J. R. D. Merril Specialty Equipment, Logan, UT) read by a Campbell Scientific CR7 data logger. A 5-mm diameter disk was excised from the midpoint along the length of the leaf blade and placed in a thermocouple psychrometer. Leaf water potential, in mV, was read after equilibrium in a water bath at 25°C for 4 hr (Morgan 1980). The psychrometers with sample were frozen in liquid nitrogen for 10 min and thawed; ψ_π in mV was measured after temperature equilibrium in a water bath for 1 hr at 25°C (Morgan 1980). Before sampling date, psychrometer was calibrated using a series of standard NaCl solutions (0, 0.1, 0.2, 0.5, and 1.0 Mol/L), and a linear relationship was calculated between solution potential and psychrometer output voltage (Morgan 1980, Brown and Osterhuis 1992). After calibration, voltage output from the excised samples was converted into ψ_w in bars.

Statistical analyses of greenbug abundance leaf water parameters, and temperature data consisted of analysis of variance (ANOVA) for a completely randomized design with sub-sampling (SAS 1996). Greenbug abundance and leaf water parameters were analyzed among treatments within a sampling time and across sampling times within a treatment. Temperature data recorded by the IRTs was modified by subtracting the ambient temperature recorded by the thermocouples to yield the temperature difference between the wheat canopy and greenhouse. These data were subjected to the same ANOVA techniques as the greenbug abundance and leaf water parameter data. Significant means were separated using the Student-Newman-Keuls test (p= 0.05).

Data for differences in temperature from ambient, ψ_π, and ψ_w between infested normally-watered and infested water-stressed treatments were regressed on greenbug abundance per plant as single-variable regressions for each treatment (SAS 1996). For the temperature data, a linear regression ($y = \beta_0+\beta_1 x$) provided the best fit, while for ψ_π and ψ_w the best fit was found with a second-order polynomial ($y = \beta_0+\beta_1 x+\beta_2 x^2$). To test if the regressions for the two treatments were significantly different from each other, the combined data for each parameter were analyzed by regression by using an indicator variable as a slope shift coefficient to test for a significant difference between two given regressions (Freund and Littell 1991).

RESULTS

Leaf water potentials, ψ_w and ψ_π, increased with time until the last date (Table 1). This was expected because the plants were growing but confined to the same physical space and amount of water throughout the experiment. As the wheat plants grew, more root mass per plant was competing for the same amount of water; therefore, the plants were still considered to be normally-watered. In most cases, ψ_w and ψ_π were significantly higher than when sampled previously. With the exception of the first and last days, all treatments were significantly different from each other on a given sampling date. In addition, all treatments fell in the same order, with the least stress in noninfested normally-watered plants, followed by infested normally-watered, noninfested water-stressed, and infested water-stressed, respectively. On the first and last days sampled, there were no significant differences among treatments. This was expected because at the first sampling time plants had not yet been water stressed or infested with aphids, and at the last sampling date aphids had been chemically removed and all plants had been thoroughly watered at the same rate.

TABLE 1. Plant Water Parameters, Greenbug Abundance, Temperature Differentials, and Average Ambient Air Temperatures in an Experiment to Remotely Sense the Effects of Greenbug Infestations and Water Stress in Winter Wheat.

Treatment [1]	Day of year					
	256	270	277	286	291	319
			Leaf water potential (Bars), 0900 hours			
NN	-0.3 A a[2]	-0.3 A a	-1.4 B a	-2.1 C a	-3.0 D a	-0.3 A a
IN	-0.4 A a	-0.7 B b	-2.5 C b	-3.4 D b	-3.9 E b	-0.4 A a
NS	-0.4 A a	-2.7 A c	-3.6 B c	-4.6 C c	-5.4 D c	-0.4 A a
IS	-0.3 A a	-3.5 B d	-4.8 C d	-5.5 D d	-6.5 E d	-0.4 A a
			Leaf osmotic potential (Bars), 0900 hours			
NN	-1.2 A a	-1.2 A a	-2.5 B a	-3.2 C a	-4.3 D a	-1.3 A a
IN	-1.2 A a	-2.6 B b	-4.5 C b	-5.2 D b	-6.2 E b	-1.4 A a
NS	-1.3 A a	-5.1 B c	-6.4 C c	-6.7 C c	-7.5 D c	-1.3 A a
IS	-1.3 A a	-6.1 B d	-7.4 C d	-8.6 C d	-9.4 D d	-1.4 A a
			Greenbugs per plant			
IS	0.0 na	16.0 A b	27.3 A a	62.9 B b	95.9 C b	0.0 na
IN	0.0 na	6.5 A a	17.3 B a	36.3 C a	69.2 D a	0.0 na
			^{0}C difference from ambient, 0100 hours			
IS	-1.9 A a	-2.1 A a	-1.9 A a	-2.4 A a	-2.0 A a	
IN	-1.3 A a	-2.5 A a	-2.3 A a	-3.0 B a	-2.6 A a	
NS	-1.3 A a	-2.5 A a	-2.5 A a	-3.4 B a	-2.3 A a	
NN	-2.2 A a	-3.0 A a	-2.6 A a	-3.3 B a	-2.4 A a	
Ambient	21.1	24.4	21.7	18.8	16.8	
			^{0}C difference from ambient, 0900 hours			
IS	0.2 C a	1.7 B a	2.0 B a	2.7 B a	3.7 A a	
IN	-1.1 C a	-0.6 C b	0.8 B ab	0.2 B b	1.6 A b	
NS	-0.2 A a	-0.7 B b	-0.9 B bc	-0.9 B b	1.6 A b	
NN	-1.4 A a	-2.9 B c	-2.0 B c	-2.9 B c	-1.1 A c	
Ambient	22.6	22.2	20.8	22.8	21.7	
			^{0}C difference from ambient, 1500 hours			
IS	-7.2 B a	-6.0 A a	-5.9 A a	-5.8 A a	-7.5 B a	
IN	-7.9 B a	-8.0 B b	-7.3 A ab	-7.2 A ab	-8.6 B a	
NS	-7.0 A a	-7.9 B b	-8.4 B b	-8.2 B b	-8.9 B a	
NN	-8.2 A a	-11.4 B c	-11.4 B c	-11.2 B ca	-10.9 B b	
Ambient	32.1	32.1	26.3	29.5	30.2	

[1]NN-noninfested, normally-watered; NS-noninfested, water-stressed; IN-infested, normally-watered; IS-infested, water-stressed. Ambient is the average air temperature at the specified time and day.

[2]Means followed by the same upper-case letter in a row or lower-case letter in a column and subheading are not significantly different, Student-Newman-Keuls mean separation test, $p = 0.05$.

Temperature data for the last sampling date was lost because of a problem in the SM192 data storage module.

Aphid abundance increased over time on the normally-watered and water-stressed plants infested with greenbugs (Table 1). On normally-watered plants, greenbug abundance was significantly higher at each subsequent sampling time when greenbugs were present. Greenbug abundance on water-stressed plants increased and differed significantly between all sampling dates but DOY 270 and 277. At all but DOY 277, there were significantly more greenbugs per water-stressed plant than per normally-watered plant.

The automated trolley sensor system sampled continuously and stored sensor readings hourly for each flat in the experiment. However, we report results from only three selected times on the dates when we sampled leaf water parameters and aphid abundance. We report temperature results from 0100, 0900, and 1500 hours. We assumed data collected at 0100 hours would be useful if plants recovered from water and insect stress during the night. We selected 0900 hours to coincide with our other data collection efforts keyed toward a time when the plants would not be subjected to the highest temperatures of a given day, but would be actively transpiring. Finally, 1500 hours was selected as a time when the plants were subjected to the maximum temperature for a given day, and the ability to compensate for water and insect stress would be most severely tested.

Because plants were not infested or water-stressed yet, results for DOY 256 were omitted from the following discussion on temperature differences among treatments and sampling dates. At 0100 hours there were no significant differences among treatments at any sampling date, indicating the plants reached equilibrium by this time of day. On DOY 286 all treatments, except the infested water-stressed plants, were significantly cooler than the preceding or subsequent dates. The reason for this difference is unclear. There is a possibility, since the temperature difference is uniformly lower than on previous or subsequent dates, that the difference could have been caused by changes in the weather on that date, especially if relative humidity increased as a front passed Bushland, TX.

Within sampling dates, at 0900 hours (Table 1, Fig. 1) noninfested normally-watered plants were significantly cooler than all other treatments except noninfested water-stressed plants on DOY 277. There were no significant differences on any sampling date between noninfested water-stressed and infested normally-watered plants. On all but DOY 277, infested water-stressed plants were significantly warmer than infested normally-watered plants. Across sampling dates at 0900 hours, there was an interesting trend. The noninfested plants generally became cooler than ambient temperatures, and infested plants were warmer than ambient temperatures. The effect was more pronounced in infested than noninfested plants. On the last sampling date (DOY 291), all treatments were significantly warmer than on the previous date.

Similar results were found at 1500 hours. Within sampling dates, noninfested normally-watered plants were significantly cooler than plants of the other three treatments. On all but the last sampling date (DOY 291) infested water-stressed plants were warmer than plants of any other treatment. There were no significant differences at any sampling date between infested normally-watered plants and noninfested water-stressed plants.In the regression analyses, temperature difference was strongly related to greenbug abundance on both normally-watered and water-stressed plants (Table 2, Fig. 2). The regressions were significant in both cases (Prob. > F = 0.0524 and 0.0155, respectively, for normally-watered and water-stressed plants), and the estimate of the slope shift coefficient for the combined data also was significant (Prob. > $|t|$ = 0.004). This significant slope shift coefficient indicated that water-stressed plants were significantly "warmer" than normally-watered plants across greenbug abundance. The light gray line in Fig. 2 indicated the temperature difference between normally-watered and water-stressed plants. This value, gradually decreased from 1.50 or 1.25 °C over the time of the experiment.

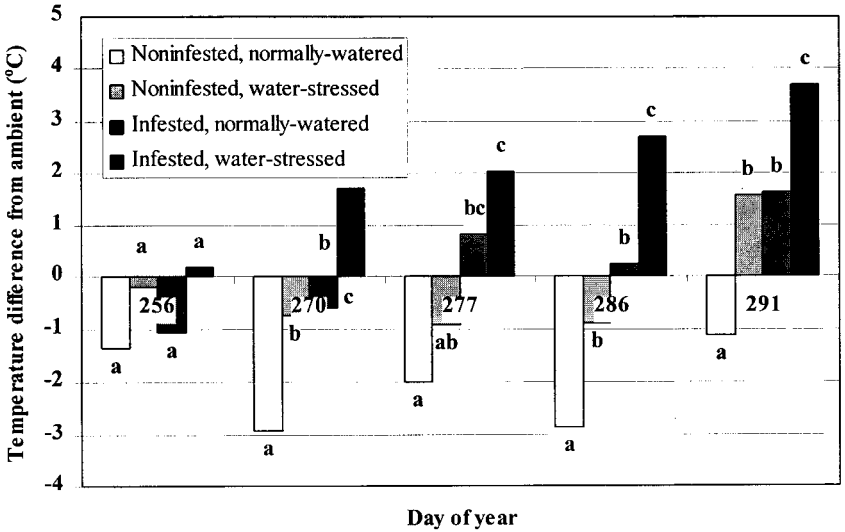

FIG. 1. Wheat canopy temperature difference (°C) from ambient at 0900 hours for four water and greenbug treatments on five sampling dates. Bars within a day of the year with the same letters are not significantly different, Student-Newman-Keuls test, p = 0.05.

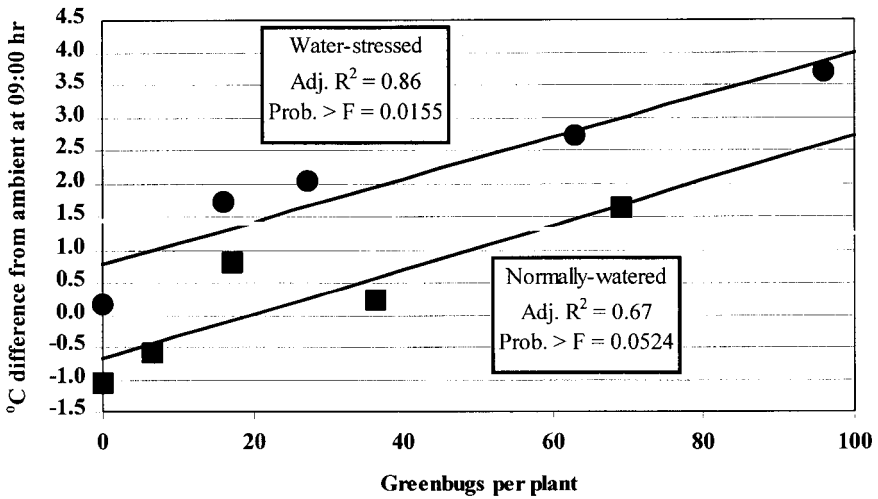

FIG. 2. Wheat canopy temperature (°C) difference from ambient vs. greenbug abundance on normally-watered and water-stressed plants. Gray line indicates the difference between regression line values.

TABLE 2. Regression Parameters Associated with Greenbug Abundance, Plant Temperatures, and Plant Water Parameters in an Experiment to Remotely Sense the Effect of Greenbugs And Water Stress on Winter Wheat.

Coefficient	Estimate	Std. error	Prob. > |t|	Adj. R^2	Coefficient	Estimate	Std. error	Prob. > |t|	Adj. R^2	Coefficient	Estimate	Std. error	Prob. > |t|	Adj. R^2
Plant temperature (°C) = $\beta_0 + \beta_1$ * Greenbug abundance														
Infested, normally watered				0.686	Infested, water-stressed				0.857	Combined				0.884
β_0	-0.671	0.388	0.182		β_0	0.782	0.339	0.105		β_0	-0.640	0.267	0.048	
β_1	0.034	0.011	0.052		β_1	0.032	0.006	0.016		β_1	0.033	0.005	0.001	
										Shift[1]	1.397	0.332	0.004	
Leaf water potential (MPa) = $\beta_0 + \beta_1$ * Greenbug abundance + β_2 * Greenbug abundance^2														
Infested, normally watered				0.953	Infested, water-stressed				0.858	Combined				0.882
β_0	-0.260	0.274	0.443		β_0	-0.850	0.795	0.397		β_0	-0.055	0.482	0.912	
β_1	-0.130	0.023	0.030		β_1	-0.149	0.045	0.082		β_1	-0.119	0.027	0.004	
β_2	0.001	0.000	0.071		β_2	0.001	0.000	0.164		β_2	0.001	0.000	0.040	
										Shift[1]	-1.362	0.489	0.032	
Leaf osmotic potential (MPa) = $\beta_0 + \beta_1$ * Greenbug abundance + β_2 * Greenbug abundance^2														
Infested, normally watered				0.919	Infested, water-stressed				0.842	Combined				0.854
β_0	-1.524	0.460	0.080		β_0	-2.093	1.125	0.204		β_0	-1.118	0.709	0.166	
β_1	-0.167	0.039	0.050		β_1	-0.214	0.064	0.080		β_1	-0.164	0.039	0.006	
β_2	0.001	0.001	0.110		β_2	-0.001	0.001	0.146		β_2	0.001	0.000	0.042	
										Shift[1]	-1.890	0.720	0.039	

[1] Shift is an indicator variable used to determine a slope shift significance between two regressions (Freund and Littell 1991).

Results for the regression analyses for ψ_w and ψ_π (Table 2) were almost identical to each other and closely followed the results found by regression of temperature difference. For normally-watered plants, regressions for ψ_w and ψ_π were significant (Prob. > F = 0.0235 and 0.0406, respectively, for ψ_w and ψ_π), and for water-stressed plants regressions were not significant (Prob. > F = 0.0709 and 0.0790, respectively, for ψ_w and ψ_π). However, when slope shift coefficients were calculated, both ψ_w and ψ_π had significant estimates of the coefficient ((Prob. > $|t|$ = 0.032 and 0.039, respectively, for ψ_w and ψ_π). This result indicated, as did the temperature difference, that water stress in terms of ψ_w and ψ_π in normally-watered plants was significantly lower than in water-stressed plants across greenbug abundance.

DISCUSSION

The temperature differences between normally-watered infested and noninfested plants in this experiment were similar to those reported by other researchers (Pinter et al. 1979, Fuchs 1990, Hatfield 1990.). As an example, Pinter et al. (1979) noted that leaves of sugar beets (*Beta vulgaris* L.) infested with *Pythium aphanidermatum* (Edson) Fitzp. were 2.6-3.6° C warmer than healthy plants.

Results of this experiment support the hypotheses that there is a significant, distinguishable temperature difference between noninfested and greenbug-infested wheat and that the difference increases with abundance of greenbug. Once the greenbug infestation was initiated, noninfested, normally-watered plants always were significantly cooler than infested, normally-watered plants, and infested, normally-watered plants became increasingly warmer than the ambient air temperature as the experiment continued and greenbug increased in abundance (Table 1, Fig. 1). However, except for the absence of greenbugs, the same can be said for the differences between noninfested, normally-watered plants and noninfested, water-stressed plants. Therefore, IRTs alone cannot be used easily to distinguish water stress and greenbug infestation in wheat because the thermal response of the plant is similar for both stress factors. This would agree with Boring and Patrick (1990) who found that visual greenbug damage can be confused with water stress in a field.

Intuitively, it would seem that greenbug infestation and drought are different kinds of stress that should manifest themselves in different ways. Water stress, particularly at the levels in this study, is not a continuous or necessarily continually-increasing stress factor. Wheat can recover overnight from light to moderate water stress, and continued water deprivation causes plants to reduce shoot growth and increase root growth (Klepper et al. 1973). Greenbug-induced stress, however, is continuous and continually increasing because there is little a plant can do to ameliorate this stress. Burton (1986) found that in as little as two weeks after infestation, as few as 20 greenbugs per plant significantly reduced both root and shoot growth in winter wheat. Six weeks after infestation, 10 greenbugs per plant significantly reduced both root and shoot growth. Burton et al. (1985) found that greenbug damage to winter wheat in the fall significantly affected wheat yield in the spring even though the greenbugs were chemically removed in late fall. Results of this experiment are similar to those from previous research. Plants affected by both greenbugs and water stress showed an additive, possibly synergistic effect, often being very much warmer than any other treatment at all sampling dates after infestation and becoming progressively warmer as the experiment continued (Fig. 1). This may indicate that greenbug damage accelerates the thermal response of the plant under water stress because greenbug damage could prevent the plant from recovering from water stress during the night-time hours. Dorschner et al. (1986) and Holmes et al. (1991) agreed that greenbug feeding on wheat caused cell membrane injury that resulted in solute leakage and reduced turgor. This damage interrupts membrane transport of photosynthates and disrupts the

mediation of solute allocation patterns within the plant during drought stress. Although this may be the basis for the results this experiment, there is no indication that the combined stresses can be partitioned out by comparison to plants only water stressed or infested by greenbug infestation. Infested, normally-watered plants were warmer than noninfested water-stressed plants, however this difference was not significant at any sampling date.

Surprisingly, temperature differences between infested normally-watered and infested water-stressed plants remained relatively constant (beginning at 1.50 and decreasing to 1.25° C) across the experiment as greenbug increased in abundance (Fig. 2). The two lines represented by these regressions also were significantly different from each other (Table 2), and would not intersect except at temperature and greenbug levels far outside reasonable limits. These results indicate there may be a statistically robust interaction between water stress and greenbug abundance in winter wheat. Although of interest, and in need of additional research, this would not be distinguishable in the field unless it was known that the wheat was either not under water stress or that the water stress was uniform across the field.

In conclusion, the use of IRTs to remotely sense greenbugs in a field may be possible if certain conditions are met. First, plants in the field must not be water stressed, the level of water stress must be constant across the field, or portions of the field under water stress must be known. Secondly, the field should be sampled shortly after sunrise to mid-morning when plants are actively transpiring, but not during the heat of the day. Water stress will be a confounding factor typically indistinguishable from greenbug infestations when using temperature differences as the stress measurement. To use IRTs as a remote sensing tool for greenbug infestations, additional sensors, such as multi-spectral radiometers, probably will be needed. IRTs by themselves measure too little of the electromagnetic spectrum to make them useful for more than a confirmation that a crop is undergoing stress of some kind. Finally, the results of the experiment say little about the combination of the temperature differences among the treatments and circular patterns caused by greenbug infestations. It is possible that the results reported here, coupled with imagery equipment to detect geometric patterns in a field, will allow reliable determination of greenbug infestations.

ACKNOWLEDGMENT

This research was funded, in part, by the 1997 Texas High Plains Precision Agriculture Legislative Initiative. The authors thank J. B. Bible, J. K. Burk and M. L. Fahnert for their technical assistance.

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PINK BOLLWORM (LEPIDOPTERA: GELECHIIDAE): DIAPAUSE LARVAL EXIT FROM COTTON BOLLS, LARVAL AND PUPAL DEVELOPMENT AND MORTALITY, AND SPRING MOTH EMERGENCE IN THE INSECTARY AND IN THE FIELD

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ABSTRACT

The number of days that pink bollworm (PBW), *Pectinophora gossypiella* (Saunders), diapause larvae remained in harvested immature cotton bolls, larval and pupal mortality after exiting bolls, and spring moth emergence under insectary and field conditions was studied at Phoenix, AZ. In the insectary, diapause larvae exited immature bolls sporadically during January, February, and early March. Thereafter, exit from the bolls was more consistent and increased to peak numbers in late April, May or early June. Larval and pupal mortality was highest in January and February, decreased in early March, and increased again in late June and July. Larvae remained in immature bolls as long as 319 days after harvest. Duration of the larval stage after exiting bolls between 28 December and 16 March averaged 34 to 135 days but was shorter for larvae exiting bolls after mid-March. Days in the pupal stage averaged 6 to 21 days over the entire experimental period. Moth emergence in the insectary and in the field was significantly correlated to accumulated heat units (12.8 and 30.6°C lower and upper developmental thresholds). In the insectary, shading, reduced temperature, and dry conditions delayed moth emergence as compared to moth emergence under unshaded field conditions and following spring irrigations.

INTRODUCTION

The pink bollworm (PBW), *Pectinophora gossypiella* (Saunders), has been an economic pest of cotton in Arizona and Southern California since the mid-1960's. PBWs overwinter as diapause larvae. This stage of the life cycle is vulnerable to cultural control methods and has been targeted for manipulation in early- and late-season PBW management systems. For example, most of the PBW spring moth emergence occurs during mid-March through mid-June. Cotton flower buds are present in AZ and southern CA cotton between mid-May and early June for cotton planted between 20 March and 20 April. Under these conditions, early-spring suicidal emergence that occurs when moths emerge in the absence of a reproductive host can range from 57 to 86% (Wene et al. 1961; Watson and Larsen 1968; Watson et al. 1970; Rice and Reynolds 1971; Slosser and Watson 1972; Bariola 1978). Delayed planting can prolong the period of suicidal emergence (Adkisson et al. 1962; Henneberry et al. 1982). In AZ, a planting date window is based on a degree-day scale to delay and reduce spring infestations by

balancing the timing of 75% suicidal emergence against the first occurrence of fruiting forms suitable for PBW reproduction (Brown et al. 1992).

Late-season water management optimizes cotton yield and controls late-season cotton growth and fruiting, and when water management is coupled with early harvest and crop residue plowdown very effective PBW cultural control results (Watson et al. 1973, Watson 1980, Chu et al. 1996). These activities focus on reducing fruiting forms for development of diapause larvae or destroying the developed diapause larvae in the soil. Additionally, plant growth regulator treatments have been developed to remove late-season green bolls that do not contribute to yield but do serve as host material for development of the diapause larval generation (Bariola et al. 1976, Henneberry et al. 1988).

The possible refinement of these effective strategies that exploit the vulnerability of the PBW diapause generation and the potential for development of additional methods prompted our studies to gain further information on PBW diapause larvae. Diapause larvae overwinter in cotton bolls, plant litter on the ground, or in the soil, but little is known about larval activity from the time of diapause initiation until moth emergence occurs in the spring of the following year. We conducted studies in the insectary to determine patterns of diapause larval exit from cotton bolls, diapause larval mortality, numbers of days after exiting bolls until pupation, pupal mortality, patterns of adult emergence and relationships to heat unit accumulations. We also determined patterns of spring moth emergence in the field from immature green bolls in screen cages to compare with insectary moth emergence patterns.

MATERIALS AND METHODS

Insectary. Approximately 38 and 27 thousand immature green cotton bolls were picked in cotton fields at Phoenix, AZ on 22 November 1994 and on 19 October 1995, respectively. Each year 1500 bolls were randomly selected from the harvested bolls, and 50 were placed in each of 30 ventilated plastic sweater boxes (Fye 1976). The boxes with bolls were held in an outdoor, screen-covered, open-wall insectary. In 1994, boxes were first examined for PBW emergence from bolls on 21 December and in 1995 on 31 October. Thereafter in each year, each box was checked weekly until 15 August 1995 and 13 September 1996. All living and dead larvae and pupae and adults were recorded. Living larvae and pupae were collected and placed individually in 28-ml capacity plastic cups with about 15 ml of agar base artificial PBW diet (Bartlett and Wolf 1985) to provide moisture. Plastic cups were examined each week and mortality, pupation, or adult emergence recorded.

Field - Adult Emergence. On 21 December 1994, approximately 1500 of the green bolls collected on 22 November 1994 were placed in each of 24 aluminum screen covered 2 x 2m PBW pyramid emergence cages (Shiller 1946). In 1995, approximately 800 of the green bolls collected on 19 October were placed on 31 October in each of 32 of the same type cages. In both years, bolls were covered with about 5.0 cm of soil, also irrigations at 14 day intervals beginning 1 May to 1 June were made to simulate cotton growing conditions. Moth collection jars attached to the tops of the cages to monitor moth emergence were inspected daily beginning on 28 December 1994 and ending on 28 June 1995 and beginning on 8 November 1995 and ending on 20 July 1996.

Heat Units. Maximum-minimum and mean daily temperatures and heat units per day were obtained from the University of Arizona's AZMet database. The lower and upper thresholds of PBW development were 12.8°C and 30.6°C, respectively. The PBW developmental thresholds used were suggested by Huber (1981).

Statistical Analysis. Living and dead larvae and pupae were totaled for all 30

ventilated plastic sweater boxes. Average numbers of days in the diapause larval stage ± standard errors (S.E.) were calculated for each weekly sampling date. The same calculations were made for pupae obtained from the larval collections. Numbers of dead larvae and dead pupae on each sampling date were recorded and graphed along with the percentage mortalities of the total numbers of PBW for that date. Accumulated percentages of emerged moths in the insectary and the field were regressed on heat units accumulated beginning on 1 January of each year to determine potential temperature relationships to adult PBW moth emergence.

RESULTS AND DISCUSSION

Insectary - Numbers of PBWs Exiting from Bolls, Percentages of Larval and Pupal Mortality and Moth Emergence Per Exit Date: 1994 - 1995. From 22 November to 21 December 1994 (29 days following boll harvest), 51 PBWs exited the bolls, 46 and 3 were found as living and dead larvae, respectively (Fig. 1A). One dead and one live pupae were also found. Seventeen of the 46 larvae died. The single live pupae found and three of the pupae that developed from larvae also died before adult emergence. Approximately 19% of the total PBW larvae remained in the bolls for 29 to 155 days, 40% from 162 to 197 days; and 41% from 204 to 252 days (Table 1). Beginning the week of 28 December 1994, numbers of PBW larvae exiting from bolls through the week of 16 March varied from 6 to 25. Numbers increased to 36 - 87 during the weeks of 16 March to 19 April and in general increased thereafter to a peak of 418 the week of 14 June and decreased to 0 during the week of 9 August and thereafter.

1995 - 1996. From 19 to 31 October 1995 (first 12 days following boll collection), 121 live larvae (14% of the total PBW recovered from bolls) exited the bolls (Fig. 2A). Twenty three of the larvae died before pupation, and 30 of the pupae that developed from larvae died. Thereafter, 5% of the larvae remained in bolls for 20 to 169 days, 78% from 179 to 274 days, and 3% from 281 to 319 days (Table 2). Small numbers (4, 1, and 1 respectively, exited bolls on 8, 15, and 22 November 1995). No larvae exited the bolls from 22 November through 11 January 1996, and larval exit from bolls was sporadic until the week of 5 April. Larvae exited bolls during each week from 5 April through 3 September with the exception of the week of 23 August.

Larval and Pupal Mortality: 1994 - 1995. For larvae exiting bolls from 22 November 1994 through 21 December 1994 and the weeks of 28 December 1994 through 8 March 1995, larval mortality ranged from 39 to 71%, pupal mortality 0 to 47%, and moth emergence 27 to 69% (Fig. 1B). Mortality in the larval and pupal stages decreased to 0 - 18 percent beginning the week of 22 March and remained at low levels through the week of 20 June. Sixty-six to 100% of the larvae that exited from the bolls during that period survived to pupate and produce moths. Larval and pupal mortality increased thereafter with reduced adult emergence occurring through 1 August.

1995 - 1996. Larval and pupal mortalities were variable and ranged from 0 to 50% for larvae exiting bolls from 31 October 1995 to 10 May 1996 and 0 to 100% for pupae during the same periods (Fig. 2B). Larval mortality was higher for larvae exiting bolls from 10 May to 3 September compared with pupal mortality on the same dates.

Duration (Days) of Larval and Pupal Stages After Exiting Bolls: 1994 - 1995. Surviving larvae (29) that exited the bolls from 22 November 1994 to 21 December 1995 averaged 126.0 ± 8.0 days before pupating (Fig. 3A). For larvae that exited the bolls weekly from 28 December through 16 March the duration of the larval stage ranged from 34.1 to 127.0 days (Fig. 3A) and 6.0 to 14.4 days for larvae exiting the bolls on 22 March and thereafter. Pupal stage ranged from 7 to 21 days over the entire experimental period (Fig. 3B), and the total numbers of days from larva to moth emergence, during 21

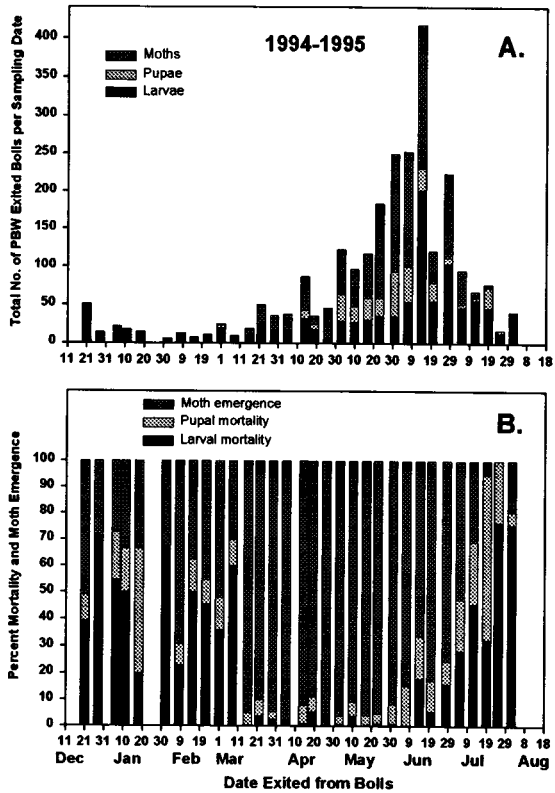

FIG. 1. Numbers of pink bollworm larvae, pupae, and moths from cotton bolls on indicated sampling dates (A) and percentages of larval and pupal mortality, and moth emergence for each larval exit date (B). (1994-1995).

December to 16 March, was 53.4 to 150.0 days and 16.8 to 32.0 days for the period from 22 March to 12 July (Fig. 3C).

1995 - 1996. The average number of days to pupation for 98 larvae exiting the bolls between 19 and 31 October was 196.8 (Fig. 4A). All of the live pupae that were found survived, and the average number of days to moth emergence was 10.6 (Fig. 4B). Four, 1, and 1 larvae exited the bolls during the weeks of 8, 15, and 22 November 1995 and remained in the larval stage 183, 142, and 149 days, respectively, before pupating. Larvae exiting bolls during 11 January to 13 March averaged 32.0-99.4 days in the larval stage. After the week of 13 March and through the week of 21 June, average numbers of days in the larval stage ranged from 4-13. Days in the pupal stage for the entire experimental period ranged from 4-21 days (Fig. 4B), and the total developmental period (larvae to moths) from 10-202 days (Fig. 4C).

Total Days PBW Larvae Remained in Bolls After Harvest and Days of Larval and Pupal Development After Exiting Bolls: 1994 - 1995 and 1995 - 1996. Number of days

TABLE 1. Numbers of Days and Numbers and Percentages of the Total Diapause Pink Bollworm Larvae that Remained in Cotton Bolls Following Boll Harvest (1994 to 1995).

Sampling date		Days in bolls after harvest (no.)	PBW Exited from Bolls	
			No.	% of total
1994				
December	21	29[a]	51	2
	28	36	14	1
1995				
January	06	45	22	1
	11	50	18	1
	18	57	15	1
February	01	71	6	<1
	08	78	13	1
	15	85	8	<1
	22	92	11	<1
March	01	99	25	1
	08	106	10	<1
	16	114	19	1
	22	120	50	2
	29	127	36	1
April	05	134	38	2
	14	143	87	3
	19	148	36	1
	26	155	46	2
May	03	162	123	5
	10	169	97	4
	17	176	117	5
	23	182	184	6
	31	190	250	9
June	07	197	253	9
	14	204	418	15
	20	210	121	5
	28	218	224	8
July	05	225	95	4
	12	232	68	3
	19	239	77	3
	25	245	17	1
August	01	252	41	2
	09	260	0	0
	15	266	1	< 1
Total	--	--	2590	100

[a] Bolls harvested 22 November, number of PBW represents accumulated exit from bolls for 29 days. All other data are for 6 to 8 days, except for 6 January and 1 February, between sampling periods.

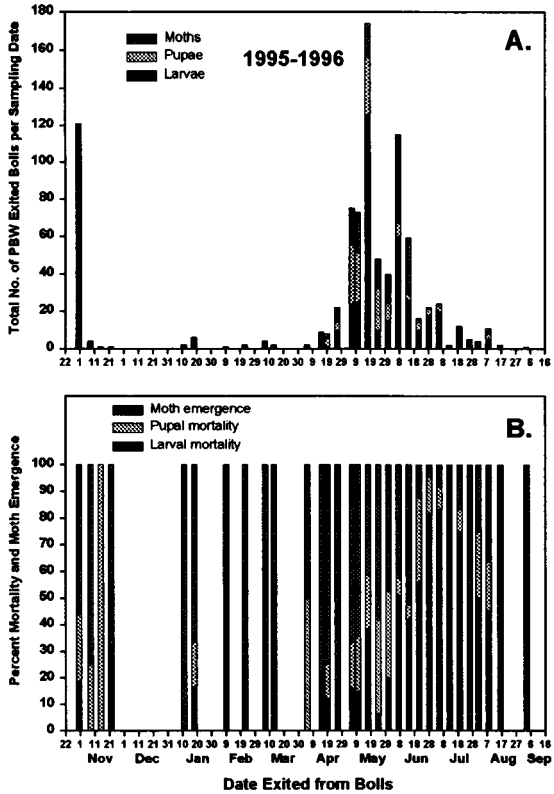

FIG. 2. Numbers of pink bollworm larvae, pupae, and moths from cotton bolls on indicated sampling dates (A) and percentages of larval and pupal mortality, and moth emergence for each larval exit date (B). (1995-1996).

for larvae remaining in the bolls following harvest plus larval and pupal development periods are shown in Fig. 5A and averaged 180 days for larvae exiting bolls between 22 November and 21 December 1994 and 252 days for larvae exiting bolls at weekly intervals from 21 December 1994 to 1 August 1995. Total time periods for larvae remaining in the bolls in 1995 to 1996 following harvest plus larval and pupal developmental periods averaged 190-230 days (Fig. 5B).

Numbers of Days to Pupation in Relation to Time of Larval Exit from Bolls and Numbers of Larvae Pupating: 1994 - 1995 and 1995 - 1996. Seventeen of 150 larvae (Fig. 6A) exiting the bolls during the period of 22 November 1994 until 14 April 1995 pupated within 7 to 21 days (Fig. 6B). The numbers of days for remaining larvae (133) to pupate averaged from 29-175. After 14 April, all larvae pupated within 6-21 days after exiting bolls. The results were similar for numbers of larvae (Fig. 7A) exiting bolls 31 October 1995 to 15 April 1996 (length of days to pupation, 18-248) (Fig. 6B). Also, larvae exiting bolls on 26 April or later pupated in 7-14 days.

TABLE 2. Numbers of Days and Numbers and Percentages of the Total Diapause Pink Bollworm Larvae that Remained in Cotton Bolls Following Boll Harvest (1995 to 1996).

Sampling date		Days in bolls after harvest (no.)	PBW Exited from Bolls	
			No.	% of total
October	31	12	121	14
November	08	20	4	<1
	15	27	1	<1
	22	34	1	<1
	30	42	0	0
December	07	49	0	0
	14	56	0	0
	21	63	0	0
	28	70	0	0
January	04	77	0	0
	11	84	2	<1
	18	91	6	1
	25	98	0	0
	31	104	0	0
February	09	113	1	<1
	16	120	0	0
	22	126	2	<1
March	01	134	0	0
	07	140	4	<1
	13	147	2	<1
	22	155	0	0
	28	161	0	0
April	05	169	2	<1
	15	179	9	1
	19	183	8	1
	26	190	22	3
May	03	197	75	9
	10	204	73	8
	17	211	174	20
	24	218	48	6
	31	225	40	5
June	07	232	115	13
	14	239	59	7
	21	246	16	2
	28	253	22	3
July	05	260	24	3
	12	267	2	<1
	19	274	12	1
	26	281	5	<1
August	01	286	4	<1
	08	293	11	1
	16	301	2	<1
	23	308	0	0
September	03	319	1	<1
	13	329	0	0
Total	--	--	868	100

[a] Bolls harvested 19 October, number of PBW represents accumulated exit from bolls for 12 days. Other data, except for 3 and 13 September are for 6 to 8 days between sampling periods.

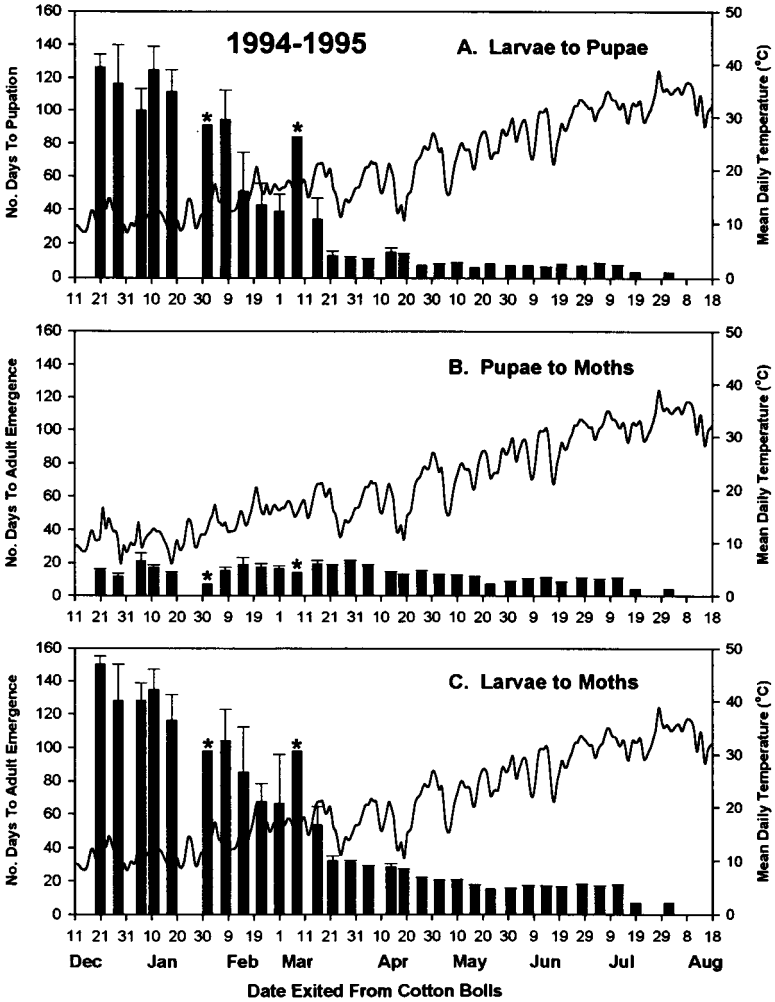

FIG. 3. Mean daily temperatures (°C) and mean ± S.E. (standard error) numbers of days for diapause PBW larvae to pupate (A), develop from pupae to moths (B) and total larval to moth development (C) periods after exiting from cotton bolls. * = one individual. (1994-1995)

Adult Emergence: *1994 - 1995*. The first moth emergence for larvae exiting bolls between 22 November and 21 December 1994 occurred on 6 January 1995, 45 days after the bolls were harvested (Fig. 8A). No moth emergence occurred the weeks of 11 and 18 January. One, 2, 1 and 1 moths emerged during the weeks of 1, 8, 15 and 22 February (71, 78, 85, and 92 days after boll harvest). Thereafter, beginning on 1 March, moth emergence occurred every week through 1 August. Numbers of moths emerged

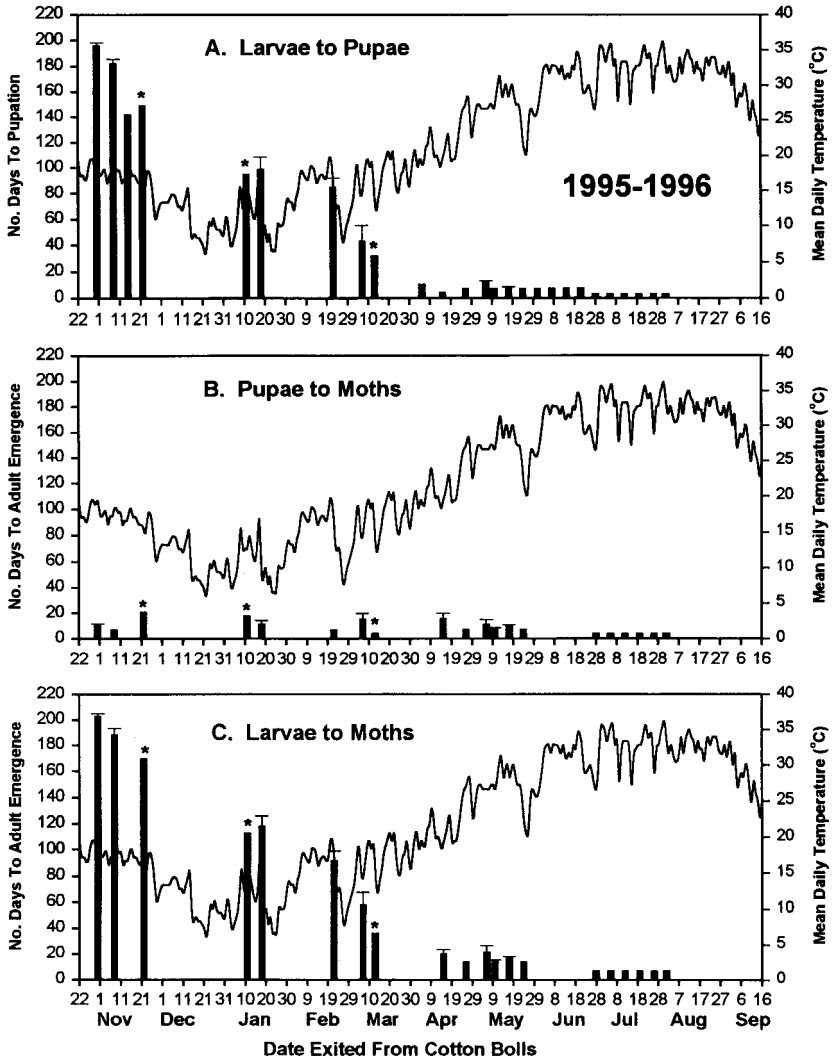

FIG. 4. Mean daily temperatures (°C) and mean ± S.E. (standard error) numbers of days for diapause PBW larvae to pupate (A), develop from pupae to moths (B) and total larvae to moth development (C) periods after exiting from cotton bolls. * = one individual. (1995-1996)

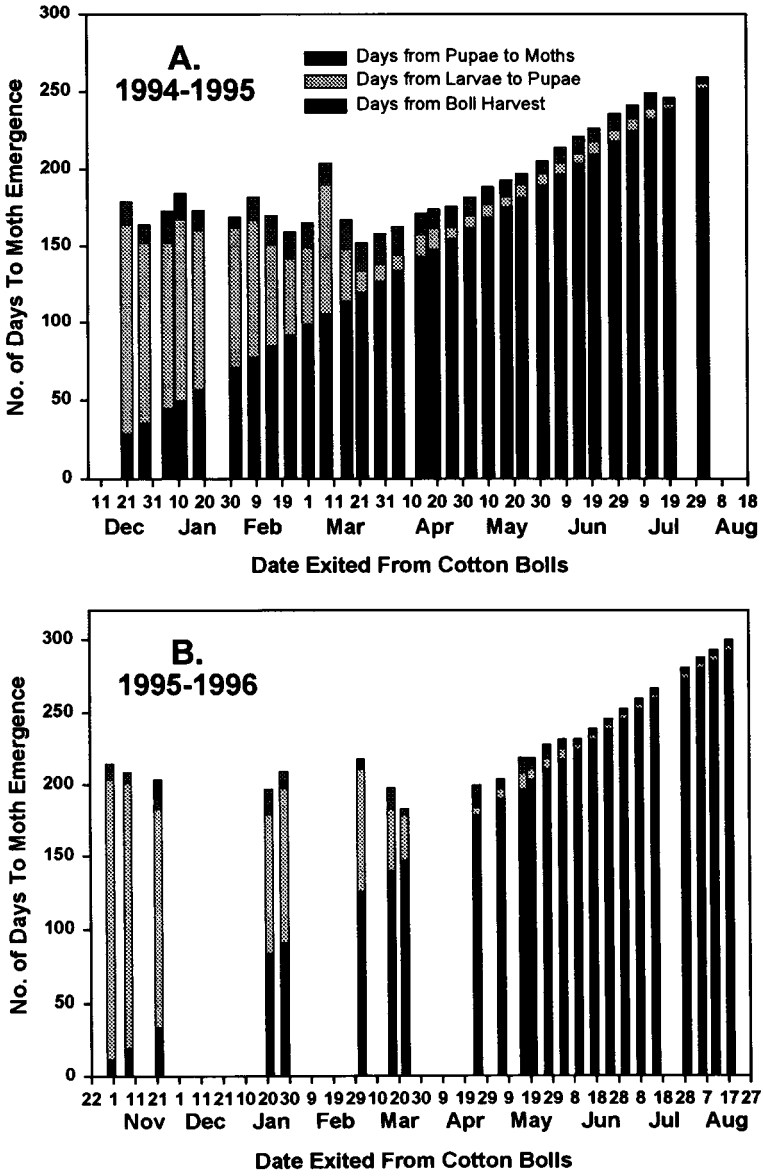

FIG. 5. Numbers of days pink bollworm larvae remained in cotton bolls following boll harvest and numbers of days for diapause larvae to pupate and develop from pupae to adults for each larval boll exit date. (1994-1995 and 1995-1996)

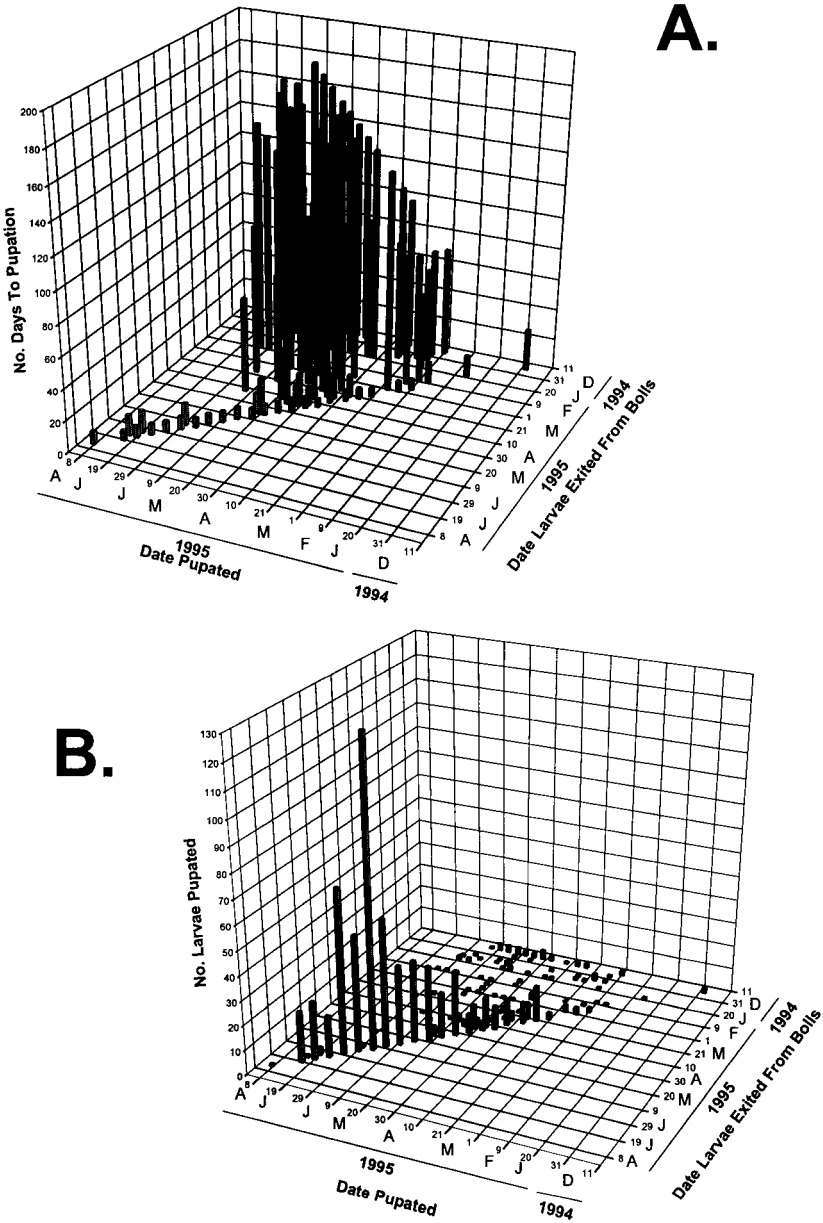

FIG. 6. Numbers of days for pink bollworm larvae to pupate in relation to the time of year larvae exited from cotton bolls (A) and numbers of larvae pupated (B). Insectary 1994-1995.

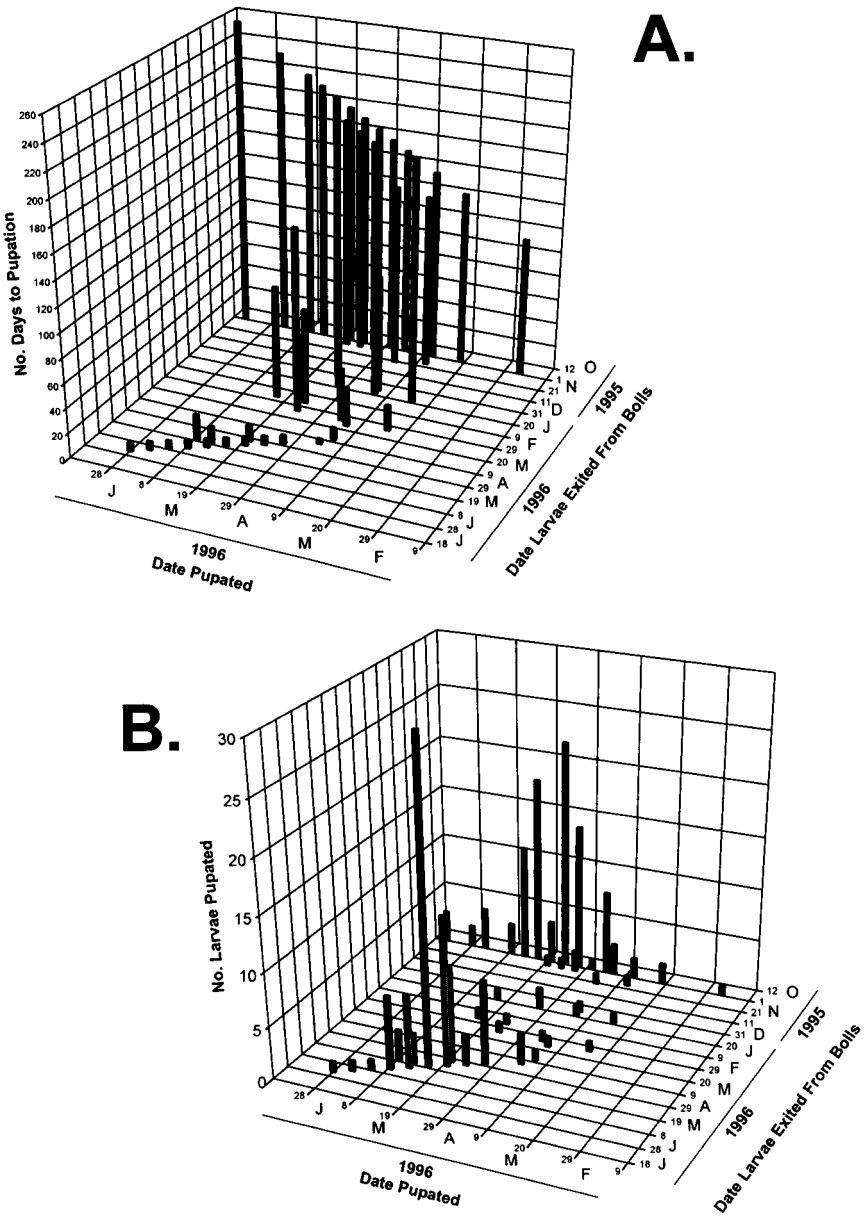

FIG. 7. Numbers of days for pink bollworm larvae to pupate in relation to the time of year larvae exited from cotton bolls (A) and numbers of larvae pupated (B). Insectary 1995-1996.

increased gradually through April; peak moth emergence occurred on 14 June and decreased thereafter.

1995 - 1996. Single moths emerged on 11 January, 9 February and 29 March (Fig. 8B). Thereafter, beginning on 13 April, increasing numbers of moths emerged to a peak of 85 on 7 June. Numbers of moths emerging decreased thereafter, and the last moth emergence occurred on 23 August.

Accumulated Heat Unit Relationships to PBW Moth Emergence: 1994 - 1995 and 1995 - 1996. Cumulative percentages of moth emergence were significantly correlated ($r = 0.99$ and $r = 0.96$, for 1995 and 1996, respectively) to the numbers of heat units accumulated beginning 1 January of each year. The regression relationships ($r^2 =$ 0.99 and 0.99, for 1995 and 1996, respectively) were also highly significant ($P \leq 0.01$; n = 30; df = 1, 28 for 1995 and $P \leq 0.01$; $n = 33$; df = 1, 31 for 1996) (Fig. 9A). Fifty percent moth emergence occurred at 784 accumulated heat units (°C) in 1995 and 849 accumulated heat units (°C) in 1996.

Field - Adult Emergence: 1994 - 1995 and 1995 - 1996. In 1994, the first PBW moth emergence in field cages occurred on 22 February (Fig. 10A). Thereafter, moth emergence increased to a peak on 5 April and decreased thereafter to 28 June. In 1995, PBW moths emerged in field cages on 15 November and weekly thereafter through 31 January (Fig. 10B). No emergence occurred 9 February through 1 March. Beginning 7 March, small numbers of moths (2-7 per cage/week) emerged through 4 April. Numbers of moths emerging increased rapidly to a peak on 1 May and decreased thereafter with the last moth emergence on 31 May.

Accumulated Heat Unit Relationship to PBW Moth Emergence. Accumulated heat units in both years (°C) were significantly correlated ($r = 0.99$ and 0.99 for 1995 and 1996, respectively) to cumulative percentages of moth emergence (Fig. 9B). The regression relationships were also highly significant ($r^2 = 0.99$ and 0.95 for 1995 and 1996, respectively), and 50% moth emergence occurred at 348 and 494 accumulated heat units (°C) in 1995 and 1996, respectively.

DISCUSSION

PBW larvae of generations 1 to 3 (non-diapause), during the growing season under acceptable environmental conditions, develop to last instar larvae (13 - 21 days, Butler and Hamilton 1976) and exit immature green bolls on plants to pupate in the soil or on the soil surface. Hormone mediated diapause, as defined by Tauber et al. (1986), enables PBW larvae (in generations 4 and 5) to avoid adverse conditions of low temperature and lack of host material during winter months and to survive to reenter reproductive cycles under more favorable conditions during cotton cultivation. About 64% of the PBW larvae in cotton fields near El Paso, TX were reported to overwinter in cocoons in the soil (Chapman et al. 1960). At several locations in AZ, Wene and Sheets (1966) reported that 89% of the PBW larvae left bolls to overwinter in cocoons. The results of these reports have been further supported by studies showing that diapause PBW larvae exit bolls after burial in the soil in March under laboratory conditions (Henneberry and Clayton 1983) and the results of the present study. Also, Fullerton et al. (1975) reported that only 52 and 1% of the PBW larvae in infested bolls buried in the soil in the field in December remained in the bolls after burial for 1 and 5 months, respectively. The reason that PBW larvae leave the protection of bolls in winter and early spring remains unknown and is difficult to explain. Because, as temperature and photoperiod conditions that induce diapause begin to occur (during generation 4 or 5), increasing numbers of larvae remain in the bolls (Crowder et al. 1975, Henneberry et al. 1980). This is presumably for protection from adverse environmental conditions since

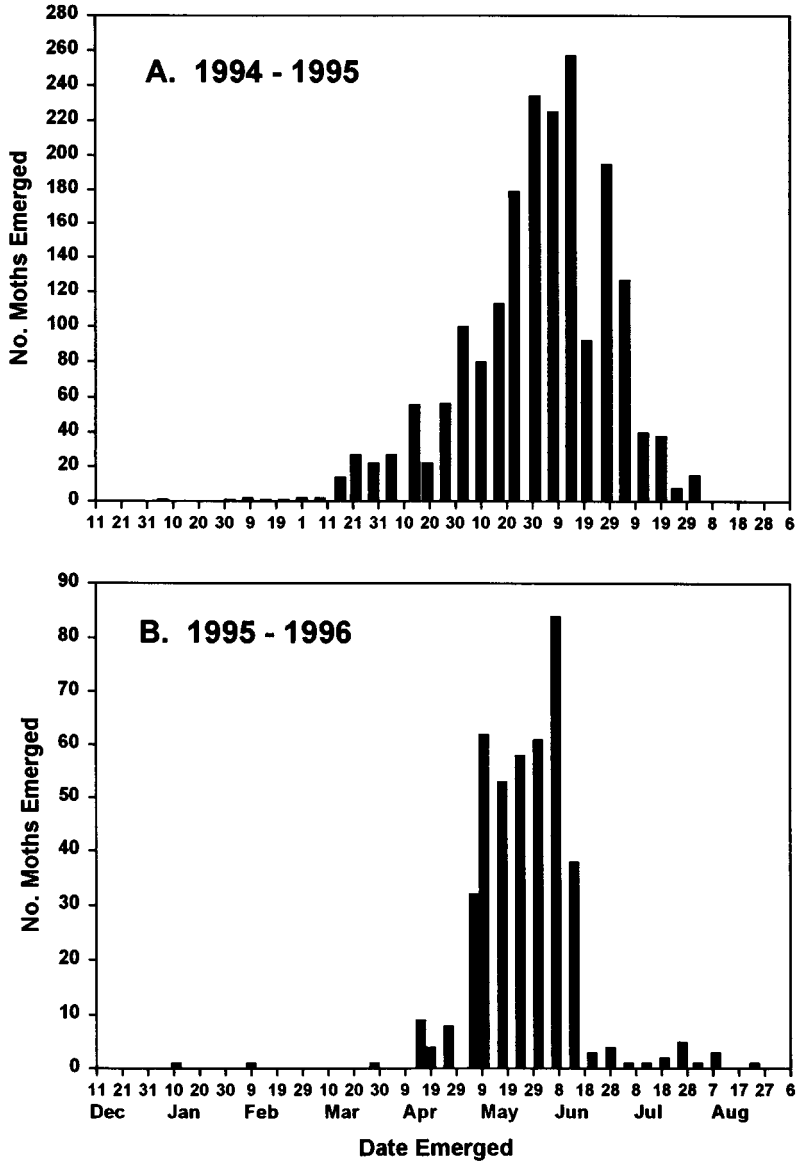

FIG. 8. Total number and dates of pink bollworm moth emergence in the insectary in 1995 (A) and 1996 (B) from diapause larvae exited from immature green cotton bolls collected in November 1994 and October 1995, respectively. (1500 bolls each year, 50 bolls in each of 30 emergence boxes)

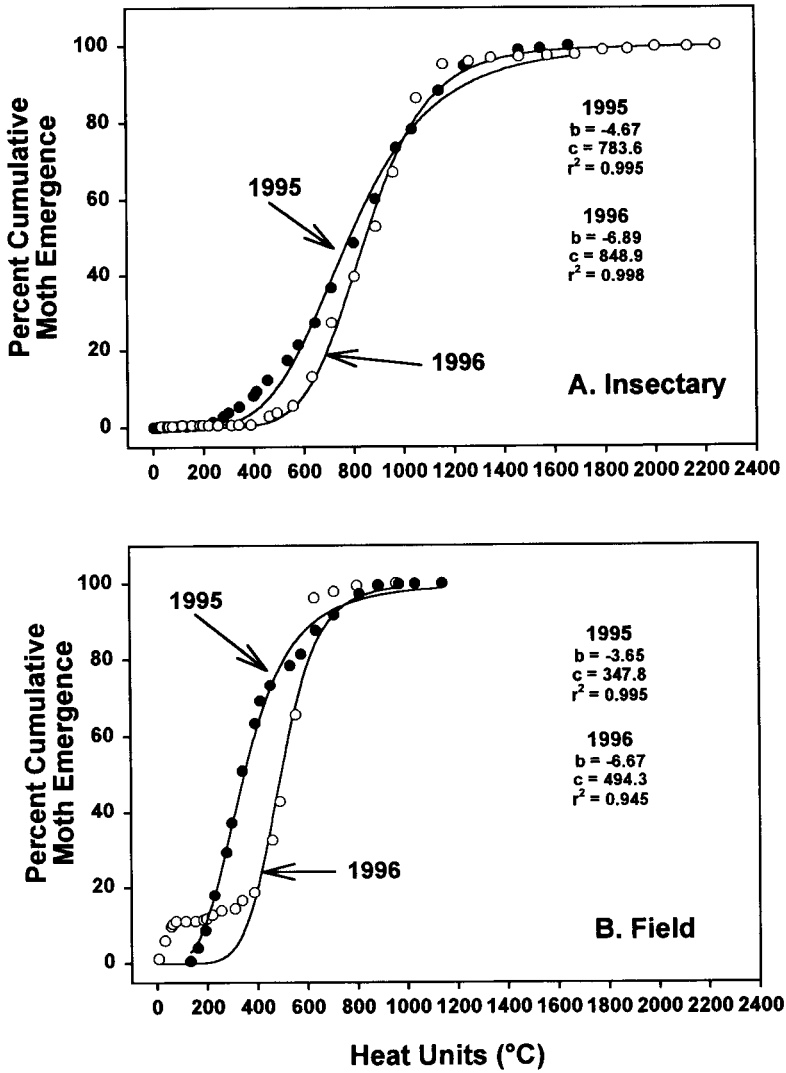

FIG. 9. Accumulated percentages of spring pink bollworm moth emergence in the insectary (A) and field (B) in relation to accumulated heat units. Upper and lower development thresholds were 30.6 and 12.8 °C, respectively. The emergence curves were effectively described by the formula:

$$Y = \frac{100}{1+(x/c)^b}$$

Y = cumulative percent moth emergence, b = regression coefficient, x = cumulative heat units and c = median cumulative heat units for 50% emergence.

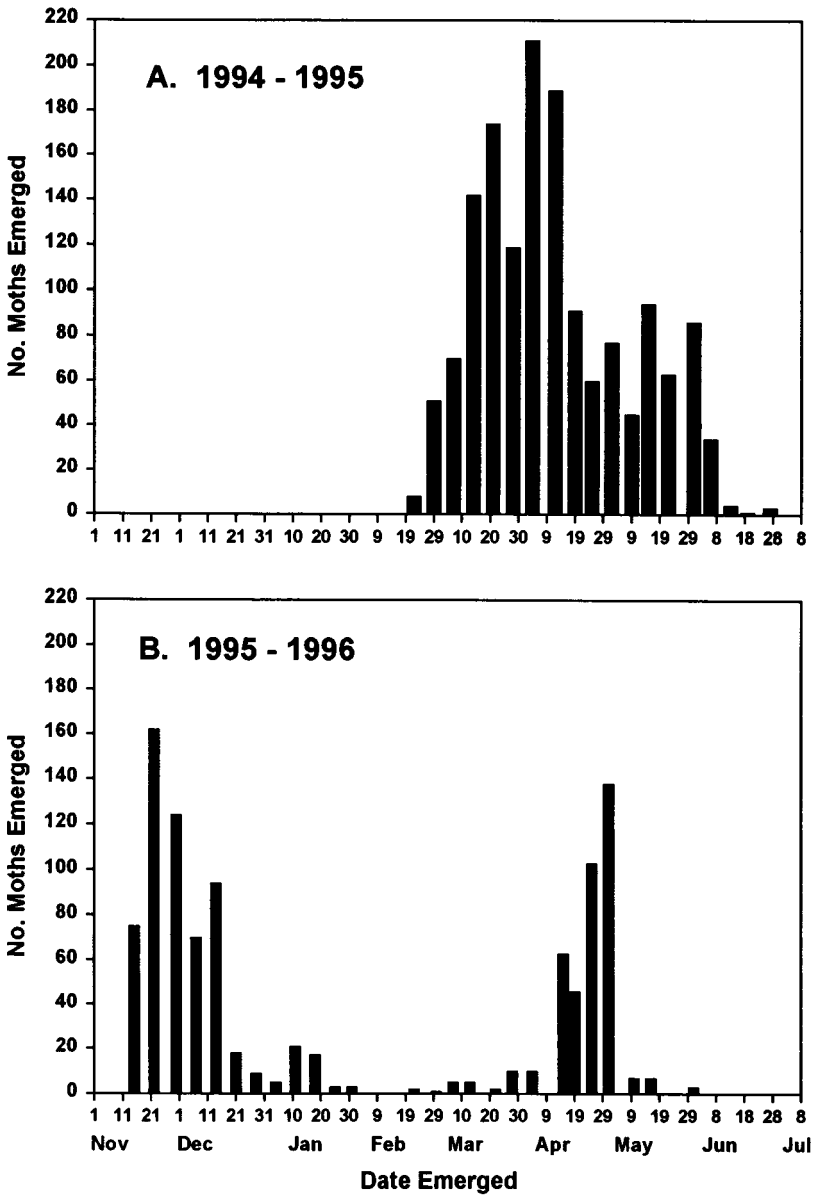

FIG. 10. Total number and dates of pink bollworm moth emergence in the field in 1995 (A) and 1996 (B) from diapause larvae exited from immature green cotton bolls collected in November 1994 and October 1995, respectively. (1500 bolls in each of 24 cages in 1994, 800 bolls in each of 32 cages in 1995)

higher overwintering survival occurs for those in bolls compared with those occurring in cocoons outside bolls (Wene et al. 1961). PBW larval exit from bolls in January to early March did not appear related to diapause termination since pupation did not occur thereafter for 34 to 135 days for 1994 - 1995 and 32 to 197 days for 1995 – 1996, respectively. Possibly, the stimulation for exiting bolls is related to locating near the soil surface to facilitate spring moth emergence.

Male moths have been caught in pheromone-baited traps every month of the year in AZ (Henneberry et al. 1980) and in Southern California's Coachella Valley, (Kaae et al. 1977). In our studies, as well as those of Fye (1979), some moth emergence occurred in January and February from infested immature bolls collected in October and November of the previous year. These results may partially explain the moth trap catches in winter in the absence of cotton crops. Whether these moths were from diapause or non-diapause larvae remains unknown. Decreasing photoperiods and lower temperatures beginning in mid-September in AZ induce PBW diapause (Butler et al. 1978, Gutierrez et al. 1981). Non-diapause larvae have been well documented in the equatorial region between 10° north and south latitudes (Lukefahr et al. 1964). In more northern latitudes percentages of PBW diapause from November collected larvae is often less than 100% (Crowder et al. 1975, Beasley and Adams 1997). Total development times for PBW larvae and pupae at 15° and 20°C were reported to be 85 and 35 and 36 and 18 days, respectively (Butler and Henneberry 1976, Butler and Hamilton 1976). Also, PBW moths have been reported to live over 110 days at constant temperatures of 17.2°C and over 74 days at fluctuating temperatures averaging 19.2°C (Butler and Foster 1979). Thus, PBW moths caught in December, January and February may be from early-terminated diapause larvae or from PBW larvae that do not diapause but develop at low rates because of low winter temperatures (Butler and Henneberry 1976). In the present studies, almost all larvae found in bolls were in the 4th instar for bolls harvested on 22 November 1994 and 19 October 1995. Moths emerged during the weeks of 6 January, 1, 8, and 15 February. These dates were 45, 71, 78, and 85 days, respectively, after bolls were harvested. Average daily temperatures (°C) for the months of November, December, January and February in 1994 and 1995 were 12.2, 11.1, 10.6, and 15.0, and 16.7, 11.1, 10.6, and 15.0, respectively. These results support both premises: (1) PBW moth emergence from larvae that do not diapause but develop slowly, or (2) moth emergence from larvae that diapause but break diapause under lower temperatures and shorter photoperiods than has been reported (Butler et al. 1978, Gutierrez et al. 1981, Gutierrez et al. 1977). The identification of a protein associated with PBW diapause larvae (pectinophorin) (Salama and Miller 1992, 1993) and the antibody serology technique make it possible to determine diapause in individual PBW larvae (Miller et al. 1993) and these techniques should be used in future studies.

The relationships between heat unit accumulations and PBW emergence have been reported by several authors (Huber 1981, Sevacherian et al. 1977) and used in AZ to develop cotton planting windows to maximize suicidal emergence that occurs when PBW moths emerge without available cotton fruiting forms as a source of oviposition and larval food (Brown et al. 1992). Our data showing 50% moth emergence under field conditions at 348 and 494 accumulate heat units (°C) agree with the data of Brown et al. (1992) and Beasley and Adams (1996). In contrast, under shaded, cooler conditions occurring in the outdoor insectary, emergence was extended over longer periods of time and 50% accumulated moth emergence did not occur until 784 and 849 accumulated heat units. Sevacherian et al. (1977) reported a similar delay in PBW emergence and accumulated heat unit relationships when comparing temperature inside and outside emergence cages in the field. Additionally, earlier emergence in our field studies was stimulated by the May irrigations which is similar to results reported by Beasley and

Adams (1995) and Fife (1961).

Some of the research from extensive studies with diapause PBW larvae have been implemented in some growing areas as highly successful cultural practice components of PBW management systems (Watson and Larsen 1968, Watson et al. 1973, Chu et al. 1996). These management systems have not been totally acceptable in all cotton growing areas because of yield limitations. Thus, there is a need for continuing studies to refine and improve existing cultural methods and look for new approaches or modifications of crop management systems that are agronomically robust and more acceptable in more demanding and less flexible cotton production situations. The results of our current studies provide additional information on PBW diapause larval behavior, habitat relocation from harvested immature bolls, mortality of larvae and pupae and spring moth emergence. This information broadens our growing knowledge of PBW biology and ecology that is essential to the development of ecologically oriented control methods.

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A DISCRIMINATING-DOSE BIOASSAY FOR DETECTING PYRETHROID RESISTANCE IN TARNISHED PLANT BUG (HETEROPTERA: MIRIDAE) POPULATIONS

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ABSTRACT

A discriminating-dose bioassay for monitoring pyrethroid resistance in adult tarnished plant bug, *Lygus lineolaris* (Palisot de Beauvois), populations was developed and tested on field populations. In the bioassay, adult plant bugs were placed into glass vials (20 ml, two adults per vial) that had been treated with 15 μg of permethrin. A minimum of 50 adults were tested from each population, and mortality was determined after 1.5 to 3.0 h of exposure. Mortality was 95% at 3.0 h of exposure in the susceptible laboratory colony used to develop the bioassay, while mortality was 98% at 3.0 h exposure in a susceptible field population. Mortalities less than 10% were found for a selected pyrethroid-resistant laboratory colony used to develop the bioassay and also for a field population collected near cotton at Wainwright, LA. Field populations were also tested that had mortalities in the discriminating-dose bioassay of 30, 48, and 78%. These were pyrethroid-resistant populations with varying numbers of susceptible plant bugs in each population. Mortality in the discriminating-dose bioassay was directly related to pyrethroid resistance as determined with a glass-vial bioassay, and as mortalities in the discriminating-dose bioassays declined, the LC_{50} values for permethrin for the plant bug populations increased. In spray chamber tests designed to simulate field conditions, three pyrethroid-resistant plant bug populations were tested that had mortalities of 9, 64, and 78% in the discriminating-dose bioassay. All three resistant populations had significantly lower mortalities when caged on cotton terminals treated with permethrin (Pounce) than susceptible plant bugs (92% mortality in the discriminating-dose bioassay). These results indicated that the discriminating-dose bioassay could detect pyrethroid-resistant plant bug populations, and predict possible control problems from using pyrethroid insecticides against resistant populations. The discriminating-dose bioassay developed in the study should be very useful to researchers, growers, and consultants in detecting plant bug populations with pyrethroid resistance.

INTRODUCTION

The tarnished plant bug, *Lygus lineolaris* (Palisot de Beauvois), is a key early-season pest of cotton in the mid-South (Scott et al. 1985). It is controlled in cotton with insecticides and has developed insecticide resistance, especially to pyrethroid insecticides (Hollingsworth et al. 1997, Pankey et al. 1996, Snodgrass and Elzen 1995, Snodgrass 1996a). Snodgrass (1996a) used a glass-vial bioassay (Snodgrass 1996b) to determine that plant bug populations with pyrethroid resistance also had multiple resistance to some organophosphorus and

cyclodiene insecticides. The glass-vial bioassay determined insecticide resistance levels in plant bug populations by use of dose-response regression lines developed from bioassay data. The bioassay was developed mainly for use in research, since it was relatively slow (24 h are required to determine mortality), required treatment of vials at several different concentrations of the test insecticide, and usually required at least 180 adult insects (30 for each concentration tested) to test each insecticide. Roush and Miller (1986) found that testing an insect sample at a discriminating dose was more efficient than estimating resistance with dose-response regression lines. Discriminating-dose bioassays have been used extensively in monitoring resistance in other cotton insect pests such as the tobacco budworm, *Heliothis virescens* (F.) (Plapp et al. 1987, Plapp et al. 1988, McCutchen et al. 1989). A discriminating-dose bioassay is not available for the tarnished plant bug; however, a residual bioassay to detect resistance in plant bug populations which used a glass vial coated with a discriminating dose would be very useful and easy to use. The vials could be pretreated, a short exposure time for bugs placed in treated vials could be used, and treated vials could be used by growers or consultants to rapidly test for resistance. Sample size could also be fairly small for a discriminating-dose bioassay. Roush and Miller (1986) found that resistance present at a frequency of >10% could be documented with 95% accuracy by testing a sample of 50 insects.

The present study was conducted to develop a discriminating-dose bioassay for detecting pyrethroid resistance in plant bugs.

MATERIALS AND METHODS

Bioassays. The discriminating doses tested were near the LC_{90} value for susceptible plant bugs. This reasoning followed that of McCutchen et al. (1989) who found that using a discriminating concentration less than the LC_{99} would allow most resistant insects to survive the treatment, while most susceptible insects would die. The susceptible and pyrethroid-resistant plant bugs used to develop the bioassay were from colonies maintained at the Southern Insect Management Research Unit, USDA-ARS, Stoneville, MS. The susceptible colony (S) was started from adults originally collected from weeds near Crossett, AR, in an area where row crops were not grown and exposure to insecticides was minimal. It has been in culture since 1989. The pyrethroid-resistant colony (RL) was originally collected from cotton in 1994 and was selected with permethrin every two to three generations in the laboratory to maintain a high level of resistance. Wild populations of susceptible and pyrethroid-resistant plant bugs were also collected and used for comparison of results obtained in the discriminating-dose bioassays and in the spray chamber tests described below. The susceptible plant bugs were collected from weeds near Monticello, AR (SM), while pyrethroid-resistant plant bugs were collected in cotton at Stoneville (RS), and from weeds by cotton at Indianola, MS (RI), Wainwright, LA (RW), and Dermott, AR (RD).

Concentration-mortality lines were estimated using technical grade permethrin (94.7% purity; FMC Corporation, Princeton, NJ) and the glass-vial bioassay developed by Snodgrass (1996b). All laboratory colonies and field collected populations of plant bugs used in the discriminating-dose bioassays and in the spray chamber tests were tested with permethrin using the glass-vial bioassay. Use of permethrin should be applicable to all pyrethroid insecticides since previous research showed that when plant bugs are resistant to one pyrethroid, they are resistant to others (Snodgrass 1996a). Data from glass-vial bioassays were analyzed with the PROC Probit option of SAS, and LC_{50} values for permethrin obtained for the laboratory colonies and field populations were compared to their percentage mortalities in the discriminating-dose bioassay using simple linear correlation (SAS Institute 1989).

In addition to the concentration of the discriminating dose, time of exposure to the dose was tested to find the best length for the exposure period. Adult plant bugs were placed into glass liquid scintillation vials (20 ml) that had been treated with 10, 15, or 20 μg of technical grade permethrin. The inner surface of the vials was coated with permethrin dissolved in acetone using the procedure of Plapp and Vinson (1977). The rate of 15 μg per vial represented the approximate LC_{90} of the susceptible bugs for permethrin. Two adults were placed in the vials and confined by placing a cotton ball in the vial opening. Five replications were used for each of the three concentrations of permethrin tested. Each replication consisted of 10 permethrin treated vials and 10 acetone treated vials (untreated check). Adults were placed in the vials and mortality was recorded at 0.5 h intervals for 3.0 h. Adults were considered dead when they could no longer move or were unable to right themselves.

Spray Chamber Tests. Plant bugs with four different levels of pyrethoid resistance (as determined with the glass vial bioassay and discriminating-dose bioassay developed in the current study) were used in two tests to see if the discriminating-dose bioassay would predict potential control problems with pyrethroids. Adults were placed in cages with cotton terminals after the terminals were treated using a spray chamber as described by Elzen et al. (1992). The spray chamber was calibrated to deliver 56 liters/ha at 2,109 g/cm2 with one hollow-cone nozzle (TX, Spraying Systems, Hammond, LA) at 3.2 km/h. The spray nozzle was positioned 30.5 cm above the spray surface. Ventilated paper cups (0.295 liter) were used as cages, and mortality was determined 24 h after placement in the cups. Ten cages (two adults per cage) were used in each replication. Controls were adults caged on terminals which had been treated with water. In both tests cotton terminals were treated with permethrin [Pounce 3.2 (EC), FMC Corporation, Philadelphia, PA] at 0.11 kg (AI)/ha. In the first test, pyrethroid-resistant hybrid adult offspring (RH) obtained by crossing the pyrethroid resistant (RL) and susceptible (S) laboratory colonies described earlier were tested along with adults from the parent resistant and susceptible colonies. In the second test, pyrethroid-resistant adults collected from weeds by cotton fields near Dermott, AR, were tested along with adults from the susceptible laboratory colony. Data from both spray chamber bioassays were analyzed using analysis of variance (ANOVA), and means were separated by least significant difference (LSD) at $P < 0.05$ (SAS Institute 1989). Treatment mortality was corrected based on mortality in the control groups using Abbot's (1925) formula before data were analyzed.

RESULTS AND DISCUSSION

Bioassays. The best separation in mortality between the susceptible and resistant plant bugs was obtained with 15 μg/vial of permethrin (Table 1). Differences in mortality between resistant and susceptible plant bugs at this rate with 1.5 to 3.0 h of exposure were 85-86%, and during this time period less than 10% of the resistant bugs and >90% of the susceptible bugs were killed. Mortality in the susceptible colony was too low (a maximum of 80%) at the 10 μg/vial rate, while mortality was too high in the resistant bugs (14-28%) from 1.5 to 3.0 h of exposure using the 20 μg/vial rate of permethrin. Mortalities in the resistant and susceptible plant bugs at 1 h of exposure at the 20 μg/vial rate were acceptable (9 and 91%, respectively). However, the increase in mortality in the resistant bugs in time periods longer than 1 h made this dose less useful than the 15 μg/vial dose. Based on the results of this test, the best discriminating dose and exposure period for detecting pyrethroid resistance in adult plant bugs was 15 μg of permethrin for 1.5 to 3.0 h. Two adults per vial were used in the present study, it is possible that three or more adults per vial might work as well or better than two, however, this was not investigated.

TABLE 1. Mortality Time of Pyrethroid-Resistant and Susceptible Tarnished Plant Bugs Exposed in 20-ml Glass Vials to Three Rates of Permethrin.

Colony	Rate permethrin (μg/vial)	Mean % mortality[a] Time (h) 0.5	1.0	1.5	2.0	2.5	3.0
Resistant	10	0	0	0	2	2	2
Susceptible	10	6	45	56	69	79	80
Resistant	15	0	1	5	7	7	9
Susceptible	15	37	86	91	92	93	95
Resistant	20	2	9	14	23	28	28
Susceptible	20	71	91	93	94	95	95

[a] Mortality at each rate is based on 5 replications with 10 permethrin treated vials and 10 vials treated only with acetone (check) per replication. Two adults were used per vial, and check mortality in all replications of all rates was 0.

Comparison of concentration-mortality lines for permethrin from pyrethroid resistant and susceptible field and laboratory populations (Fig. 1) showed that the use of the LC_{90} dose of 15 μg produced the best separation by mortality in the resistant and susceptible populations.

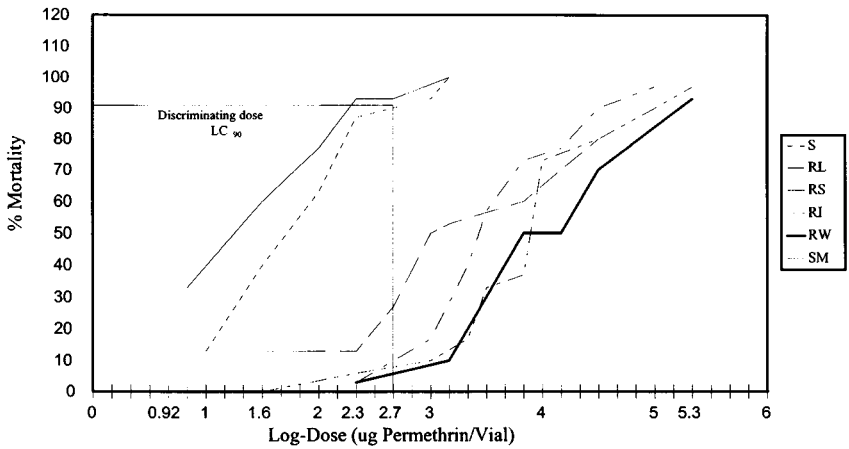

Figure 1. Concentration-mortality lines for susceptible and resistant adult tarnished plant bugs exposed to permethrin. Plant bugs tested were from susceptible (S) and pyrethroid-resistant (RL) laboratory colonies; a susceptible population collected from weeds near Monticello, AR (SM); and from pyrethroid-resistant populations collected from cotton or weeds near cotton at Stoneville, MS (RS), Indianola, MS (RI), and Wainwright, LA (RW).

On the concentration-mortality line for the field collected susceptible population from Monticello (SM), the LC_{90} dose of 15 μg of permethrin produced a mortality of 93%, which was close to the 90% mortality for the susceptible laboratory colony (S). Mortality at the LC_{90} dose in one (RW) of the three field collected resistant populations was 6%, which was close

to the 8% mortality for the resistant laboratory colony (RL) used to develop the discriminating-dose bioassay. Mortalities for the resistant field populations from Indianola (RI) and Stoneville (RS) were 27 and 12%, respectively, at the LC_{90} dose. Insecticide resistance to pyrethroids in plant bugs is a recessive trait, and offspring of crosses between resistant and susceptible parents have reduced expression of pyrethroid resistance with an LC_{50} value for permethrin closer to that for the susceptible parent (Snodgrass 1996a). Thus, tarnished plant bug populations collected in or near cotton could have varying numbers of individuals expressing resistance. The number with resistance would depend on a variety of factors such as the selection pressure from pyrethroid insecticide applications made to the fields in the collection area, the number of susceptible plant bugs found in the area on weeds or crops, and the genetic makeup of individuals that migrate into the area. In years where pyrethroid use in crops is frequent, plant bug populations could be highly selected for resistance and be made up of mostly resistant individuals (as was the population from Wainwright, LA, in the present study).

Differences in pyrethroid resistance levels among the four populations can also be seen in their LC_{50} values for permethrin (Table 2). The LC_{50} values for permethrin for the RL and RW populations were moderately high at 52.1 and 62.0 μg/vial, respectively (4.6 μg/vial for the susceptible colony), which indicated that both populations were selected for resistance. The RS and RI populations had more moderate levels of resistance with LC_{50} values of 37.3 and 29.1, respectively. Results from testing the four resistant populations with the discriminating-dose bioassay developed in the current study (Table 2) showed that those populations with the highest levels of pyrethroid resistance had the lowest mortalities in the discriminating-dose bioassay. Pyrethroid resistance (LC_{50} values) and mortality in the discriminating-dose bioassay were significantly correlated with a correlation coefficient (r) of -0.9796, $P<0.0001$.

TABLE 2. Mortality of Adult Tarnished Plant Bugs Exposed to Permethrin in a Glass-Vial Bioassay.

Strain[a]	N	Slope±SE	LC_{50}[b]	95% CL	χ^2	$P>\chi^2$	% mortality dis. dose[c]
S	210	1.46+0.19	4.6	(3.8-5.4)	2.1	0.7	95
SM	210	1.23+0.18	3.8	(2.8-4.6)	2.2	0.7	98
RL	300	1.25+0.13	52.1	(44.9-60.4)	8.6	0.3	9
RH	240	1.42+0.17	11.1	(9.5-13.0)	5.4	0.4	64
RS	270	1.34+0.15	37.3	(31.9-42.9)	2.0	0.9	30
RI	240	0.70+0.11	29.1	(22.4-39.8)	4.6	0.5	48
RW	210	1.15+0.16	62.0	(50.8-75.6)	3.2	0.5	2
RD	240	1.25+0.16	9.6	(8.1-11.3)	2.3	0.7	78

[a] Adults tested were from susceptible (S) and pyrethroid-resistant (RL) laboratory colonies reared at Stoneville, MS. A cross between the resistant (RL) and susceptible (S) colonies produced the hybrid colony (RH) with pyrethroid resistance. A susceptible population was collected from weeds near Monticello, AR (SM), and pyrethroid-resistant adults were also collected from cotton or weeds near cotton at Stoneville (RS), Indianola, MS (RI), Wainwright, LA (RW), and Dermott, AR (RD) for testing.

[b] Insecticide concentrations are micrograms of permethrin per vial; survival was scored at 24 h.

[c] Based on exposure of at least 50 adults (2 adults per vial) for 3 h in 20-ml glass vials treated with 15 μg of permethrin.

Spray Chamber Tests. The hybrid adult plant bugs (RH) in the first test had a mortality of 64% in the discriminating-dose bioassay, with an LC_{50} value for permethrin of 11.1 μg/vial (Table 3). Mortality from caging hybrid adults on cotton terminals treated with formulated permethrin in the spray chamber was only 3.6%, which was significantly lower ($F = 8.36$; df = 2, 4; $P = 0.04$) than the 41.9% mortality obtained in the test with the susceptible bugs. No mortality occurred in the plant bugs from the highly pyrethroid-resistant laboratory colony (RL) caged on the cotton terminals. In the second test, the pyrethroid resistant adult plant bugs collected from weeds in AR had a mortality of 78% in the discriminating-dose bioassay and an LC_{50} for permethrin of 9.6 μg/vial. Their mortality when caged on the treated cotton terminals was 8.7% which was significantly lower ($F = 25.29$; df = 1, 2; $P = 0.04$) than the 50.8% mortality obtained in the test with the susceptible bugs. These results showed that the discriminating-dose bioassay could detect pyrethroid resistant populations, and that mortality in the pyrethroid resistant populations was significantly lower than mortality in susceptible populations when exposed to a formulated pyrethroid insecticide under simulated field conditions.

TABLE 3. Mortality of Adult Tarnished Plant Bugs Caged (24 h) on Cotton Terminals Treated in a Spray Chamber with Pounce (Permethrin). Different Pyrethroid Resistance Levels in the Colonies are Shown as LC_{50} Values for Permethrin (Determined in a Glass-Vial Bioassay), and as Mortality in a Discriminating-Dose Bioassay.

Colony[a]	LC_{50}[a]	% mortality discriminating dose[b]	% mortality spray chamber[c]
Test 1			
RH	11.1	64	3.6b
S	4.6	95	41.9a
RL	52.1	9	0.0b
Test 2			
RD	9.6	78	8.7b
S	4.6	95	50.8a

[a] See footnotes a and b Table 2.

[b] Mortality based on 100 adults of each colony exposed for 3 h (2 adults per vial) in vials treated with 15 μg of permethrin.

[c] In each test means in a column followed by a common letter are not significantly different ($P=0.05$; least significant difference test [SAS Institute 1989]). Mortality corrected for check mortality using Abbot's (1925) formula. Percent mortality is the mean for 3 replications with 20 adults per replication caged on cotton terminals (2 adults per cage) treated with Pounce (0.11 kg AI/ha).

In summary, a discriminating-dose bioassay for detecting pyrethroid resistance in populations of adult tarnished plant bugs was developed and tested. The bioassay required testing of at least 50 adults from each test population by exposing them two per vial in glass vials treated with 15 μg of permethrin per vial. The bioassay is easy to use and results are obtained rapidly in 1.5 to 3.0 h. Three tarnished plant bug populations identified as having pyrethroid resistance with the discriminating-dose bioassay were caged on cotton terminals treated with a formulated pyrethroid (Pounce). Mortalities in the pyrethroid-resistant plant bug populations were significantly lower than the mortalities obtained with the susceptible

bugs. These results indicated that the discriminating-dose bioassay could be used to predict possible control problems with pyrethroid-resistant bugs and pyrethroid insecticides.

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AMITRAZ OR COUMAPHOS EFFICACY TESTS IN GUATEMALA FOR CONTROL OF *VARROA JACOBSONI*[1] IN HONEY BEES[2]

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ABSTRACT

Field studies in southwestern Guatemala demonstrated that plastic strips impregnated with either amitraz or coumaphos controlled *Varroa jacobsoni* Oudemans in colonies of honey bees, *Apis mellifera* L. In the spring of 1997, four groups of 12 colonies each were given two plastic strips in the broodnest containing one of four concentrations of amitraz (5, 7.5, 10 or 12.5%). A fifth group was untreated. All four amitraz treatments were highly effective (97 to 99% mite mortality) in reducing numbers of varroa over a 47-day treatment period. In the fall of 1997, a similar study was done with coumaphos-impregnated plastic strips placed in the broodnest or attached to the hive entrance. Four groups of ten colonies each received treatment, and the remaining six colonies were untreated controls. Broodnest treatments were one, 10% strip; or two, 10% strips; or two, 1% strips per colony. Two 10% strips were attached to the hive entrance in the fourth group. One or two 10% strips were effective against varroa (91- 97% reduction) when placed in the broodnest or across the hive entrance. The 1% strips and controls were ineffective and failed to reduce the mite population.

INTRODUCTION

Currently only one acaricide, Apistan(R)(tau-fluvalinate-impregnated plastic strip), has full EPA registration for control of *Varroa jacobsoni* Oudemans in the United States[7]. It is widely known that beekeepers need alternative acaricides to protect honey bee, *Apis mellifera* L., colonies from varroa (Milani and Vedova 1996). The need for additional acaricides became critical with the discovery of fluvalinate-resistant varroa in Italy (Milani 1994, 1995), and now in the U.S. (Eischen 1997; Baxter et al. 1998; Elzen et al. 1998,1999).

Varroa mites were first detected in the U.S. in 1987. Unfortunately, they spread rapidly

[1]Mesostigmata: Varroidae

[2]Mention of a trade name does not mean endorsement by USDA.

[3]Apicultura, MOSCAMED, Coatepeque, Quetzaltenango, Guatemala

[4]Bayer Corp., Agric. Div., P. O. Box 390, Shawnee Mission, KS 66201-0390, USA

[5]Y-Tex Corp., P. O. Box 1450, Cody, WY 82414 USA

[6]USDA-APHIS Regional Office, Guatemala City, Guatemala

[7]Coumaphos plastic strip granted Section 18 approval January 1999

across the entire country because of frequent transport of colonies, package bees and queens. Acaracides have emerged as the only cost effective way of controlling this parasite. Since acaricide use has become so prevalent in the U.S., there is a clear need for efficacy testing in other countries where varroa populations are still increasing (Wilson et al. 1998), acaricide application is less frequent and there is no evidence of fluvalinate resistance. Thus, the research reported in this article was done in southwestern Guatemala in the spring and fall of 1997.

MATERIALS AND METHODS

Two separate studies were conducted to evaluate the efficacy of amitraz- and coumaphos-impregnated plastic strips for varroa control. Both tests were accomplished in cooperation with Guatemalan beekeepers and personnel from the Moscamed Program (a joint effort between Mexico, Guatemala and the United States to control the Mediterranean fruit fly, *Ceratitis capitata* Wiedemann). Each bee colony used in the tests was housed in a single deep Langstroth hive body full of bees and brood with a laying queen and adequate stores of honey and pollen. There were no detectable brood diseases in the colonies. All colonies (n=106) were located near the town of Coatepeque in the state of Quetzaltenango, Guatemala. Colony placement within an apiary reduced the amount of potential bee drift by providing 3-4 m of space in all directions. At the beginning of study, all bee colonies were heavily infested with adult varroa mites.

In one study, two amitraz-impregnated plastic strips (each 2.5x18x0.3 cm) were hung in the center of the broodnest of each colony. On 17 April 1997, 60 colonies were divided into five groups of 12. Four groups received strips from one of four concentrations: 5%, 7.5%, 10% and 12.5%. The fifth group of 12 colonies served as untreated controls. An apron trap was assembled in front of each colony to retain dead adult bees (see Rhodes and Wilson 1978). All colonies were fitted with a screen cover over a DeWill(R) sticky board placed in the bottom of the colony to trap dead and dying varroa. After 24 h, the sticky boards were removed and dead adult mites counted. After 45 days, the amitraz treatments were removed and the bees left untreated for 48 hours. On day 47, a new single Apistan(R) strip along with a screen and sticky board were placed in each of the 60 colonies. The sticky boards were removed after 24 h and the final mite count made. Data are shown in Table 1. Amitraz strips were provided by the Y-Tex Corp.

The percentage reduction in the varroa population was calculated from the first and the final number of varroa mites captured on sticky boards. The mite knockdown resulted from the varroa that were parasitizing adult bees during a period when two or more complete cycles of bee brood emerged. Calculating the percentage varroa reduction was similar to the parasite reduction formula developed by Lloyd et al. (1983) for livestock research and listed for bees in Wilson and Collins (1994). The final mite-drop number was divided by the first mite-drop number to give the percentage reduction for each colony. A mean was determined for each treatment group and compared to the mean for untreated controls by a statistical program. If the final mite count was higher than the first, the mite population had increased which indicated a lack of efficacy in the test chemical.

A second study determined the efficacy of one or two coumaphos-impregnated plastic strips (3x20x0.3 cm) per colony with strip placement in the center of the broodnest or at the hive entrance. On 20 August 1997, 40 bee colonies were divided into four groups of ten each, and six additional colonies were left as untreated controls. Two groups of ten received either one or two 10% strips hung in broodnest. The third group had two 10% strips stapled to the bottom board of each colony immediately inside the entrance where bees walked across the strip when going in or out of the hive. The fourth group received two 1% strips in the broodnest.

Screens and sticky boards were placed under the broodnest of all 46 colonies on day 1 and removed 24 h later for the initial varroa count (mite numbers ranged from several hundred to >1,000 varroa per sticky boards). After 45 days, all strips were removed and the colonies left untreated for 48 h. On day 47, one new Apistan strip was placed in the broodnest of all colonies for 24 h along with sticky boards to determine how many mites remained on the adult bees. This was the final mite count. These data were used in calculating the percentage reduction in the mite population. Data are shown in Table 2. Coumaphos strips were provided by the Bayer Corp.

Test results were evaluated by the general linear model (GLM) procedure. Treatment means were compared using least significant differences (LSD), (P=0.05) (SAS System for Windows_Release 6.11, 1989-1995).

TABLE 1. Varroa Control in Guatemalan Honey Bee Colonies Using Plastic Strips Impregnated with Amitraz.

Treatment	% Varroa Change[a]	Range	SD
5% 2-strips	-97% a	-88 to -100	4.68
7.5% 2-strips	-98% a	-94 to -100	2.11
10% 2-strips	-98% a	-94 to - 99	1.92
12.5% 2-strips	-99% a	-97 to -100	1.73
Control	+76% b	+65 to +84	8.56

[a]Means with the same letter are not significantly different.

TABLE 2. Varroa Control in Guatemalan Honey Bee Colonies Using Plastic Strips Impregnated with Coumaphos.

Treatment	% Varroa Change[a]	Range	SD
in broodnest			
10% 1-strip	-91% a	-78 to -98	7.02
10% 2-strips	-97% a	-90 to -100	3.61
1.0% 2-strips	-48% b	-26 to -90	41.53
on bottom board			
10% 2-strips	-91% a	-77 to -99	6.53
Control	+71% b	+53 to +86	12.22

[a]Means with the same letter are not significantly different.

RESULTS AND DISCUSSION

The percentage change in mite infestation levels over the 47-day test period (Tables 1 and 2) showed that both amitraz and coumaphos in plastic strips provided excellent control (91-99%) of *Varroa jacobsoni* in active honey bee colonies without causing obvious damage to adult or immature honey bees. During the 45 days of acaricide exposure, there were no accumulations of dead bees in the apron traps (general observations by J. R. Baxter). Our field studies closely agree with laboratory results in Italy. Recently Milani and Iob (1998) report that coumaphos in plastic gave 85-100% control of varroa in a laboratory study, and that coumaphos was more toxic to varroa than fluvalinate (Milani and Vedova 1996).

All four doses (5, 7.5, 10 and 12.5%) of amitraz were highly effective in killing varroa mites with 97-99% mite reduction (Table 1). The effectiveness of amitraz became apparent when the untreated control colonies showed an increase of 76% in the varroa population. These findings are supported by earlier studies on the value of using amitraz for varroa control (Ritter 1988, Lupo and Gerling 1990, Jelinski et al. 1994).

Plastic strips impregnated with coumaphos provided effective control of varroa mites in the tropical climate of southwestern Guatemala with a varroa reduction of 91-97% in colonies treated with 10% strips (Table 2). Control levels above 90% prevent rapid recurrence of varroa and are considered necessary by commercial beekeepers in successful parasite management programs. Mite control benefitted numerically but not statistically from adding a second 10% coumaphos strip per colony. One 10% coumaphos strip per colony gave an average of 91% varroa control while two 10% strips yielded 97% control (Table 2).

Two 10% coumaphos strips mounted across the base of the entrance gave a 91% reduction. The advantage of this type of placement is that treatments can be added or removed without opening the hive. According to the manufacturer (Bayer Corp.), the entrance treatment may lessen the chance of coumaphos residues in beeswax compared to placing the strips in the broodnest.

The 1% strip did not contain an adequate quantity of acaricide, and therefore was ineffective with only a 48% reduction in the varroa population. The controls had a mean increase in varroa of 71%.

ACKNOWLEDGMENT

The authors wish to thank Noe Buenrostro, Raul Rivera (USDA-ARS), Armando Girón, Saúl Perez, Antonio Paz, Roger Rubio, Onofre Pospoy and Aida Lucrecia De León Rivadeneira (MOSCAMED, Guatemala) for their technical assistance. We thank the following for administrative support: G. Santamarina, F. Hamdy, C. Lira, and Analisa Salinas. Grants to cover travel costs were received from Y-Tex Corp., Cody, WY. and Bayer Corp., Agriculture Division, Shawnee Mission, KS.

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ATTRACTION OF *PHYLLOPHAGA CONGRUA, PHYLLOPHAGA CRASSISSIMA, PHYLLOPHAGA CRINITA,* AND *CYCLOCEPHALA LURIDA* (COLEOPTERA: SCARABAEIDAE) ADULTS TO SELECTED VOLATILE COMPOUNDS

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ABSTRACT

Several plant volatiles are known to be powerful attractants for adults of a few phytophagous scarabs; however, no such information is available for *Phyllophaga* spp. in the southern United States or for *Cyclocephala lurida* Bland. In 1996 and 1997, we used standard Japanese beetle traps to field-capture beetles in Dallas, Texas, in replicated tests of 12 volatile compounds, two combination treatments and an unbaited control. The traps captured 3,473 *P. crassissima* (Blanchard), 596 *P. congrua* (LeConte), 378 *P. crinita* Burmeister, and 229 *C. lurida* Bland. Different species of beetles responded to different lures. *Phyllophaga crassissima* preferred anethole over any other lure, with second preference being either geraniol or eugenol. The 1:1:1 mixture of phenyl propionate: eugenol: geraniol (PEG); the 1:1 mixture of eugenol: geraniol (EG); and phenyl propionate by itself also were preferred over the unbaited control. Linalool, cinnamyl alcohol, 1-nonanol, 1-octanol, n-hexanoic acid, valeric acid, octyl butyrate, and butyl sorbate were statistically equivalent to the unbaited control. *Phyllophaga congrua* responded strongest to phenyl propionate, the EG mixture, and linalool, with the latter two being statistically indistinguishable from geraniol, eugenol, and the PEG mixture. Butyl sorbate apparently was repellant to *P. congrua*. Neither *P. crinita* nor *C. lurida* responded significantly to any of the tested lures. Although the relative attractiveness of the various chemicals was the same for males and females of any given species, significantly more males were collected for *Phyllophaga* spp. (23.4 M: 4.2 F *P. crassissima*; 2.8 M: 1.7 F *P. congrua*; 2.9 M: 0.1 F *P. crinita*); in *C. lurida*, more females (0.8 M: 1.0 F) were collected. These results indicate that different species of phytophagous scarabs respond very differently to potential lures. Responsiveness to these lures (most of which are found in plants) was related to whether the adult beetle is a feeding stage.

INTRODUCTION

Several types of attractants are used to lure phytophagous scarabs to traps. The most commonly employed attractant is light, usually ultra-violet (UV) light. Among the obvious advantages of light traps are their ready availability and their attractiveness to a broad

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range of nocturnal scarabaeids. Unfortunately, light traps also are highly attractive to numerous moths and other non-target species, are not effective for diurnal species, and need a source of electricity. Sex pheromones are too specific for many situations because they normally attract only one sex of a single species. Volatile plant-based chemicals used by insects to identify a potential food source generally have the advantages of attracting both sexes of several target species, having little effect on most non-target arthropods, being cheap to produce, and having no requirement for a source of electricity. They also can enhance the effectiveness of pheromone traps, as has been done for the Japanese beetle, *Popillia japonica* Newman (Ladd 1986).

Research on the use of plant-based chemical attractants in trapping of adult Japanese beetles (*P. japonica*) began in 1919. Over the intervening years, a broad range of plant-based chemicals with various levels of attractiveness when used alone or in combination has been identified (reviewed in Fleming 1969). Langford et al. (1940) found that chemical-baited traps reduced crop damage by Japanese beetle, and Hamilton et al. (1971) reported that an isolated Japanese beetle population was reduced 50% by such trapping.

Metzger and Sim (1933) noted that many other species of scarabs are captured in Japanese beetle traps, either due to attractiveness of the chemicals (geraniol and eugenol) in the bait or as a result of physical properties of the traps themselves. They reported 24 assorted species of scarabs to have been recovered incidentally from one set of nearly 400 traps run in 1932, near Woodstown, N.J. Among those species were *Phyllophaga ephilida* (Say) (200 specimens), *P. futilis* LeConte (125 specimens), *P. fervida* (Fab.) (15 specimens), and *P. hirticula* (Knoch) (120 specimens). Proprawski and Yule (1992) tested several compounds as attractants for *P. anxia* (LeConte), and found that only n-hexanoic acid lured more beetles than did his arbitrary standard lure, a mixture of § -phenylethyl propionate : eugenol (9:1; v/v). No related work is known for *Phyllophaga* spp. in the southern United states or for *Cyclocephala* spp.

The purpose of the present research was to determine whether selected chemicals known to lure some other scarab adults would attract adults of certain *Phyllophaga* spp. [(principally *P. congrua* (LeConte), *P. crassissima*(Blanchard), and *P. crinita* (Burmeister)], *Cyclocephala lurida* Bland, or other scarabs present in northern Dallas, Dallas County, Texas.

MATERIALS AND METHODS

The study was conducted at the Texas A&M University Research and Extension Center in Dallas from 3 May to 10 July in 1996 and from 18 April to 23 July in 1997. Chemicals were tested using Trécé Japanese beetle traps fitted with lure dispensers (Klein and Edwards 1989). Each trap was baited by adding 5ml of the chemical attractant to the lure dispenser to saturate the absorbent material. The charged lure-holder was placed upside down on top of the trap. Dispensers were refilled weekly. Trapping sites were in a large grassy area, part of which contained small oak (*Quercus* spp.) trees. Traps were hung on rebar poles with cable ties so that the lure-holders were 50cm above the ground. The rebars were hammered into the ground at ca. 45° angle and were placed 3-meters apart. In each location (replication), traps were arranged in two rows with 3 meters between the rows and between traps within rows. Traps in adjacent rows were offset from each other. In 1996 and 1997, respectively, five and six replications in a randomized complete block design were employed, with about 30 meters between replications. Ten and 12 different chemicals or combinations of chemicals plus an unbaited control (Table 1) were tested in 1996 and 1997, respectively. Insects captured in the traps were collected either daily or twice a week, according to the intensity of beetle flight. Beetles were stored in a freezer until they could be identified later to family or species.

Ultraviolet light insect traps commonly are used to monitor scarab flights; however, catch size is dependent on trap location. In the interest of avoiding competition from having a light trap near the chemical traps, no ultraviolet traps were used as a standard in this test.

Total numbers of beetles of each species captured per year per trap were subjected to analyses of variance using SuperAnova® version 1.11, with treatment separations determined by Fisher's Protected LSD test (Gagnon et al. 1989).

RESULTS AND DISCUSSION

Four species of phytophagous scarabs were captured in 1996 and 1997; those include 3,473 *P. crassissima*, 596 *P. congrua*, 378 *P. crinita*, and 229 *C. lurida*. Light trap catches (Crocker unpub. data) have shown all four of these beetles to be abundant in this general area every year for the past 19 years (Crocker unpub. data).

TABLE 1. Average Total Numbers of Phytophagous Scarabs Captured per Trap per Season (1996 and 1997) in Trécé Japanese Beetle Traps Baited with Selected Chemical Lures.

	Number of Scarabs/Trap/Season[a]			
Chemical	*P. crassissima*	*P. congrua*	*P. crinita*	*C. lurida*
Butyl sorbate	0.3 a	0.2 a	0.0 a	0.0 a
Octyl butyrate	0.9 a	0.9 ab	0.0 a	0.0 a
Valeric acid	1.9 a	1.0 ab	1.4 a	0.5 a
N-hexanoic acid	2.0 a	1.0 ab	1.2 a	0.8 a
1-Octanol	2.8 a	1.6 bc	0.9 a	2.0 a
1-Nonanol	2.8 a	2.3 c	1.9 a	0.6 a
Cinnamyl alcohol	3.0 a	1.4 bc	2.1 a	0.6 a
Untreated check	3.7 a	1.8 bc	1.7 a	0.1 a
Linalool	5.2 ab	4.0 de	2.0 a	0.5 a
EG[b]	9.7 b	3.9 de	1.7 a	0.2 a
Phenyl propionate	10.7 b	4.9 e	1.8 a	0.0 a
PEG[c]	10.7 b	3.4 d	1.6 a	3.2 a
Eugenol	31.7 c	3.3 d	1.8 a	1.2 a
Geraniol	35.3 c	3.2 d	1.7 a	0.0 a
Anethole	80.0 d	2.2 c	1.5 a	1.4 a

[a] ANOVA statistics for treatment (TRT), sex (SEX), replication (REP), and residual (RESID) (based on square-root transformed data) were *P. crassissima* ($F_{TRT} = 31.43$, $P_{TRT} \leq 0.0001$; $F_{SEX} = 105.92$, $P_{SEX} \leq 0.0001$; $F_{REP} = 6.19$, $P_{REP} \leq 0.0001$), *P. congrua* ($F_{TRT} = 3.64$, $P_{TRT} \leq 0.0001$; $F_{SEX} = 21.29$, $P_{SEX} \leq 0.0001$; $F_{REP} = 19.02$, $P_{REP} \leq 0.0001$), *P. crinita* ($F_{TRT} = 0.48$, $P_{TRT} = 0.94$; $F_{SEX} = 129.43$, $P_{SEX} \leq 0.0001$; $F_{REP} = 6.07$, $P_{REP} \leq 0.0001$), and *C. lurida* ($F_{TRT} = 0.78$, $P_{TRT} = 0.69$; $F_{SEX} = 11.59$, $P_{SEX} \leq 0.0001$; $F_{REP} = 14.15$, $P_{REP} \leq 0.0001$); for all species, $df_{TRT} = 14$, $df_{SEX} = 1$, $df_{REP} =$, and $df_{RESID} = 226$. Treatments followed by the same letter are not significantly ($P \leq 0.05$) different, according to Fisher's Protected LSD test (Gagnon et al. 1989).

[b] EG = Eugenol-Geraniol (1:1).

[c] PEG = Phenyl propionate: eugenol: geraniol (1:1:1).

Beetles' preferences among the lures differed according to the species of beetle (Table 1). *Phyllophaga crassissima* preferred anethole over any other lure, with second preference being for geraniol or eugenol. The 1:1:1 mixture of phenyl propionate: eugenol: geraniol (PEG), the 1:1 mixture of eugenol: geraniol (EG), and phenyl propionate by itself also were preferred over the unbaited control. The other chemicals (linalool, cinnamyl alcohol, 1-nonanol, 1-octanol, n-hexanoic acid, valeric acid, octyl butyrate, and butyl sorbate) were statistically equivalent to the unbaited control. No significant differences in the preferences were noted when data for either sex were examined separately.

Phyllophaga congrua responded strongest ($P = 0.05$) to phenyl propionate, the EG mixture, and linalool, with the latter two being indistinguishable from the response to geraniol, eugenol, and the PEG mixture. One-nonanol, anethole, 1-octanol, cinnamyl alcohol, n-hexanoic acid, and valeric acid were not attractive to *P. congrua*. Butyl sorbate apparently was repellant, in comparison to the unbaited control. Separate examination of the data for either sex yielded similar results to those obtained for both sexes grouped together. Neither *P. crinita* nor *C. lurida* reacted significantly to any of the lures.

Overall, significantly ($P = 0.05$) more males per trap were collected of the *Phyllophaga* spp. (23.4 M: 4.2 F *P. crassissima*; 2.8 M: 1.7 F *P. congrua*; 2.9 M: 0.1 F *P. crinita*) and significantly more females per trap were collected of *C. lurida* (0.8 M: 1.0 F). These sex-related differences may be due primarily to an overall greater abundance of males in flights of the four species, especially for those species that were not significantly attracted to any of the lures.

Our results indicate that even congeneric scarab adults responded very differently to various potential lures. The two species that reacted most strongly to some of the chemicals (*P. crassissima* and *P. congrua*) both feed intensively in the adult stage; whereas, *P. crinita* and *C. lurida* feed little if at all as adults (Crocker, unpublished data). Thus, it appears that response to these chemicals, most of which are found in plants, was related to whether the adult is a feeding stage. A broad range of chemicals, alone and in combinations, has been found to be attractive to the Japanese beetle (Fleming 1969). Further testing of the present compounds and other chemicals is needed in order that a broad understanding may be gained of the role of volatile plant compounds on host plant selection and the potential usefulness of chemical lures in scarab management.

ACKNOWLEDGMENT

We thank Allen E. Knutson, Michael E. Merchant, James A. Reinert, and Edgar A. Rojas (Texas A&M University REC, Dallas, Texas) for critical review of this manuscript.

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BASIC BIOLOGY OF *MELANOPLUS ARIDUS*[1], A FORBIVOROUS GRASSHOPPER COMMON ON SOME SOUTHWESTERN RANGELANDS

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ABSTRACT

Melanoplus aridus (Scudder) (Orthoptera: Acrididae) is a common southwestern rangeland grasshopper that has rarely been described. Laboratory and field studies were initiated to determine morphological characteristics and to better understand the basic biology of this grasshopper. *M. aridus* has five nymphal instars. Definitive morphological characteristics were determined for each instar. These included the number of antennal segments, body and hind femur length, and wing pad shape and size. Phenologies at the study site and egg pod characteristics of laboratory-reared grasshoppers were similar to other Melanopline grasshoppers. *M. aridus* survived equally well on two species of snakeweed and can significantly reduce above ground biomass of these weeds. This grasshopper preferred threadlleaf snakeweed, *Gutierrezia microcephala* (DeCondolle) Gray, to all other plants tested in choice and no-choice experiments; however, it was able to survive on a variety of other plants.

INTRODUCTION

Melanoplus aridus (Scudder) (Orthoptera: Acrididae), is associated with threadleaf snakeweed, *Gutierrezia microcephala* (DeCondolle) Gray, in south central Arizona (Gardner 1996, Gardner and Thompson 1998). This species was the most abundant grasshopper present on *G. microcephala* dominated rangeland during 1994 and 1995 at this site; however, basic biological information of *M. aridus* is lacking.

Helfer (1953) listed *M. aridus* in his collection of Orthopterans in Arizona. The species is known from Texas to California and is found from the lower Sonoran desert up to elevations of 2,900 m. Adults range in size from 13 to 20 mm and are brachypterous. Although *Dalea, Eriogonum, Baccharis,* and "field crops" were listed as host plants (Helfer 1953), little information is available on specific hosts and damage potential of *M. aridus.*

The genus *Melanoplus* is a very diverse group of grasshoppers. Many, such as *M. bowditchi* Scudder, are specialists feeding only on limited host species (Mulkern et al. 1969). Others are generalists, although populations rarely cause enough damage to be considered pests. However, several species, such as *M. sanguinipes* (Fabricius), are serious pests of crops and rangelands throughout the Western United States (Pfadt 1994).

[1]Orthoptera: Acrididae

G. microcephala and the closely related broom snakeweed, *G. sarothrae* (Pursh) Britton & Rusby, are common in many arid and semi-arid habitats of western North America (Lane 1982) and are considered undesirable by ranchers because they are toxic to livestock and compete with other desirable forages (McDaniel and Sosebee 1988). Greater than 400 species of insects have been collected from these snakeweed species (Foster et al. 1981, Richman and Thompson 1999). The grasshopper, *Hesperotettix viridis* (Thomas), is capable of reducing snakeweed biomass and density (Thompson et al. 1996), and in high numbers can cause significant mortality (Thompson et al. 1995). Gardner and Thompson (1998) suggested that *M. aridus* might play a role in suppressing *G. microcephala* when their population densities are high. This study was designed to collect basic biological information and to determine the host specificity of *M. aridus*.

METHODS

Descriptive Characteristics. Wing pad characteristics of each instar were determined from laboratory-reared grasshoppers. Overall grasshopper length (measured from the anterior most point of the vertex to the posterior most point of the abdomen with the grasshopper in a resting position) and femur length (measured from anterior tip to posterior tip) were measured using a micrometer (±0.1 mm). Antennal segments were counted using a 40X dissecting scope. Measurements were taken on ten grasshoppers from each instar.

Phenology. M. aridus phenology was estimated by collecting grasshoppers bimonthly at the Buenos Aires National Wildlife Refuge (BANWR) in Pima County, Arizona, using sweep samples. A standard 38-cm sweep net was used to collect a minimum of 100 grasshoppers during each sampling period. Bimonthly sampling began 21 April 1994 and 17 May 1995 and ended 10 September 1994 and 22 September 1995. All grasshoppers collected were stored in plastic bags and frozen at -10°C. Grasshoppers were later sorted to species. All *M. aridus* were sorted to instar using differences in wing pad characteristics as determined from laboratory-reared grasshoppers. Stadium duration of the grasshoppers was determined using the stage frequency analysis option of POPSYS (Manly 1990) as in Gardner (1996). Degree-days were calculated from daily maximum and minimum temperatures recorded by a National Oceanic and Atmospheric Administration weather station located ≈3 km from the site. The minimum developmental temperature used for degree-day calculations was 21°C (Gardner 1996).

Development. Mean stadium length of instars was determined at 35°C in the laboratory. Both *G. sarothrae* and *G. microcephala* were tested to determine if stadium length of *M. aridus* varied when fed the two species of snakeweed. Grasshoppers were collected from the BANWR on 12 May 1996. First-instar *M. aridus* were placed randomly in individual cages as described by Gardner (1996). Each cage was then placed over ≈10-cm high individual snakeweed stems cut with a razor blade from plants in the Las Cruces, New Mexico, vicinity. Cages were set on wire mesh (mesh size=0.8 cm x 0.8 cm) supports covered with water resistant paper. Large blocks of water saturated Instant Prime® florist foam (Greenleaf Wholesale Florists Inc., Albuquerque, NM) were set under the supports and were contained in plastic cafeteria trays (Consolidated Plastics, Twinsburg, OH). Individual stems were pushed through the paper and into the florist foam. Trays were then placed in an environmental chamber at 35°C with a 12:12 photoperiod. Stems were changed daily and distilled water was added to the trays so that the food supply remained fresh.

Daily observations were made to determine if molting had occurred. Presence of exuvia in the cages was evidence of molting. When an exuvium was found, the date was recorded and the exuvium removed. Grasshopper deaths were also recorded. Data were analyzed using least significant means (PROC GLM, LSMEANS; SAS, Version 6.10, Cary, NC).

Egg Pod Characteristics. Egg pods were collected from caged grasshoppers in the laboratory. Grasshoppers were captured from the field as either second or third instars and reared to the adult stage in the laboratory. Five males and five females were placed in 0.25 m^2 cages and fed fresh *G. microcephala* stems daily. Five 30 ml cups, set flush with the bottom of the cages, were filled with sand for egg deposition. Egg pods were dry sieved after all grasshoppers had died. Length and width of egg pods were measured using a micrometer (±0.1 mm) and the number of eggs in each pod was counted. Egg color and shape were also recorded.

No-choice Host Test. Laboratory no-choice host tests were conducted in cages identical to those used for the development experiments. Thirteen individual stems of ten plant species were cut from plants in the field for each of five replicates. Plants used included three common field crops (alfalfa, *Meticago sativa* L., chile, *Capsicum annuum* L., and cotton, *Gossypium hirsutum* L.); two grasses (Sudan grass, *Sorghum* sp., and mesa dropseed, *Sporobolus flexuosus* (Thurb. ex. Vasey) Rydb.); and five weed species common to the refuge where the grasshoppers were collected (threadleaf snakeweed, burroweed, *Aplopappus tenuisectus* (Greene) Blake, desert broom, *Baccharis sarothroides* Gray, redroot pigweed, *Amaranthus retroflexus* L., and Russian thistle, *Salsola iberica* Sennen & Pau).

One adult *M. aridus* grasshopper was enclosed in each of ten cages containing an individual stem of one of the test plants per replication. Stems were placed in water saturated florist blocks to maintain freshness during the feeding trial. Three additional cages per replication were used as controls with no grasshopper feeding to determine water loss for each species of plant. Green weight of each stem was determined (±0.1mg) using a Mettler HR35 balance prior to being used in the experiment. Following the feeding period (usually 1-2 days) the stems were removed and re-weighed. Stems were then oven dried for 48 hours at 60°C and weighed again. Ten feeding trials were conducted.

Choice Host Test. Choice tests were conducted in 0.25 m^2 screen cages in a laboratory rearing room at 30°C and a 12:12 photoperiod. Plants used in the choice test were dictated by grasshopper survival in the no-choice tests. These included *G. microcephala*, *Baccharis sarothroides*, *Aplopappus tenuisectus*, *Meticago sativa*, *Salsola iberica,* and *Sorghum* sp. Individual stems of each of these plants were weighed in the same manner as the no-choice test to determine the amount consumed. Stems were maintained in water saturated florist blocks to maintain freshness and changed at the end of each feeding period (2-3 days). The six species of plants were presented randomly to eight, same age adult *M. aridus* grasshoppers in each cage. Grasshopper deaths were recorded so that the amount consumed per day per grasshopper could be determined. Feeding trials were replicated five times during each of 11 feeding periods. Three additional cages were set up without grasshoppers to determine water loss as in the no-choice tests.

Green to dry weight ratios for both the choice and no-choice tests were determined using regression analyses. Daily consumption of individual grasshoppers was determined using linear equations from the regressions. Means were separated using least significant means (PROC GLM, LSMEANS; SAS, Version 6.10, Cary, NC).

RESULTS

Descriptive Characteristics. Wing pad size and shape was determined for each instar (Fig. 1). These characteristics were used to differentiate between age classes for the phenology study. Differentiation between first- and second-instar *M. aridus* is difficult and time consuming because of the magnification required to see differences in wing pad shape and to count antennal segments. Therefore, first and second instars were not separated in the phenology study.

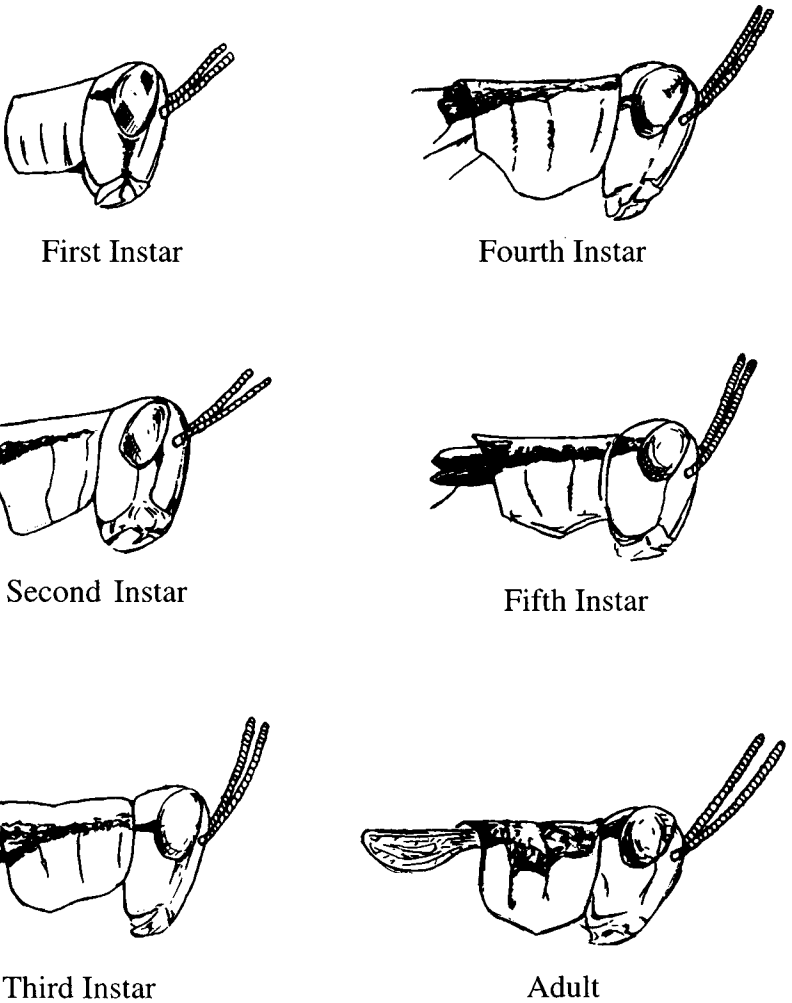

FIG. 1. *Melanoplus aridus* wing pad size and shape in relationship to the head and thorax. Drawings by Patrick Long.

Femur and body length, as well as the number of antennal segments of *M. aridus* (Table 1), are similar to that of other small Melanopline grasshoppers described by Pfadt (1994). However, these characteristics may be useful in helping to identify and age this species. The number of antennal segments may be of particular importance. These don't tend to overlap between instars so differentiation between age classes is possible. Magnification of 40X is necessary to count individual antennal segments of first- and second-instar *M. aridus*.

Phenology. *M. aridus* typically have seven life stages including egg, five nymphal instars and adult; however, an additional molt is somewhat common (28 of 569), at least in laboratory-reared grasshoppers. The Kempton model for multi-cohort data was used to

TABLE 1. *M. aridus* Descriptive Measurements (n=10 for each).

	Antennal Segments		Femur Length	Body Length
Instar	Mean ± SE	Range	Mean (mm) ± SE	
1st	13.1 ± 0.18	12-14	3.42 ± 0.2	6.14 ± 0.71
2nd	16.3 ± 0.26	15-17	4.55 ± 0.18	7.19 ± 0.21
3rd	22.5 ± 0.23	21-23	7.28 ± 0.27	11.71 ± 0.97
4th	23.7 ± 0.15	23-24	8.56 ± 0.22	12.55 ± 0.25
5th	25.6 ± 0.17	25-26	9.53 ± 0.21	13.51 ± 0.38
Adult male	26.6 ± 0.22	26-28	9.98 ± 0.11	16.83 ± 0.22
Adult female	25.9 ± 0.18	26-28	11.93 ± 0.02	19.45 ± 0.39

TABLE 2. Mean Stadium Length (Days) ± SEM of *M. aridus* Instars in South-Central AZ During 1994 and 1995.

Instar	1994[a]	1995[b]
1st and 2nd (combined)	44.51 ± 2.76	48.58 ± 2.85
3rd	38.25 ± 1.21	20.19 ± 1.04
4th	14.75 ± 0.89	15.82 ± 0.99
5th	10.82 ± 0.72	9.42 ± 0.88

[a] SEM adjusted using heterogeneity factor of 10.76 (279.78 deviance/26df)

[b] SEM adjusted using heterogeneity factor of 6.25 (137.42 deviance/22df)

determine stadium durations for each instar (Table 2). Stadium duration was similar for all instars between years other than the extended third instar in 1994. First and second instars also appear longer than other Melanopline grasshoppers. However, these long stadium durations may be due to long hatching periods. *M. aridus* began hatching at BANWR between 22 accumulated degree days (ADD) (28 March) and 64 ADD (17 May), and continued to hatch through July. The extension of the third instar in 1994 does not appear to be related to degree-day accumulation differences between years (Fig. 2). Degree-days accumulated similarly between years until mid-April, then accumulated faster in 1994. Grasshopper development should have occurred at an accelerated rate in 1994; but because of an unknown factor, it appears that third instar development was slowed for several weeks in June (Fig. 3). The maximum developmental threshold for *M. aridus* is unknown although the maximum for *H. viridis*, the second most common grasshopper at this site, is 40°C (Gardner 1996). The average maximum temperature in June was 37.38°C and 33.49°C in 1994 and 1995, respectively. It is unlikely that development of this desert species would be slowed at these temperatures.

Food quality may also have been a contributing factor in the slowed development in 1994. Precipitation from 1 November 1993 through 30 June 1994 was 16.05 cm; whereas, precipitation from 1 November 1994 through June 30 1995 cm was 33.02 cm (NOAA 1993,

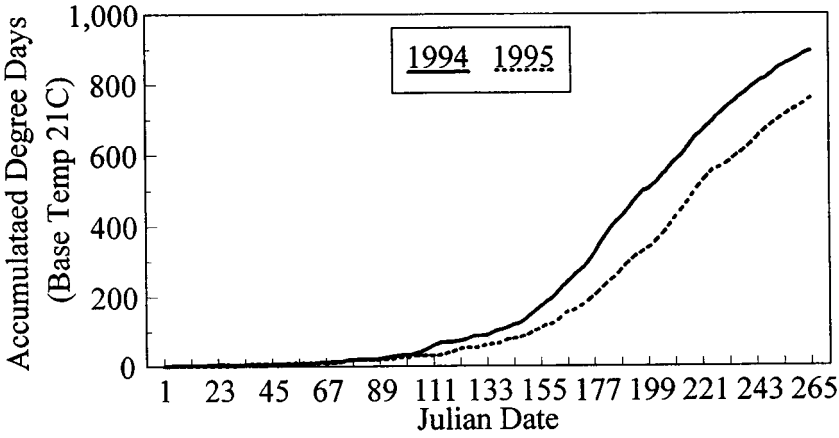

FIG. 2. Accumulated degree days at study site in 1994 and 1995.

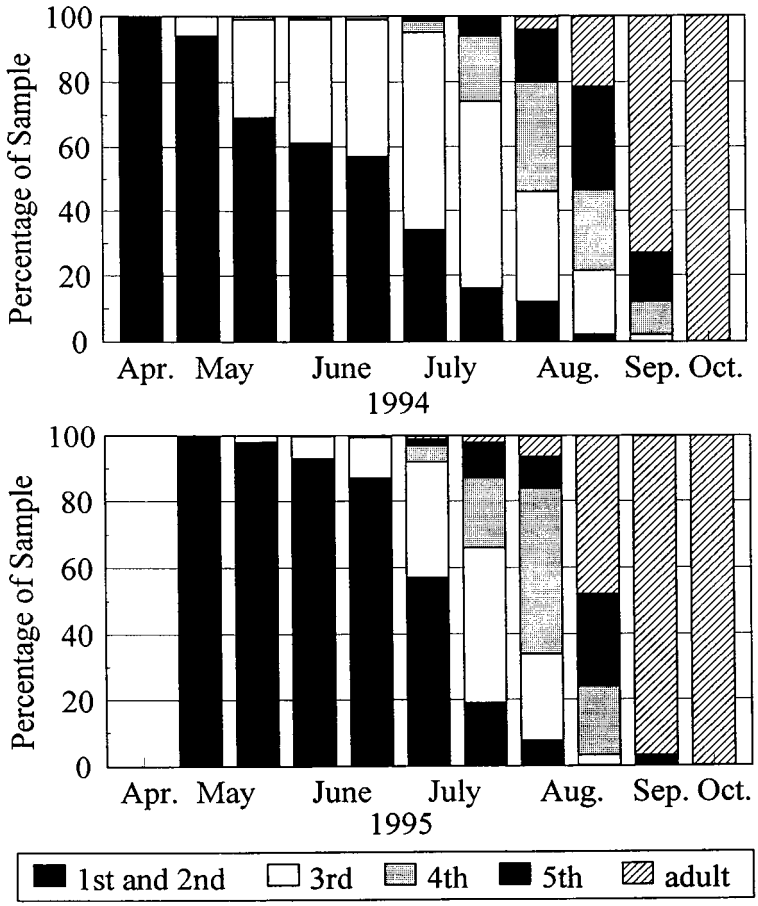

FIG. 3. *M. aridus* phenology as percent of sample in 1994 and 1995 in south central Arizona

1994, 1995). Snakeweeds are capable of photosynthesis (and therefore growth) in the winter (Depuit and Caldwell 1975, Pieper and McDaniel 1989). Plants capable of producing more photosynthetic tissue from increased precipitation should provide better nutrition to herbivores utilizing them, thus they should develop at a faster rate.

Development. M. aridus stadium lengths were not significantly different ($P<0.05$) between *G. microcephala* and *G. sarothrae* (Table 3) suggesting that this grasshopper is capable of developing on both species equally well. Both species of snakeweed are distributed throughout the southwestern United States, but it is unknown whether this grasshopper prefers one or the other where both occur.

TABLE 3. Mean Stadium Durations (days) ± SEM of *M. aridus* Reared at 35°C on *G. microcephala* and *G. sarothrae*.

		Means (days) ± SE		
Instar	n	*G. sarothrae*	n	*G. microcephala*
2nd	52	11.35 ± 0.66	69	11.30 ± 0.52
3rd	82	11.76 ± 0.66	75	11.75 ± 0.71
4th	73	12.32 ± 0.65	70	12.41 ± 0.68
5th	57	13.98 ± 0.89	63	13.76 ± 0.51
6th (additional)	14	15.07 ± 2.48	14	13.50 ± 0.80

TABLE 4. *M. aridus* Egg Pod Characteristics from a Laboratory Reared Population.

	n	Mean ± SE
Length (mm)	15	10.05 ± 0.99
Width (mm)	15	4.17 ± 1.14
Number of eggs per pod	11	9.28 ± 1.65

Egg Pod Characteristics. M. aridus egg pods are similar in size and shape to many other Melanopline grasshoppers and contain a similar number of eggs per pod (Table 4). The number of egg pods per female was not determined.

Choice and No-choice Tests. M. aridus is a polyphagous grasshopper based on both choice and no-choice tests (Table 5). Although it preferred snakeweed over all other plants tested, it was able to develop on many of the other plants, except for chile, cotton, and redroot pigweed. Both the chile and cotton plants fed to the test grasshoppers were known to be free of pesticides. First- and second-instar *M. aridus* were observed feeding in stands of pigweed in the field; however, it is unknown why they did not survive on redroot pigweed in the laboratory. Pigweed was common at BANWR only in low lying areas that collected and stored precipitation. Due to the rainfall patterns of the region, these areas provide more lush vegetation in the spring when the upland vegetation tends to be dry. *M. aridus* may have been feeding on pigweed only for the higher moisture content it provided.

TABLE 5. Daily Consumption (mg Dry Weight) of Various Host Plants by Individual Adult *M. aridus* in Choice and No-choice Tests.

Plant	No-Choice (Mean ± SE)[a]	Choice (Mean ± SE)[a]
Gutierrezia microcephala	46 ± 4 a	19 ± 4 a
Aplopappus tenuisectus	34 ± 3 b	13 ± 2 b
Baccharis sarothroides	25 ± 3 c	7 ± 1 cd
Meticago sativa	23 ± 2 c	12 ± 1 bc
Salsola iberica	16 ± 2 c	1 ± 1 e
Sorghum sp.	9 ± 1 c	3 ± 1 de

[a]Means within columns followed by the same letter are not significantly different (GLM; $P < 0.05$).

DISCUSSION

M. aridus was the most abundant grasshopper at the BANWR study site and is common at other sites in Arizona and New Mexico. *M. aridus* densities at this site ranged from 6.6/m^2, representing 73% of all grasshoppers present, in 1994 to 4.2/m^2, representing 65% of all grasshoppers present, in 1995. These grasshoppers were a major part of the total arthropod biomass and were an important food item for the vast population of insectivorous birds at this site (Gardner and Thompson 1998).

Although *M. aridus* is polyphagous, it is primarily a forbivore. In a rangeland ecosystem, where *M. aridus* is most commonly found, potential damage to valuable forage is very small unless forbs are an important part of the grazing plan. *M. aridus* and *H. viridis* are capable of influencing vegetative structure and can decrease above ground biomass of their host plants. Individual *M. aridus* in this study consumed similar amounts of snakeweed per day (46 mg/day) compared to *H. viridis* grasshoppers in a field study (47 and 85 mg/day at two different sites) conducted by Thompson et al. (1996). Damage to snakeweed stands at the BANWR site from these grasshoppers was obvious. Together or separately, *M. aridus* and *H. viridis* may play an important role in stabilizing snakeweed populations, and may suppress populations of snakeweed in some cases where populations are large enough.

To the best of our knowledge, these are the highest densities of *M. aridus* reported in the literature. These grasshoppers are native insects and are just one component of the biota common to most rangelands. Conservation biological control techniques can be used to influence weed populations by manipulating native insect populations (Newman et al. 1998). Knowledge of the grasshopper species composition in an area prior to the initiation of weed management strategies such as burning or insect management strategies such as insecticide applications is very important. When spray programs are initiated on grasshopper densities alone, managers run the risk of weed resurgence if beneficial weed-feeding grasshoppers are eliminated (Thompson et al. 1996). In another example, populations of the grasshopper, *H. viridis*, can be dramatically increased by altering the dates of prescribed burns (Newman et al. 1998). *Melanoplus aridus* populations undoubtedly would respond similarly.

ACKNOWLEDGMENT

We would like to thank the staff of Buenos Aires National Wildlife Refuge, U.S. Fish and Wildlife Service, Sasabe, Az, for their cooperation and access to field plots. We also thank the many students at the Range Insect Laboratory for their work with the grasshopper colony. This research was supported in part by the USDA/CREES and the New Mexico Agricultural Experiment Station, New Mexico State University, Las Cruces.

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EVALUATION OF CHLORFENAPYR EAR TAG EFFICACY AND SUSCEPTIBILITY OF HORN FLIES[1] TO CHLORFENAPYR

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ABSTRACT

During 1996 and 1997, the efficacy of experimental chlorfenapyr ear tags for horn fly control was evaluated under field conditions at St. Gabriel, LA. In 1996, the 30, 40, and 40% TT (thicker tags) chlorfenapyr tags provided ≥90% control for 11, 10, and 16 weeks, respectively. In 1997, fly reduction of ≥90% was observed for chlorfenapyr tags after two weeks post-treatment, and was maintained until week 8, 8, and 18 by the 20, 30, and 30% TT tags, respectively. At least 90% control was obtained with 40% diazinon tags for 23 weeks in 1996 and 16 weeks in 1997. The susceptibility of flies to chlorfenapyr, diazinon, and *lambda*-cyhalothrin was measured at seven locations in LA. All seven horn fly field populations were more susceptible to chlorfenapyr than was a reference susceptible strain. Both pyrethroid- and organophosphate-resistant flies were highly susceptible to chlorfenapyr, with resistance ratios ranging from 0.07 to 0.21.

INTRODUCTION

Since WWII, a novel insecticide class for controlling horn flies, *Haematobia irritans irritans* (L.), has been marketed approximately every decade (Sparks et al. 1985, Drummond et al. 1988). Except for the avermectins, resistance in horn flies has been described for all of these insecticides (Byford et al. 1985, Sparks et al. 1985). Although a relatively large number of insecticide products currently are available commercially for horn fly control, the active ingredient for the majority of these products is either a pyrethroid or an organophosphate (OP) insecticide.

A new insecticide class known as the pyrroles was discovered by the American Cyanamid Company in the 1980's as a result of the synthetic improvement of a natural fermentation product from a *Streptomyces fumanus* culture (Kuhn et al. 1993, Treacy et al. 1994). The lead compound of this new class, a cyanopyrrole known as chlorfenapyr (AC303630), is a pro-insecticide, which is activated in insects by oxidative metabolism (Treacy et al. 1994). Chlorfenapyr interferes with cellular respiration by uncoupling oxidative phosphorylation, thus impairing ATP production in the mitochondria (Black et al. 1994, Treacy et al. 1994). Laboratory studies have shown that chlorfenapyr has both contact and oral activity against the tobacco budworm, *Heliothis virescens* (F.) (Treacy et al. 1991). In field studies, chlorfenapyr has been found to provide acceptable control of several major insect and

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mite pests of cotton (Farlow et al. 1991). Bioassays have shown that an increased susceptibility to chlorfenapyr can occur in pyrethroid-resistant insects such as the tobacco budworm (Pimprale et al. 1997) and the horn fly (Sheppard and Joyce 1998). In this study, we evaluated the efficacy of experimental ear tags containing chlorfenapyr for control of horn flies under field conditions and measured the susceptibility of field populations of both susceptible and resistant (OP- and pyrethroid-resistant) horn flies to chlorfenapyr.

MATERIALS AND METHODS

During 1996 and 1997, experimental ear tags containing chlorfenapyr (4-bromo- 2-(4-chlorophenyl)-1-(ethoxymethyl)-5-(trifluoromethyl) pyrrole-3-carbonitrile) were evaluated under field conditions at the St. Gabriel Research Station (St. Gabriel, LA), a unit of the Louisiana Agricultural Experiment Station (LAES). In 1996, three chlorfenapyr ear tags, containing 30% AI, 40% AI, or 40% AI in thicker tags (TT), and 40% diazinon tags (Patriot®, Boehringer Ingelheim Animal Health, St. Joseph, MO) were tested. Forty mixed-breed adult cows were randomly assigned to the four tag groups and an untreated control group (eight animals each). In 1997, three chlorfenapyr tags, containing 20% AI, 30% AI, or 30% AI TT, and 40% diazinon tags were tested. Fifty mixed-breed adult cows were randomly assigned to the four tag groups and an untreated control group (ten animals each). In both trials, tags were applied at two per animal (one per ear); the treatment periods were 10 June-27 November 1996 and 22 May-30 October 1997. All tags were provided by Fort Dodge Animal Health (FDAH), Princeton, NJ. Cattle were maintained on pastures ranging from 15 to 40 acres. All treatment groups were separated by at least one pasture (about 30-40 m) and were separated from the untreated control group by greater than 1 km.

Both years, fly counts were conducted weekly from about one week prior to tagging until tags were removed. In 1996, the pre-treatment count was conducted before animals were assigned to treatment groups. In 1997, the pre-treatment count was taken after treatment groups were assigned to their pastures. The number of flies on one side of each animal in each group was estimated before 0900 h cdst by one observer at a close range. Efficacy of control provided by the tags is discussed in terms of percentage of reduction of fly numbers, which was calculated as follows:

$$\text{Percentage Efficacy} = \frac{(\text{control group mean} - \text{treated group mean})}{\text{control group mean}} \times 100$$

Fly count data were transformed by logarithmic transformation ($y=\log_{10}(1+\text{count})$) before analyses were performed. First, analysis of combined post treatment data was conducted using a two-way analysis of variance (PROC GLM) (SAS Institute 1989) using the model Flycount = Week Treatment Week*Treatment. Then, a one-way analysis of variance was performed with treatment groups for each week using the model Flycount = Treatment. Differences in fly counts were considered statistically significant at the 5% significance level.

Susceptibility of fly populations to chlorfenapyr (99% purity, FDAH) was assessed using the impregnated filter paper method (Sheppard and Hinkle 1987). Chlorfenapyr-impregnated filter papers (from 0.1953 to 50 $\mu g/cm^2$) were provided by FDAH. Flies were collected from untreated animals using hand nets, and the bioassays were performed immediately after collection. The 1996 pre-season bioassay at St. Gabriel was conducted before animals were tagged. Post-season bioassays were conducted at least two weeks after tags were removed. Three replicates (approximately 30 flies each) per concentration were used, and flies unable to walk after a 4 h exposure period were considered dead.

Susceptibility of flies to chlorfenapyr was measured in the fall of 1997 at seven LAES

locations: Ben Hur (Baton Rouge), Hill Farm (Homer), Red River (Bossier City), Rosepine (Rosepine), St. Gabriel (St. Gabriel), and two locations of the Northeast Research Station (i.e. Northeast [St. Joseph] and Macon Ridge [Winnsboro]). Susceptibility of flies from these locations to *lambda*-cyhalothrin (93% purity, Mallinckrodt Veterinary, Mundelein, IL) and diazinon (87.5% purity, Fermenta Animal Health, Kansas City, MO) also was assessed using the impregnated filter paper method. St. Gabriel was the only location where chlorfenapyr tags were used.

Flies from the Knipling-Bushland U.S. Livestock Insects Research Laboratory, USDA-ARS (Kerrville, TX) were used as the reference susceptible strain. Resistance ratios (RRs) were calculated as LC_{50} from field populations/LC_{50} from the reference strain. Mortality data were corrected using Abbott's formula (Abbott 1925) when necessary, and bioassay results were analyzed by probit analysis using POLO-PC (LeOra Software 1987). The LC_{50}'s were considered significantly different when the 95% fiducial limits did not overlap. RRs to chlorfenapyr were not calculated for 1996 because bioassays were not conducted with the reference strain.

RESULTS

In 1996, the average number of flies per side on untreated cattle ranged from 44 to 275 (Table 1). Numbers of horn flies were significantly reduced after treatment in all treated groups. Average control efficacies for the entire study period (24 weeks) were 89, 77, 91, and 95% for chlorfenapyr 30, 40, 40% TT, and diazinon tags, respectively. The number of flies was reduced at least 90% for at least 10 weeks for all treatments. The 40%TT chlorfenapyr tags provided $\geq$90% control until week 16 (except for weeks 12 and 13) and provided $\geq$80% control until week 18. Ear tags containing 30% chlorfenapyr reduced fly numbers $\geq$80% until week 16, except for week 13. At least 90% control was achieved for 23 weeks with diazinon tags, except for week 19.

In 1997, the average number of flies per side before treatment was 210, 253, 190, and 269 for the chlorfenapyr 20, 30, 30% TT, and diazinon groups, respectively. The average number of flies/side on untreated cattle during the study ranged from 35 to 305 (Table 2). Average control efficacies for the entire study period were 63, 72, 82, and 78% for chlorfenapyr 20, 30, 30% TT, and diazinon tags, respectively (Table 2). Reduction in fly numbers $\geq$90% did not occur until after the second week post-treatment with the chlorfenapyr tag treatments. After the second week (except for weeks 5 and 7), efficacy of 20% chlorfenapyr tags remained $\geq$90% until week 8. Efficacy of 30% chlorfenapyr tags $\geq$ 90% was observed until week 8 and above 80% until week 15, except for weeks 9 and 10. The 30% TT chlorfenapyr tags reduced fly numbers $\geq$90% until week 18, except for weeks7 and 15. Control using the diazinon tags was $\geq$90% from the first week after treatment until week 16 (except week 10).

Results from pyrethroid and OP bioassays at St. Gabriel indicated that flies were susceptible to both *lambda*-cyhalothrin and diazinon at the beginning of both trials (Table 3). Initial RRs were 0.6 for *lambda*-cyhalothrin for both years and 0.5 and 0.1 for diazinon in 1996 and 1997, respectively. Results from pyrethroid and OP bioassays conducted at all locations in 1997 are presented in Table 3. Flies from all sites except St. Gabriel were resistant to pyrethroids; RRs to *lambda*-cyhalothrin ranged from 16.7 (Rosepine) to 2,625.0 (Winnsboro). Susceptibility to diazinon was less variable than that observed for pyrethroids, and RRs ranged from 0.1 (St. Gabriel) to 6.6 (Rosepine). Lower LC_{50}'s to chlorfenapyr were measured for field populations than for the reference susceptible strain (Table 3). The LC_{50} (36.39 $\mu g/cm^2$) observed for the reference strain was 4.9-fold higher than the highest LC_{50} (7.46 $\mu g/cm^2$) measured among field populations.

TABLE 1. Mean Number of Horn Flies per Side on Control Cattle and Percentage Reduction on Treated Animals by Insecticidal Ear Tags[a] at St. Gabriel, LA, in 1996

Date	Week post-treatment[b]	Control flies/side	Chlorfenapyr			Diazinon
			30%	40%	40% TT[c]	40%
05 Jun	-	209	-	-	-	-
19 Jun	1	219	99	96	89	100
27 Jun	2	275	100	100	100	100
03 Jul	3	244	100	100	100	100
10 Jul	4	213	100	100	100	100
17 Jul	5	150	100	100	100	100
24 Jul	6	165	95	98	99	100
31 Jul	7	172	99	95	98	99
08 Aug	8	238	97	95	97	99
14 Aug	9	228	98	95	100	98
20 Aug	10	228	95	94	99	95
27 Aug	11	166	94	74	94	99
03 Sep	12	109	89	61	86	90
10 Sep	13	131	65	56	85	96
17 Sep	14	231	89	68	96	92
26 Sep	15	172	95	80	93	95
03 Oct	16	141	87	93	95	95
10 Oct	17	100	78	76	89	93
19 Oct	18	197	81	70	81	92
24 Oct	19	88	64	34[d]	74	72
31 Oct	20	188	86	75	83	98
06 Nov	21	66	89	69	72	96
14 Nov	22	109	96	39[d]	95	99
21 Nov	23	44	46[d]	16[d]	63[d]	90
27 Nov	24	46	72	34[d]	85	88

[a] Fly number and percentage reduction based on arithmetic means; all animals were tagged on 10 June and tags were removed on 27 November

[b] Number of weeks were calculated as close as possible from the real dates

[c] TT= thicker tags

[d] Mean number of flies on treated animals did not differ significantly from control group (α=0.05)

DISCUSSION

Ear tags containing 30% or 40% chlorfenapyr were effective for horn fly control. The diazinon tag treatment was more effective than the chlorfenapyr treatments during the first year of the study. However, in the second year, efficacy of 30% chlorfenapyr tags was equivalent to that observed using the diazinon tags, and efficacy of 30% TT chlorfenapyr tags was higher than that recorded for diazinon tags. Similar levels of horn fly control for diazinon tags have been reported elsewhere (Cocke et al. 1990, Crosby et al. 1991, Byford et al. 1988).

In 1997, pyrethroid resistance was detected at all sites except St. Gabriel, which is consistent with reports of its widespread occurrence (Sparks et al. 1985, Kunz and Schmidt 1985). If a RR of >2 for diazinon is considered an indicator of an OP-resistant population (Barros 1998), then four of the seven populations tested were considered OP-resistant. This is

TABLE 2. Mean Number of Horn Flies per Side on Control Cattle and Percentage Reduction on Treated Animals by Insecticidal Ear Tags[a] at St. Gabriel, LA, in 1997

Date	Week post-treatment[b]	Control flies/side	Chlorfenapyr			Diazinon
			20%	30%	30% TT[c]	40%
22 May	0	263	-	-	-	-
29 May	1	131	78	49	40[d]	93
05 Jun	2	138	62	53[d]	29[d]	92
12 Jun	3	145	94	94	96	100
20 Jun	4	178	98	97	99	100
27 Jun	5	91	89	94	97	100
03 Jul	6	65	96	99	92	94
10 Jul	7	35	88	96	85	94
17 Jul	8	173	91	99	98	99
25 Jul	9	118	33	75	92	90
31 Jul	10	84	44	37[d]	95	74
07 Aug	11	146	76	84	94	95
14 Aug	12	305	95	98	100	100
21 Aug	13	133	59	87	90	96
28 Aug	14	178	56	97	97	98
04 Sep	15	170	49	90	73	91
11 Sep	16	155	62	79	91	99
18 Sep	17	93	75	64	95	68
26 Sep	18	133	97	97	98	64
03 Oct	19	193	49[c]	79	86	74
10 Oct	20	228	41[d]	59[d]	76	60
16 Oct	21	103	-61[d]	2[d]	46	-7[d]
23 Oct	22	106	72	50[d]	96	57[d]
30 Oct	23	61	54[d]	50[d]	79	12[d]

[a] Fly number and percentage reduction based on arithmetic means; all animals were tagged on 22 May and tags were removed on 30 October

[b] Number of weeks were considered as close as possible from the real dates

[c] TT= thicker tags

[d] Mean number of flies on treated animals did not differ significantly from control group (α=0.05)

consistent with control failure of OP tags observed at Rosepine, St. Joseph, and Winnsboro prior to this study (Barros 1998).

All field populations tested were more susceptible to chlorfenapyr than the reference strain, independent of their level of susceptibility to pyrethroid or OP insecticides. The highest LC_{50} to chlorfenapyr observed among field strains was about five-fold lower than that found for the reference flies. Similar results were reported by Sheppard and Joyce (1998), who found that pyrethroid-resistant horn flies in Georgia were five times more susceptible to chlorfenapyr compared with susceptible flies. Sheppard and Joyce (1998) suggested the enhanced chlorfenapyr toxicity found in pyrethroid-resistant horn flies was a consequence of higher activation of this insecticide by mixed-function oxidases (MFO). This assumption is supported by laboratory studies, which have showed that oxidative metabolism is the major route of activation of chlorfenapyr (a pro-insecticide) to its *N*-dealkylated analog, which is actually the toxic uncoupling agent (Black et al. 1994, Treacy et al. 1994). Metabolic activation of

TABLE 3. Susceptibility (LC_{50} Expressed as $\mu g/cm^2$) and Resistance Ratios for Horn Fly Populations in Louisiana

Year	Chlorfenapyr		λ-cyhalothrin		Diazinon	
Site	LC (95% F.L.)	RR[d]	LC_{50} (95% F.L.)	RR	LC_{50} (95% F.L.)	RR
1996						
St. Gabriel[a]	6.3 (-)[e]	-	0.1 (0.1-0.2)[f]	0.6	0.2 (-)[e]	0.5
1997						
St. Gabriel[b]	5.0 (-)[eg]	-	0.4 (0.2-0.7)	2.3	0.2 (-)[e]	0.7
St. Gabriel[a]	-	-	0.2 (0.1-0.3)	0.6	0.1 (-)[e]	0.1
St. Gabriel[b]	2.9 (2.1-3.4)	0.1	0.04 (0.01-0.1)[f]	1.6	0.4 (0.4-0.5)	1.0
Rosepine[b]	4.0 (2.7-5.1)	0.1	0.4 (0.3-0.5)	16.7	2.8 (-)[e]	6.6
Ben Hur[b]	2.6 (1.9-3.3)	0.1	2.5 (2.1-3.0)	100.4	0.4 (0.2-0.7)	0.9
Red River[b]	4.9 (4.2-5.5)	0.1	2.5 (1.5-4.2)	100.4	0.4 (0.3-0.5)	0.9
Hill Farm[b]	3.1 (2.4-3.9)	0.1	10.2 (8.6-12.0)	413.6	1.1 (0.9-1.3)	2.6
St. Joseph[b]	6.2 (-)[e]	0.2	42.7 (24.5-239.5)	1738.7	1.2 (-)[e]	2.8
Winnsboro[b]	7.5 (6.5-8.7)	0.2	64.5 (38.5-223.0)	2625.0	1.2 (0.8-1.5)	2.9
Kerrville[c]	36.4 (-)[e]	-	0.02 (-)[e]	-	0.4 (0.4-0.5)	-

[a] Pre-season (May/June) bioassays

[b] Post-season (September/October) bioassays

[c] Reference susceptible colony (Knipling-Bushland U. S. Livestock Insects Research Laboratory, USDA-ARS, Kerrville, TX)

[d] Resistance ratio (LC_{50} from field population / LC_{50} from reference susceptible strain)

[e] Data too heterogeneous to calculate 95% fiducial limits (LeOra Software 1987)

[f,g] Control mortality in the bioassay between 15% and 22%([f]), and 30.9% ([g]); control mortality <15% in all other bioassays

insecticides by MFO activity in pyrethroid-resistant horn flies also has been proposed to occur with diazinon (Cilek et al. 1995). Pimprale et al. (1997) reported a negative correlation between pyrethroid and chlorfenapyr toxicity for the tobacco budworm. In our studies, field populations with varying levels of pyrethroid resistance were uniformly more susceptible to chlorfenapyr than the reference strain. The apparent lack of correlation between pyrethroid and chlorfenapyr toxicity found in our studies indicates that there may be differing mechanisms conferring resistance in the different populations.

Pyrethroid resistance in horn flies has been widespread in the southern United States since the mid-1980s (Kunz and Schmidt 1985, Sparks et al. 1985). Although pyrethroid-resistant horn flies have been reported to be more susceptible to diazinon than pyrethroid-susceptible flies (Sheppard and Marchiondo 1987, Cilek and Knapp 1993, Cilek et al. 1995), reports of resistance to diazinon are increasing (Cilek et al 1991, Steelman et al. 1994, Barros 1998). Furthermore, Barros (1998) demonstrated that seven years of annual rotation between OP and pyrethroid tags failed to improve pyrethroid efficacy or prevent further development of resistance to the pyrethroid. Also, development of resistance to both insecticides has been observed in flies under a pyrethroid-OP rotation in laboratory studies (McKenzie and Byford 1993). Therefore, a need exists for alternate chemicals to control horn flies. The results of our study indicate that chlorfenapyr will be useful in controlling horn flies, regardless of their susceptibility to pyrethroids or OPs. Furthermore, availability of an alternate insecticide class with toxicity against resistant populations provides a potentially valuable tool for improving resistance management.

ACKNOWLEDGMENT

We thank Pat DeRouen for technical assistance and Fort Dodge Animal Health for partial support for this study. Published with approval of the Director of the Louisiana Agricultural Experiment Station as Manuscript No. 98-17-0530.

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SOUTHWESTERN ENTOMOLOGICAL SOCIETY

PERSPECTIVE

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The Southwestern Entomologist provides this section for contributions of general interest with an emphasis on papers of a theoretical or historical nature.

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REVIEW AND ANALYSIS OF DAMAGE FUNCTIONS AND MONITORING SYSTEMS FOR PINK BOLLWORM (LEPIDOPTERA: GELECHIIDAE) IN SOUTHWESTERN UNITED STATES COTTON

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ABSTRACT

Pink bollworm, *Pectinophora gossypiella* (Saunders), continues to be a significant pest of cotton worldwide. Two factors essential for effective integrated pest management (IPM) programs for pink bollworm include: 1) quantification of damage-yield relationships, and economic injury levels, and 2) practical sampling plans for estimating pest density, or classifying the pest population above or below an economic threshold. Research conducted over the past 30 years is reviewed in an effort to quantify damage functions, and to assess the advantages and disadvantages of several common monitoring methods for pink bollworm. Composite results of five independent studies from 1969 to 1991 show that yields are not reduced until the average seasonal larval infestation in bolls approaches 15%. Each study shows that insecticide applications are significantly reduced when economic thresholds (ETs, based on moth catches in pheromone traps, or egg or larval infestations in bolls) are used. Compared with scheduled applications, the average reduction in insecticide use, based on ETs, ranged from 23 to 37% with no reduction in yield. Although monitoring adult moth flights with pheromone traps continues to be a popular tool, the recent development of binomial (presence-absence) sequential sampling plans for pink bollworm eggs offers several advantages, including an indirect indicator of female moth activity and earlier detection of subsequent larval infestations in bolls. Recent commercialization of Bt-cotton, genetically transformed to express an insecticidal toxin from the bacterium, *Bacillus thuringiensis*, provides considerable promise for consistent control of pink bollworm. However, its continued use will depend upon rapid development and adoption of resistance management plans by growers. Moreover, the use of Bt-cotton is likely to be preserved longer if it is implemented within the context of an integrated pest management (IPM) program, rather than as a single tactic.

INTRODUCTION

Many cotton growers in the southwestern U.S. have experienced the decimating impact of the pink bollworm, *Pectinophora gossypiella* (Saunders), despite the application of multiple insecticide treatments. After losing one or two fields to the pink bollworm, it is understandable why cotton growers develop strong aversions to risk associated with this pest. This scenario was most evident among growers in the Imperial Valley of southeastern California who experienced ≅ 95% reduction in total cotton acreage from 142,000 ac in 1977 to ca. 9,000 ac in 1994 (Burrows et al. 1982, Chu et al. 1996), primarily because of problems associated with the pink bollworm. Increasing infestations of the whitefly complex (*Bemisa*

spp.) have also been attributed to insecticide use targeted for pink bollworm (Butler et al. 1986). Because of the pest management problems associated with full-season cotton production, more cotton in the southwestern U.S. is now produced in a short-season, single boll-set system (Chu et al. 1996).

To minimize the risk of local population outbreaks, growers have historically relied on insecticides for pink bollworm management. However, as evidenced by significant reductions in cotton acreage in the Imperial Valley, this strategy has not always been successful in the long-term. Indeed, the Imperial Valley experience continues to support the need for integrated pest management (IPM) programs for long-term sustainability (e.g., Henneberry 1986). transgenic cotton, genetically engineered to express various toxins produced by *Bacillus thuringiensis* (Bt), has the potential to significantly suppress pink bollworm in many growing regions (e.g., Wilson et al. 1992, 1994; Flint et al. 1996). However, as with other Bt-crops, long-term effectiveness of Bt-cotton will depend upon whether resistance to Bt can be avoided or delayed (Bartlett 1995, Gould and Tabashnik 1998). Bt cotton should therefore become a complementary tactic within an IPM system (e.g., Henneberry 1986).

Irrespective of the control technology employed, the *timing* of each control tactic should be optimized to provide maximum long-term profits to growers. Successful IPM and resistance management programs for the pink bollworm will require the use of more information than most conventional insecticide approaches. The purpose of this article is to review two types of information that are necessary for effective use of many control strategies for pink bollworm. Specifically, research conducted over the past 30 years is reviewed in an effort to quantify damage functions, and to assess the advantages and disadvantages of several common monitoring methods for pink bollworm. Where possible, published data on economic thresholds and damage functions were collated for a composite, or meta-analysis of multiple studies (e.g., Hunter and Schmidt, 1990).

DAMAGE FUNCTIONS

For over 40 years, the economic threshold (ET) has been a guiding paradigm for the development of IPM programs. Development of the ET concept is generally attributed to Stern et al. (1959) who were the first to articulate a working definition: "the density at which control measures should be applied to prevent an increasing pest population from reaching the economic injury level (EIL)". The EIL was defined as "the lowest pest population density that will cause economic damage" (Stern 1973), where the ET is always less than EIL. Headley (1972) provided a more quantitative definition of the ET: "the population that produces incremental damage equal to the cost of preventing that damage." Although these original definitions have been criticized for oversimplification and alternative terms such as "action threshold" or "treatment threshold" have been introduced (e.g., Pedigo et al. 1986, Wilson 1986), the underlying concept remains the same. For most crop/pest interactions there is some minimum population density where control activity becomes economically justified.

In this section, I review three studies designed to evaluate economic thresholds for pink bollworm in short-staple cotton, including ETs based on egg, larval and adult population density. Based on data from these and other studies, I then examine the major components which contribute to the pink bollworm damage-yield relationship. Given the damage-functions, a range of ETs are calculated based on fluctuations in market price for cotton lint and cost of insecticides. Although much of the early work on the pink bollworm-yield interaction was done in Texas (e.g., Brazzel and Gaines 1956), the following analysis is limited primarily to research conducted under irrigated conditions in the southwestern U.S., specifically southern Arizona and southeastern California.

Field Studies. Field research on economic thresholds for the pink bollworm reflects a diversity of studies where three different criteria and subsequent sampling methods were used

to determine when insecticides should be applied (Table 1). Also, for each of the studies reviewed, a variety of insecticides were applied, including carbamates, organophosphates, pyrethroids and chlordimeform. Watson and Fullerton (1969), in Arizona, used an initial treatment threshold of 20% larval-infested bolls. Toscano et al. (1974), in the Imperial and Palo Verde Valleys of California, used a threshold of 3.5-4.0 moths/trap/night (Hexalure-baited traps). Hutchison et al. (1988a, 1991), in the Palo Verde Valley, used 6-8% egg-infested bolls as their treatment threshold. In each study, significant reductions in insecticide use ranged from 23-37% when thresholds were used (Table 1). Fields treated based on action thresholds were compared with fields receiving scheduled weekly applications (Watson and Fullerton 1969, Toscano et al 1974) or fields monitored by pest control advisors where treatments were applied based on larval infestations combined with scheduled applications (Hutchison et al. 1988a, 1991). Despite significant reductions in insecticide use, no significant yield loss was observed in any study for fields managed by specific treatment criteria. Hutchison et al. (1988a, 1991) also found no significant differences in seed damage or lint quality in fields receiving 23-35% less insecticide. In summary, the data collected from each of these studies (Table 1) under a variety of experimental conditions, strongly suggest that when monitoring systems are consistently employed, insecticide use can be reduced significantly.

Components of Pink Bollworm Damage: Lint Yield. The impact of pink bollworm on cotton is most often measured in terms of total fiber and lint quality. Fry et al. (1978) quantified the relationship between lint yield ('Deltapine-16' cotton) and larval density where bolls were infested with 0-7 larvae each (Fig. 1A). Fig. 1A also illustrates the decline in pickable lint, the amount of lint actually available to machine harvesters. The decline in pickable lint, particularly at a larval densities > 1.0/boll, results from larvae moving between carpel walls of the boll as they feed, which precludes normal boll opening (Noble 1969). Although Fry et al. (1978) suggested that much of the unpickable lint can be recovered if a stripper harvester is used, the quality of this lint would undoubtedly be reduced (*see also* Lint Quality).

Fig. 1A, based on Fry et al. (1978), is presented to show the impact of maximum pink bollworm damage. However, the highlighted area (upper left corner) of the graph reflects pink bollworm larval infestations typically observed in commercial cotton (i.e., those where the seasonal average density $\leq$ 1.0 larva/boll). In fact, it is only at densities of 0.4 - 0.6 larvae/boll that pickable and total lint, respectively, are reduced to < 90% of maximum weight. A larval density of 0.4/boll is $\approx$30% boll infestation (i.e., % of bolls with one or more larvae) and 0.6/boll is $\approx$40% boll infestation (Table 2). Most commercial fields are usually protected to prevent average infestation levels exceeding 5% (e.g., Hutchison et al. 1991, Staten et al. 1987).

Fig. 1B illustrates a normalized yield response for a range of average seasonal pink bollworm larval infestations from 1.3 to 49.5% that are more typical of commercial cotton (Watson and Fullerton 1969; Toscano et al. 1974; Henneberry et al. 1977, 1978; George and Wilson 1983; Hutchison et al. 1988a, 1991). Here, a range of pink bollworm densities and yields resulted from the use of a variable number of insecticide applications. Lint yields were estimated from Watson and Fullerton's (1969) data by assuming a 39% gin turnout of seed cotton weights. Normalized yield (Fig. 1B) was calculated by comparing maximum lint yields (=100%, Fig. 1B) from fields receiving a maximum number of insecticide applications (10-19/season) to those of fields receiving fewer insecticide treatments.

With the exception of a few low-yield (< 90% of maximum) observations where pink bollworm infestations were $\leq$ 15%, most of the significant reductions in yield did not occur until average seasonal larval infestations approached 15% or more. Beyond the 15% infestation level, the probability of maximum yield declined quickly and lint yield estimates

TABLE 1. Comparison of Mean Number of Insecticide Applications for Pink Bollworm Control, Mean Percent Reduction in Insecticide Use, Mean Seasonal Larval Infestations and Mean Yield, Where Three Different Economic Thresholds Were Evaluated.[a]

Source	Threshold[b]	No. Applications	% Reduction	% Infestation[c]	Bales/ac[d]
Watson & Fullerton	Larvae (20%)	6.0	33	22.9	2.62
(1969), AZ	vs. Scheduled	9.0	--	15.3	2.63
Toscano et al. (1974)	Moths (3.5-4/Trap)	8.0	35	4.7	2.68
Imperial Valley, CA	vs. Scheduled	12.3	--	3.9	2.70
Toscano et al. (1974)	Moths/Trap	6.0	37	6.8	2.29
Palo Verde Valley, CA	vs. Scheduled	9.5	--	6.4	2.29
Hutchison et al. (1988a)	Eggs (@ 6%)	5.4	35	13.6	2.92
Palo Verde Valley, CA	vs. Larvae (@ 5%) + Scheduled	8.3	--	14.6	3.18
Hutchison et al. (1991)	Eggs (@ 6%)	6.2	23	8.8	3.09
Palo Verde Valley, CA	vs. Larvae (@ 5%) + Scheduled	8.1	--	4.6	3.09

[a]See individual references for actual materials applied.

[b]Larval and egg thresholds refer to % of bolls infested with one or more larvae or eggs, respectively.

[c]Percentage of bolls infested with one or more larvae based on 'boll-cracking' method, except for Hutchison et al. (1988a) which is based on boll incubation method which tends to yield higher estimates.

[d]Lint yields were estimated from Watson and Fullerton's (1969) published seed-cotton weights, and by Hutchison et al. (1991).

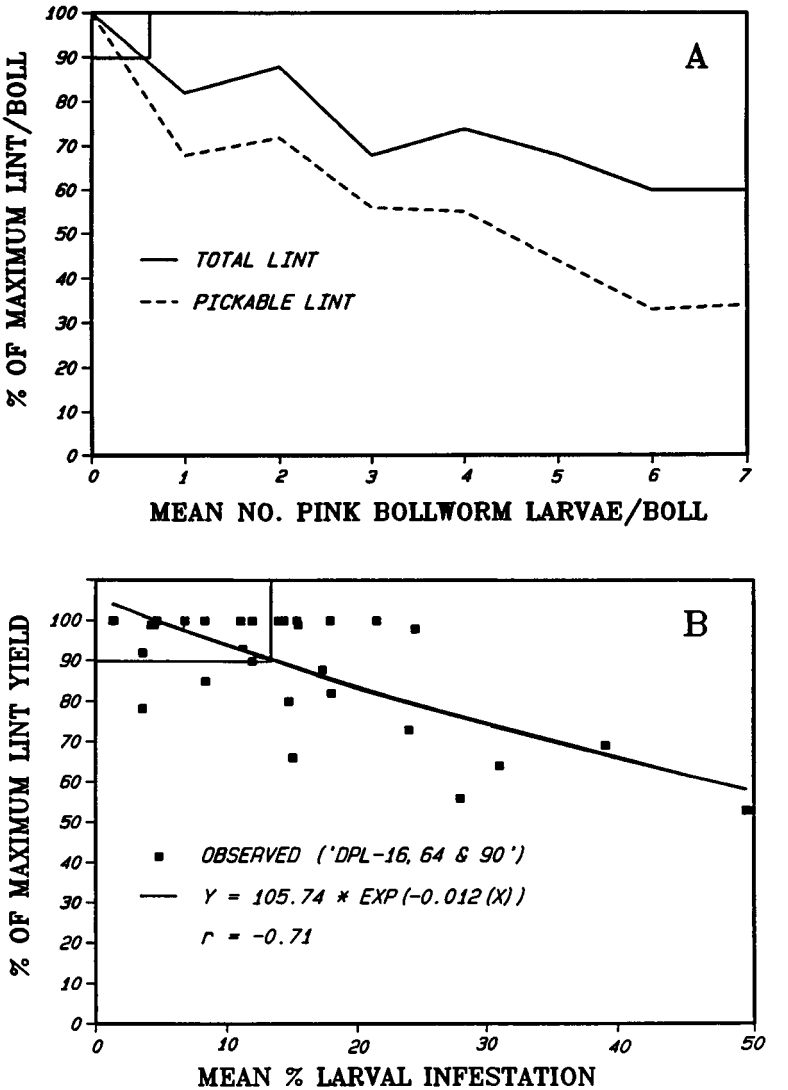

FIG. 1. (A) Normalized total lint and pickable lint in relation to pink bollworm density; data from Fry et al. (1978) for 'Deltapine 16' upland cotton. (B) Normalized yield response in relationship to mean seasonal larval infestations ($r^2 = 0.51$) (see Table 1 for source of data).

become more variable. Based on the exponential function fit to these data, only 51% (r^2 = 0.51, r = -.71) of the variability in yield is explained by larval infestation level. Variable yield results undoubtedly reflect the myriad of agronomic, edaphic, and climatic factors, as well as other insect pests (including resurgence of secondary pests) that affect cotton yield.

TABLE 2. Relationship Between Percentage of Bolls Infested with Pink Bollworm Eggs and Larvae and the Number of Eggs and Larvae per Boll, Respectively.

Eggs[a,b]		Larvae[b]	
% Bolls Infested	Mean No./Boll	% Bolls Infested	Mean No./Boll
2	0.15	2	0.02
4	0.25	4	0.05
6	0.35	6	0.07
8	0.46	8	0.09
10	0.58	10	0.12
15	0.92	15	0.19
20	1.32	20	0.26
25	1.81	25	0.33
30	2.42	30	0.41
35	3.15	35	0.49
40	4.03	40	0.58
50	6.40	50	0.78
60	9.94	60	1.00
70	14.85	70	1.24
80	22.23	80	1.52
90	33.18	90	1.83
100	49.00	100	2.15

[a]Based on data from Stroschein et al. (1988).
[b]Based on data from Hutchison et al. (1986b).

Components of Pink Bollworm Damage: Seed damage. Although the impact of pink bollworm on lint yield is the most commonly measured and important component of crop damage, final yields are an indirect result of seed damage (Henneberry et al. 1994). Seeds developing in the boll are the primary nutritional source for pink bollworm larvae (Noble 1969). Because optimal lint production is dependent upon seed quality throughout the boll maturation period (Fry et al. 1978), seed damage resulting from pink bollworm causes reductions in both lint yield and quality.

The relationship between larval density and seed damage is shown in Fig. 2A. These data were taken from Fry et al. (1978), Henneberry et al. (1978), George and Wilson (1983), and Hutchison et al. (1988a, 1991). Most of these results were obtained using the x-ray method (Wilson and Wilson 1975, Wilson and George 1985) for rapid detection of damaged or larva-infested seeds. As pointed out by Wilson and Wilson (1975), seed damage is the most consistent indicator of pink bollworm damage. In contrast to the variability present in the yield response shown in Fig. 1B, the seed damage relationship (Fig. 2A) corroborates the results of several independent studies (Wilson and Wilson 1975, Wilson and George 1985, Henneberry et al. 1994).

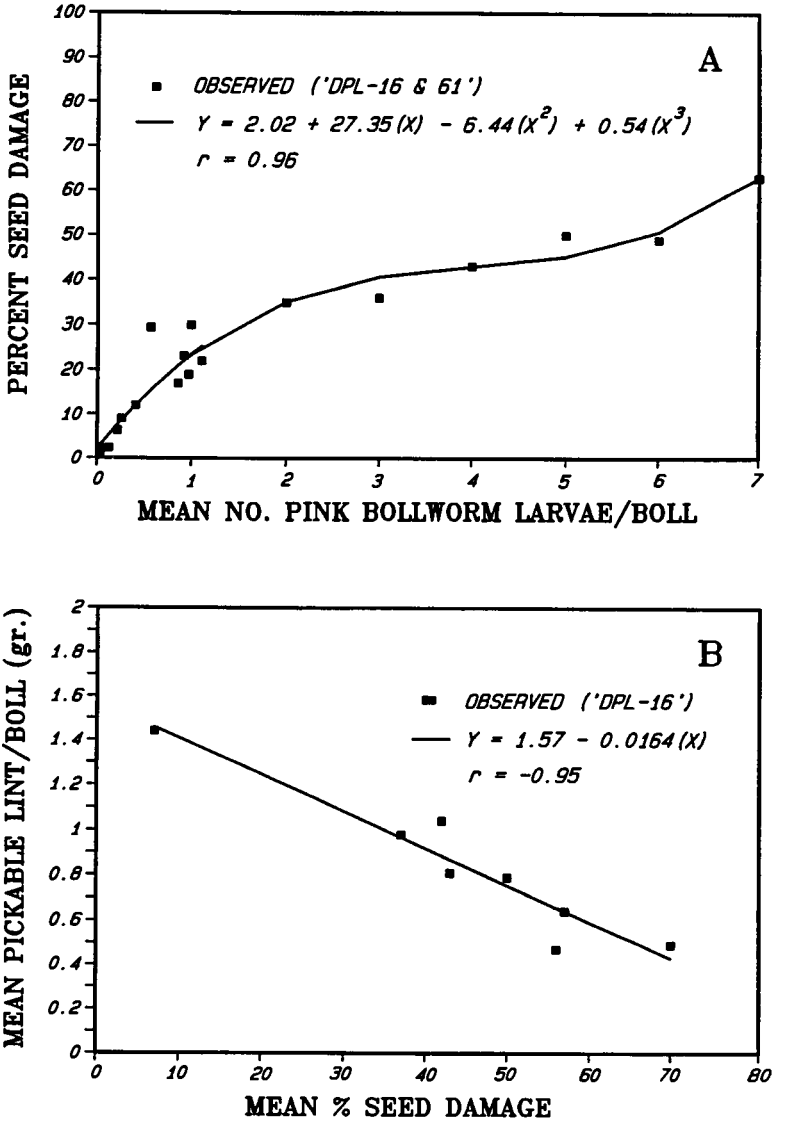

FIG. 2. (A) Percent seed damage as a function of pink bollworm larval density, and (B) the impact of seed damage on the amount of pickable lint/boll; data from Fry et al. (1978).

The corresponding impact of increasing seed damage on reducing pickable lint per boll is shown in Fig. 2B. Though most of these data (Fry et al. 1978) were available only for larval infestations > 38% (i.e., > 0.54 larvae/boll, Table 2), they demonstrate the importance of maintaining healthy seed for optimal lint production.

Components of Pink Bollworm Damage: Lint Quality. Although extremely high pink bollworm populations will cause significant reductions in lint quality (e.g., Brazzel and Gaines 1956, Fry et al. 1978), there are only a few documented cases of lint quality reductions in commercial cotton in Arizona or California (Hutchison et al. 1988a, 1991; Henneberry et al. 1994). For example, Fry et al. (1978) did not observe significant reductions in most lint quality parameters [fiber length, fiber strength, micronaire (fineness)] until an average of about 1.5 larvae/boll were present (Fig. 3). More recently, Henneberry et al. (1994), with 'Deltapine 90', 'Deltapine 5415' and 'Deltapine 5461', did show some reduction in selected lint quality parameters. Most of these reductions occurred when larval infestations exceeded 0.5 larvae/boll, or seed damage was >15%. Based on the relationship between larval density and percentage boll infestations (Table 2), 0.5 to 1.5 larvae/boll is approximately equal to 35 to 80% boll infestations, respectively, which are much higher than those normally tolerated in commercial cotton.

The greatest potential for reductions in lint quality occurs when growers attempt to maximize yield by continuing to push the crop through late October. Cotton left in the field from late September through November becomes more susceptible to pink bollworm-induced lint quality losses and there is increasing potential for lint exposure to inclement weather.

Another factor affecting lint quality late in the season is the incidence of boll rot caused by the fungus, *Aspergillus flavus* (Russell et al. 1976). *A. flavus* also produces the carcinogen, aflatoxin, which accumulates in the seed. Most pathologists primarily attribute high *A. flavus* and aflatoxin counts to late-season pink bollworm populations, which facilitate proliferation of the disease and toxin through exit holes made by larvae when they exit bolls (e.g., Russell et al. 1976, Simpson and Batra 1984). Scavenger beetles (Nitidulidae) and mites are also known to disseminate *A. flavus* among bolls via pink bollworm exit holes (Simpson and Batra 1984). High aflatoxin content in the seed (usually > 20 ppb) requires an ambient-pressure-ammonia treatment before marketing (Burrows et al. 1984). Because of the history of aflatoxin problems in southeastern California, and the fact that most seed is sold to dairy farms, the aflatoxin treatment has become an automatic, added cost to most producers.

Damage Functions and Economic Injury. Another important relationship that has been previously documented is the effect of insecticide application frequency on average larval infestation levels, and the corresponding impact on final yield. Fig. 4 is useful for comparing numerous studies to quantify yield loss in relation to the number of insecticide applications (e.g., Gutierrez and Wang 1984). This approach was selected for analysis because of the nature of most published data. That is, nearly all studies provide average seasonal larval infestation and insecticide use data, while some provide larval density and yield relationships (Table 1) (Henneberry et al. 1994).

The yield response, as a function of insecticide applications (Fig. 4), is variable among studies (Table 1). However, the variability was not unexpected based on the yield-larval density response (Fig. 1B), and the fact that many factors can influence final yield. Clearly, most of the variability occurred at ≤ 5 insecticide applications. Alternatively, for most of the fields, representing a wide range of larval infestation levels, applying more than six insecticide treatments during the season had little impact on yield or larval infestations. In fact, there was a slight decline in the average yield response reflecting the variability among fields and larval infestation levels. This modest yield decline (at ten applications) may also reflect insecticide-induced problems such as indirect negative effects on cotton plant physiology, or resurgence of secondary pests (e.g., Naranjo et al. 1996).

Summary statistics (Table 3) for fields receiving six or more insecticide applications (Fig. 4) confirm a higher expected average yield (95 vs. 83% of maximum) and lower larval

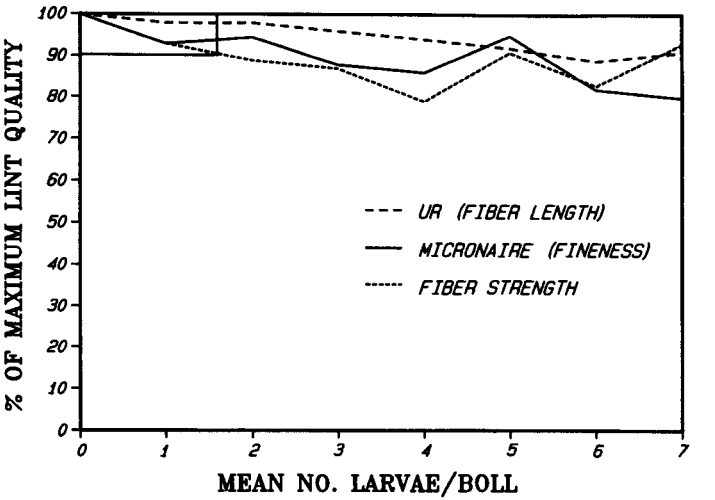

FIG. 3. Normalized lint quality as a function of pink bollworm larval density; data from Fry et al. (1978).

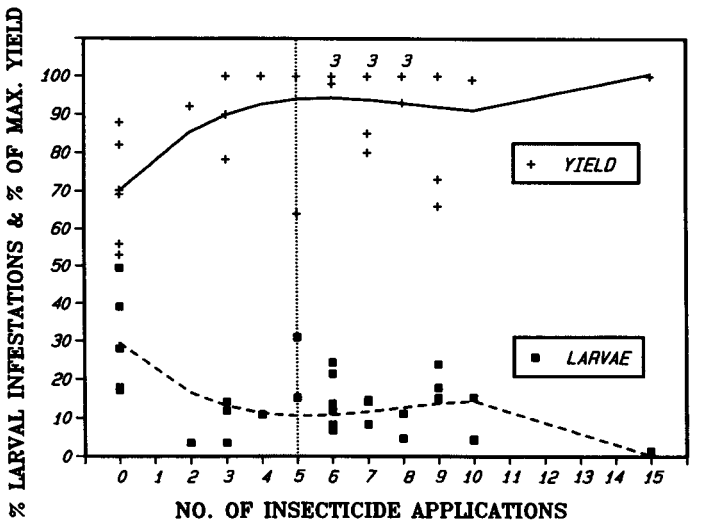

FIG. 4. Normalized yield response and seasonal pink bollworm larval infestations in relation to total number of insecticide applications. Yield response regression: $Y = 70.2 + 10.12(x) - 1.331(x2) + 0.053(x3)$, $r^2 = 0.59$. Percent larval infestation regression: $Y = 105.7 * EXP(-0.012(x))$, $r^2 = 0.71$ (See Table 1 for sources of data; '3' refers to 3 observations for given data point).

infestation level (12 vs. 24%). Yields and larval infestations were also much less variable (see 95% CI, Table 3) than for fields receiving ≤ 5 applications/season. More specifically, the less variable yield response was also associated with a larval infestation upper limit of 15% (95% CI: 8.7-15.2%). Because of the increased variability in yield response associated with larval infestations (95% CI: 14.6-34.3%), and the increased probability of unpickable lint, reduced lint quality, and aflatoxin problems, these data strongly suggest that the mean 12% infestation level (and upper 95%CI limit of 15% for ≥ 6 applications) could be a reasonable EIL.

TABLE 3. Summary Statistics for Yield Response and Pink Bollworm Larval Infestations in Fields Receiving ≤ 5 and ≥ 6 Insecticide Applications.[a]

		No. Insecticide Applications	
		≤ 5	≥ 6
Yield	Mean	83.1%	94.6%
(% of Max. Lint)	SE	4.4	2.3
	95%CI	(73.5-92.5%)	(90.0-99.4%)
% Bolls	Mean	24.4%	11.9%
infested	SE	4.5	1.6
w/larvae	95%CI	(14.6-34.3%)	(8.7-15.2%)
No. of Fields (*n*)		13	18

[a]Data from Watson and Fullerton (1969), Toscano et al. (1974), Henneberry et al. (1977, 1978), George & Wilson (1983), Hutchison et al. (1988a, 1991); *n* = no. of fields or experimental plots.

Less variability in larval infestation levels and expected yield response (narrower 95% CI) reflects less management risk to the grower and explains why many growers will often apply six or more treatments/season (Beasley et al. 1985, Staten et al. 1987). However, it is important to emphasize that these results are for the "average" field with "average" pink bollworm pressure and assume that all applications are optimally timed. Because of the yield variability illustrated in Fig. 4, I developed three additional larval density-yield scenarios that reflect some of the variability observed in commercial field situations. In addition to the "average pink bollworm pressure" obtained from published data (solid line, Fig. 4) "low pressure," "high pressure with good (optimal) timing" of applications, and "high pressure with poor timing" were also calculated based on the observed variability. All four scenarios are illustrated in Fig. 5A, where the "average" pressure yield response is identical to the average yield response in Fig. 4. The "low" pressure response is based on the upper limit of the yield response variability in Fig. 4 and the "high (poor timing)" response is based on the lower limit. "High pressure (good timing)" response is based on the lower limit. "High pressure (good timing)" is in between and represents a realistically increasing yield response due to optimum timing (either fortuitous or planned). Low pressure fields are also fields where pink bollworm infestations do not typically develop until late in the season after peak-boll whereas high infestations are those that are considered to develop early (e.g., ≥ 30% larval infestation at first-boll or any time before peak-boll).

In addition to the larval density and insecticide yield relationships (Figs. 4 and 5A),

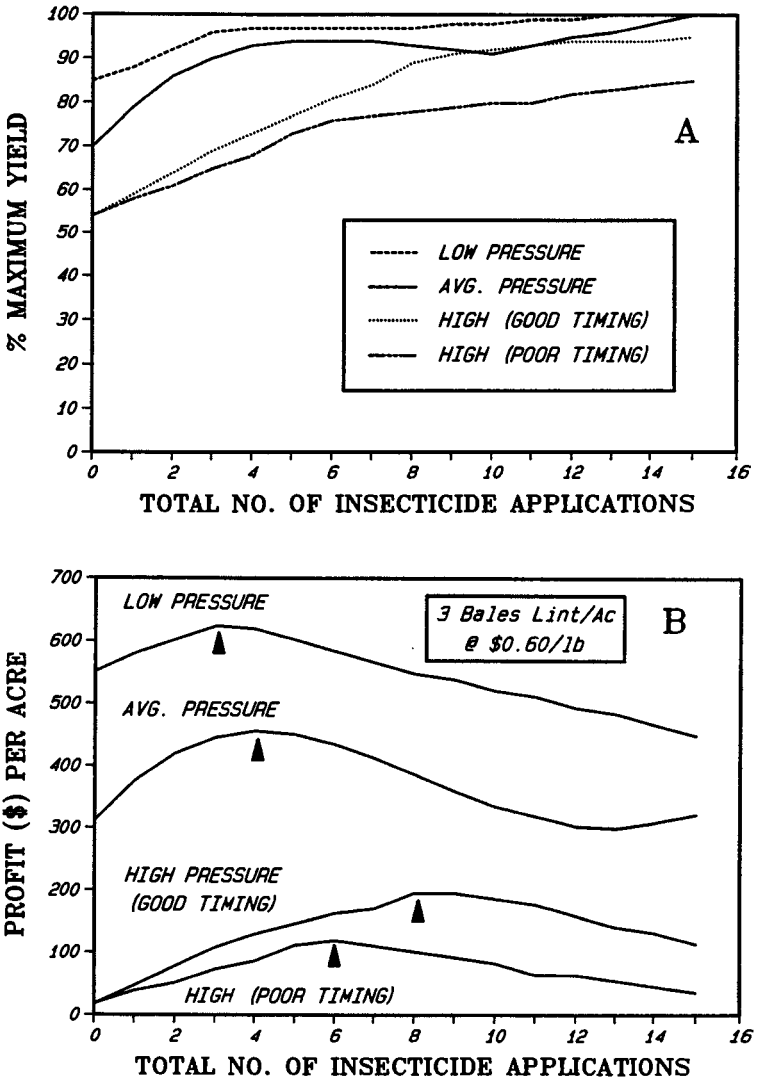

FIG. 5. (A) Pink bollworm pressure scenarios used for estimating optimum number of insecticide applications, and (B) Profit/ac for each scenario.

the cost of insecticide applications and market prices for cottonseed and lint are needed to calculate ETs. In the following analysis, I consider lint prices ranging from $0.50 to $0.70/lb. Calculations are made for expected lint yield varying from 2.0 to 3.5 480-lb bales/ac (960-1680 lbs/ac). Income from seed was based on a gross of $0.0625/lb minus aflatoxin treatments costing $20.00/ton, resulting in a net return of $0.0525/lb. Based on Fig. 2A and larval density/seed weight data of Wilson and George (1985), larval infestation levels < 20% are also assumed to reduce seed production (and revenue) by 6% whereas those > 35% are assumed to reduce seed production by 10%. Each insecticide application is assumed to cost $10.00/acre, plus $3.00/acre for application costs. Calculations are therefore based on an average treatment cost of $13.00/ac. However, an additional cost of $5.00/acre for acaricides to control spider mite (*Tetranychus* sp.) infestations developing after the first four applications was also added, reflecting this scenario observed during the 1987 season in the Palo Verde Valley (Hutchison et al. 1991).

Using the optimization formulas presented by Gutierrez and Wang (1984): Net Revenue (Profit) = B ($\underline{x}$,N) - C ($\underline{x}$,N), where B = benefits associated with $\underline{x}$ amount of pesticide, N is some function of population density, and C are corresponding costs. Specifically, costs include cost of pesticide + cost of unprevented damage,

$$C(\underline{x},N) = P_L,H(\underline{x}) + (Y(Max.) * PCIMX(\underline{x} = 0) \bullet P_2$$

where: P_L = low cost of pesticide for x = 1 to 4, P_H = high cost of pesticide for $x \geq 5$ (includes additional cost for acaracides), PCIMX (x=0) = % of maximum yield Y(Max) with no insecticide applications based on Figs. 4 and 5A for specific pink bollworm infestation scenarios, and P_2 = market price of lint and seed. Benefits of gross revenue were calculated as $B(\underline{x},N) = P_2 \bullet Y(Max.) \bullet PCIMX(\underline{x})$. The economic threshold, in terms of $\underline{x}$, then becomes $B(\underline{x}) - C(\underline{x}) \geq B(\underline{x}=0)$ and the optimum amount of $\underline{x}$ is $\underline{x}^*$ such that Max. Profit = $B(\underline{x}^*) - C(x^*)$.

As shown in Fig. 5B, profit varies considerably for each pink bollworm pressure scenario, from $600 (low pressure) to $100/ac (high-poor timing). For the low, average, high-good timing and high-poor timing scenarios, the optimum number of insecticide applications/field was 3,4,9 and 6, respectively. Considering the relatively broad profit peaks for each scenario, ± 1 application per infestation scenario would be a reasonable estimate of the variability associated with these results (i.e., variance within this range would not likely alter the final yield analysis). In addition, calculations based on the range of lint, seed and market prices all resulted in the same profit response patterns for a given pink bollworm pressure scenario (Fig. 6). Furthermore, for the average pressure scenario, Fig. 7 shows how the increasing cost of additional insecticide applications limit profit beyond 6 applications.

Simulation Studies. As indicated by the previous analysis, it is difficult to incorporate all the necessary variables of a biological system into conventional optimization analysis. One alternative to conventional regression model analysis is the use of computer simulation models of insect pest-crop dynamics. Stone and Gutierrez (1986), and Stone et al. (1986), conducted extensive ET simulation studies using a computer simulation model of cotton and pink bollworm population dynamics. They were specifically interested in the economics of applying insecticide and/or pheromone (gossyplure) treatments, where pheromone is applied as a mating-confusion tactic designed to inhibit mating, and minimize development of economic infestations (Stone and Gutierrez 1986). They also examined different criteria for timing insecticide and pheromone (gossyplure) treatments and evaluated these strategies for five different initial (early-season emergence) pink bollworm population densities.

Strategies which provided maximum average profits as well as minimum profit variability included insecticides applied using an economic threshold of $\leq$ 8% larval-infested bolls with two early-season (pre-boll) treatments and insecticide applied at a 5% larval infested threshold, plus pheromone applied early. Both early-season pheromone and

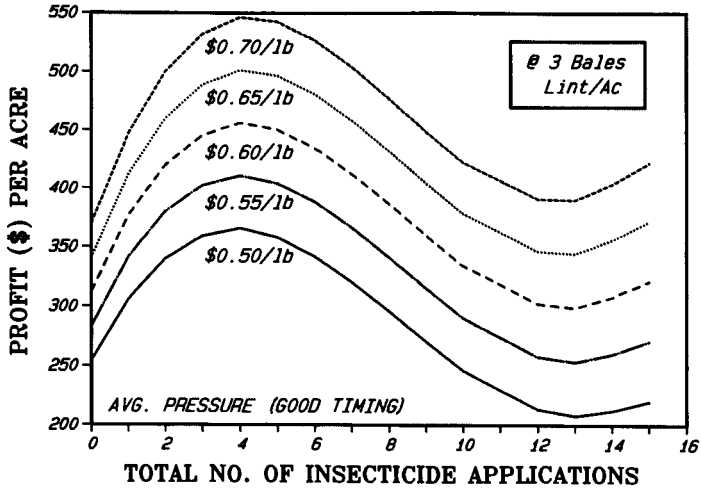

FIG. 6. Profit comparisons for specific prices for the "average pink bollworm pressure" scenario.

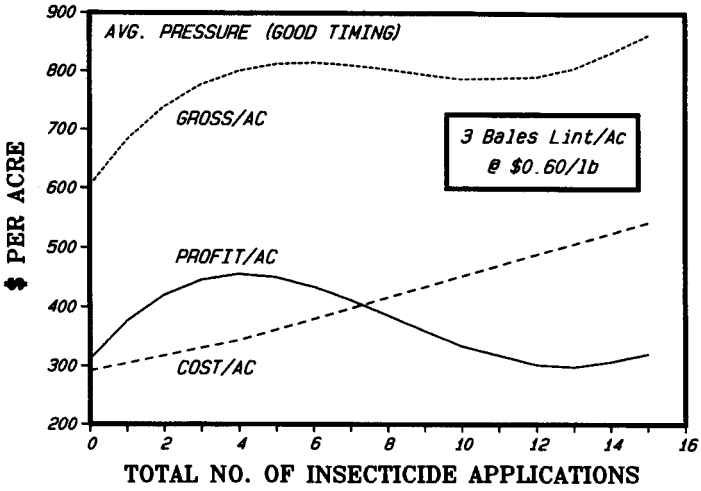

FIG. 7. Gross revenue, cost and profit/ac for the average pink bollworm pressure" scenario, for an average yield of 3 bales/ac, and $0.60/lb.

insecticide applications had the effect of delaying subsequent population growth and reducing the total number of insecticide applications required.

Although these simulation studies indicated that 5% larval ET provided the most consistent results, which is reasonable assuming an EIL of 12-15%, the average number of applications per season was much higher than those shown in Fig 5B. For example, Stone et al. (1986) usually obtained maximum profits when at least ten or more treatments were applied. As indicated by these authors, the results are theoretical in nature and must be interpreted based on the assumptions inherent to the simulation model and calculations employed. One aspect that was not included in these calculations was any penalty for early and continuous insecticide use, such as resurgence of secondary pests such as whitefly. Given such a penalty, the pheromone plus insecticide combination would likely provide a more environmentally and economically sound policy.

Hutchison (1988) also developed a simulation model of pink bollworm population dynamics which has been useful for studying various action thresholds, as well as the timing of insecticide applications. The model has been used to conduct full-season validations and to perform insecticide timing studies with egg infestation levels (i.e., $\geq$ 6% of bolls infested; Hutchison et al. 1991) as the treatment criterion. This model incorporates age-specific development rates (Hutchison et al. 1986a), the impact of insecticide applications on oviposition timing and magnitude (Hutchison et al. 1988b), and egg-larval survivorship in the field (Hutchison et al. 1988c). These simulations have shown that the most economical timing for any given increasing moth flight is when 6% of the bolls are infested with eggs. This timing had more impact on an increasing infestation than applying an insecticide when first adults were present even though the 6% egg infestation was usually not reached until 6-8 days after first moth emergence. For pink bollworm generations which occur in August (usually F_{3-4}), treating at the 6% egg infestation level and automatically treating 5 days later, was also more effective than treating once and waiting until the egg population again reached the 6% level.

SAMPLING

Successful implementation of economic thresholds is highly dependent on the development of practical sampling methods that can be utilized by consultants and growers in the field (Hutchison et al. 1991, Naranjo and Hutchison 1997). Over the past 20 years, several methods for monitoring pink bollworm infestations have been proposed. Here, I review the advantages and disadvantages of larval, adult and egg sampling.

Larvae. Historically, the method most commonly used to monitor pink bollworm for deciding when insecticides should be applied has been checking, or "cracking" bolls for larvae (e.g., Fye 1976, Toscano and Sevacherian 1980, Fry and Henneberry 1983). When monitoring larval infestations, bolls usually 14 to 21 days old (for upland cotton), generally considered most susceptible to pink bollworm attack, should be collected at random in each quarter of the field. It is usually recommended that 25 bolls be examined/quadrant (e.g., Ellington et al. 1985). This is especially true of 80-ac fields, but two to three 25-boll samples are usually sufficient for 40-ac fields

Although the magnitude of larval infestations in bolls at any given time of the season is indicative of the potential for yield loss, there are several disadvantages in using larval counts as a criterion for deciding when to initiate control measures. Using larval infestations to determine when to treat for adults has virtually no impact on larvae already present in the bolls. More important, however, the use of a larval threshold results in a significant time lag between the period of increasing adult populations and the time when insecticides are actually applied. Hutchison et al. (1988a) estimated a total time lag of 6-9 days based on an adult preoviposition period of 1-2 days, and an average egg developmental time of 4-6 days. Additional delays may also result from the difficulty of early detection of small instars in bolls

and timely application of an insecticide treatment.

As indicated by the simulation study of Stone et al. (1986), a 5% larva-infested boll ET is probably optimal, but because of the sampling error associated with estimating such a low threshold, it is also possible for pest control advisors to miss infestations increasing beyond the 5% level. This phenomenon adds yet another element of risk in the decision-making process and indicates why larval ETs as low as 1% have been reported (Staten et al. 1987). Data shown in Table 2 can be used to estimate mean larval density per boll, based on the percentage of bolls infested with one or more larvae (see also Hutchison et al. 1986b).

Adults. As with many other agricultural insect pests, the discovery of the pink bollworm pheromone in the late 1960s facilitated the development and proliferation of a variety of pheromone-baited traps for monitoring purposes. The obvious advantage of pheromone traps is that moth populations, the targeted stage for insecticidal control, can easily be monitored by pest control advisors and growers. Toscano et al. (1974), using hexalure-baited traps, were the first to experiment with moth trap catch as a criterion for making treatment decisions. They found that a treatment threshold of 3.5-4.0 moths/trap/night worked well in large-scale field tests in the Imperial and Palo Verde valleys from 1971 to 1973. To minimize some of the maintenance costs associated with trap monitoring, Toscano et al. (1979) later developed a thermal summation (degree-day) program for predicting generation peaks and the time when traps should be monitored closely.

Hexalure, however, was subsequently replaced by gossyplure (Hummel et al. 1973), 1:1 mixture Z, Z & Z Z, E isomers of 7, 11-hexadecadienyl acetate, which proved to be more efficient at attracting male moths in the field (Flint et al. 1974, 1976). Since the late 1970's Delta traps (Sandia Die and Cartridge Co., Albuquerque, NM), baited with 1.0 mg of gossyplure, have been used extensively by pest control advisors and researchers for monitoring moth populations (e.g., Beasley and Adams 1994a, Beasley et al. 1985). Beasley and Adams (1994b) also used a Multi-Pher 'non-overloading' trap, baited with 1 mg of gossyplure, to estimate times of peak capture. They found that peak capture was most correlated with minimum night temperature, time when wind speed fell below 2.7 m/s, and moon age. An extensive analysis of alternative degree-day models, using the Delta trap, was conducted by Beasley and Adams (1996). Their results indicated considerable agreement between field-based developmental thresholds and those estimated from constant temperature studies in the laboratory. They found that a lower and upper threshold of 13.9 and 32.8°C, respectively, provided the best fit to field data. These thresholds for the degree-day model, along with the Delta trap, can be very useful tools for detecting initial and peak flights, and thus optimizing actual field sampling time to verify infestation levels in bolls (egg or larva sampling).

Although pheromone traps provide an index of moth activity and early-season trap catches (at first "hostable" or susceptible square, i.e., flower bud at ¼-inch diameter) have been correlated with late-season larval infestations (Adams et al. 1986), trap catch can still be limited as the only criterion for making within-season treatment decisions. For example, once two to three insecticides have been applied, post-treatment moth catches are not consistently indicative of actual larval infestations in bolls (Flint et al. 1976, Henneberry and Clayton 1982). Inconsistent results may occur because the response of males to pheromone-baited traps may be affected just as sublethal insecticide exposure effects the chemical communication between male and female moths (Haynes and Baker 1985). The combination and diversity of insecticide materials applied also contributes to the variability observed among studies of pheromone trap catches of moths and subsequent larval infestations. For example, Huber and Chesser (1984) found that trap catches of male moths increased after two organophosphate insecticides (azinphosmethyl and methyl parathion) were applied, and decreased after two applications of permethrin. Larval infestations in both sets of fields, however, were maintained at similarly low levels.

An additional problem with using pheromone traps for making treatment decisions is

that the traps catch only male moths. Although one can usually assume a 50:50 sex ratio for pink bollworm (Henneberry and Clayton 1982), male trap catches may not necessarily be indicative of female moth dynamics. This result may especially be true after one or more insecticides have been applied. Although a malaise trap has been developed (Butler 1966, Butler et al. 1983) for monitoring both male and female moths, the traps are most effective only for relatively high population densities and therefore most useful for research purposes. Their size (6x4x12 ft) and nonspecificity for pink bollworm moths also precludes implementation on a large-scale basis.

Despite the problems associated with pheromone trap catch data during the season, more recent studies by Adams et al. (1986) and Chu and Henneberry (1990) found that early-season trap catches (at first susceptible square) were often correlated with mid-to late-season larval infestations in flowers and bolls. These relationships may be particularly useful as an early-season, advance warning for pest control advisors, indicating high-risk fields where one or two early-season (pre-boll) insecticide applications may be justified.

Egg Sampling. Because of the problems associated with using larval sampling or moth trap catches as criteria for deciding when insecticide applications should be applied, Hutchison et al. (1986b, 1988a, 1991) evaluated egg sampling as an alternative treatment threshold criterion. The primary rationale for sampling eggs was that the egg stage presently represents the only index of female moth activity that can be efficiently measured in the field.

As mentioned previously, Hutchison et al. (1988a, 1991) observed 23-35% reductions in insecticide use compared with fields monitored by the 5% larva-threshold method and/or scheduled applications. Computer simulations (Hutchison 1988; unpublished data) also support these results; for any given increasing pink bollworm infestation, delaying insecticide application until the egg infestation reaches 4-6% is preferable to treating at first adult or after larvae enter the boll. Assuming an average egg-larval mortality rate of 80% for eggs laid on bolls in insecticide-treated fields (Hutchison et al. 1988c), 4-6% egg infestation, should on average, result in a maximum 4-6% larval infestations, which is close to the larval ET (Hutchison et al. 1991). Percentage boll infestations are similar, despite the 80% larval mortality rate, because eggs laid on bolls are much more aggregated compared with surviving larval infestations (Table 2). In a detailed study of the spatial pattern of pink bollworm oviposition on cotton bolls, 63% of all eggs were found in egg masses of 2-8 each; 25% were laid singly and mean egg mass size was 4.43 (Hutchison et al. 1986b).

To minimize the time required to sample egg populations, a sequential sampling plan was developed and field-tested (Stroschein et al. 1988) using an egg treatment threshold of 6% infested bolls (i.e., percentage with one or more eggs/boll). Based on Green's (1970) sequential sampling plan, typically used to estimate population density, the plan was modified to classify egg infestations above or below the action threshold. These plans are developed to control for making one of two errors. The type I error (α) is the probability of taking control action when the pest population is below action threshold, whereas type II error (β) is the probability of not taking control action when the pest population is above the action threshold.

The probability of an α error, or treating a field when a treatment is not justified (i.e., actual egg infestation < 6%), was considered less critical for the pink bollworm-cotton system since pink bollworm egg sampling has been shown to minimize insecticide use over conventional larval monitoring methods (Hutchison et al. 1988a, 1991). Thus, the plan by Stroschein et al. (1988) was evaluated by estimating only the β error rate. The probability of making an incorrect treatment decision (β) averaged only 6.4% for the season (Table 4). The highest error rate (9.7%) occurred in August, which is still reasonable for pest management purposes.

Stroschein et al. (1988) also found a highly significant ($P<0.001$; $z = 7.84$; $df = 131$) correlation ($r = 0.60$; 95% CI = 0.48 to 0.70) between sequential- and fixed-sample estimates of pink bollworm egg infestations. The observed average sequential sample size averaged 97 bolls/field, typically requiring three to four 25-boll samples. However, further analysis of the

sequential sampling data, showed that the plan was more conservative than necessary for maintaining the same error rates shown in Table 4. Subsequent analysis indicated that approximately 95% of all treatment decisions could have been made after sampling only 25 to 50 bolls while still maintaining an average seasonal error rate of 6.4%. A final adjusted average sample size of 46 bolls/field required an average of only 15 minutes/field, which is

TABLE 4. Monthly and Seasonal Number of Insecticide Treatment Decisions for Pink Bollworm Control and % Incorrect Treatment Decisions Made by Sequential Sampling of Pink Bollworm Eggs, Palo Verde, CA, 1987.

Month	No. Treatment Decisions	No. Incorrect Decisions[a]	% Incorrect
June	16	2	6.2
July	68	2	2.9
August	72	7	9.7
Season	156	10	6.4

[a]Decision to not treat when the actual egg infestation > 6% [type II error(β)].

approximately a 70% reduction in sampling time over the fixed sample size of 160 bolls/field.

More recently, two additional binomial sequential sampling plans for pink bollworm eggs were developed using a resampling validation analysis (Naranjo and Hutchison 1997). With these plans, an action threshold of 12% bolls infested (ca. 0.75 eggs/boll; Table 2) was used. For both plans, the probability of making α (type I) and β (type II) errors were pre-set at 0.10 (e.g., Wald 1947). With Wald's binomial plan (Wald 1947), maximum sample size approached 75 bolls, near the action threshold. However, for most data sets, average sample size was only about 10-15 bolls. A binomial plan based on fixed sample sizes of 25 or 50 bolls was also acceptable, resulting in similar operating characteristic (OC) functions and comparable to the OC for Wald's plan. For all plans, α was held to very low levels, averaging 0.004. For the fixed-sample binomial plan, a fixed sample size of 50 bolls reduced the β error rate from 0.084 to 0.033 (where β was pre-set at 0.10). In summary, the sample size and error rate results observed for the resampling analysis of each binomial plan were similar to the field validation of the previous plan developed by Stroschein et al. (1988). The binomial egg sampling plans, which provide early detection of pink bollworm infestations, with low error rates, may therefore become a practical sampling alternative for both IPM and research purposes.

PRODUCER GOALS AND DECISION-MAKING

In the present analysis, I have made the assumption that the goal of most IPM programs is to maximize profit within the current season. However, producers' goals often become more complex, particularly with longer planning horizons (e.g., > 10 years), and current financial constraints. Complexity also arises when subsidy programs are involved, which encourages cotton growers to maintain maximum yield (e.g., Lacewell et al. 1986) over a period of several years. Although maximizing yield and maximizing profit are not necessarily mutually exclusive, they often become conflicting goals in cotton growing regions

of the southwestern U.S. where yields can exceed 3.0 bales/acre (Hutchison et al. 1991).

Lacewell et al. (1986) provide an accurate picture of the role of cotton subsidy programs in influencing producers pest management decisions. In addition to fluctuating market prices and profit maximization, the authors emphasize that most cotton growers also consider the additional goals of high yields, controlled risk, and long-term corporate growth and survival. All goals are influenced by the government program and the planning horizon of the producer. Given the multiple variables influencing producer decisions, the authors suggested IPM be evaluated in terms of continued "profit over time (year to year) subject to a 'tolerable' level of risk, and an IPM strategy that stabilizes yield over time and is associated with acceptable profit; this might be preferred to one that maximizes yield or profit in any one year."

Most IPM programs developed for cotton tend to maintain yield and reduce costs rather than increase yield (Lacewell et al. 1986). As indicated by this review, consistently adhering to an ET for pink bollworm management should result in reducing insecticide cost while maintaining yield (Table 1). The field results summarized here, and those of Stone et al. (1986), also suggest that an ET approach can be implemented without subjecting the grower to increased risk over the long term. This strategy, from a regional economic viewpoint, may in fact be preferred over the ever present concern for increasing yields. Lacewell et al. (1986) also discuss the paradoxical impact of increasing cotton yields; despite the positive economic effects of locally higher prices, excess supplies may lower regional prices.

Ideally, if more research-based information (tools) for the pink bollworm/cotton system is made available to the grower, information should replace unnecessary insecticide applications (Feder 1979). However, to implement decision aids that will actually be adopted by producers, one should consider the behavioral and practical elements of new technology (Mumford and Norton 1984). During the past 15 years, computers have played a significant role in the development of our understanding of how dynamic pest/crop systems function, primarily through the use of simulation modeling and optimization techniques (Gutierrez and Wang 1984, Stone et al. 1986, Venette and Hutchison 1999), as well as expert systems (Lemmon 1986), and comprehensive decision support systems (Geyer et al. 1994, Sequeira et al. 1996). Although a variety of computer-based decision aids have been implemented at the farm level, further adoption of data-intensive models has been more difficult to sustain (Wilson 1989).

More recently, the deployment of Bt-cotton will likely have a major impact on reducing pink bollworm populations over entire production regions (Wilson et al. 1992, 1994; Flint et al. 1996). However, the degree to which growers adopt effective resistance management plans (Bartlett 1995, Gould and Tabashnik 1998) will be critical for ensuring long-term viability of the technology. Appropriate use of Bt cotton, coupled with other complementary tactics such as sterile-male release (Bartlett and Staten 1996), pheromone-based mating disruption (Staten et al. 1987, Miller et al. 1994), short-season production (Chu et al. 1996), enhanced biological control (Naranjo et al. 1995), and fall irrigation (Beasley and Adams 1995, Beasley 1997), should permit growers to be well positioned for successful pink bollworm management well into the next century.

ACKNOWLEDGMENT

I thank Tom Henneberry, Doug Wilson (USDA-ARS, Phoenix, Arizona), and Robert Venette (Department of Entomology, University of Minnesota), for their helpful reviews of an early draft. This publication was supported by the USDA-ARS, USDA-APHIS, and the University of Minnesota Agricultural Experiment Station. This is publication 99117-0016 of the University of Minnesota Agricultural Experiment Station.

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United States Postal Service

Statement of Ownership, Management, and Circulation

1. Publication Title	2. Publication Number	3. Filing Date
Southwestern Entomologist	3 2 4 _ 0 5 0	October 1, 1999
4. Issue Frequency	5. Number of Issues Published Annually	6. Annual Subscription Price
Quarterly (March, June, September, December)	4	$10.00 Individual $20.00 Institution

7. Complete Mailing Address of Known Office of Publication *(Not printer) (Street, city, county, state, and ZIP+4)*
Southwestern Entomological Society, c/o Allen Knutson
17360 Coit Road, Dallas, Texas 75252

Contact Person: Allen Knutson
Telephone:

8. Complete Mailing Address of Headquarters or General Business Office of Publisher *(Not printer)*
Southwestern Entomological Society, c/o Allen Knutson
17360 Coit Road, Dallas, Texas 75252

9. Full Names and Complete Mailing Addresses of Publisher, Editor, and Managing Editor *(Do not leave blank)*

Publisher *(Name and complete mailing address)*
Southwestern Entomological Society, c/o Darrell Bay
Department of Entomology, Texas A&M University, College Station, Texas 77843-2475

Editor *(Name and complete mailing address)*
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Department of Entomology, Texas A&M University, College Station, Texas 77843-2475

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(non-profit)	Texas A&M University
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PS Form **3526**, October 1999 *(See Instructions on Reverse)*

13. Publication Title: Southwestern Entomologist			14. Issue Date for Circulation Data Below: June	
15. Extent and Nature of Circulation			Average No. Copies Each Issue During Preceding 12 Months	No. Copies of Single Issue Published Nearest to Filing Date
a. Total Number of Copies *(Net press run)*			525	525
b. Paid and/or Requested Circulation	(1)	Paid/Requested Outside-County Mail Subscriptions Stated on Form 3541. *(Include advertiser's proof and exchange copies)*	468	468
	(2)	Paid In-County Subscriptions Stated on Form 3541 *(Include advertiser's proof and exchange copies)*	3	3
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c. Total Paid and/or Requested Circulation *(Sum of 15b. (1), (2),(3),and (4))* ▶			471	471
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g. Total Distribution *(Sum of 15c. and 15f)* ▶			482	482
h. Copies not Distributed			43	43
i. Total *(Sum of 15g. and h.)* ▶			525	525
j. Percent Paid and/or Requested Circulation *(15c. divided by 15g. times 100)*			98%	98%

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