INFLUENCE OF SEX PHEROMONE TRAP PLACEMENT RELATIVE TO FIELD EDGE ON CATCH OF BOLLWORM[1] MALES IN COTTON[2]

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ABSTRACT

Tests were conducted to compare the practical operation of bollworm, *Helicoverpa* (=*Heliothis*) *zea* (Boddie), sex pheromone baited traps situated adjacent to and directly within cotton, *Gossypium hirsutum* L., fields. Although the numbers of male moths caught were reduced in traps outside the field, numbers were adequate to allow inferences regarding comparable numbers caught within the field. A correction factor was developed to adjust for differences in location, allowing traps to be placed near the perimiter of the cotton field to avoid conflicts with tillage, irrigation and pesticide application.

INTRODUCTION

Sex pheromone baited traps provide a sensitive method for detecting bollworm, *Helicoverpa* (=*Heliothis*) *zea* (Boddie) (Hartstack et al. 1979, Lopez et al. 1987). Recent studies concerning the potential of traps for estimating pest population density levels in cotton, *Gossypium hirsutum* L., have been designed with trap placement some distance within the field itself (Hayes et al. 1988, Lopez et al. 1988). During practical application, however, it would be more efficient to service traps located adjacent to a field. Eliminating the need for workers servicing traps to enter

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[1]Lepidoptera: Noctuidae

[2]This research was conducted in cooperation with the Texas Agricultural Experiment Station, Texas A&M University, College Station, TX 77843. This paper presents the results of research only. Mention of a proprietary product does not constitute an endorsement of this product by the U.S. Department of Agriculture.

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fields minimizes potential damage to the crop and reduces concern of contact with pesticides. Placement of traps outside the fields also avoids conflicts with field machinery and irrigation operations. This paper reports the results of studies conducted to determine variations in numbers of male moths caught in traps situated inside, directly on and outside the perimeter of a cotton field.

Similar tests have been carried out for other species and crops. Aliniazee (1983), for example, studied the catch of filbertworm, *Melissopus latiferreanus* (Walsingham), in filbert orchards with sex pheromone baited sticky traps. He found that traps placed within an orchard caught significantly more moths than traps placed outside. At distances of 10 m and greater from the orchard, however, very few moths were caught. Thus, no relationship could be derived to predict numbers caught within the orchard from those outside the orchard.

Tingle and Mitchell (1979) studied factors affecting catches of fall armyworm, *Spodoptera frugiperda* (J. E. Smith), in sex pheromone baited traps. In a study conducted in corn, no significant difference between catches in traps placed at the edge of the crop (10.8 per trap-night) and traps placed 25 m into the crop (13.4 per trap-night) was demonstrated. However, traps 25 m within a peanut field did catch significantly more males (49.2 per trap night) than traps at the field edge (29.8 per trap-night).

MATERIALS AND METHODS

This study was conducted in two relatively square, 16-ha commercial cotton fields on opposite sides of Hwy 60 in Burleson County, TX, *ca.* 15 km west of College Station from 17 May to 15 July 1988. Orientation of the highway was northeast to southwest (45°-225° azimuth), and the prevailing winds were from the SSE (160° azimuth). Cotton was planted in rows perpendicular to the highway with a cultivated turn-row of 3-4 m between the end of the rows and the highway right-of-way. Total distance between perimeters of the planted areas in the two fields was *ca.* 28 m. Routine practices of cultivation, irrigation and pesticide application were carried out throughout the study. Native vegetation which was periodically mowed covered the highway right-of-way during the test period.

Wire mesh, cone-75-50 traps (Hartstack et al. 1979) were baited with laminated plastic dispensers manufactured by Hercon Environmental Co., Emigsville, PA 17318 (Lopez et al. 1987). Baits, 1.27 x 2.54 cm, contained 1.25 mg of a four component mixture in the ratios identified by Klun et al. (1980). Wind speed and direction at 3 m above ground level were monitored and recorded hourly by an automatic weather station *ca.* 5 km north of the test site. The weather station and test site were in a relatively flat river bottom with *ca.* 1 m difference in elevation.

Three traps were placed at each of three locations parallel to the field perimeter: 5 m inside, at the perimeter, and 5 m outside. The 5 m distance within the field was selected to coincide with the USDA-ARS Pilot Test on Pheromone Trap Calibration (Lopez et al. 1988). The two positions outside the field were selected to bracket a range

at which traps might be operated, depending on the existence of turn rows, field roads or fence lines adjacent to cotton to be monitored. Therefore, traps were situated on a line perpendicular to the field perimeter at 100-m intervals, and their positions relative to the perimeter within each replicate were rotated daily, so that each treatment was situated at each location by the end of three days. This basic design was replicated three times so a total of nine traps were used, and all were spaced at 100 m intervals. The test was conducted for five 3-day periods, or blocks, in the field on the northwest side of the highway; traps were then moved to the southeast side of the highway and the test was conducted for five additional 3-day periods (blocks).

Analysis of variance was computed and the means for each treatment compared using Duncan's Multiple Range Test with α=0.05 (SAS Institute 1988). Regression analysis was computed using a nonlinear regression technique (STSC 1987).

RESULTS AND DISCUSSION

There were no significant differences in male moths numbers caught between any of the nine locations when the data from each field for each trap position were pooled. During the first five time periods, winds were from the southeast for six nights, the northwest for one night, variable for three nights, and light (< 0.5 m/s) for five nights. During the second five time periods, winds were from the southeast for 13 nights, the northwest one night, and light (< 0.5 m/s) for one night. Overall, winds were not from the prevailing direction (160°) only five of 30 nights, and wind conditions did not influence the statistical significance of the results.

A comparison of the treatment means calculated from the complete 10 block data set is shown in Table 1. Traps located 5 m inside the field perimeter resulted in significantly greater catches of males than the other two positions. There was no significant difference between numbers of males caught in traps at the perimeter and those caught in traps placed 5 m outside the perimeter.

TABLE 1. Bollworms Caught in Pheromone Traps at Three Positions Relative to Cotton Field.

Trap Position	Males Per Trap-Night[ab]
5 m inside	91.4±86.5 A
perimeter	63.1±53.1 B
5 m outside	59.6±49.0 B

[a] Mean ± standard deviation for 10 blocks (N=90) (9 replicates per block, 3 temporal X 3 spatial).
[b] Means followed by the same letter are not significantly different (α=0.05, Duncan's Multiple Range Test).

Data for traps at the perimeter of the field and traps outside the perimeter were pooled since they were not

significantly different. These trap positions are referred to collectively as "adjacent" for remaining analysis. A predictive relationship between the numbers caught in traps adjacent to the field and numbers caught inside was found to be:

$$Ti = Ta / (0.842 - 0.00176 \cdot Ta)$$

where Ti is number caught in traps 5 m within the field perimeter and Ta is number caught in traps 0-5 m adjacent to the crop. The correlation coefficient (r^2) was 97%. This function is plotted together with the data in Fig. 1. The resulting model allows the estimation of number that would be caught inside the field from the catch obtained in traps operated outside the field within 5 m of the crop perimeter. The adjustment factors extrapolated from the model are 1.0 for catches of 10 or less, 1.25 for catches near 60, 1.5 for catches near 100 and 2.0 for catches of near 200.

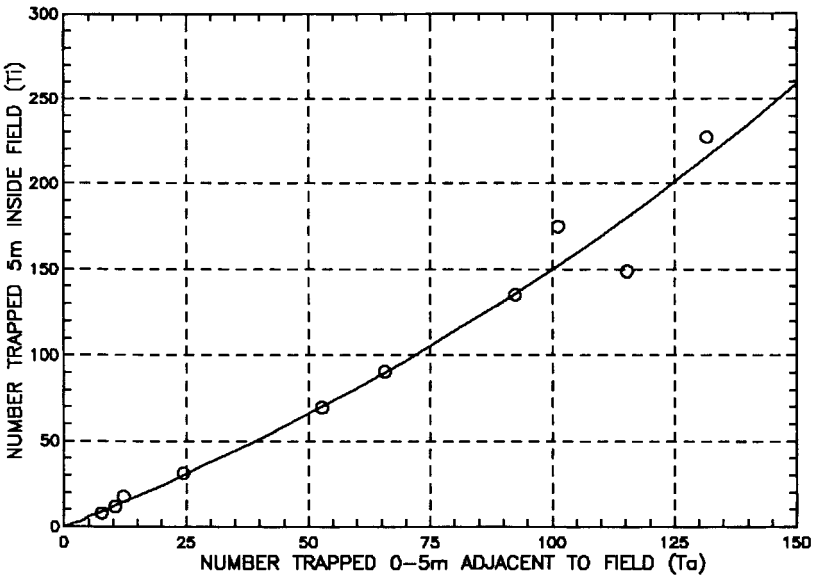

FIG. 1. Regression results for numbers of bollworms caught in traps placed 5 m inside cotton field compared to numbers caught 0 to 5 m adjacent to field.

Further statistical analysis demonstrated a significant difference between blocks (3-day time periods), primarily due to an increase in the numbers of males caught as the experiments progressed. Test of significant differences between means grouped by block, as shown in Table 2, indicated a tendency for less difference in numbers caught at lower population densities than at higher densities.

TABLE 2. Bollworms Caught in Pheromone Traps at Three Positions Relative to Cotton Field by Block.

Block	Males per trap-night[ab] at indicated treatment		
	5 m inside	perimeter	5 m outside
	Northwest Field		
1	7.7± 3.7 A	8.4± 3.4 A	6.9± 2.9 A
2	11.6± 8.8 A	12.4± 7.7 A	8.3± 4.6 A
3	17.4± 5.2 A	11.7± 3.0 B	12.6± 4.3 B
4	31.1± 7.8 A	25.1± 8.3 A	23.8± 6.3 A
5	69.4± 13.2 A	54.9±13.5 B	50.4±17.3 B
	Southeast Field		
6	148.9± 52.8 A	123.3±39.6 A	107.2±45.3 A
7	90.4± 28.7 A	67.1±16.4 B	64.1±15.9 B
8	227.2±110.4 A	147.3±36.2 B	115.9±19.7 B
9	174.9± 54.5 A	89.1±27.7 B	113.2±34.8 B
10	135.0± 61.4 A	91.1±49.7 A	93.8±40.7 A

[a] Mean ± standard deviation of nine replicates per block (3 temporal x 3 spatial).
[b] Means in the same row followed by the same letter are not significantly different (α=0.05, Duncan's Multiple Range Test).

Based on the results on Table 2, we would not have found a significant difference between trap positions relative to the field perimeter if our experiments had been conducted only during periods when catches were below approximately 50 males per trap-night. Tingle and Mitchell (1979) reported a significant difference in peanuts with average in-field catches of fall armyworm near 50 per trap night, but no difference in corn with catches of about 13 per trap night. Our results indicate that the failure to detect a significant difference between moth catches in traps placed inside or outside corn fields may have been due to population density rather than crop species. Without suggesting any relationship between the two species involved, we recommend that future tests involving the effects of pheromone trap location on captures should include considerations of population levels.

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AN ECTOPARASITE SURVEY OF MAMMALS IN BREWSTER COUNTY, TEXAS, 1982-1985

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ABSTRACT

A total of 26 species of small to medium-sized mammals collected in an ectoparasite survey in Brewster County, TX were found to be infested with 11 species of ticks, 20 species of fleas and five species of lice. Plague was not evident in blood samples of 22 mammalian species collected. Rickettsial and trypanosomal infections were found in packrats, ringtails and desert cottontails.

INTRODUCTION

An ectoparasite survey was initiated in Brewster County, TX during 1982. The principal objectives of this survey were (1) to determine the species of potential vectors of arthropod-borne diseases on small to medium sized mammals and (2) to determine which etiological agents might be found in the vectors and hosts. In addition to our own field work, additional blood and ectoparasitic specimens were obtained by graduate students working on other projects whenever the opportunity arose. An incident of plague in a bobcat near Alpine in Brewster County in May 1981 (Tabor and Thomas 1986) served an impetus for this study.

Brewster County is the hub of a rather large tourist-based economy in sparsely populated Trans-Pecos Texas. Big Bend National Park, Guadalupe Mountains National Park, and a number of state parks within the region draw many people into the area for outdoor activities. This provides the opportunity for closer contact with wild animals, their denning sites, and their ectoparasites. Also, more homes are being constructed on small acreage ranchettes that, along with the already large rural population, increases the chance of exposure of humans to arthropod-borne diseases.

MATERIALS AND METHODS

Study area. Brewster County, TX lies within the northern extension of the Chihuahuan Desert in the Trans-Pecos Texas area. Elevations range from ca. 800-2100 m. Collections were made from mammals inhabiting intermontane grassland sites and from shrub desert sites. Intermontane grasslands are sloping plains and mountainsides (ca. 1500 m) below the juniper-oak woodlands of higher elevations. These grasslands are interspersed with woody shrubs, cacti, and a few trees. Typical shrubs are *Ephedra*, *Yucca*, *Larrea*, and *Koeberlina*. Drainage areas are lined with junipers, *Prosopis* spp., and sumacs (*Rhus* spp.). Species of

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Bouteloua, *Aristida*, and *Sorghastrum* are prevalent grasses. The shrub desert vegetation consists of woody shrubs, cacti, and a few grasses. Predominant shrubs are *Larrea*, *Agave lechuguilla*, *Fouquieria splendens*, and *Dasylirion leiphyllum*. *Bouteloua breviseta*, *Hilaria mutica*, and *Erioneuron pulchellum* are the prevalent grasses.

The shrub desert area is largely uninhabited by humans and the intermontane grasslands are grazed extensively by cattle and are settled by ranchers. Alpine, TX (population 7835) and Marathon, TX (population 800) are the only towns of size lying within the grassland areas. Lajitas, TX (population 6) and Study Butte, TX (population 120) lie within shrub desert areas. The population of humans in this area varies throughout the year due to visitors to the Big Bend National Park and Davis Mountains area.

Collection techniques. Small mammals were caught in live traps and larger mammals were caught in snap traps or harvested by gun. Live mammals were euthanized in plastic bags with chloroform soaked cotton at the collection sites and examined at the research laboratories at Sul Ross State University. Ectoparasites collected were either stored in 70% ethanol or live arthropods were sent to Texas Department of Health Laboratories (TDH) in Austin to test for tularemia, *Rickettsia rickettsii*, and other diseases.

Whole blood was taken by heart puncture using a 3 cc disposable syringe with heparin with a 2.5 cm, 21-gauge needle on selected mammals. Blood was transported on ice to our laboratory. Thin and thick blood smears stained with Wright's stain were examined for haematoprotozoans. The rapid slide latex linked agglutination blood serum assay (Hechemy 1983) was used for screening of *Rickettsia rickettsii* and *Francisella tularensis* agglutination in lagomorph blood sera.

Nobuto blood sampling paper strips (Toyo Roshi Kaisha, Ltd. Tokyo, Japan) were used in the field to collect blood for plague antibody testing. Strips were soaked in blood from the host animal, air dried, and sent to the Plague Branch, Center for Vector-borne Viral Disease, Fort Collins, CO for plague antibody testing (Passive Hemagglutination Inhibition assay).

Species identification of the ectoparasites and mammalian hosts were based upon published keys of Babcock and Ewing 1938, Kohls 1940, Ewing and Fox 1943, Cooley and Kohls 1944, Hubbard 1947, Eads 1950, Ferris 1951, Eads et al. 1956, Smit 1958, Allred et al. 1960, Peterson 1960, Clifford et al. 1961, Price and Emerson 1971, Price and Hellenthal 1975, Schmidly 1977, Anonymous 1978, Kim and Ludwig 1978, Eads et al. 1987, and Jones et. al. 1988. Vouchers specimens for all ectoparasites were placed in the Invertebrate Collection at Sul Ross State University.

RESULTS AND DISCUSSION

Eleven species of ticks, 20 species of fleas and 5 species of lice were found on the 26 species of mammalan hosts examined (Table 1). None of the 143 (22 species) mammals tested for plague were positive. Four ringtails and one coyote, negative for plague, were taken in Brewster County where plague infected ringtail, bobcat, and coyotes had been collected previously (Tabor and Thomas 1986). Drought triggered local reductions in rodent populations during the course of this survey. This reduction may account for the absence of plague positive animals in our survey.

Echidnophaga gallinacea (Westwood) was the most commonly collected flea on 16 of 26 host species followed by *Pulex simulans* Baker and *Orchopeas sexdentatus* (Baker), respectively. Both *Geomydoecus expansus* (Duges) and *Hoplopleura hirsuta* (Ferris) were the most commonly collected lice from sampled host species. *Ixodes scapularis* Say and *Haemaphysalis leporis-palustris* (Packard) were the most commonly collected ticks.

Only three (*Dermacentor parumapertus* Neuman and *H. leopris-palustris*) of 16 ticks tested positive for *Rickettsia rickettsii*. Rapid slide latex linked agglutination blood sera assay tests were negative for RMSF and tularemia. Trypanosomes were found in blood smear

TABLE 1. Ectoparasites Recovered from Mammalian Hosts Collected in a Survey of the Trans-Pecos Texas Area Between September 1982 to August 1985.

Ectoparasite	Host	Prevalence	Range	Mean
ACARI				
Ixodidae				
Amblyomma americanum (L.)	*Lepus californicus* (Gray) (Black-tailed Jack Rabbit)	4/40	2-11	4
	Peromyscus maniculatus (Wagner) (Deer Mouse)	1/19	1	1
	Sylvilagus audubonii (Baird) (Desert Cottontail)	3/19	2-12	7
	Bassariscus astutus (Lichtenstein) (Ringtail)	1/16	2	2
Amblyomma inornatum (Banks)	*Lepus californicus* (Gray) (Black-Tailed Jack Rabbit)	1/40	1	1
	Sigmodon hispidus (Say and Ord) (Hispid Cotton Rat)	1/3	1	1
	Canis latrans (Say) (Coyote)	1/3	1	1
	Neotoma micropus (Baird) Southern Plains Wood Rat)	1/13	11	11
Dermacentor albipictus (Packard)	*Ammotragus lervia* (Pallas) (Aoudad Sheep)	1/2	1	1
Dermacentor parumapertus Neumann	*Peromyscus maniculatus* (Wagner) (Deer Mouse)	1/19	2	2
Dermacentor variabilis (Say)	*Lepus californicus* Gray (Black-Tailed Jack Rabbit)	1/40	3	3
	Sylvilagus audubonii (Baird) (Desert Cottontail)	3/19	1-2	1
	Dipodomys merriami Mearns (Merriam's Kangaroo Rat)	1/12	4	4
Haemaphysalis leporis-palustris (Packard)	*Lepus californicus* Gray (Black-Tailed Jack Rabbit)	16/40	1-10	8
	Sylvilagus audubonii (Baird) (Desert Cottontail)	4/19	2-8	5
Ixodes cookei Packard	*Conepatus mesoleucus* (Lichtenstein) (Hog-Nosed Skunk)	1/1	3	3
	Bassariscus astutus (Lichtenstein) (Ringtail)	1/6	2	2
	Spilogale gracialis (L.) (Western Spotted Skunk)	1/1	2	2
Ixodes scapularis Say	*Lepus californicus* Gray (Black-Tailed Jack Rabbit)	1/40	1	1
	Sylvilagus audubonii (Baird) (Desert Cottontail)	3/19	1-4	2
	Urocyon cinereoargentatus (Schreber) (Gray Fox)	2/7	1-14	7
	Bassariscus astutus (Lichtenstein) (Ringtail)	1/6	2	2
Ixodes woodi Bishop	*Neotoma micropus* Baird (Southern Plains Wood Rat)	1/13	4	4
Rhipicephalus sanguineus (Latreille)	*Lepus californicus* Gray (Black-Tailed Jack Rabbit)	12/40	1-3	2
	Canus latrans Say (Coyote)	1/3	1	1
	Sylvilagus audubonii (Baird) (Desert Cottontail)	3/19	1-9	4
	Dipodomys merriami Mearns Merriam's Kangaroo Rat	1/12	1	1
Argasidae				
Otobius megnini (Duges)	*Ammotragus lervia* (Pallas) (Aoudad Sheep)	1/2	3	3

SIPHONAPTERA				
Pulicidae				
Xenopsyllus cheopis (Rothschild)	*Mus musculus* (L.) (House Mouse)	1/2	1	1
Echidnophaga gallinacea (Westwood)	*Lepus californicus* Gray (Black-Tailed Jack Rabbit)	13/40	1-17	7
	Sylvilagus audubonii (Baird) (Desert Cottontail)	4/19	1-5	3
	Sylvilagus floridanus (Allen) (Eastern Cottontail)	9/15	1-94	21
	Peromyscus boylii (Baird) (Brush Mouse)	2/7	3-7	5
	Sigmodon hispidus Say and Ord (Hispid Cotton Rat)	2/3	1-5	3
	Canus latrans Say (Coyote)	2/3	1-37	19
	Peromyscus maniculatus (Wagner) (Deer Mouse)	1/19	3	3
	Urocyon cinereoargentatus (Schreber) (Gray Fox)	2/7	1	1
	Chaetodipus hispidus Baird (Hispid Pocket Mouse)	4/10	2-19	6
	Mus musculus L. (House Mouse)	1/2	1	1
	Dipodomys merriami Mearns (Merriam's Kangaroo rat)	4/12	2-26	8
	Bassariscus astutus (Lichtenstein) (Ringtail)	3/6	1-3	2
	Neotoma micropus Baird (Southern Plains Wood Rat)	1/13	10	10
	Spermophilus spilosoma (Bennett) (Spotted Ground Squirrel)	1/4	19	19
	Spermophilus variegatus (Rock Squirrel)	3/9	1-24	11
	Ammospermophilus interpres (Merriam) (Texas Antelope Squirrel)	2/3	3-84	34
Pulex simulans Baker	*Lepus californicus* Gray (Black-Tailed Jack Rabbit)	8/40	1-5	3
	Sylvilagus audubonii (Baird) (Desert Cottontail)	4/19	2-8	5
	Sylvilagus floridanus (Allen) (Eastern Cottontail)	7/15	1-14	8
	Canus latrans Say (Coyote)	2/3	1-5	3
	Urocyon cinereoargentatus (Schreber) (Gray Fox)	5/7	1-36	16
	Conepatus mesoleucus (Lichtenstein) (Hog-Nosed Skunk)	1/1	2	2
	Bassariscus astutus (Lichtenstein) (Ringtail)	4/6	1-2	1
	Neotoma micropus Baird (Southern Plains Wood Rat)	3/13	2-9	5
	Spilogale gracialis (L.) (Western Spotted Skunk)	1/1	1	1
	Spermophilus spilosoma (Bennett) (Spotted Ground Squirrel)	1/4	2	2
	Neotoma albigula Hartley (White-Throated Wood Rat)	3/10	1	1
Euhoplopsyllus glacialis affinis (Baker)	*Lepus californicus* Gray (Black-Tailed Jack Rabbit)	1/40	3	3
	Sylvilagus floridanus (Allen) (Eastern Cottontail)	10/15	2-9	4
	Peromyscus maniculatus (Wagner) (Deer Mouse)	2/19	1-5	3
	Peromyscus pectoralis Osgood (White-Ankled Mouse)	1/16	1	1

Hoplopsyllus anomalus (Baker)	*Spermophilus variegatus* (Rock Squirrel)	3/9	2-12	7
	Ammospermophilus interpres (Merriam) (Texas Antelope Squirrel)	1/3	2	2
	Peromyscus pectoralis Osgood (White-Ankled Mouse)	1/16	1	1
Rhopalopsyllidae				
Polygenis gwyni (Fox)	*Sigmodon hispidus* (Say and Ord) (Hispid Cotton Rat)	1/3	2	2
Hysterichopsyllidae				
Anomiopsyllus hiemalis Eads and Menzies	*Neotoma micropus* Baird (Southern Plains Wood rat)	1/13	2	2
	Neotoma albigula Hartley (White-Throated Wood Rat)	1/10	1	1
Anomiopsyllus nundatus	*Neotoma micropus* Baird (Southern Plains Wood Rat)	1/13	1	1
Meringes arachis (Jordan)	*Onychomys arenicola* (Coues) Mearn's Grasshopper Mouse)	1/21	1	1
Meringes vitabilis Eads	*Dipodomys merriami* Mearns (Merriam's Kangaroo Rat)	2/12	2-4	3
Leptopsyllidae				
Peromyscopsylla hesperomys (Baker)	*Peromyscus maniculatus* (Wagner) (Deer Mouse)	2/19	2-3	2
	Peromyscus pectoralis Osgood (White-Ankled Mouse)	1/16	2	2
	Peromyscus leucopus (Rafinesque) (White-Footed Mouse)	1/15	3	3
Ceratophyllidae				
Dactylopsylla percernis Eads and Menzies	*Chaetodipus hispidus* Baird (Hispid Pocket Mouse)	1/10	2	2
	Bassariscus astutus (Lichtenstein) (Ringtail)	1/6	2	2
	Onychomys arenicola (Coues) (Mearn's Grasshopper Mouse)	1/2	12	2
	Cratogeomys castonops (Baird) Yellow-Faced Pocket Gopher)	2/17	2-5	3
Diamanus montanus (Baker)	*Spermophilus variegatus* (Rock Squirrel)	1/9	2	2
	Ammospermophilus interpres (Merriam) (Texas Antelpoe Squirrel)	1/3	1	1
Monopsyllus exilis (Jordan)	*Onychomys arenicola* (Coues) (Mearn's Grasshopper Mouse)	3/21	4-11	7
	Spermophilus spilosoma (Bennett) (Spotted Ground Squirrel)	1/4	2	2
Monopsyllus wagneri (Baker)	*Peromyscus maniculatus* (Wagner) (Deer Mouse)	1/19	2	2
Opisocrostis bruneri (Baker)	*Peromyscus maniculatus* (Wagner) (Deer Mouse)	2/19	1-2	1
	Peromyscus pectoralis Osgood (White-Ankled Mouse)	1/16	1	1
	Neotoma micropus Baird (Southern Plains Wood Rat)	2/13	1-2	1
Opisocrostis hirsutus (Baker)	*Onychomys arenicola* (Coues) (Mearn's Grasshopper Mouse)	1/21	2	2
Orchopeas leucopus (Baker)	*Peromyscus maniculatus* (Wagner) (Deer Mouse)	1/19	1	1
	Neotoma albigula Hartley (White-Throated Wood Rat)	1/10	2	2
Orchopeas sexdentatus (Baker)	*Peromyscus boylii* (Baird) (Brush Mouse)	2/7	2	2
	Peromyscus maniculatus (Wagner) (Deer Mouse)	2/19	2-4	3
	Peromyscus pectoralis Osgood (White-Ankled Mouse)	6/16	1-6	3

	Peromyscus leucopus (Baird) (White-Footed Mouse)	4/15	3-17	8
	Onychomys arenicola (Coues) (Mearn's Grasshopper Mouse)	1/21	2	2
	Canus latrans Say (Coyote)	1/3	1	1
	Sylvilagus audubonii (Baird) (Desert Cottontail)	1/19	2	2
	Conepatus mesoleucus (Lichtenstein) (Hog-Nosed Skunk)	1/1	1	1
	Spilogale gracialis (L.) (Western Spotted Skunk)	1/1	2	2
	Dipodomys merriami Mearns (Merriam's Kangaroo rat)	1/12	2	2
	Bassariscus astutus (Lichtenstein) (Ringtail)	1/6	1-4	2
	Neotoma micropus Baird (Southern Plains Wood Rat)	2/13	2-5	3
	Neotoma albigula Hartley (White-Throated Wood Rat)	2/10	1-5	3
	Spermophilus spilosoma (Bennett) (Spotted Ground Squirrel)	2/4	1-4	2
Thrassis pansus (Jordan)	*Neotoma micropus* Baird (Southern Plains Wood Rat)	1/13	2	2
Thrassis fotus (Jordan)	*Onychomys arenicola* (Coues) (Mearn's Grasshopper Mouse)	1/21	1	1
DIPTERA				
Nycteribiidae				
Basilaria antrozoi (Townsend)	*Antrozous pallidus* (LeConte) (Pallid Bat)	2/4	2-4	3
ANOPLURA				
Hoplopleuridae				
Hoplopleura hirsuta Ferris	*Sigmodon hispidus* Say and Ord (Hispid Cotton Rat)	2/3	2-52	28
Polyplacidae				
Neohematopinus neotomae Ferris	*Neotoma micropus* Baird (Southern Plains Wood Rat)	2/13	1-3	2
Pecaroecidae				
Pecaroecus javalii Babcock and Ewing	*Tayassu tajacu* (L.) (Collared Peccary)	1/1	15	15
MALLOPHAGA				
Trichodectidae				
Bovicola ovis (Schrank)	*Ammotragus lervia* (Pallas) (Aoudad Sheep)	1/2	21	21
Geomydoecus expansus (Duges)	*Cratogeomys castonops* (Baird) (Yellow-Faced Pocket Gopher)	9/17	6-74	17

slides from the 16 of 38 specimens of two species of *Neotoma*, one ringtail and one desert cottontail.

Recent reports of plague, Lyme disease and rickettsial infections in Brewster County have appeared since this survey (Newman and Moore 1987, Teltow 1988, Moore 1989). Transmission of these zoonotic diseases to humans is most likely to occur to hunters, skinners and processors of wild game and those involved with handling hosts for research purposes. While the Trans-Pecos area of west Texas has several zoonoses present, the human population is sufficiently small to reduce the risk of human cases. Plague has been reported from human cases in west Texas but was associated with hunters and trappers, a high risk group. There have been several reported die-offs of rodent populations in the area with no human cases reported. However, there is a risk of increased incidence of infections in

humans during periodic increase in tourism and visitors in the region coming in contact with the wildlife reservoirs.

Adult and larval stages of *Amblyomma americanum* (L.) were collected from a ringtail [*Bassariscus astutus* (Lichtenstein)]. Adults of *Dermacentor variabilis* (Say) were found on *Dipodomys merriami* Mearns. Larval stages of *Ixodes scapularis* Say were abundant on gray fox [*Urocyon cinereoargentatus* (Schreber)]. These ticks have been found on a wide variety of hosts including humans. They represent some of the ectoparasites prevalent in Brewster County which have the potential for transmitting to humans via feral hosts a number of diseases.

Dactylopslla percernis Eads and Menzies and *Ixodes cookei* Packard were the only ectoparasites found on ringtail previously not reported (Toweill and Price 1976, Custer and Pence 1979). *Thrassis fotus* (Jordan) from a southern grasshopper mouse was previously found on thirteen-lined ground squirrel in Kleberg County, TX (Roberts and Homer 1980). Prince (1944) described *Thrassis pansus* (Jordan) from Brewster County, TX from *Onychomys* spp. Based upon descriptions of Prince (1944) and Stark (1970), the species conforms to that of *Th. fotus*. Fleas found on deer mice were previously reported from deer mice in Texas County, OK (Wyman and Schaefer 1972). However, we did not recover any *Hoplopleura hesperomydus* on the 19 *Peromyscus maniculatus* (Wagner) sampled in our survey. *Polygenis gwyni* (Fox) was reported from hispid cotton rat (Eads 1950) but not by Pfaffenberger and De Bruin (1988) in their survey in Roosevelt County, NM. Populations of *E. gallinacea* on lagomorphs and wood rats was less than that reported by Hightower et al. (1953). This may be due to the drought related mortality of rodent populations in Brewster County, TX during the survey. *Xenopsylla cheopis* (Rothschild) was collected on *Mus musculus* L. in a warehouse infested with this flea's normal host, *Rattus rattus* L.

Borrelia burgdorferi, causitive agent of Lyme disease, was reported from auodad sheep in Brewster County and from jackrabbits, *Lepus californicus*, in Presidio and Pecos counties in a 1985/1986 survey (Newman and Moore 1987). These authors also reported *D. parumapertus* positive for *B. burgdorferi* by monoclonal antibody test. No cases of Lyme disease in humans has been reported for Brewster County.

The absence of plague detection by our survey prior to additional periodic outbreaks in the area is unexplained. The Nobuto strips may not have been saturated with blood sufficient for analysis or some other failure of the strips. It is also possible that our regional plague outbreaks are extremely isolated and animals infected with the disease die without coming into contact with other individuals. It does appear that periodic drought conditions reduces mammalian populations and die-offs from droughts aid in the isolation of surviving individuals. The connection between known incidence of plague and the native wildlife populations has sometimes proved difficult in the Southwest (Caten and Kartman 1968). Extensive trapping of rodents in the immediate area where a bobcat was found dead of plague in 1981 (Tabor and Thomas 1986) failed to produce a single rodent testing positive for plague. Plague was documented to reoccur in Brewster County in 1988 following a known outbreak to the north in Midland and Ector Counties (Moore 1989) when surveillance personnel of Region 3, Texas Department of Health, secured a woodrat, *N. albigula*, east of Alpine infected with plague. No cases of plague in humans have been reported for Brewster County as yet.

Many of the ectoparasites reported from our area are well known as vectors of zoonoses (e.g., plague, tularemia, Rocky Mountain spotted fever, encephalitides, Lyme disease). It is important to continue to monitor the prevalence of these diseases and the populations of the reservoir hosts and vectors.

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GREENHOUSE REARING AND FIELD INFESTATION OF RUSSIAN WHEAT APHID[1] USING TRITICALE AS AN EXAMPLE

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ABSTRACT

Russian wheat aphid, RWA [*Diuraphis noxia* (Mordvilko)] is a serious pest of several small-grain cereals in many countries. Plant resistance represents a practical management option. A method is described for rearing RWA in the greenhouse and for subsequent infestation and assessment of material in the field. Screening spring triticales (X *Triticosecale* Wittmack) of good agronomic type for RWA resistance is used as an example. Resistant and susceptible entries were best separated at growth stage 30. Lines with good resistance to RWA in the field included selections from Brumby, BGLT/LNC//DGO 4/3/TATU 4, and LASKO//2*REH/HARE 212-11. Susceptible lines included GAUR 3 and HARE 286/ELK 32//STIER 19-1.

INTRODUCTION

The Russian wheat aphid, RWA [*Diuraphis noxia* (Mordvilko)] continues to be a serious pest of wheat, *Triticum aestivum* L., and barley, *Hordeum vulgare* L., in many parts of the world. RWA feed within tightly rolled leaves of infested plants and the impact that contact insecticides and predators/parasitoids might have is reduced. Management of this aphid is being sought through plant resistance. Resistance has been identified in a range of species including wheat (Du Toit 1987, Harvey and Martin 1990), barley (Webster *et al.* 1991, Robinson *et al.* 1991), triticale, X *Triticosecale* Wittmack (Frank *et al.* 1989, Webster 1990, Scott *et al.* 1991a) and grass species (Kindler and Springer 1991).

In most instances resistance has been identified and characterized from testing seedlings in the greenhouse and growth chamber. Seedling resistance in the greenhouse/growth chamber may not necessarily reflect field conditions (Benedict and Hatfield 1988). Moreover, material with good field resistance may easily be passed over in greenhouse/seedling tests which tend to be severe in their effect. The consequences of this may be serious in a situation where few sources of resistance exist. Large scale field screening of material for resistance to RWA may not be an attractive or viable option in many instances, but ultimately selected material has to have field resistance.

Barley and wheat have been tested for field resistance to RWA (discontinuously) at CIMMYT (International Maize and Wheat Improvement Center) headquarters at El Batan since 1983 and triticale and rye, *Secale cereale* L., since 1989. Although natural infestations of RWA are observed every year in this location, selection has been done in artificially infested plots. This ensures a uniform distribution of the aphid within the material being screened, and equally important, guarantees a standard selection pressure from year to year.

This paper describes the methods used at CIMMYT for greenhouse rearing of RWA and subsequent field infestation and damage assessment. Data from a two year field screening assessment of spring triticale for RWA resistance are used to illustrate the methodology.

[1] Homoptera: Aphididae

MATERIALS AND METHODS

Greenhouse rearing. RWA are reared in a greenhouse maintained at a temperature between 16 and 20°C and a relative humidity of 70% under conditions of natural daylight. The greenhouse is located at CIMMYT's El Batan headquarters in Mexico (19° 31'N, 98° 50'W, 2249 masl). 'Centinela', a Mexican barley cultivar, which is susceptible to the aphid, is used as the host. The barley is grown in plastic flats (29.5 x 22.5 x 7.5 cm) in a soil mixture (2 soil:1 peat:1 sand). Barley seeds are sown in six hills around the edge of each flat at a rate of approximately 15 seeds per hill. Thirty flats are kept on a drained metal table (1 m x 2.42 m) which is caged (1 x 2.4 x 1 m) with fine gauge nylon mesh on a metal frame.

Flats are watered daily but not to such an extent that the soil becomes saturated. When seedlings are at growth stage (GS) 11 (Tottman and Makepeace 1979), they are infested with RWA from stock colonies of a single clone. These aphids are maintained in pots (12.5 cm diam.) of Centinela (12-15 plants per pot) caged with clear plastic tubes (61 x 9.5 cm diam.) with mesh covered ventilation holes along the sides of the tubes (13 holes x 4.5 cm diam.). Leaves are harvested from these plants and placed on the seedlings in the flats. Approximately 60 leaves, each supporting about 50 aphids of various instars are used per cage. RWA colonies are allowed to develop for three weeks until alates are noted. At this stage, the flats are removed and the plants cut at soil level. The aphids are shaken off the leaves onto large paper sheets on which talcum powder has been sprinkled to prevent the aphids sticking together and collected in plastic containers for transport to the field in a cool-box. The aphids can be kept overnight in a refrigerator at > 4°C with limited mortality.

Field infestation and damage assessment. In the field, RWA of various instars are mixed with corn cob grits in plastic bottles by gently rotating them until a homogeneous mixture is obtained. The bottles screw into an applicator originally designed for infesting maize with lepidopteran larvae (Mihm 1982). The applicator delivers 0.382 cm^3 of the aphid/corn cob grit mixture and is calibrated by delivering test doses on paper and adding appropriate amounts of aphids/corn cob grits until the desired concentration is achieved. The maximum number of mixed instar aphids used per application is about 50.

Plant material for testing is sown in the field at El Batan in January and grows during the dry winter season. For general screening of germplasm, seeds are planted in hill plots of 8-10 seeds per hill with 50 cm between hills on beds 75 cm wide. Plots are furrow irrigated. Plants at GS 11 are infested with between 90 and 110 aphids in two applications corresponding to about 10 aphids per plant. Scoring is done three times during the season - at tillering (≈GS 20), jointing (≈GS 30), and post-heading (≈GS 60) - using an ascending scale of 1-6 (Table 1) adapted from Du Toit (1987) to indicate increasing damage.

TABLE 1. Russian Wheat Aphid Damage Scores.

Score	Damage	Classification
1	No apparent damage	Resistant
2	Chlorotic striping/ flecking on few leaves	Resistant
3	Striping on several leaves, minimal leaf rolling	Intermediate resistant
4	Severe striping and obvious leaf rolling	Intermediate susceptible
5	Striping, severe leaf rolling, necrosis	Susceptible
6	Severe necrosis, plant death imminent	Susceptible

Field screening triticale for RWA resistance. A spring triticale nursery (GAT TCL), comprising 50 good agronomic types (entries), was field screened for RWA resistance in 1990 and 1991, according to the methods described in this paper. RWA damage scores were analyzed separately for each year using a SAS PROC ANOVA model for a randomized block design (SAS Institute 1985); the entry effect was calculated using entry by replicate as the error term (Gomez and Gomez 1984). Data for both years were combined and analyzed according to the model of Gomez and Gomez (1984) with entry effects calculated using the same error term as for the individual analyses. Significantly different mean scores for entries, for the combined data, were separated with least significant difference at $P = 0.05$ (SAS Institute 1985).

RESULTS AND DISCUSSION

The method for mass rearing RWA has been successful in supplying sufficient numbers of aphids for infesting field-sown small-grain cereal screening plots . At least 100,000 aphids, sufficient to infest 1,000 hill plots, are produced from the plants growing in the 30 flats on one table. Few problems have been experienced using this methodology, but care is taken not to over-water the flats and not to plant too densely. RWA seems less prone to attack from entomophagous fungi than other cereal aphid species. Exclusion of other aphids from the greenhouse is essential. *Rhopalosiphum padi* (L.) is very aggressive, easily contaminates RWA colonies, and reproduces faster than RWA.

Barley, wheat, triticale, and rye have been successfully screened in the field at CIMMYT using the hill plot method. Using four replicates of each accession has indicated that uniform aphid pressure is maintained across the trials. Moreover, the applicator can be accurately adjusted to deliver ten aphids, suitable for infesting single plants. Large plots have also been infested by evenly distributing RWA with the applicator. There is no natural way of excluding other aphid species, which are able to colonize plants after infesting with RWA, from such large scale screenings. It has been our experience that other aphids do not have any significant influence on the outcome of the screening under our conditions. Were the screening to be done during the rainy season, this might not be the case.

Webster *et al.* (1991) used a 1-9 scale for scoring RWA damage on single plants in greenhouse tests and Smith *et al.* (1991) scored leaf rolling, leaf folding, and chlorosis separately. Although many cereal diseases are scored on a 1-9 scale for the GRIN system it is suggested that use of a single 1-6 scale is more rapid and enables discrimination between resistant and susceptible accessions to a similar extent. This scale has also been successfully employed for scoring RWA damage on single plants in the greenhouse and in the field (CIMMYT, unpublished data). Although scores of 1-3 classify plant accessions as resistant, selection of material for further investigation, and possibly crossing, is restricted to accessions with means scores below 2.5.

Table 2 contains information on selected triticales from the GAT TCL nursery screened for RWA resistance in 1990 and 1991. Twenty-one entries had mean scores below 2.5 and the scores ranged between 4.4 and 1.3 in the first year (Calhoun *et al.* 1991). In the second year, 11 entries had mean scores below 2.5, and there was a range in scores from 4.0 to 1.6. Combining data for the two years, 15 entries had mean scores below 2.5 and the range of scores was between 4.0 and 1.5. Entries that were resistant in the first year were resistant in the second, and the most susceptible in year one were the most susceptible in year two. Ten of the 15 resistant entries, identified from analysis of the combined data for the two years, are given in Table 2 with their pedigrees, and the two most susceptible entries are included for comparison. In years one and two, the entry effects were significant (Year 1; $F = 19.83$, df = 49, 147, $P < 0.001$: Year 2; $F = 12.42$, df = 49, 147, $P < 0.001$). Error variances for the two years were homogeneous ($F = 1.30$, df = 147, $P < 1.44$) and the data for the two years were combined. There was a significant year effect ($F = 102.5$, df = 1, 147, $P < 0.001$). The entry effect in the combined analysis was also significant ($F = 22.53$, df = 49, 147, $P < 0.001$). In years one and two, the timing of damage scores was significant (Year 1: $F = 34.74$, df = 1, 300, $P < 0.001$; Year 2: $F = 31.20$, df = 1, 300, $P < 0.001$). Mean scores were highest at jointing. By post-heading, RWA damage symptoms on the lower leaves were less apparent than at tillering and jointing.

TABLE 2. RWA Damage Scores for Selected Spring Triticales.

Entry	Pedigree	Classification[a]	Score±SE[b]
813	Brumby 11-1	R	1.50±0.10
815	Brumby 12	R	1.54±0.10
814	Brumby 11-3	R	1.58±0.13
812	Brumby 7	R	1.67±0.13
811	Brumby 4	R	1.75±0.11
818	BGLT/LNC// DGO 4/3/TATU 4	R	1.79±0.13
848	LASKO//2*REH/ HARE 212-11	R	1.88±0.11
816	BGLT/LNC//DGO 4/ 3/TATU 4	R	2.04±0.19
847	LASKO//2*REH/ HARE 212-11	R	2.08±0.12
817	BGLT/LNC//DGO 4 /3/TATU 4	R	2.08±0.16
839	GAUR 3	S	3.63±0.18
843	HARE 286/ELK 32 //STIER 19-1	S	4.04±0.18
Least significant difference between scores at $P = 0.05$			0.94

[a] R = resistant, S = susceptible.
[b] Mean damage scores from four replicates, three times and two years.

Spring triticales having good resistance to RWA include selections from Brumby, BGLT/LNC//DGO 4/3/TATU 4, and LASKO//2*REH/HARE 212-11. These were not given by Frank *et al.* (1989), Scott *et al.* (1991a), and Scott *et al.* (1991b) in their reports on resistance to RWA in CIMMYT triticales, and therefore represent new sources of resistance.

The triticale screening trial in the first year was adjacent to a spring wheat screening trial of 546 entries planted in two replicate blocks. All entries in both replicates of the latter were severely damaged by RWA. Two scores were taken on the material and the mean score for the trial was > 5.0. The triticales were therefore generally less susceptible to RWA than the spring wheats.

The RWA production and screening methodology described has proved practical and reliable. That we observe severe damage in all spring wheat accessions while observing a range of damage symptoms in other cereals makes us confident that the methods are sound. The principal benefit of these methods is that plants grow under natural conditions where artificial stresses, as may exist in the greenhouse and growth chamber, are absent, and natural populations of predators and parasitoids ensure that RWA colony development is subjected to natural control. Having identified resistance to RWA in the field the components of resistance are studied in more detail in the greenhouse (Robinson *et al.* 1991).

It is not suggested that the methods described in this paper will have universal application but they could represent a basis on which suitable methods for different circumstances can be developed.

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BEHAVIOR OF COTTON APHID[1] EXPOSED TO SUBLETHAL DOSES OF THREE INSECTICIDES

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ABSTRACT

Cotton aphids, *Aphis gossypii* Glover, that were not exposed to insecticides spent a greater percentage of time feeding than walking. Aphids, treated topically or exposed to residues of LC_{10} doses of cypermethrin or dicrotophos, spent equal percentages of time walking and feeding. Topical applications and residual exposure to LC_{10} concentrations of sulprofos increased aphid activity with more time spent walking than feeding. In laboratory tests using leaf discs with half their surface area treated with LC_{10} doses of cypermethrin, dicrotophos, or sulprofos and the other half with no treatment, cotton aphids did not demonstrate a preference for either treated or untreated surfaces.

INTRODUCTION

Frequent failures of insecticides to control cotton aphid, *Aphis gossypii* Glover, have been reported and attributed to insecticide resistance (King et al. 1987, 1988, Hardee and Herzog 1991). Although insecticide resistance has been identified with topical applications (Grafton-Cardwell 1991, Kerns and Gaylor 1992), levels of resistance have not been correlated with control failures in the field. Aphids may be able to avoid lethal doses of insecticide as a result of inadequate coverage, insufficient dosage, or active avoidance of residues. Although an insecticide dose may be sublethal, aphid behavior and survival may still be affected. Haynes (1988) reviewed many of the positive and negative effects of sublethal insecticide doses on insect behavior, but effects of sublethal doses on cotton aphid survival have yet to be determined. Cotton aphids exposed to sublethal doses may actively avoid insecticide treated surfaces, thus avoiding lethal exposure. Alternatively, because of irritancy or repellency, increased aphid locomotion may result in increased exposure to residual insecticide droplets, thus potentially increasing mortality.

In this paper, we report the effects of topical and residual applications of sublethal doses of insecticides on the activity of cotton aphid.

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MATERIALS AND METHODS

The three insecticides, cypermethrin (a pyrethroid), dicrotophos, and sulprofos (organophosphates), used in this study are all recommended for use in cotton by the Alabama Cooperative Extension Service (Smith et al. 1991). Insecticides were obtained from ICI Americas Inc., Goldsboro, N.C (cypermethrin); E. I. DuPont De Nemours & Co., Wilmington Del. (dicrotophos); and Mobay Corp., Kansas City, Mo. (sulprofos). Pre-determined LC_{10} doses of technical grade insecticides (cypermethrin 3 ppm, dicrotophos 17 ppm, and sulprofos 840 ppm) (Kerns & Gaylor, 1992) were dissolved in acetone and diluted with equal portions of distilled water containing 0.02% v/v Triton X-100 wetting agent. The control solution consisted of equal portions of acetone and wetting agent solution.

The aphid colony was established from a single parthenogenetically reproducing female collected from an Auburn University greenhouse that had not been treated with insecticides since 1986. The colony was maintained on 'Deltapine 90' cotton growing in an environmental chamber maintained at 22 $\pm$ 3°C and a 14:10 (L:D) photoperiod. Cotton leaf discs (3.14 cm^2) were cut from fully expanded leaves located near the plant terminal. Discs were taken from an area approximately half the distance between the petiole and the leaf tip, centered on the mid-vein. Cutting was performed using a cork borer while the leaves were submerged in distilled water to prevent desiccation. One leaf disc was taken per leaf.

To determine if insecticide residues on leaf surfaces affect aphid behavior, entire leaf discs were dipped in either insecticide or control solutions. Leaf discs were then floated ventral side up in a 60 x 15-mm petri dish containing 15 ml of Hoagland's nutrient solution (Hoagland and Arnon 1950). The exposed portion of the leaf disc was allowed to dry at room temperature for 30 min. Petri dishes were uncovered for the duration of the experiment. The effect of topical exposure of aphids was also determined. Adult apterous aphids were placed in 20 x 20-mm glass cylinders, covered on one end by 0.25-mm mesh, and drenched with approximately 2 ml of insecticide or control solution. The bottoms of the cylinders were then patted dry with tissue paper. Aphids were transferred with a camel's hair brush to a glass petri dish where they remained for 15 min before being transferred to leaf discs treated as previously described with control solution. Treatments for both the topical and residual exposure experiments included cypermethrin, dicrotophos, sulprofos and a control, each replicated eight times.

To determine if aphids preferred insecticide treated or untreated portions of leaf discs, half of each leaf disc was dipped in prepared insecticide solution perpendicular to the mid-vein for 1 sec. The remaining half of each leaf disc was dipped in control solution, thus providing the aphid with a choice between an insecticide-treated or untreated substrate. Both halves of control leaf discs were treated with control solution and handled as previously described. After the leaf discs dried, adult apterous aphids were randomly placed in the center of either the treated or untreated portion of each disc. Eight replicates of each treatment were included.

Activity was measured with a hand-held stop watch for 1 h. Time spent walking or feeding was recorded in seconds. For the preference study, time allocation on either the insecticide-treated or untreated portion of the leaf was also recorded. An aphid was considered to be in a walking mode when in visible locomotion and in a feeding mode when sedentary, whether or not active probing or feeding was visible.

Data were converted to percentages and transformed using an arc-sine transformation for analysis. The data presented in the text are the non-transformed percentages. Transformed data were subjected to analysis of variance (ANOVA) using

general linear model (GLM) procedures (SAS Institute 1988). Means were separated ($P < 0.05$) using pairwise t tests protected by an overall F test ($P < 0.05$) (SAS Institute 1988).

RESULTS AND DISCUSSION

Aphids treated either topically or exposed to insecticide residues exhibited distinct behavioral differences between treatments and the control (Table 1). Aphids treated by topical exposure with cypermethrin or dicrotophos did not differ significantly from the control in percentage of time feeding or walking. Although the control aphids spent a significantly greater percentage of time feeding than walking, walking and feeding times of aphids topically exposed to cypermethrin or dicrotophos did not differ. Aphids topically exposed to sulprofos spent a significantly greater percentage of time walking than feeding, and spent more time walking and less feeding than did aphids in the other treatments. Increased movement at the expense of less feeding might be expected to be associated with reduced fecundity. However, sulprofos induced larger cotton aphid outbreaks than did cypermethrin (Kerns and Gaylor 1991). Rice et al. (1983) reported that *Myzus persicae* (Sulz.), were more active on leaf discs treated with deltamethrin (a pyrethroid), than on untreated discs or discs treated with demeton-S-methyl (an organophosphate) or pirimicarb (a carbamate). Thus, increased walking behavior elicited by different organophosphorus insecticides is not consistent.

TABLE 1. Mean Percentage of Time[a] Spent Feeding or Walking by Cotton Aphids after Topical or Residual Exposure to LC_{10} Doses of Three Insecticides and a Control.

	Topical Exposure[b]		Residual Exposure[b]	
Treatment[c]	%Feeding[d]	%Walking[d]	%Feeding[d]	%Walking[d]
Cypermethrin	55.79aA	44.21aA	54.64aA	45.36aA
Dicrotophos	53.52aA	46.48aA	50.37aA	49.63aA
Sulprofos	35.57bB	64.43aB	41.94bA	58.06aA
Control	56.34aA	43.66bA	67.88aB	32.12bB

[a]Actual percentages shown, but statistical differences are based on arc-sine transformed values.
[b]Topical exposure represents treatments applied to aphids; residual exposure was to leaf discs.
[c]Insecticide treatments were applied at LC_{10} doses.
[d]Means in a row within an exposure followed by the same lower case letter and means in a column followed by the same capital letter are not significantly different based on pairwise t tests protected by an overall F test ($P \geq 0.05$).

In the residual exposure treatments (Table 1), control-treated aphids spent a significantly larger percentage of time feeding and a smaller percentage walking than did aphids in any of the insecticide treatments. Topical exposure to either synthetic pyrethroids or organophosphorus insecticides induced activity in cotton aphids. Increased activity caused by sublethal doses can be beneficial because aphids spending a greater

proportion of time moving on a treated surface have a greater probability of contacting a lethal dose or coming into contact with a predator or parasitoid. Also, because the aphids are spending a smaller percentage of their time feeding, they are probably causing less damage to the plant.

We could detect no significant differences in behavior among any of the treatments in the percentage of time aphids spent walking or feeding on either insecticide treated or untreated portions of leaf discs (data not presented). Thus, cotton aphids did not preferentially avoid nor were they attracted to leaf surfaces treated with LC_{10} concentrations of these insecticides when presented in close proximity to an untreated surface. Similarly, Adams et al. (1990) showed that apterous cotton aphids did not avoid spray droplets of the pyrethroid, bifenthrin. They also demonstrated that bifenthrin had vapor activity resulting in cotton aphid hyperactivity or death. However, *M. persicae* dispersed to untreated potato leaflets from those treated with the pyrethroids deltamethrin or fenvalerate, the carbamate pirimicarb, or the organophosphate methamidophos, but not from leaflets treated with the organophosphate, dimethoate (Lowery and Boiteau 1988). They also reported that *Aphis nasturtii* (Kaltenbach) dispersed from leaflets treated with deltamethrin or fenvalerate, but not from pirimicarb, methamidophos or dimethoate-treated leaflets. Because of the close proximity of the treated and untreated surfaces in our experiment, vapor effects from the treated portion of the leaf discs may have irritated the aphids and masked any repellent effect, thus resulting in the lack of significant differences.

Because of insecticide resistance, consideration of sublethal effects of insecticides on cotton aphid behavior is an important concern. These data suggest that cotton aphids do not avoid insecticide treated surfaces, and that some insecticides have an irritating effect, resulting in increased locomotor activity, which could increase the aphid's exposure to the insecticide. Because traditional dose versus mortality studies do not consider sublethal effects, it is evident that many studies underestimate insecticidal impacts.

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INCIDENCE OF ARTHROPODS FOUND IN PACKED NECTARINE FRUIT IN CENTRAL CALIFORNIA[1]

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ABSTRACT

About 18,000 fruit of each of six cultivars of nectarine, *Prunus persica* (L.) Batsch v. *nucipersica*, fruits were sampled (326,625 packed nectarines) yearly for three years and examined individually for the presence of arthropods. Twenty-three different species representing 2 classes, 11 orders and 16 families of insects, mites, and spiders were found. Pests of economic significance were represented by seven lepidopterous species, five species of Homoptera, and one species each of Thysanoptera, Heteroptera, Neuroptera, Coleoptera, and Acari. Of the 17 species identified, 12 were found to be recorded on nectarine as a host in a comprehensive survey of literature and of California state records for arthropods. Species with relatively high incidence (10-60/100,000 fruit) included omnivorous leafroller, *Platynota stultana* (Walsingham); oriental fruit moth, *Grapholita molesta* (Busck); walnut scale, *Quadraspidiotus juglandsregiae* (Comstock); San Jose scale, *Q. perniciosus* (Comstock); *Chrysopa* sp.; and Pacific spider mite, *Tetranychus pacificus* McGregor.

INTRODUCTION

Several species of insects and mites infest California-grown nectarines, *Prunus persica* (L.) Batsch v. *nucipersica*, in the field. Some of these species are of economic importance requiring management and the application of pesticides for their control (Barnett et al. 1988, Barnett and Rice 1989). However, there has been no controlled qualitative and quantitative sampling to elucidate the array of field arthropods found on or in packed nectarine fruit. During a study to determine the incidence of codling moth, *Cydia pomonella* (L.), in packed nectarines sampled from packinghouses located in the San Joaquin Valley of California (Curtis et al. 1991), we also determined the presence of other arthropods and their infestation levels. We also made a comprehensive survey of the literature and of California state host/pest records to determine the host/California status for the arthropods found.

[1] This article reports the results of research only. Mention of a proprietary product does not constitute an endorsement or a recommendation for its use by the USDA.

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MATERIALS AND METHODS

Fruit from 25-lb shipping boxes coming off the packing lines of three different nectarine packinghouses were examined individually for the presence of live arthropods or evidence of arthropod damage each year for three consecutive years. The cultivars sampled (harvest date) at each packinghouse included: 'May Grand' (early June), 'Firebrite' (mid June), 'Spring Red' (mid June), 'Red Diamond' (late June), 'Summer Grand' (early July), and 'Fantasia' (mid July). Although there are about 130 commercial cultivars of nectarines grown in California, these six were selected because they represent the major portion of nectarines entering export markets. Three packinghouses were selected to represent the Tulare and Fresno districts which produce 95% of the state's total nectarine crop.

Typically, harvest of a given cultivar covers about a 10-day period, and the product is stored at 1°C for a maximum of three weeks before reaching the consumer. Fruits that had been washed, sized and sorted were taken from shipping boxes packed under commercial conditions prior to cold storage. All fruits from any selected box were examined. We sampled about 18,000 fruit of each of the six cultivars per year (i.e., a total of 105,700 - 112,625 packed nectarines each growing season) for a three-year total of 326,625 fruit.

Nectarines suspected of containing insects were removed from shipping boxes and taken to the laboratory for closer examination under an illuminated 3-diopter magnifier (Glidelite, Hackensack, N.J.). Any arthropod found was removed, counted, and identified. Very small or other difficult to identify lepidopterous larvae were reared to the adult stage for identification. Some species were sent to specialists at the California Department of Food and Agriculture (CDFA) for identification or verification. Damaged fruits, with no live arthropods present, were also categorized according to the kind of arthropod which caused the damage. This was possible by observing the type of damage associated with a given arthropod when present and/or identifying head capsules that were found.

Count data (incidence) and percent damage data were subjected to analysis of variance (ANOVA) using years as a random effect (n=3); means were separated with Duncan's multiple range test (Duncan 1951, SAS Institute 1988). Reported table values were calculated from untransformed data. ANOVA and mean separations for incidence data were performed using the square root transformation to stabilize the variances among cultivars.

RESULTS AND DISCUSSION

Species of arthropods representing 2 classes, 11 orders, 16 families, and at least 23 species (17 species were identified) were found in nectarines (Table 1). Numbers (± SEM) of live individuals per 100,000 fruit are recorded for each species. Some aphids, psocids, carabids and spiders, representing only 2.6% of the live arthropods collected, were not identified.

If we rank the arthropods into three groups, those with relatively high incidences from 10 to 60 per 100,000 fruit (Table 1) included *Platynota stultana* (Walsingham), *Grapholita molesta* (Busck), *Quadraspidiotus perniciosus* (Comstock), and *Tetranychus pacificus* McGregor, all of which are recognized economic pests of nectarine. *Quadraspidiotus juglandsregiae* (Comstock) and a beneficial species of *Chrysopa* also occurred in large numbers.

Species occurring in relatively low numbers from 1 to 10 per 100,000 nectarines (Table 1) were three lepidopterous insects, *Cydia latiferreana* (Walsingham), *Anarsia lineatella* (Zeller), and *Amyelois transitella* (Walker); a scale insect, *Diaspidiotus ancylus* (Putnam); thrips, *Frankliniella occidentalis (Pergande)*; a nitidulid beetle, *Carpophilus freemani* Dobson; a species of *Drosophila*; as well as psocids and spiders. *Anarsia lineatella* is a recognized economic pest of nectarine.

Finally, those species with extremely low incidence of less than one per 100,000 fruit (Table 1) included *Cydia pomonella (L.)*, *Cadra figulilella* (Gregson), *Rhopalosiphum padi* (L.), *Pseudococcus affinis* (Maskell), *Euschistus conspersus* Uhler, *Forficula auricularia* L., and a species of Carabidae.

Other observations made of the 326,625 nectarine fruits were as follows: 0.20% (666) contained live arthropods, an additional 0.26% (834) contained lepidopterous damage, 0.05% (161) contained empty lacewing cocoons, and 0.05% (170) contained spider retreats. Also, there were 1,786 fruits with split pits, 2,791 fruits with mechanical damage including gum, and 17 fruits with bird pecks; the total damage for all of these categories was 6,425 fruits (1.97%). Split pits harbored a few species of relatively low incidence; e.g., one adult of *C. freemani*, one specimen of an unidentified sp. of Carabidae, and two adults of *F. auricularia*.

A comparison of occurrence of six of the lepidopterous species for each cultivar sampled is shown in Fig. 1. *Platynota stultana* was the most prevalent species of Lepidoptera from nectarine. However, *G. molesta* also occurred in relatively high numbers, particularly in earlier maturing cultivars ('May Grand', 'Firebrite', and 'Spring Red'). The decreasing incidence of this species in later maturing cultivars may be related to timing of a spray application made in May to control second generation *G. molesta*. The other four species were found infesting nectarines only at very low levels. However, *A. transitella* and *A. lineatella* showed a relatively higher incidence on 'Fantasia' compared to the other cultivars.

Incidence, by cultivar, for *P. stultana* and *G. molesta* is shown in Table 2. There was no significant difference in incidence among cultivars for either *P. stultana* ($\underline{F}$ = 1.11; df = 5, 10; $\underline{P}$ = 0.415) or *G. molesta* ($\underline{F}$ = 1.23; df = 5, 10; $\underline{P}$ = 0.364). *Platynota stultana* occurred in higher numbers compared to *G. molesta* in all cultivars, except 'Firebrite'.

Damage caused by *P. stultana* compared to that by all of the lepidopterous species is shown in Table 3. Damage levels were not significantly different among cultivars for either *P. stultana* ($\underline{F}$ = 0.73; df = 5, 10; $\underline{P}$ = 0.620) or for total lepidopterous damage ($\underline{F}$ = 0.77; df = 5, 10; P = 0.592). Damage by *P. stultana* accounted for 51-85% ($\bar{x} \pm$ SEM = 75 ± 5) of that caused by lepidopterous insects.

Recognized pests of nectarine that may require management and arthropods identified by CDFA as being recorded in their current nectarine host records are footnoted in Table 1. The following discussion elucidates the California and host records found in our literature search and also notes the type of damage or manner of occurrence on the fruit for most of the arthropods recorded in our studies.

Playnota stultana (Walsingham) was first found in California in 1913 on citrus nursery stock and has been found associated with over 80 host plants including nectarine (Barnett and Rice 1989). In our study, larvae and pupae were always found in a webbed retreat in the stem cavity from which the larvae fed on the surface of the fruit.

Grapholita molesta (Busck) was first found in California in 1942 (Mackie 1942) in fruit collections from Orange County and was considered to be an established pest of deciduous fruits one year later (Mackie 1944a). It had been

TABLE 1. Live Arthropods Found in Packed Nectarine Cultivars (n = 6) Sampled from Packinghouses in Central California , and Incidence per 100,000 Fruit, 1985-1987

Order	Family	Genus, species (common name)	Mean ± SEM incidence per 100,000 fruit	Stage(s) found (%)[1]
Class Insecta				
Lepidoptera	Tortricidae	*Platyota stultana* (Walsingham)[2,3] (omnivorous leaf roller)	40.1 ± 8.4	L(91), P(9)
		Grapholita molesta (Busck)[2,3] (oriental fruit moth)	11.3 ± 3.6	L(95), P(5)
		Cydia latiferreana (Walsingham)[3] (filbertworm)	1.8 ± 0.9	L(83), P(17)
		Cydia pomonella (L.)[3] (codling moth)	0.9 ± 0.5	L(100)
	Gelechiidae	*Anarsia lineatella* (Zeller)[2,3] (peach twig borer)	4.0 ± 1.8	L(92), P(8)
	Pyralidae	*Amyelois transitella* (Walker)[3] (navel orangeworm)	4.9 ± 1.8	L(94), P(6)
		Cadra figulilella (Gregson)[3] (raisin moth)	0.6 ± 0.4	L(100)
Homoptera	Diaspididae	*Quadraspidiotus juglandsregiae* (Comstock)[4] (walnut scale)	32.5 ± 13.1	A(100)
		Quadraspidiotus perniciosus (Comstock)[2,3] (San Jose scale)	13.2 ± 5.3	A(100)
		Diaspidiotus ancylus (Putman)[4,5] (Putman scale)	1.5 ± 0.6	A(100)
	Aphididae	*Rhopalosiphum padi* (L.)[4] (bird cherry-oat aphid)	0.3 ± 0.2	N(100)
		unidentified spp.	0.3 ± 0.2	N(100)

TABLE 1. (Continued)

Order	Family	Genus, species (common name)	Mean ± SEM incidence per 100,000 fruit	Stage(s) found (%)[1]
	Pseudococcidae	*Pseudococcus affinis* (Maskell)[4] (obscure mealybug)	0.3 ± 0.3	N(100)
Thysanoptera	Thripidae	*Frankliniella occidentalis* (Pergande)[2,3] (western flower thrips)	8.6 ± 2.9	N(75), A(25)
Heteroptera	Pentatomidae	*Euschistus conspersus* Uhler[2] (consperse stinkbug)	0.6 ± 0.4	N(100)
Neuroptera	Chrysopidae	*Chrysopa* spp.	60.3 ± 35.7	L(50), P(50)
Diptera	Drosophilidae	*Drosophila* spp.	3.1 ± 2.9	E(100)
Coleoptera	Nitidulidae	*Carpophilus freemani* Dobson[3] (driedfruit beetle)	3.1 ± 2.1	E(60), A(40)
	Carabidae	unidentified sp.	0.3 ± 0.3	A(100)
Dermaptera	Forficulidae	*Forficula auricularia* L. (European earwig)	0.6 ± 0.6	A(100)
Pscoptera	Trogiidae	unidentified spp.	1.2 ± 1.2	A(100)
Class Arachnida				
Acari	Tetranychidae	*Tetranychus pacificus* McGregor[2] (Pacific spider mite)	11.0 ± 8.2	N(50), A(50)
Araneae	Salticidae	unidentified spp.	3.4 ± 0.5	A(100)

[1] E = egg; L = larva; N=nymph; P = pupa; A = adult.
[2] Recognized pests of nectarine which may require management (Barnett *et al.* 1988, Barnett and Rice 1989).
[3] Postively identified by CDFA as being found on nectarine in their current host records listing.
[4] Identified by CDFA as being found on deciduous fruits in general in their current host records listing.
[5] Near *D. ancylus* (Putman), Putnam scale (a complex).

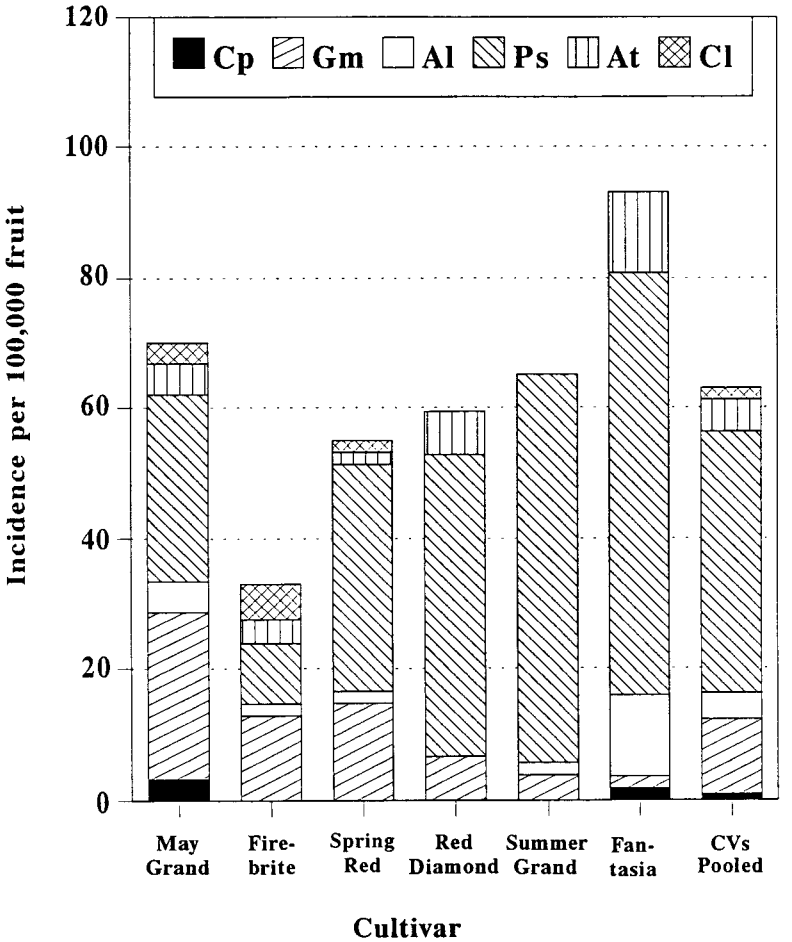

FIG. 1. Incidence of six lepidopterous pests found in six cultivars of nectarines in central California (1985 - 1987); Cp = *C. pomonella*, Gm = *G. molesta*, Al = *A. lineatella*, Ps = *P. stultana*, At - *A. transitella*, Cl = *C. latiferreana*.

TABLE 2. Mean (± SEM) Incidence (no. per 1,000 Fruit) of *G. molesta* and *P. stultana* for Selected Nectarine Cultivars, 1985-1987[a]

Cultivar	n (years)	*G. molesta*	*P. stultana*
May Grand	3	0.263 ± 0.215 A	0.287 ± 0.166 A
Firebrite	3	0.128 ± 0.037 A	0.092 ± 0.067 A
Spring Red	3	0.146 ± 0.048 A	0.349 ± 0.130 A
Red Diamond	3	0.068 ± 0.044 A	0.382 ± 0.346 A
Summer Grand	3	0.040 ± 0.040 A	0.602 ± 0.182 A
Fantasia	3	0.018 ± 0.018 A	0.650 ± 0.221 A

[a] Numbers followed by the same letter, within columns, are not significantly different ($P = 0.05$; Duncan's multiple range test [Duncan 1951, SAS Institute 1988]).

TABLE 3. Mean (± SEM) Percent Damage by *P. stultana* and by All Lepidopterous Pests for Selected Nectarine Cultivars, 1985-1987[a]

Cultivar	n (years)	*P. stultana*	Total Lepidoptera
May Grand	3	0.064 ± 0.034 A	0.110 ± 0.048 A
Firebrite	3	0.076 ± 0.032 A	0.148 ± 0.064 A
Spring Red	3	0.125 ± 0.031 A	0.193 ± 0.037 A
Red Diamond	3	0.414 ± 0.399 A	0.487 ± 0.472 A
Summer Grand	3	0.164 ± 0.071 A	0.207 ± 0.100 A
Fantasia	3	0.281 ± 0.160 A	0.361 ± 0.173 A

[a] Numbers followed by the same letter, within columns, are not significantly different ($P = 0.05$; Duncan's multiple range test [Duncan 1951, SAS Institute 1988]).

intercepted numerous times in imported fruits from 1935 to 1941 (Hunt 1941); stone fruit hosts included nectarine (Wood 1918, Mackie 1944b, Gonzalez 1983). Summers (1966) described the life history and biology of *G. molesta* in California. In our study, larvae were found in tunnels bored into the fruit, particularly at or near the stem end; pupae occurred in the stem cavity.

Cydia latiferreana (Walsingham) was described by Walsingham (1879) based upon specimens collected in Mendocino County, California, and also appeared in a checklist of California insects (Woodworth 1912). We observed that larvae bored a single tunnel to the pit of the fruit in a manner similar to that of codling moth. Pupae were located in the stem cavity of the fruit.

Cydia pomonella (L.), as a pest on its usual hosts (apple, pear, and Persian walnut), has been extensively studied (Butt 1975). It was first found in California in

1873 (Cooke 1883, Essig 1931). Only brief mention of nectarine as a host was made in four publications (Mackie 1930, Smith 1940, Borden and Madsen 1949, Madsen and Borden 1954). In our study, only three fruit were found infested with one live codling moth larva per fruit. Two were found in 'May Grand' fruit and another inside a 'Fantasia' nectarine. All were fourth instars and were reared to be one male and two females. No eggs, pupae or diapausing larvae were found. Larvae were found in tunnels bored directly to the pit. Interestingly, *C. pomonella*, which has been identified as a quarantine pest in several hosts including nectarine, was among the insects with extremely low incidence. Nectarine may be considered to be a nonpreferred host at such low levels.

Anarsia lineatella Zeller is of European origin. Its first observation in California was as early as 1881 (Anonymous 1884), but the first official record appears to be 1882 (Cooke 1883). It reportedly injured several kinds of fruit trees including nectarine (Cooke 1883, Marlatt 1908). Marlatt reported on injury to new shoots and the short stems bearing the fruit, but took exception to reports of fruit damage. Jones (1935) and Cooke (1883) reported damage to nectarine fruits. Bailey (1948) stated that damage was twofold - killing twigs and directly injuring fruit. Furthermore, damage to plum and nectarine was usually less severe and more localized than damage to other stone fruits. We observed that larvae tended to feed at the surface of the fruit and that pupae were usually located within the frass in the damaged area.

Amyelois transitella (Walker) was first recorded in California in 1942 (Mackie 1942) and has been recorded on nectarine (Wade 1961, Anonymous 1969). In our study, larvae tended to excavate a chamber beneath the surface of the fruit, and pupae were webbed within the frass of the damaged area.

Cadra figulilella (Gregson) was first reported in the area of Fresno, California, in 1928 on muscat raisins (Simmons and Nelson 1975). Larvae were found feeding on the shoulder of the fruit near the stem cavity.

Quadraspidiotus juglandsregiae (Comstock) was first recorded in 1881 from walnut trees in Los Angeles (Cooke 1883). It was reported in the host index to California Coccidae (Baker and Essig 1912) on almond and several stone fruits, but nectarine was not mentioned as a host. We found individuals scattered about on the surface of the fruit; nearly all (97%) were on the later maturing 'Summer Grand' and 'Fantasia' nectarine cultivars.

Quadraspidiotus perniciosus (Comstock) was noticed by growers soon (1870) after the establishment of deciduous fruit orchards in California (Essig 1931). It was also listed in the host index to California Coccidae (Baker and Essig 1912) on almond, cherry, plum, prune, and peach. We found 87% of *Q. perniciosus* on the early maturing cultivars 'May Grand' and 'Firebrite'. We also found a species of scale insect identified as near *Diaspidiotus ancylus* (Putnam), but only five specimens were taken during the course of the study. This species was observed in California on apricot in 1937 and apricot and plum in 1946 (Armitage 1946).

Frankliniella occidentalis (Pergande) has been detected throughout California on a great variety of plants (Jensen et al. 1981). LaRue et al. (1972) reported damage to nectarines in California. We observed nymphs and adults in the stem cavity of the nectarines.

Tetranychus pacificus McGregor was noted on grapes in Fresno, California, in 1876 (Essig 1931). Many California orchards, including almond, apple, pear, plum, and cherry, were seriously infested (Cooke 1881), and chemical control recommendations have been published for nectarine by Barnett et al. (1988). We

found *T. pacificus* in webbing in the stem cavity; most (69%) were found in one lot of 'Summer Grand' nectarines in 1987.

Rhopalosiphum padi (L.), *Pseudococcus affinis* (Maskell), and *Euschistus conspersus* Uhler were represented in our survey by only a single specimen of each species. *Pseudococcus affinis* is shown in the host index to California Coccidae (Baker and Essig 1912) on *Anemone* species. Distribution of *E. conspersus* was described by Van Duzee (1904, 1917) as California, Washington, and Vancouver Island. However, the economic importance of this bug on deciduous fruits was first recognized in California by Madsen (1950). In the western United States, *E. conspersus* is a sporadic pest of deciduous fruits (Borden et al. 1952, Hoffmann et al. 1987) and chemical control recommendations are published for peach and nectarine (Barnett et al. 1988).

Carpophilus freemani Dobson, *C. hemipterus* L., and *Drosophila melanogaster* Meigen are widespread pests found on a great variety of hosts. All were captured in California by Michailides and Spotts (1990) in tests with the fungus, *Mucor piriformis*. We found eggs and adults of *C. freemani* and eggs of *Drosophila* sp. on packed nectarines.

Forficula auricularia L. was first reported in Berkeley, California, in 1923 by Essig (1923). We found only two adults, and each was in a split pit.

Chrysopa spp., known to be widespread predators of aphids, mealybugs, scales, and mites, were found in relatively large numbers in our study. Live cocooned larvae and pupae were found in the stem cavity of nectarines.

No previous record of occurrence on nectarine in California could be found for the following seven arthropods: *Q. juglandsregiae, D. ancylus, R. padi, P. affinis, F. auricularia, Chrysopa* spp., and *Drosophila* spp. All but the last three were reported by CDFA as probably occurring on deciduous fruits. Only *Q. juglandsregiae* and *Chrysopa* spp. occurred in relatively high numbers (>10 per 100,000 fruit).

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METAMORPHOSIS OF THE MALPHIGIAN TUBULES OF *DIACRISIA OBLIQUA* WALKER[1]

Abir Kabir[2], Mahmud-ul Ameen[2], and Shirlee Meola[3]

ABSTRACT

During metamorphosis of *Diacrisia obliqua* Walker, the cryptonephric region of the larval Malpighian tubules degenerated completely through histolysis, losing its association with the rectum. The epithelial nuclei of the remaining portion of the larval tubule became enlarged, with some nuclei disintegrating while the remainder underwent endomitosis to become the nuclear complement of the adult tubule epithelium. The microvilli of the apical surface of the epithelium were absorbed in the prepupal stage and reappeared in the late pupa. In the adult, the brush- border gradually became indistinct and the diameter of the tubules enlarged along with a subsequent enlargement of the lumen.

INTRODUCTION

Metamorphosis of the Malpighian tubules of Lepidoptera has been studied by a number of workers. Cholodkowsky (1887) demonstrated that in *Tineola biselliella* (Hummel) the larval Malpighian tubules disappear gradually by histolysis, while the basal trunk elongates to form the imaginal tubules. A diametrically opposite view was held by Samson (1908), who stated that in *Heterogenea limacodes* Hafn. the Malpighian tubules of the larva directly transform into imaginal ones. Ito (1921) observed in detail the metamorphic changes of the Malpighian tubules in *Bombyx mori* (L.) where the tubules directly passed from the larval to the imaginal stage. The larval Malpighian tubules in *Vanessa urticae* (L.) (Henson 1937), and in *Philosamia ricini* (Hut) (Srivastava and Khare 1966, Khare 1977) persist in the adult with little differentiation. Recently, Ryerse (1979), investigating the metamorphosis of the proximal (absorptive) region of the Malpighian tubules of *Calpodes ethlius* (Stoll), reported that although the epithelial cells of this region persist through metamorphosis, they undergo considerable structural alteration. Although the biology and principal structural features of the larva, pupa and adult of *D. obliqua* are known, little information exists concerning the morphological and histological changes of the Malpighian tubules during metamorphosis. Earlier, the anatomy and histology of the Malpighian tubules in the various developmental stages of *D. obliqua* were described by Kabir and Ameen (1986). In the present paper, we describe changes that occur in the Malpighian tubules during metamorphosis.

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MATERIALS AND METHODS

Larvae of *D. obliqua* were collected from infested jute fields near Dhaka. They were reared in the laboratory to larval, prepupal, pupal, and adult stages. Malpighian tubules of the various developmental stages were dissected in physiological saline (0.9% NaCl) solution under a binocular microscope. For histological study, specimens were fixed in hot Bouin's fluid and cut in half 30 min later to facilitate fixation. The body wall of each life stage was completely removed. Specimens were then dehydrated in grades of ethyl alcohol and passed through 1% celloidin in methyl-benzoate followed by benzene before being embedded in paraffin at 50°C. Serial 5-6 μm longitudinal and transverse sections were cut and stained with hematoxylin and eosin.

RESULTS AND DISCUSSION

As seen in Fig. 1, the six Malpighian tubules of *D. obliqua* are arranged in two groups of three and arise from two ampullae at the posterior pylorus. In the larva, the distal ends of the tubules impinge upon the rectum, forming a cryptonephridial complex around the anterior part of the rectum. This complex consists of the distal ends of the Malpighian tubules and rectum, enclosed by a perinephric membrane (Ramsay 1976). Proximal regions of the tubules in different larval instars did not exhibit any appreciable metamorphic changes except in growth and development of the constituent parts (Table 1). The Malpighian tubules reached their maximum length at the end of the 6th instar. With each subsequent instar, there was a gradual increase in the size and number of nuclei per Malpighian tubule cross section, indicating endopolyploidy.

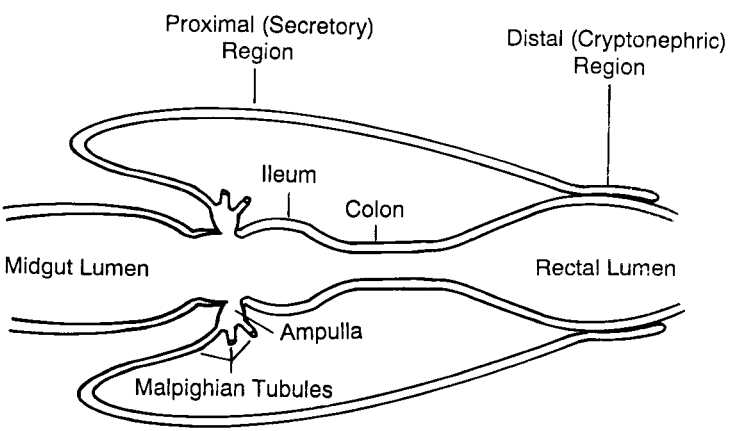


FIG. 1. Diagram showing the relationship of the larval Malpighian tubules to the alimentary canal.

Changes During the Prepupal Stage. Changes began to occur in the Malpighian tubules with initiation of the prepupal stage. The free portion of the tubules in the body cavity decreased in length, and at certain points the epithelial wall of the tubules detached from the basement membrane. Compared to the larva (Fig. 2), the tubule lumen of the prepupa became extremely narrow and elongated (Fig. 3). Masses of granules and a few hemocytes appeared around the Malpighian tubules.

TABLE 1. Measurements of the Proximal Region of the Malpighian Tubules of *D. Obliqua* During Different Stages of Metamorphosis.

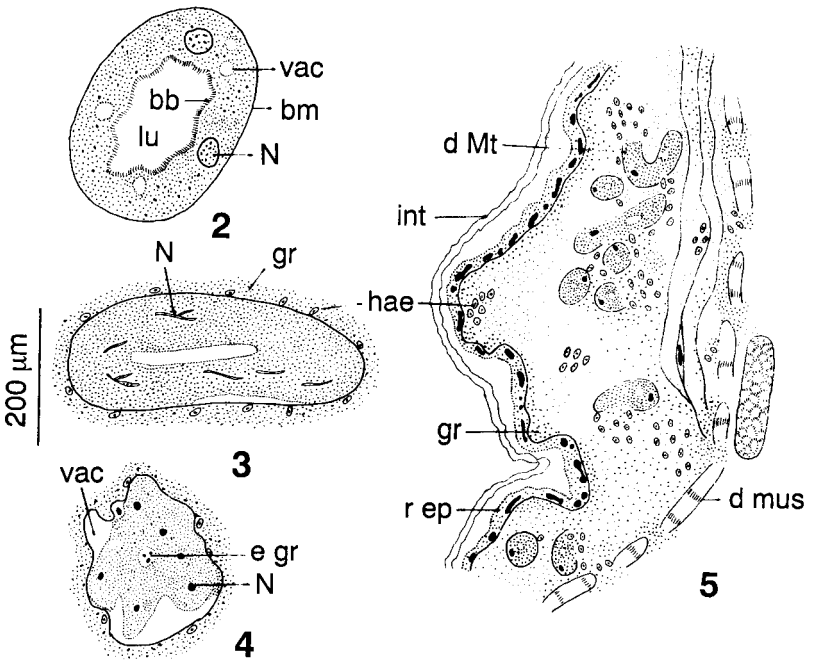
Stage	Malpighian tubule dia. (μm)	Nuclei number in X-section	Nuclei Size (μm)	Nuclei Shape
1st-instar	19.5-30.0	1-2	3 x 3	Spherical
2nd-instar	27.0-34.5	1-2	5 x 4	Spherical, oval
3rd-instar	42.0-60.0	2-3	5-6 x 4.6	Oval, oblong
4th-instar	45.0-75.0	2-4	7.5-9 x 6.0	Spherical, elongated
5th-instar	72.0-99.0	4-11	6-12 x 6.9	Spherical, elongated
6th-instar	76.9-153.8	4-11	12-60 x 7.5-15.0	Spherical, elongated
Prepupa	60.0-96.0	---	---	Elongated, branched, broken
2-day pupa	27.0-30.0	10-13	1.5-3.0	Almost spherical
4-day pupa	45.0-50.0	14-16	2.4-4.5	Spherical
6-day pupa	45.0-60.0	12-17	5.4-6.0	Spherical
7-day pupa	45.0-66.0	12-16	3.0-6.0	Spherical
Adult	46.5-76.9	12-18	3.0-6.0	Spherical

The cytoplasm of the epithelial cells appeared disorganized and the brush-border of the lumen became almost imperceptible. The nuclei became prominent, elongated, and branched and the nuclear material appeared granular (Fig. 3). Some nuclei broke down and disintegrated while in others the nuclear material became pycnotic, assuming a clumped blackish structure of variable size and shape. The basement membrane histolyzed resulting in an amorphous layer of granules around the surface of the tubules. A few vacuoles appeared between the basement membrane and the plasma membrane of the epithelial cells (Fig. 4). At this time, a few strongly eosinophilic granules and globules also appeared within the lumen of the tubules. The diameter of the distal ends of the tubules gradually became reduced in size and detached from the rectal wall within 48 h.

Simultaneously, histological changes took place within the cryptonephric Malpighian tubules. Due to histolysis, the perinephric membrane began to disintegrate. Groups of hemocytes appeared in the spaces between the Malpighian tubules and rectal wall. Hemocytes attacked and destroyed the basement membrane

of the tubules and then the cytoplasm and nuclei (Fig. 5). At the end of the prepupal stage, a large number of vacuoles and granules became visible around the periphery of the rectum.

Changes During the Pupal Stage. The proximal region of the Malpighian tubules further decreased in size after dissociation from the rectal wall but remained embedded among the fat bodies which were abundant in the pupa. The basement membrane of the tubules was indistinct in 1-day old pupa; the epithelial wall became thin, irregular and delicate, and the tubules were roughly circular in outline. The

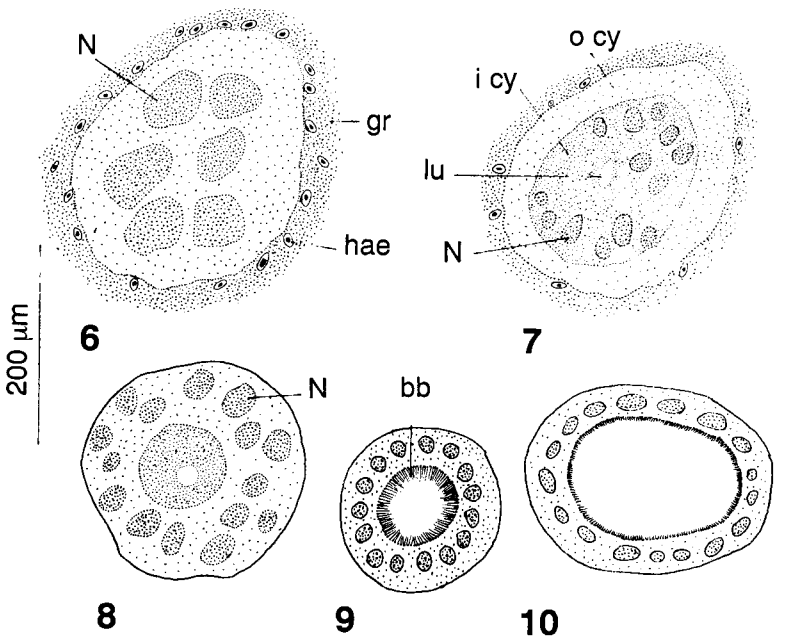


FIGS. 2-5. Distal (cryptonephric) region of Malpighian tubules of *Diacrisia obliqua*: (2) 4th instar larva, transverse section of tubule prior to initiation of histolysis. (3) Early prepupa, transverse section of a Malpighian tubule showing histolysis of the basement membrane. (4) Late prepupa, transverse section of a Malpighian tubule showing small spherical nuclei and disorganized cytoplasm. (5) Early prepupa, longitudinal section through a part of rectal wall showing histolysis of cryptonephric tubules.

bb - brush-border; bm - basement membrane; d Mt - degenerating Malpighian tubule; d mus - degenerating muscle; e gr - eosinophilic granules; gr - granules; hae - haemocyte; int - intima; lu - lumen of tubule; N - nucleus; r ep - rectal epithelium; vac - vacuole.

lumen of the tubules had become so constricted as to be almost imperceptible. In cross section, there were about 6-8 nuclei in the center of the tubule (Fig. 6). Nuclear materials remained clumped together as hematoxylin-stained bodies; cytoplasm appeared granular with a few small vacuoles. Granules and hemocytes were still present around the Malpighian tubules.

In the 2-day old pupa, a narrow lumen appeared in the center of the tubule which remained filled with an eosin-colored mass. The nuclei dispersed at this stage as dark globular hematoxylin-stained bodies of variable sizes. The cytoplasm of the tubules was differentiated into an outer peripheral eosin-colored layer and an inner hematoxylin colored layer which contained 10-13 nuclei (Fig. 7).



FIGS. 6-10. Transverse sections through proximal region of Malpighian tubules of *Diacrisia obliqua*: (6) 1-day pupa, cross section of a Malpighian tubule showing large-sized irregular shaped nuclei and lack of a well defined lumen. (7) 2-day pupa, cross section of a Malpighian tubule showing the outer and inner layer of cytoplasm, the inner layer having small round or ovoid nuclei and a definite lumen of tubule. (8) 4-day pupa, cross section of a Malpighian tubule showing the nuclei in the outer layer of cytoplasm. (9) 6-day pupa, cross section of a Malpighian tubule showing the nuclei arranged in a row and a well- developed brush-border around the lumen. (10) 2-day adult, cross section of a Malpighian tubule showing large lumen with indistinct brush-border.

bb - brush-border; gr - granules; hae - haemocyte; i cy - inner layer of cytoplasm; lu - lumen of the tubule; N - nucleus; o cy - outer layer of cytoplasm.

In the 4-day old pupa, usually 14 to 16 nuclei ranging from 2.4 to 4.5 μm in diameter, appeared as irregular globular or spherical structures (Fig. 8). These nuclei were randomly dispersed in the cytoplasm, although orientated toward the periphery of the tubule. The diameter of the Malpighian tubules at this stage was 45-50 μm while the thickness of the epithelial wall was 15-16 μm. The hemocytes and granules were now absent around the tubule wall. The basement membrane gradually became visible.

In 5- and 6-day old pupae, the diameter of the tubules increased and the lumen became spacious. A distinct brush-border appeared with filaments 0.5-0.9 μm tall. The basement membrane also became more distinct. Nuclei were spherical although quite variable in diameter (5.4-6.0 μm). In cross section, 12-17 cells formed the epithelial wall around the lumen with the nuclei arranged in a row around the central axis (Fig. 9). By days 7 and 8, the basement membrane was more distinct and the nuclei of the epithelial cells were more granular. The tubule lumen became more spacious and the brush-border reached its maximum height of 5.7-6.9 μm.

In the adult, the basement membrane of the epithelial wall is well formed (Fig. 10). About 10-18 cells were arranged around a large spacious lumen (Fig. 10). The nuclei were oblong or spherical and, unlike the pupa, were situated nearer to the lumen. The cytoplasmic filaments that formed the border of the lumen became much smaller (1.9- 2.7 μm) in the adult compared to the pupa.

Metamorphosis of Malpighian tubules in insects has been studied extensively by many authors. The published results, however, are widely divergent, especially for Lepidoptera. Cholodkowsky (1887) noted that in *T. biselliella* the larval Malpighian tubules disappear gradually by histolysis and the basal trunk forms the imaginal tubes. In contrast, after undergoing certain histological alterations, the larval Malpighian tubules transform into the imaginal tubules in *H. limacodes* (Samson 1908), *V. urticae* (Henson 1937), and *P. ricini* (Srivastava and Khare 1966). Hufnagel (1912) observed that while one part of the larval tubule disappears entirely during metamorphosis in *Hyponomeuta padella* L., the other portion persists and differentiates into the definitive adult organ. Ameen (1969) noted that two of the five larval Malpighian tubules in the fly, *Ptychoptera albimana* F., were modified to become the saccular tubes in the adult while the other three were carried over to the adult without much change in external appearance. He suggested that redifferentiation, division of larval cells, and participation of some imaginal cells are among the several processes that are involved in tubular metamorphosis.

Ito's (1921) observations that the free portion of the Malpighian tubules passes from the larval to the imaginal stage in *B. mori* and that the perirectal tubules are completely destroyed by histolysis and phagocytosis at pupation agree well with our present study of metamorphosis in *D. obliqua*. Ito's findings, however, differed from ours in that the cytoplasm of the Malpighian tubule epithelium in pupal *B. mori* has large vacuoles between the nuclei and the basement membrane. In *D. obliqua*, the vacuoles were seen at the prepupal stage between the basement membrane and the plasma membrane of the epithelial cells, whereas vacuoles were absent at the pupal stage. In the pupal stage of *B. mori*, the lumen of the tubule is very irregular in shape and contains homogeneous or granular substances. On the other hand, the lumen of *D. obliqua* was oval or rounded and did not contain any recognizable

substances. The results of the present study also differ from those of Ryerse (1979) in that the diameter of the adult Malpighian tubules of *D. obliqua* became enlarged relative to those of the larva, whereas in *Calpodes ethlius* the adult tubules have a reduced diameter. Waku (1974) noted ultrastructural differences in larval and pupal Malpighian tubules of *B. mori*. The pupal tubules lack inflections of plasma membrane, microvilli and associated mitochondria, while the adult epithelial cells assume these characteristics. In the present study, the cytoplasm of larval epithelium was coarsely granular and had small vacuoles while it appeared homogeneous in the pupa and fairly granular in the adult.

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THE INFLUENCE OF ULV MALATHION, APPLIED FOR BOLL WEEVIL CONTROL, ON OTHER PEST AND BENEFICIAL SPECIES IN ARIZONA COTTON FIELDS 1989-90

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ABSTRACT

Studies were conducted to determine the effect of ULV malathion treatments on beneficial and selected pest insect species on cotton. Higher mean numbers of thrips, *Frankliniella occidentalis (Pergande)*, and minute pirate bug, *Orius tristicolor* (White) per 200 sweeps occurred in ULV malathion-treated fields in 1989 and 1990. Predator species were reduced for two weeks following malathion treatments. *Lygus hesperus* (Knight) numbers were significantly reduced by malathion treatments for the first week after malathion treatments in 1989 but not in 1990 with 9 and 2 per 200 sweeps in the untreated fields, respectively. *Bemisia tabaci* (Gennadius), parasitic Hymenoptera, and spiders were not affected by malathion treatments.

INTRODUCTION

Wene and Sheets (1962) characterized predatory and injurious arthropods in Arizona Salt River Valley cotton fields during June, July, and August. They reported populations for some species to be highly variable over time and geographic area. Smith and Stadelbacher (1978) conducted a three-year study on the seasonal rise and decline of predator populations in Mississippi delta cotton fields. They found that predator populations peaked in July or early August and then declined sharply by mid August. Butler and Las (1983) reported that predator species suffered high mortality one day after field treatment with a mixture of acephate, trichlorfon and sulfur. In a three-year study, Scott et al. (1985) compared three rates of aldicarb treated fields to untreated control fields and found that *Nabis, Orius*, spiders, and parasitic Hymenoptera were generally not adversely affected by aldicarb, but *Geocoris* were reduced with the high rate [1.31 kg. (AI)/ha] of aldicarb during one year of the study. Scott et al. (1986) made foliar applications of dimethoate, chlordimeform, flucythrinate, and fenvalerate and reported that chlordimeform had the least effect on hemipteran predators with significant differences only on one sample date. Terry (1991) demonstrated that early season pesticide use had no long term detrimental effect on beneficial arthropods in Arizona and only two of five sites had short term reduction in arthropods.

Leggett (1989) sampled squares weekly in Arizona cotton fields to determine the damage from pest species in fields treated with ultra low volume (ULV) malathion and in untreated fields. Damage, mainly from beet armyworms, *Spodoptera exigua*

(Hübner), was highly variable among fields and was not correlated with malathion treatment. The present study was undertaken to determine if predatory or other pest species were influenced by ULV malathion treatments aerially applied by the Southwest Boll Weevil Eradication Program.

METHODS AND MATERIALS

Cotton fields, treated with (ULV) malathion, and untreated fields (ten of each) were sampled weekly in the Gilbert and Laveen areas (Maricopa County, Arizona) from 22 May to 12 June 1989 (total of 20 pairs). In 1990, each of twenty treated and untreated fields were selected in Maricopa County. Each treated field received two aerial applications of malathion [1.31 kg. (AI)/ha] at seven-day intervals to control boll weevil, *Anthonomus grandis* Boheman, populations. Treatment was based on number of boll weevils found in traps, and in each case a similar age cotton field not likely to be treated according to trap catches was also selected for sampling. Fields in a pair were less than one mile apart and of the same species of cotton. Cotton plant development was determined weekly by counting nodes on the main stem of five plants in each quadrant of each field. At the time of the second ULV malathion treatment in 1989, cotton plants in the fields were in the 12 and 7 node of plant development at Gilbert and the 10 and 9 node of plant development at Laveen for the treated and untreated cotton, respectively. In 1990, both the treated and untreated cotton fields were in the 10 node of plant development at the time of the second malathion treatment. A sample of 50 sweeps was taken with a standard 38-cm sweep net and five leaves were collected from the fifth node down on the main stem in the corners of each field. The sweep samples were frozen and insects were separated from leaves in a white pan. One person made all sweeps during a season. Fields within a pair were sampled within 30 min of each other. Leaves were examined for immature whiteflies and spider mites under a stereo microscope, and the numbers of each species per leaf were recorded. The range of treatment dates was 8 to 30 May at Gilbert, 8 to 22 May 1989 at Laveen, and 3 May to 1 June 1990 in Maricopa County. Sweep samples were summarized for the week of the second malathion treatments (week zero in 1989 figures) and weekly for three weeks after treatments (22 May to 12 June 1989). Sweep samples were summarized weekly beginning after malathion treatments from 21 May to 20 July 1990. Sweep samples from the untreated fields were summarized for the same dates as treated fields in both years. After pinhead square ULV malathion treatments, boll weevil populations in some fields triggered the need for additional treatments from one to three weeks later and/or the growers made applications for other insects. This occurred in most cases during 1989. Data from fields treated with other insecticides were not included in the summary or analysis. As a result of the later applications, an equal number of fields were not available for each weekly summary in 1989. The number of fields available at each sampling date is presented in Table 1. The mean and standard errors of sweep samples for seven individual insect species or groups of insects were examined for four and nine weeks following ULV malathion treatments in 1989 and 1990, respectively. The mean and standard errors were calculated in Sigma Plot software program, Jandel Scientific, Corte Madera, California. Student's t-test was used to compare insect numbers between treated and untreated fields for each week, MSTAT-C software program Michigan State University. Unpaired and paired t-tests were used for 1989 and 1990, respectively, to determine statistically significant difference ($P \leq 0.05$). Chi-square (equal and unequal expected frequencies) was used

TABLE 1. Number of Fields Used to Obtain Mean Number of Insects Per 200 Sweeps in 1989-90.

Sample no.	1989[a]	No. fields for means		1990	No. fields for means
		Treated	Untreated		
1	5/22	15	11	5/21	30
2	5/30	16	14	5/28	34
3	6/05	14	11	6/04	40
4	6/12	12	11	6/11	38
5	-	-	-	6/18	38
6	-	-	-	6/25	38
7	-	-	-	7/02	38
8	-	-	-	7/09	34
9	-	-	-	7/16	34

[a] No samples for 5 to 9 in 1989 due to additional insecticide application.

to compare total number of pest and beneficial insects in treated and untreated fields for 1989 (NWASTATPAK Northwest Analytical Inc., Portland Oregon).

RESULTS AND DISCUSSION

A higher percentage of the fields at Gilbert was treated in 1989 compared to Laveen which included most of the older cotton. Even though untreated fields at Gilbert were in early stages of plant growth, there was no significant difference in insect numbers between the two locations except for week 3 after treatment. At that time, Laveen untreated fields had significantly more whiteflies and spiders than the Gilbert untreated fields (Chi-square = 20.6 and 6.6 respectively at $P = 0.05$ with 1 df).

Sweep netting does not sample all species of insects with equal efficiency. Therefore, the estimate of arthropods in Figs. 2-9 should be considered relative numbers as influenced by the malathion treatments. Fig. 1 presents an estimate of cotton plant age and growth rate until first bloom in the treated and untreated fields. Figs. 2-9 present mean numbers of arthropods per 200 sweeps in the treated and untreated cotton fields for 1989 and 1990.

Thrips *(Frankliniella occidentalis)* (Pergande). In 1989 and 1990, populations were higher in treated fields on most sampling dates. Due to variability among fields, means for any sampling date were not significantly different (Fig. 2). However, means per field for three weeks in 1989 and for six weeks in 1990 were compared with a t-test, and the treated fields had significantly more thrips than the untreated fields at $P = 0.05$ with 12 df in 1989 and 19 df in 1990.

Lygus *(Lygus hesperus)* (Knight) and cotton fleahopper *(Pseudatomoscelis seriatus)* (Reuter). In 1989, malathion treatments significantly reduced populations on two sampling dates as judged by a t-test at $P = 0.05$ when 8-10 plant bugs per 200 sweeps were collected in the untreated fields; however, with lower population levels (two plant bugs per 200 sweeps on 21 May) in 1990, differences between treatments were not significant (Fig. 3).

Whiteflies *(Bemisia tabaci)* (Gennadius). Adult populations were higher in 1989 than in 1990 but there were no significant differences between treatments. In 1990,

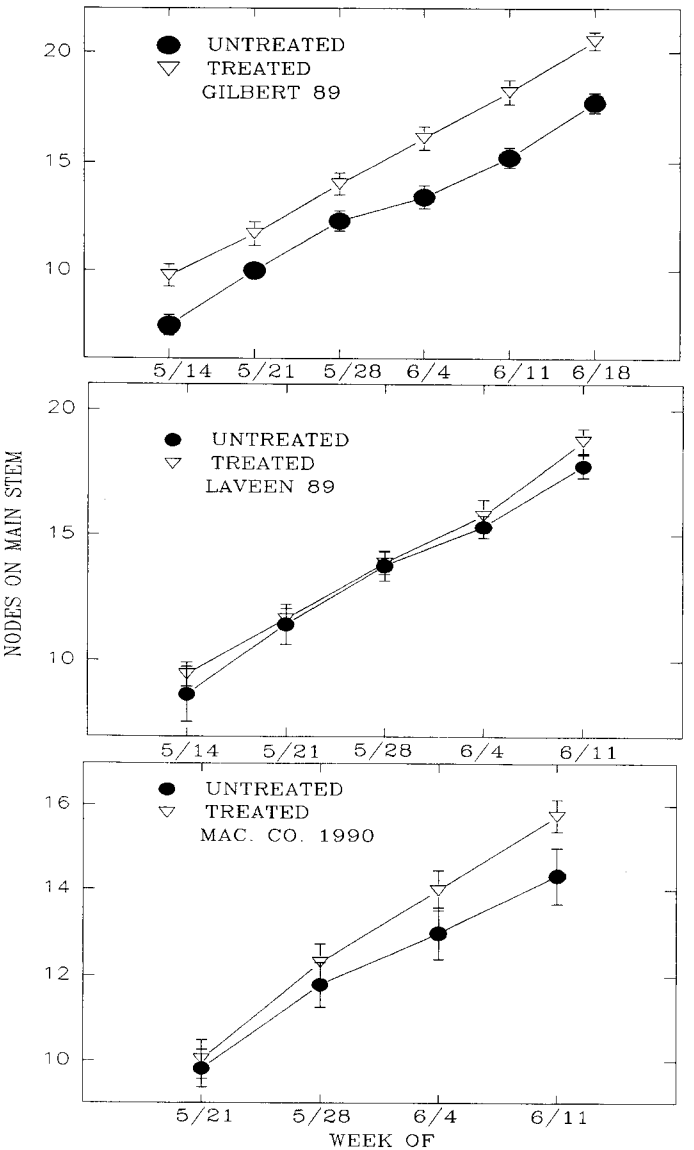

FIG. 1. Mean node (± S. E.) on main stem for untreated and ULV malathion treated fields. Maricopa County, AZ 1989-90.

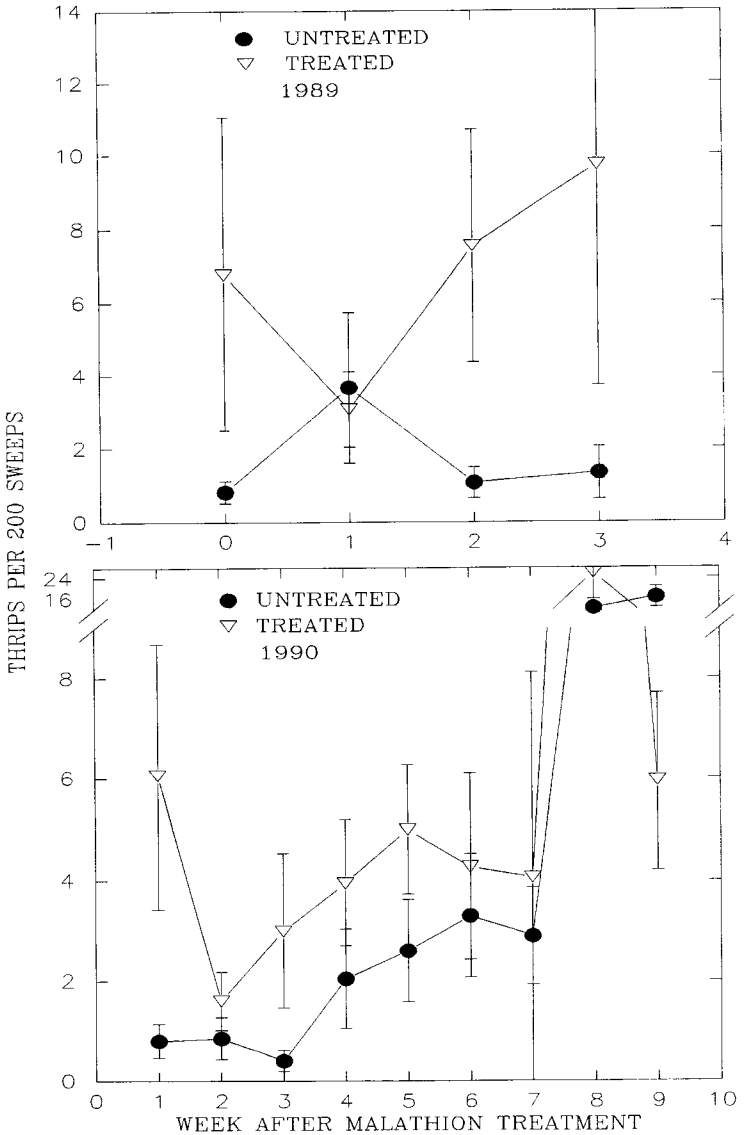

FIG. 2. Mean (± S. E.) number of thrips in untreated and ULV malathion treated fields. Maricopa County, AZ 1989-90. Week zero for 1989 = week of last malathion treatment.

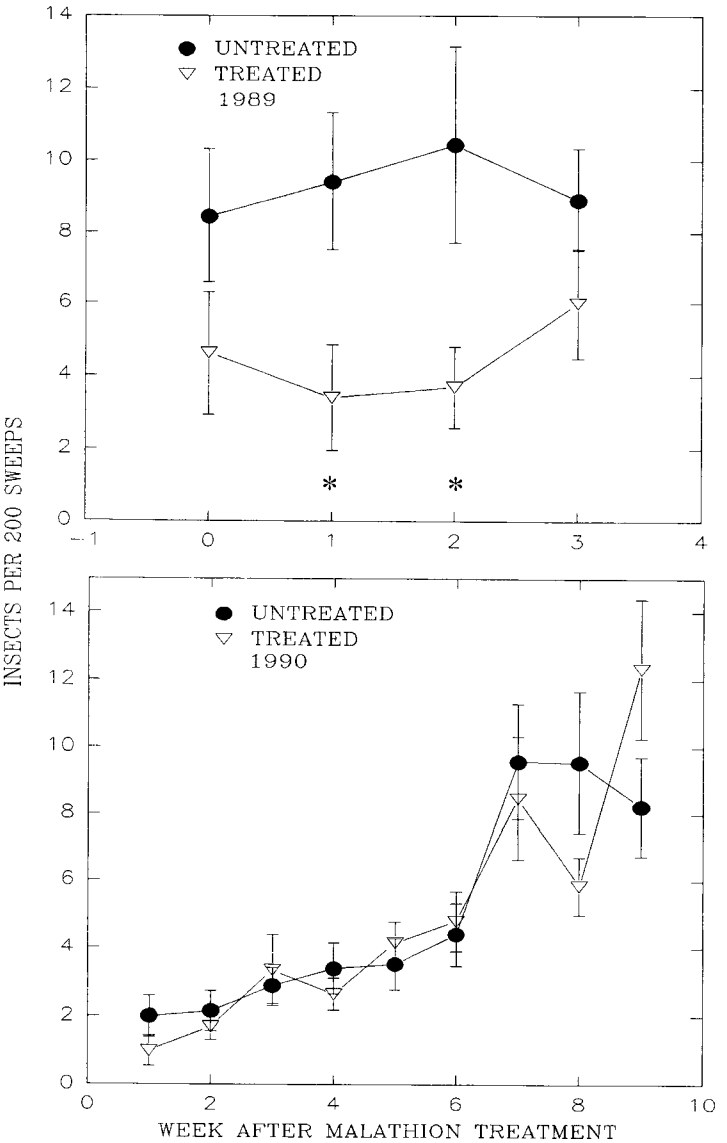

FIG. 3. Mean (± S. E.) number of *Lygus* and cotton fleahopper in untreated and ULV malathion treated fields. Maricopa County, AZ 1989-90.

* Indicates significant difference at $P = 0.05$ as judged by t-test (df=25 and 17 for week 1 and 2 respectively).

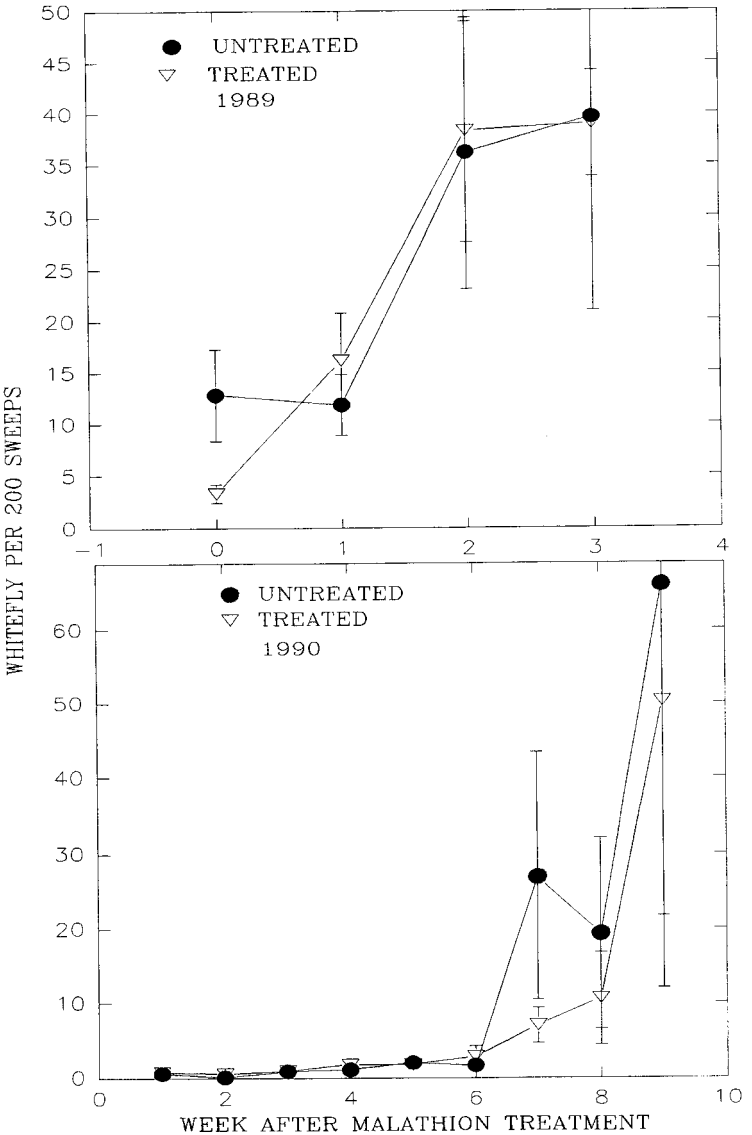

FIG. 4. Mean (± S. E.) number of whitefly adults in untreated and ULV malathion treated fields. Maricopa County, AZ 1989-90.

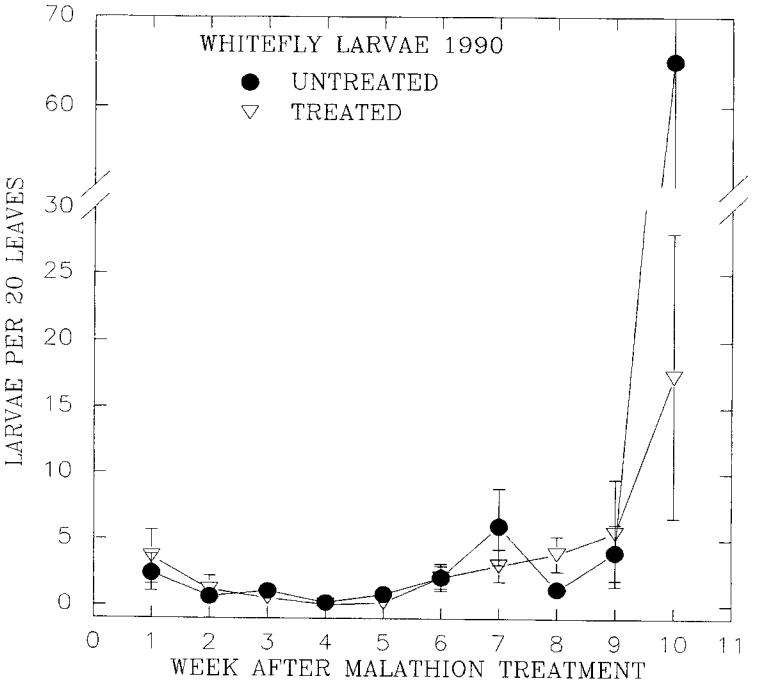

FIG. 5. Mean (± S. E.) number of whitefly immatures/field in untreated and ULV malathion treated fields. Maricopa County, AZ 1989-90.

Spiders. Samples consisted mainly of crab and jumping spiders in the families Thomisidae and Salticidae, respectively. Numbers increased gradually throughout the season in both treatments. In both 1989 and 1990, there were fewer spiders in the treated fields for the first two weeks after treatment. However, spiders were numerically greater in the treated fields from 18 June through 16 July 1990 (Fig. 9).

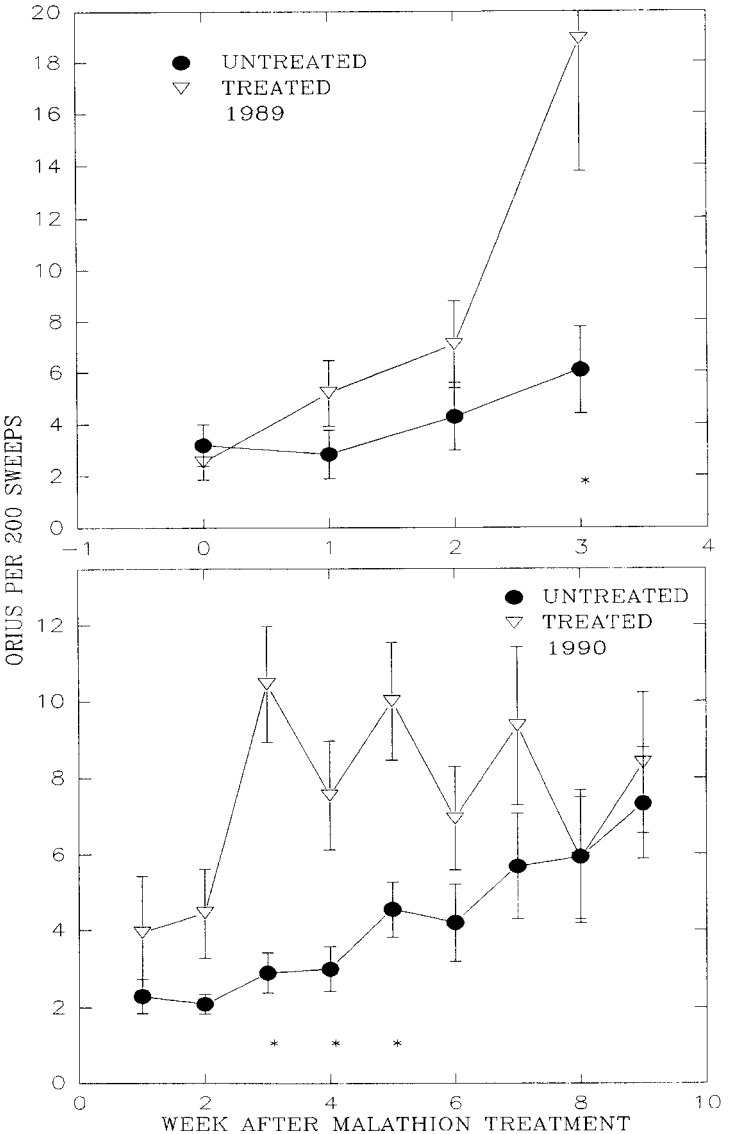

FIG. 6. Mean (± S. E.) number of *Orius* in untreated and ULV malathion treated fields. Maricopa County, AZ 1989-90.
* Indicates significant difference at $P = 0.05$ as judged by t-test (df=25 for week 3 1989 and 19 for week 3, 4 and 5 1990).

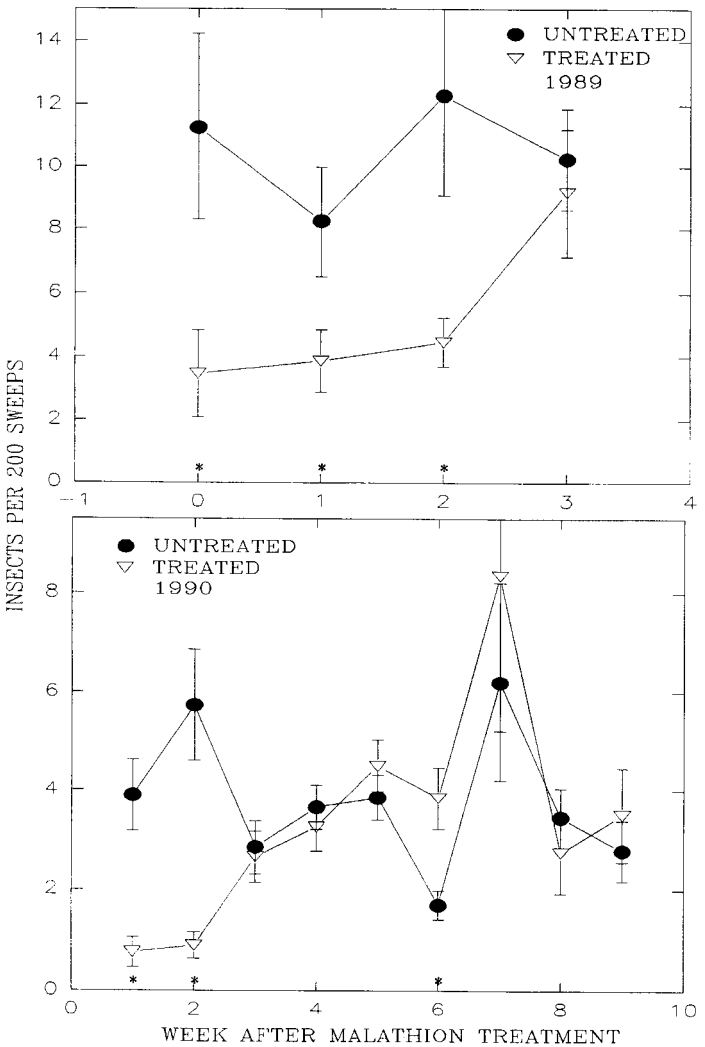

FIG. 7. Mean (± S. E.) number of assassin bugs, big-eyed bugs, nabids, lacewing, lady beetles, and *Collops* in untreated and ULV malathion treated fields. Maricopa County, AZ 1989-90.

* Indicates significant difference at $P = 0.05$ as judged by t-test (df = 14, 24, and 14 for week 0, 1, and 2 1989 respectively) (df = 14, 14 and 18 for week 1, 2 and 6 1990 respectively).

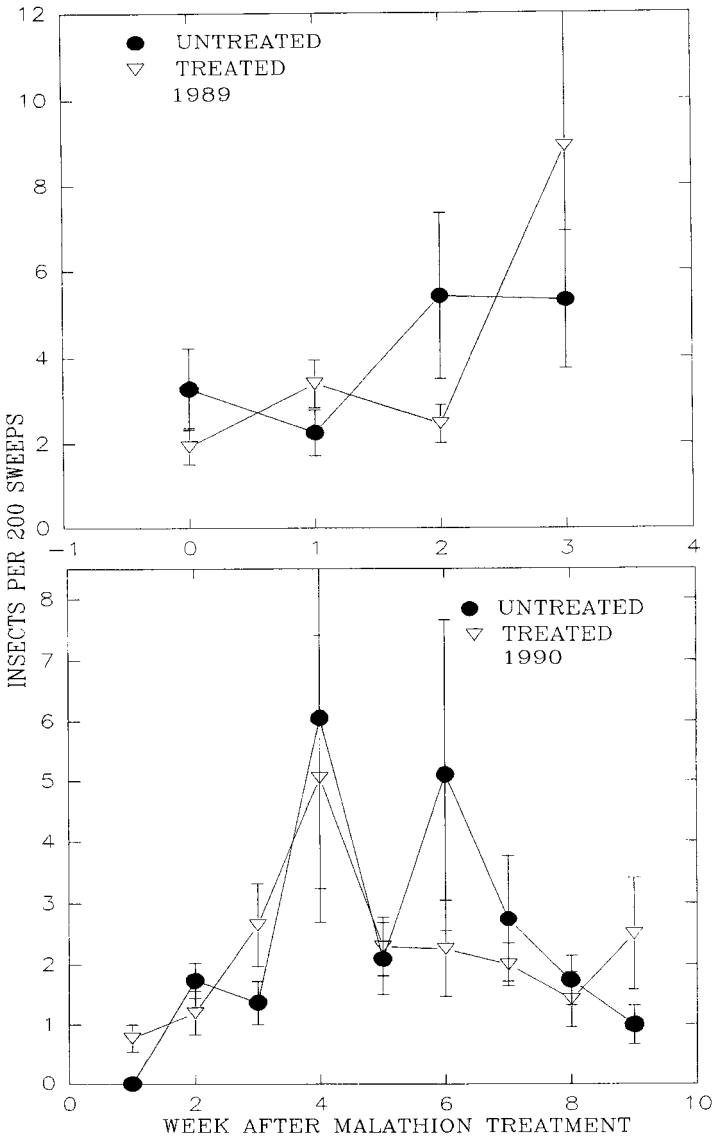

FIG. 8. Mean (± S.E.) number of parasitic Hymenoptera (Braconidae, Ichneumonidae, Chalcidoidae, and Trichogrammatidae) in untreated and ULV malathion treated fields. Maricopa County, AZ 1989-90.

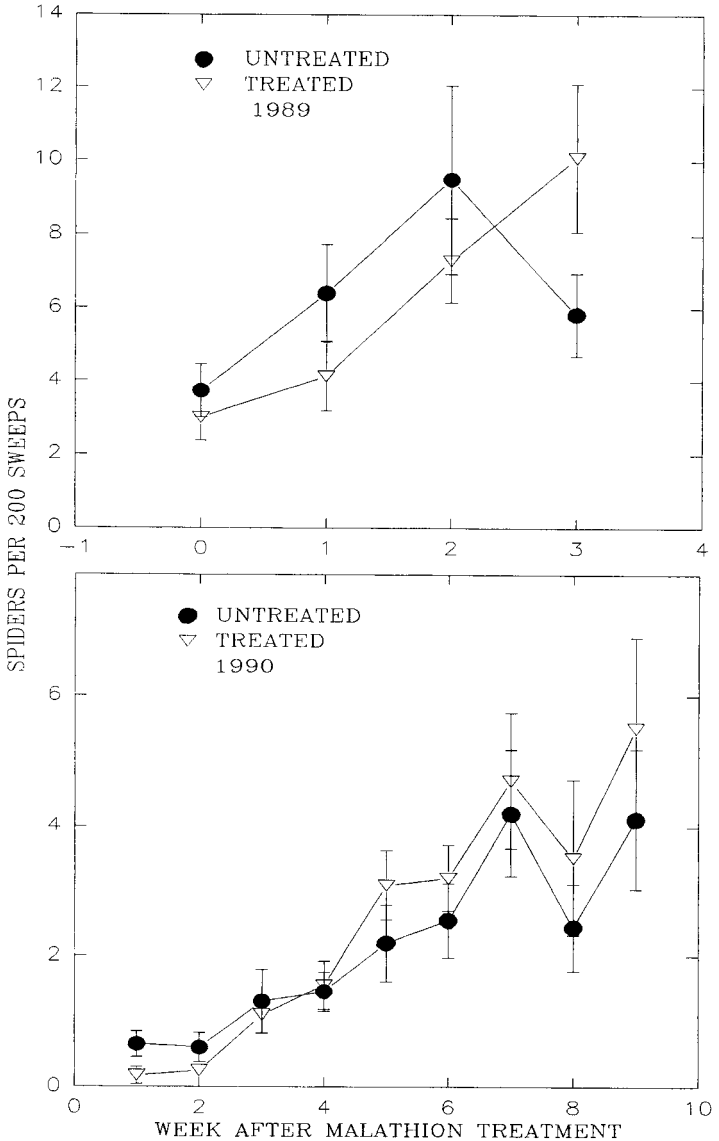

FIG. 9. Mean (± S. E.) number of spiders (mainly crab and jumping spiders) in untreated and ULV malathion treated fields. Maricopa County, AZ 1989-90.

Numbers of thrips, *Frankliniella* spp., increased after ULV malathion treatments but this occurred after cotton was squaring and the amount of damage was unknown. A more detailed study of thrips and their potential damage during mid-season would be useful. An application of ULV malathion may be beneficial if damaging populations of *Lygus* or cotton fleahoppers were present. In general, the predators were the most affected group of insects but populations completely recovered two weeks after treatment. The only cotton fields treated with malathion were those with positive boll weevil trap catches two weeks before pinhead squares are found. It is unlikely that economic damage from secondary pests would occur at this time when fruiting is at a low level. Subsequent applications of insecticide for boll weevil or other pest could result in economic damage if secondary pest were present during the two-week period following insecticide treatment. In 1988, some growers applied Guthion rather than allowing the Southwest Boll Weevil Program to apply malathion. Beet armyworm populations in fields of these growers were greater than in the malathion treated fields (Leggett 1989).

ACKNOWLEDGMENT

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SELECCION DE CEPAS DE *BACILLUS THURINGIENSIS* PARA CONTROL DE INSECTOS LEPIDOPTEROS

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Se evaluaron cepas nativas de *Bacillus thuringiensis* (Berliner) dontra el "gusano cogollero" *Spodoptera frugiperda* (Smith) y el "gusano bellotero" *Heliothis virescens (Fabricius),* lepidópteros plaga de importancia ecónomica en México. Cada cepa fué crecida en un medio con melazas y harina de soya. La espora delta-endotoxina producida fué extraida al final de cada fermentación para determinar su toxicidad. Cinco aislados producen mayor toxicidad que el estandar HD-1-S-1980 (LC_{50} = 6492 ug/ml). Las cepas GM-1, GM-8, GM-9, GM-10 y GM-11 producen LC_{50} de 92.5, 77.1, 275.1, 59.0 y 185.3 ug/ml respectivamente. La cepa GM-10 (LC_{50} = 19.3 ug/ml) superá también al estandar Internacional HD-1-S-1980 al probarse con *Heliothis virescens*. *Bacillus thuringiensis* GM-10 ofrece una gran alternativa para el control de lepidópteros en México.

ABSTRACT

Native strains of *Bacillus thuringiensis* (Berliner) were evaluated against the fall armyworm, *Spodoptera frugiperda* (Smith), and tobacco budworm, *Heliothis virescens,* lepidopteran pests of economic importance in Mexico. Isolates of each strain were grown on molasses medium supplemented with soybean flour. Spore delta endotoxins were extracted at the end of fermentation to assess their toxicities. Five isolates exhibited toxicity against fall armyworm greater than that of the International Standard HD-1-S-1980 (LC_{50} = 6492 ug/ml), Strains GM-1, GM-8, GM-9, GM-10 and GM-11 yielded LC_{50} values of 92.5, 77;.1, 275.1, 59.0, and 185.3 ug/ml, respectively. The LC_{50} of GM-10 (19.3 ug/ml) also exceeded that of the International Standard HD-1-S-1980 (21.6 ug/ml) against tobacco budworm. *Bacillus thuringiensis* strain GM-10 therefore may be considered one of the best choices for lepidoptera control in Mexico.

INTRODUCCION

El uso de bioinsecticidas del tipo de *Bacillus thuringiensis* (Berliner) para el control de plagas es una excelente alternativa al control de insectos, dado su inocuidad para aves, peces, mamiferos, plantas e insectos benéficos (Granados 1981). *Bacillus thuringiensis* es una bacteria gram positiva, con espora oblicua o esférica en posición terminal o subterminal, que produce un cuerpo paraesporal o cristal paraesporal conocidos como delta-endotoxina (Dulmage & de Barjac 1973), dentro del cual radica la fracción tóxica para lepidópteros, dípteros y coleópteros (Dulmage 1981, Krieg et al. 1989, Pfannesteil et al. 1983).

En la actualidad se sabe que la potencia de las preparaciones de *B. thuringiensis* varía de acuerdo a la cepa utilizada (aislado o serotipo), medio de propagación, condiciones de fermentación, formulación final e insecto contra el cual se utilize (Smith 1982, Salama et al. 1983). *Spodoptera frugiperda* (Smith) (Gusano cogollero) y *Heliothis virescens* (Fabricius) (Gusano bellotero) constituyen dos de los insectos plaga de mayor importancia económica para cultivos de maíz y frijol en México, los que se pueden controlar con *B.*

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thuringiensis (Beegle 1979, Couch & Ross 1980). La presente investigación se realizó con la finalidad de seleccionar de un grupo de 19 aislados de *B. thuringiensis* aquellos con mayor actividad tóxica contra el gusano cogollero y gusano bellotero.

MATERIALES Y METODOS

Manejo de las Cepas de *B. thuringiensis*. Se trabajó con 19 cepas de *B. thuringiensis* de la Colección Microbial del Departamento de Microbiología e Inmunología de la Facultad de Ciencias Biológicas, U.A.N.L.. Para su mantenimiento se efectuaron resiembras trimestrales en agar nutritivo con pH 7, incubadas por 36h a 30°C, almacenadas en refrigeración a 5°C hasta el momento de su uso. Estas cepas también fuéron liofilizadas en leche desnatada al 10% como soporte para su conservación, utilizando cultivos esporulados de cada una de las cepas de *Bacillus thuringiensis* (Vandekar & Dulmage 1982). Cada cepa fué activada en tubos con agar nutritivo inclinado con pH 7 e incubada por 24h a 30°C, posteriormente se tomó una asada para inocular matraces de 250 ml con caldo de triptosa fosfato (CTP) pH 7, los cuales se sometieron a 250 rpm de agitación rotatoria durante 12h a 30°C, finalmente se inocularon los matraces de producción con 0.5% (V/V) del inóculo.

La fermentación se realizó en matraces de 2000 ml con 400 de medio de propagación el cual contenía 5 g/l de melazas, 10 g/l de harina de soya, 1 ml/l de líquido de remojo de maíz (lrm), 0.1 g/l de CaCO3 y pH inicial de 7.0. La temperatura de incubación fué 30°C y 250 rpm de agitación rotatoria. El tiempo de fermentación fué variable hasta obtener un 85% de lisis celular o liberación de esporas y cristales (delta-endotoxina), el cual se determinó mediante observaciones microscópicas continuas. Al termino de cada fermentación se centrifugo el licor a 5000 rpm durante 20 min, del precipitado se extrajó el complejo espora cristal por coprecipitación con lactosa y acetona. Al polvo final se le determinó esporas viables (UFC) por cuenta viable en placas de agar nutritivo (Dulmage et al. 1970, Dulmage 1971, 1973).

Bioensayos de Seleccion y Toxicadad. La toxicidad y potencia de las preparaciones de *Bacillus thuringiensis* se determinó através de bioensayos con larvas del primer estadío del gusano cogollero y del gusano bellotero. SE usaron dosis preliminares de 500 ug/ml del extracto de *Bacillus thuringiensis* mezclado con la dieta. La cría, dieta y condiciones de producción de los dos insectos de ensayo se establecio según los métodos reportados por Raulsaton & Lingren (1972), Guerra & Bhuiya (1977). La dosis letal media (LC_{50}) se determinó através del análisis probit de los datos obtenidos de cada ensayo, acorde a los procedimientos reportados por Dulmage et al. (1976), Dulmage & Orlin (1977).

RESULTADOS Y DISCUSION

Los rendimientos de producto final recuperados de las fermentaciones de *B. thuringiensis* oscilaron entre 7 y 8 g de polvo activo por litro de medio de propagación, después de 36 a 48h de incubación según la cepa utilizada. En la Tabla 1 se muestra el porcentaje de mortalidad de cada una de las cepas en los insectos prueba, como se aprecia *Bacillus thuringiensis* GM-1, GM-8, GM-9, GM-10 y GM-11 provocarón entre el 76 al 100% de mortalidad en el gusano cogollero y gusano bellotero, razón por la cual fuéron seleccionadas como las más activas y se procedio a determinar su potencia en el control de estos insectos. Las demás cepas producen una delta-endotoxina inactiva o inespecifica para el control de estos insectos dada su inocuidad para ellos según a lo reportado por Krieg et al. (1989).

Como se puede observar en la Tabla No. 2 las cinco cepas seleccionadas demonstraron ser más tóxicas para *S. frugiperda* que el estandar internacional HD-1-S-1980, el cual se puede considerar prácticamente como a tóxico para el gusano cogollero. Por el motivo anterior sólo se muestra la Concentración Letal Media (LC_{50}) y no la potencia (LC_{50} del estandar/LC_{50} del extracto de fermentación X 16000 UI/mg), dado que el estandar de comparación es pobremente activo. Es factible, sin embargo, distinguir que de las cinco cepas los extractos de la GM-10 poseen los mayores niveles de actividad

TABLA 1. Bioensayos Preliminares de Toxicadad de Cepas Nativas de *Bacillus thuringiensis*.

Clave Del Aislado *B. thuringiensis*	% de Mortalidad *S. frugiperda*	A500 ug/ml *H. virescens*
GM 1*	92	96
GM 2	12	24
GM 3	4	24
GM 4	0	32
GM 5	0	36
GM 6	48	20
GM 7	56	24
GM 8[a]	84	100
GM 9[a]	80	40
GM 10[a]	100	100
GM 11[a]	76	92
GM 12	4	32
GM 13	0	36
GM 14	18	20
GM 15	8	12
GM 16	0	16
GM 17	4	28
GM 18	16	28
GM 19	8	16

[a] Cepas seeccionadas para determinar su potencia.

insecticida (LC_{50} - 59 ug/ml), por lo que para fines prácticos es recomendable su uso experimental. Con respecto a la cantidad de esporas producidas se aprecia que no existe una relación proporcional con la toxicad dado que mientras la potencia es variable, el número de esporas producidas es prácticamente la misma en todas las cepas nativas de *B.*

TABLA 2. Actividad de Aislados Nativos de *Bacillus thuringiensis* Contra *Spodoptera frugiperda.*

CEPA	UFC[a]/MG Del Extracto	LC50[b] ug/ml
HD-1-S-1980[c]	-	6491.2
GM-1	2×10^6	92.3
GM-8	2×10^6	77.1
GM-9	2×10^6	275.7
GM-10	2×10^6	59.0
GM-11	6×10^6	185.3

[a] UFC (Unidades formadoras de colonia).
[b] Concentración Letal Media de acuerdo al análisis Probit.
[c] Estandar Internacional de Referecia.

thuringiensis, lo que coincide con lo reportado por Dulmage et al. (1973, 1977), en el sentido de que las unidades de crecimiento de esta bacteria como el número de esporas no tienen una relación estricta con la toxicidad en insectos.

Al determinar la potencia de los extractos de cepas nativas de *B. thuringiensis* en *Heliothis virescens* se encontró que presentaron menor toxicidad que el estandar internacional HD-1-S-1980, con excepción de la cepa GM-10 que posee una potencia de 17,889 UI/mg del extracto final, en comparación con las 16,000 UI/mg del estandar (Tabla 3). La segunda cepa con mejor actividad correspondió a *B. thuringiensis* GM-1 con 10,097 UI/mg, 37% menos activa que el estandar internacional. Al igual que la actividad de estos aislados para el gusano cogollero, tampoco existe relación del número de esporas con la actividad insecticida para el gusano bellotero, debido a que la cantidad de esporas producidas es similar (2.5×10^6) más no así la potencia.

TABLA 3. Actividad de Aislados Nativos de *Bacillus thuringiensis* contra *Heliothis virescens*.

CEPA	UFC[a]/MG Del Extracto	LC50[b] ug/ml	Potencia UI/mg
HD-1-S-1980[c]	-	21.6	16 000
GM-1	2×10^6	34.2	10 097
GM-8	3×10^6	62.9	5 506
GM-10	2×10^6	19.3	17 889
GM-11	6×10^6	62.2	5 549

a UFC (Unidades Formadoras de Colonia).
b Concentración Letal Media de acuerdo al Análisis Probit.
c Estandar Internacional de Referecia.

CONCLUSIONES

La selección de cepas de *B. thuringiensis* debe de realizarse en las especies de insectos que se desee controlar, dado que los niveles de actividad son muy variables entre los aislados de esta bacteria. Además, la cantidad de esporas producidas al crecer en medios de propagación no corresponde con los niveles de actividad de *B. thuringiensis*. De las 19 cepas en estudio *B. thuringiensis* GM-10, es el aislado con mayor actividad insecticida que el estandar internacional H-1-S-1980 al aplicarse contra *S. frugiperda* o *H. virescens*, de ahí su gran potencialidad para aplicarse en el control de insectos plaga de importancia económica.

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SCIENTIFIC NOTE

IDENTIFICATION AND OCCURRENCE OF *LIRIOMYZA* SPECIES ASSOCIATED WITH COTTON IN ARIZONA

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Agromyzid leafminer species in the genus *Liriomyza* have become serious economic pests of several crops in North America (Parrella 1982). The polyphagous species *Liriomyza trifolii* (Burgess) and *Liriomyza sativae* Blanchard are most prevalent and have overlapping host ranges (Spencer 1973). The number of known host plants of *L. trifolii* worldwide is estimated at about 120 species in 21 families (Spencer 1981). In Arizona, *L. sativae* was first reported to cause substantial damage to melons and lettuce in the 1940's (Hills and Taylor 1951). The first occurrence of *L. trifolii* in Arizona was reported in 1987 on lettuce and celery (Rethwisch 1989). Cotton, *Gossypium* spp. has not previously been listed as a host for *L. trifolii* (Spencer 1981). However, many growers in Arizona have assumed that *L. trifolii* occurs on cotton because of the large numbers which typically infest fall lettuce in close proximity to cotton (Scott Tollefson, per. comm.). In 1991, cotton in Central Arizona became heavily infested with leafminers at mid-season. Upon close inspection, it was determined that a large population of *L. trifolii* was present. The purpose of this note is to report that two species of cotton, *Gossypium hirsutum* L. and *G. barbadense* L., are new hosts of *L. trifolii*.

Reported herein are observations of larval populations and damage to cotton grown in Arizona in the summer of 1991. The first observation involves cotton that was sampled on 23 July near Coolidge, Arizona. About 200 acres of cotton (DPL 56-90) were heavily damaged by leafminer. The leaves contained a very large population of larvae and adults that primarily consisted of *L. trifolii*. Mined leaves were distributed regularly throughout the field, and most leaves in the upper and middle plant canopy were infested with larvae. From the number of exited mines observed on leaves on the lower canopy, it was apparent that several generations had developed previously. Leaves were collected from the upper 1/3 of the plants, examined for mines and larvae, and placed in emergence cages for rearing of adults. Specimens of each species of *Liriomyza* were identified by Dr. Eric Fisher, Calif. Dept. of Food & Agric., Sacramento. Specimens of parasitoids were identified by Carl Olson, Univ. of Arizona. Infested leaves contained an average of 28.9 ± 8.9 mines and 11.2 ± 5.3 larvae per leaf. In the heaviest mined leaves, it was estimated that a large proportion of the leaf surface ($> 60\%$) was chlorotic as a result of leafminer feeding. On the field margins, about 10% of the leaves had been defoliated from the lower plant canopy due to heavy mining. Large numbers of adult *L. trifolii* flies were readily observed flying within the upper plant canopy. A total of 319 pupae, of which 180 yielded adult *L. trifolii* was collected (Table 1). Only four pupae yielded *L. sativae* adults; the remaining 139 pupae were either parasitized with the hymenopterous parasitoids, *Chrysocharis* spp. and *Dacnusa* spp., or did not yield viable insects. Pima cotton (S-6) sampled at Coolidge was not heavily infested with leafminers, but two *L. trifolii* adults were collected from the leaf samples.

Table 1. Species Composition of Populations of *Liriomyza* Infesting Cotton at Coolidge and Yuma, Arizona in 1991.

Cotton variety	Location	No. leaves sampled	Mean larvae per leaf	% *Liriomyza* adults per sample	
				trifolii	*sativae*
DPL 56-90	Coolidge	45	11.2 ± 5.3	97.8	2.2
Pima S-6	Coolidge	34	0.2 ± 0.1	40.0	60.0
DPL 90	Yuma	80	1.1 ± 0.7	38.5	61.5
Pima S-6	Yuma	80	0.1 ± 0.1	25.0	75.0

The second observation of *L. trifolii* on cotton was at Yuma, Arizona. Cotton (DPL 90) leaves were collected from a 45-acre field on 10 July 1991. Numbers of total mines and larvae per leaf were much lower than those examined from Coolidge (Table 1). Very little mining of leaves was observed, and adult flies were not detected on the plants. A total of 74 pupae was collected from the infested leaves, of which 20 yielded *L. trifolii*; 32 yielded adults of *L. sativae,* and the remainder consisted of the adult parasitoids. The number of larvae and mines in pima (S-6) leaves was very low, resulting in only a single *L. trifolii* adult.

Although *L. trifolii* has probably occurred on cotton for several years, this is the first report of *L. trifolii* infesting and damaging upland cottons in any growing region. The incidence of defoliated leaves demonstrates the capability this insect has for inflicting heavy leaf damage to fruiting cotton. Populations of leafminers (*L. sativae*) in cotton normally occur at low levels during the season. However, cotton infested at Coolidge was grown using a combination of very unique management and cultural practices. First, it was irrigated by subsurface, drip technology utilizing minimum tillage. In addition, the cotton was rotated with watermelons and lettuce, both of which have a history of *L. trifolii* outbreaks in that area. Finally, the cotton received multiple applications of broad-spectrum insecticides (methyl parathion, thiodicarb and acephate) for early season insect control. It is probable that the combination of these management practices predisposed the cotton for such an outbreak of *L. trifolii.* Infestations of *L. trifolii* reported in this note should serve as a warning that large numbers of leafminers are capable of causing damage and defoliating fruiting cotton under special circumstances.

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THE OCCURRENCE OF *CATOLACCUS HUNTERI*, A PARASITOID OF *ANTHONOMUS EUGENII*, IN INSECTICIDE TREATED BELL PEPPER

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The pepper weevil, *Anthonomus eugenii* Cano, has contributed to excessive use of insecticides on bell peppers due to difficulties in timely control of adults (Riley 1990) and through the release of secondary pest populations. The apparent lack of nonchemical control tactics and low rates of mortality due to natural enemies (Elmore et al. 1934) exacerbate the problem. Wilson (1986) reported *Catolaccus hunteri* Crawford (Hymenoptera: Pteromalidae) parasitizing pepper weevil larvae in Florida within bell pepper flowers at rates of ca. 5%. *Catolaccus* and related genera have been described (Burks 1954), the distribution of certain species in cotton recorded (Cross and Mitchell 1968), and several bruchid and anthonomine hosts listed (Chestnut and Cross 1971); however, very little is known about the habits of *C. hunteri* in peppers. The objective of the following study was to document parasitism of immature pepper weevils in untreated and insecticide treated bell pepper over two seasons at Bradenton, Florida.

In the first test, treatments consisted of weekly applications of permethrin (0.23 kg AI/ha), weekly applications of oxamyl (1.10 kg AI/ha), action threshold applications of permethrin (one threshold at 1 pepper weevil adult/200 terminal pepper buds and a second threshold in a separate plot at 2 adults/200 terminal buds), action threshold applications of oxamyl, and an untreated control. Treated plots consisted of three rows by six meters and were replicated four times in a randomized complete block design. Pepper weevil larvae and parasitoids in fallen pepper buds were surveyed in six meter sections of row replicated four times on 3, 11, 17, 24 October 1988 and 0.5 meter sections of row on 1 November 1988. In the second test, treatments consisted of weekly applications of oxamyl (0.57 kg AI/ha), weekly applications of permethrin (0.23 kg AI/ha), action threshold applications of oxamyl (one threshold at 1 pepper weevil adult/400 terminal pepper buds and a second threshold in a separate plot at 1 adult/200 terminal buds) and action threshold applications of permethrin. Treated plots consisted of three rows by 7.6 meters and were replicated four times in a randomized complete block design. Pepper weevil larvae and parasitoids were surveyed in fallen pepper buds and small fruit in 1 m of row on 13, 17, 24 April and 1 and 8 May 1989. An adjacent untreated pepper field planted at the same time as the treatment plots was also surveyed.

Parasitoid occurrence was evaluated by examining fallen pepper buds and small fruit for the presence of weevil larvae, which were subsequently held with minimal disturbance in petri dishes with moistened filter paper, until emergence of either *C. hunteri* adults or pepper weevil adults. Parasitoids were identified with descriptions by Burks (1954) and through the assistance of L.A. Stange, Dept. Plant Industry, Gainesville, Florida.

In the first test, no insecticide application was made in any threshold plot through 1 November because of low numbers of pepper weevils. Examination of 2651 fallen buds yielded 24 pepper weevil larvae and 8 parasitoids. There was no significant difference between individual treatments ($P<0.05$), but parasitoid numbers from all threshold plots

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grouped together had significantly higher numbers of parasitoids (0.3/1 m row) than calendar treatment plots (none) on 1 November ($t=2.4$; $df=31$; $P<0.05$).

In the second test, 1443 buds were examined, yielding 83 pepper weevil larvae and 6 parasitoids. More *C. hunteri* were observed in the oxamyl treated plots [0.1/1 m row (26% parasitism); $t=2.2$; $df=53$; $P<0.05$] than permethrin treated plots (none) over all sampling dates. In an adjacent untreated field, 325 buds were examined, yielding 78 pepper weevil larvae and 9 parasitoids (12% parasitism).

Insecticide action thresholds and the use of oxamyl enhanced numbers of *C. hunteri* in fallen pepper buds. In Honduras, *Catolaccus* specimens have also been found in oxamyl treated plots (Riley 1990). This is the first direct evidence of benefit to natural enemies of pepper weevil where reduced or selective insecticide treatments were used. Parasitoids were not detected in fallen fruit larger than 2.5 cm in diameter. It is possible that the thickness of the fruit pericarp in larger fallen fruit is a physical barrier to the parasitoid. Thus, the impact of *C. hunteri* on pepper weevil mortality may be greater in small fruiting buds.

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THE POTENTIAL OF NON-NATIVE SELENIUM ACCUMULATING MUSTARD PLANTS AS HOST FOR BEET LEAFHOPPER AND BEET CURLY TOP VIRUS

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Agricultural irrigation/drainage problems in the westside of central California have resulted in public concern after scientists determined that excessively high concentrations of naturally-occurring selenium (Se) accumulated in the food chain and caused deformities and reproductive problems in wildfowl (Presser and Barnes 1985, Ohlendorf et al. 1986). Consequently, environmentally sound approaches to control the amount of Se entering the agriculture ecosystem are being studied. Two suggested plans for managing drainage-induced Se problems include reusing drainage water for irrigation where possible (Rhoades et al. 1989, Ayars et al. 1987) or using a recently introduced exotic species of mustard from Pakistan, *Brassica juncea* Czern L., to remove Se from the soil (Bañuelos and Schrale 1989). Because *B. juncea* is able to absorb high concentrations of soluble Se from the soil (Bañuelos and Meek 1990), interest exists in its potential for Se removal in central California. However, there is also concern that test plantings of native and/or non-native plant species might contribute to beet curly top virus (BCTV) dissemination by serving as host plants or harboring insect vectors.

In North America, the beet leafhopper, *Circulifer tenellus* (Baker), feeds in the phloem cells of dicotyledonous plants (Magyarosy and Duffus 1977). Many plant species serve as host to BCTV including field crops such as sugarbeets, tomatoes, cantaloupe, spinach, beans, and many ornamentals (Mumford 1982). In late fall, as food crops are harvested and the favored weed hosts are drying, insects colonize alternate host plants for food and shelter.

The beet leafhopper overwinters on weed hosts in central California. Much is known about the weed host range of the beet leafhopper and BCTV (Bennett 1971), and comparisons between major weeds as hosts for beet leafhopper and BCTV have been made in central California (Mumford and Doney 1984) and elsewhere (Gracia and Feldman 1972). It is necessary therefore to determine any relationship between beet leafhoppers, BCTV, and this exotic mustard before planting area-wide to facilitate Se removal. Accordingly, plantings of *B. juncea* located adjacent to different agronomic crops in central California were sampled for beet leafhopper during the summer of 1990. In addition, *B. juncea* was experimentally inoculated with the BCTV, and the virus's rate of spread monitored with the enzyme-linked immunosorbent assay (ELISA).

Field experiments were conducted in west-central California between 15 May and 1 September 1990. *Brassica juncea* Czern L. was planted on three different field sites in two-week intervals: (1) site 1, located 25 km southwest of Fresno, consisted of a 0.1-ha parcel 25 m from corn and birdsfoot trefoil; (2) site 2, located 10 km west of Los Baños, consisted of six 0.05-ha parcels 50 m from cotton and ornamental eucalyptus; (3) site 3, located in the northeast sector of

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Fresno on California State University Fresno, consisted of a 0.1-ha parcel 25 m from turf and peach trees.

Two-week old *B. juncea* seedlings were transplanted to each growing site on beds spaced 15 cm apart. Plants were irrigated similarly to the crops grown near each respective site. Eighteen days after transplanting, the plants were sampled for leafhoppers at 10 A.M. using an insect sweep net. Each sample consisted of eight random sweeps progressing from the outside towards the middle of the plot. Collected insects were placed into a glass jar and frozen for later identification. A total of four samples, 12 days apart, were collected at each field site.

Both *Brassica juncea* and *Brassica alba* (a mustard native to California) were inoculated with different strains of BCTV (Logan, St-11, Fresno I and HRCT) and then monitored for BCTV by serological methods (ELISA) (Mumford 1982). In addition to normal inoculation procedures (10 leafhoppers/plant with a 3 to 4-day inoculation feeding period), a large number of viruliferous leafhoppers (100) were colonized on test plants for 10 days; adults were removed and developing nymphs were monitored for BCTV.

During the study we collected insects representing 10 orders and over 22 families (data not shown). *Circulifer tenellus* was taken from all three test mustard plots; however, a total of only thirteen beet leafhoppers were found at site 1, three at site 2, and twelve at site 3. A few specimens of two other leafhopper species were also collected: *Macrosteles quadralineatus* Forbes, the aster leafhopper, and an unidentified species in the genus *Empoasca*.

In the inoculation experiments, BCTV was not detected in beet leafhoppers after an acquisition period of 3-4 days on BCTV-inoculated mustard plants and also was not detected in developing nymphs after an acquisition period of 10 days (Table 1). In addition, test plants assayed for the BCTV virus by ELISA were negative (Table 1). Similar tests with *B. alba*, a known susceptible mustard host, produced positive results.

Several weeds, many of which are used by various insects as overwintering habitats, have been reported to be hosts of curly top virus (Gracia and Feldman 1972, Mumford and Doney 1984). The only insect vector of curly top virus described to date has been the beet leafhopper, which can undergo three or four generations per year in California (Magyarosy and Duffus 1977). According to Mumford (1982), an abundance of viruliferous leafhoppers is essential to cause a severe outbreak of curly top virus disease; our sampling detected relatively

TABLE 1. Detection with ELISA and Virus Recovery in *Brassica juncea* and *Brassica alba* Infected with Different BCTV Strains.

Species	BCTV Strain	No. Plants	No. Beet Leafhoppers Per Plant	ELISA[a]	Virus Recovery[b]
B. juncea	Logan	20	10	-	-
B. juncea	St-11	20	10	-	-
B. juncea	Fresno I	20	10	-	-
B. juncea	HRCT	20	10	-	-
B. juncea	Fresno I	1	100 (colony)	-	-
B. alba	Fresno I	20	10	+	+
B. alba	St-11	20	10	+	+
B. alba	Logan	20	10	+	+

[a]Leafhopper giving ELISA values >0, three times healthy control: yes(+), no(-).
[b]Infection in plant tissue: yes(+), no(-).

low numbers feeding on wild brown mustard during the study. Even after inoculation of wild mustard with BCTV, nymphs collected from leafhoppers feeding on inoculated *B. juncea* did not exhibit a positive reaction to the virus with the ELISA technique. In addition, the virus was not recovered in the plant tissue. Thus, the exotic *B. juncea* was shown to be a nonhost plant for BCTV, even though the same inoculation produced ELISA positive plants and the virus was recovered in *B. alba*. Increased cultivation of *B. juncea* along the western margin of the San Joaquin Valley in central California, where naturally high concentrations of Se are present, will modify vegetation in certain isolated areas and may influence insect patterns. If damaging levels of beet leafhopper were to occur on these plantings, control measures, including insecticide applications, might be necessary. However, multiple harvests of mustard plantings will be made during the year to remove Se accumulated in the plants. Repeated harvests would decrease population densities and reproductive capacities of beet leafhoppers and other insects. Our tests provide presumptive evidence that *B. juncea* Czern is a nonhost of BCTV under greenhouse conditions. Therefore, even if this plant becomes attractive to beet leafhopper, it is doubtful that the potential for outbreak of BCTV disease would increase due to plantings of this exotic brown mustard for the purpose of Se removal from the soil.

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PINK BOLLWORM (LEPIDOPTERA: GELECHIIDAE) MALE MOTH TRAP CATCHES IN GOSSYPLURE-BAITED TRAPS IN RELATION TO ACCUMULATED HEAT UNITS

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ABSTRACT

Studies were conducted from 1981 to 1989 to examine the relationship between pink bollworm (PBW), *Pectinophora gossypiella* (Saunders), male moth trap catches in gossyplure-baited traps and heat units (degree days) in the Imperial Valley, California. Heat unit accumulations were based on 30.0/12.8°C (86/55°F) upper/lower temperature thresholds. Results showed that the number of PBW male moths/trap/night caught in gossyplure-baited traps from early March to 31 August were estimated by the equation: $\hat{Y}$ = -1.797 + 0.988E-03 X, where $\hat{Y}$ was Log_{10} [male moths/trap/night] and X was the number of accumulated degree days beginning 1 January each year (R^2 = 0.717). Decreasing numbers of male moths/trap/night from 1 September to mid-November were estimated by the equation $\hat{Y}$ = -3.766 -0.398E-03 X, but the data fit was poor (R^2 = 0.114) because results were confounded by decreasing temperatures beginning in early September. Results of the study suggest that trap catch data, as related to degree days, may be used to describe seasonal male moth trap catch increases as a reflection of increasing PBW populations. Further, male moth trap catches and degree day relationships provide the potential for developing an economic level decision-making tool.

INTRODUCTION

The pink bollworm (PBW), *Pectinophora gossypiella* (Saunders), has been an economic pest of cotton, *Gossypium* spp., in Arizona and southern California since 1965. Estimated cotton yield losses due to PBW infestations alone have been as high as 15% in the Imperial Valley, California (Burrows et al. 1982). Because of the economic impact to the cotton industry, the state of California passed legislation regulating the cotton growing season. Mandated cotton planting, chemical fruiting termination, and cotton plowdown dates were established as 1 March, 1 September, and 1 November, respectively, in the Imperial Valley, California (Chu and Henneberry 1990).

There is an urgent need for methodology to describe and predict PBW population development, particularly under short-season growing conditions, as an aid in decision making for control action. Development of PBW eggs and growth of larvae and pupae are temperature related (Butler and Hamilton 1976, Hutchison et al. 1986). Temperature also triggers induction of diapause (Bariola and Henneberry 1980), larval survival, and spring emergence of overwintered PBW moths (Bariola

1983). Heat-unit accumulations have been used to characterize the relationships between plant growth and insect development (Fry 1983). They have also been used to estimate the time of occurrence of early-season cotton fruiting forms in relation to PBW moth overwintering emergence (Brown et al. 1990, Sevacherian et al. 1977, Silvertooth 1989). However, no studies have been reported relating heat units and PBW male moth trap catches as an indicator of seasonal population development.

The objective of the current study was to investigate this relationship to provide a potential tool for decision making in developing strategies for PBW management systems.

MATERIALS AND METHODS

PBW live traps (Lingren et al. 1980) were baited with gossyplure (0.1 mg) in methylene chloride on rubber septa (Flint et al. 1974). The capture potential of the PBW live trap exceeds that of other trap designs by 3-15 x (Lingren et al. 1980). Traps were installed each year from 1981 to 1989 in cotton fields at the Imperial Valley Irrigated Desert Research Station, Brawley, California. Traps were checked daily, except for weekends, and the number of male moths caught was recorded. Trapping began as early as 9 March (1982) and as late as 13 May (1981). Trapping was terminated as early as 31 August (1989) or as late as 12 November (1982). Number of traps installed each year ranged from 6 (1982) to 58 (1989) and averaged 16.7 traps per year.

Heat units (degree days) for each day of each year were calculated according to the sine curve method of Fry (1983) and Snyder (1985). Upper/lower temperature thresholds were 30.0/12.8°C (86/55°F) (Brown et al. 1990, Silvertooth 1989). Degree days were accumulated from 1 January to 12 November each year. Daily mean degree days and the mean accumulative degree days from 1981 to 1989 and mean nightly and cumulative mean male moth catches per gossyplure-baited trap per night were calculated. Data were subjected to correlation and regression analyses to determine relationships between nightly and cumulative male moth catches/trap/night and degree days and cumulative degree days. Logarithmic transformations of the data were also explored as a method of improving data fit relationships.

RESULTS AND DISCUSSION

The earliest date PBW moths were caught in gossyplure-baited traps was 28 March in 1986 (Fig. 1). In all other years first trap catches occurred between 30 March and 4 May. Trap catches each year through late June reflected, for the most part, low overwintering moth emergence. However, a small percentage of the late June moth catches could be a result of the F_1 generation development. Mean moth catches increased exponentially thereafter, as a result of reproducing populations in the current year's crop. Peak catches occurred in early September. Trap catches decreased exponentially thereafter, to the end of the trapping period in November. Mean daily degree days and the cumulative degree days for the years 1981 to 1989 from 1 January to 12 November in the Imperial Valley, California are shown in Fig. 2. Numbers of mean daily degree days increased to about mid-July, remained fairly constant through August, and decreased thereafter to 12 November. Cumulative mean day degrees increased from January through 12 November.

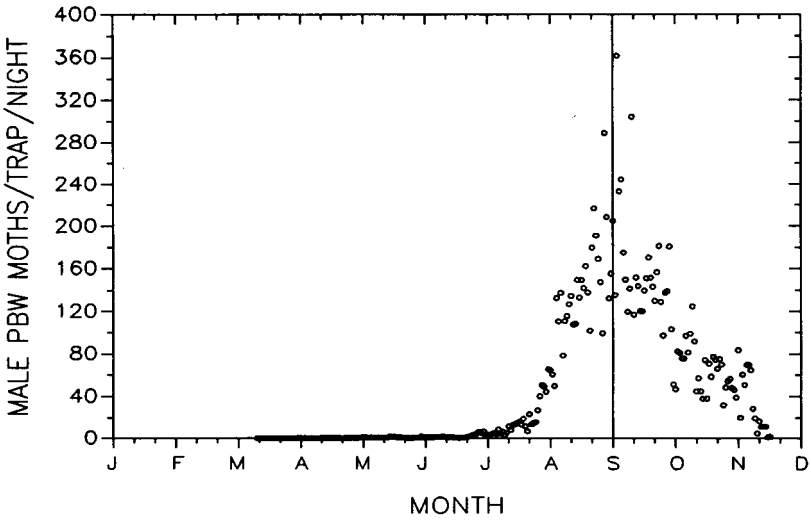

FIG. 1. Mean numbers of male pink bollworm moths caught/trap/night in gossyplure-baited traps from 1981 to 1989 in the Imperial Valley, CA.

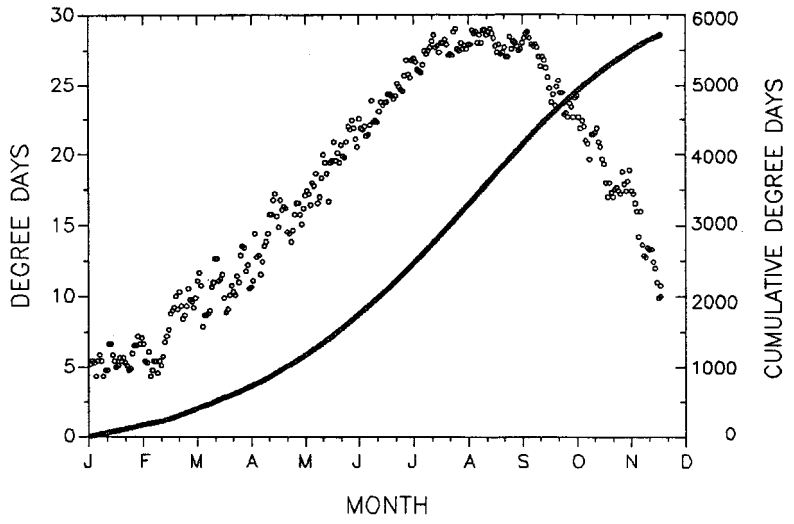

FIG. 2. Mean daily number of degree days and cumulative mean daily number of degree days from 1981 to 1989 in the Imperial Valley, CA.

Except for 1981, correlation coefficients for moth catches/trap/night versus cumulative degree days were higher than for moth catches/trap/night versus daily degree days (Table 1). These results agree with the reports of Brown et al. (1990). However, in our studies the highest correlation coefficient of 0.648 in 1987, although

TABLE 1. Correlation Coefficients for Numbers of Pink Bollworm Male Moths Caught in Gossyplure-Baited Traps in Relation to the Average Numbers of Degree Days[a] Day from 1981 to 1989 at Brawley, California.

Year	Days (No.)	Trapping period	Correlation Coefficients[b]		
			Moth catches vs. degree days	Moth catches vs. cumulative degree days	Cumulative moth catches vs. cumulative degree days
1981	162	5/13 - 10/21	0.445	0.201	0.956
1982	247	3/09 - 11/12	0.221	0.441	0.862
1983	223	3/25 - 11/02	0.282	0.631	0.864
1984	195	4/18 - 10/29	0.311	0.386	0.898
1985	193	4/09 - 10/18	0.197	0.597	0.897
1986	183	3/28 - 9/26	0.307	0.501	0.813
1987	148	4/07 - 9/01	0.510	0.648	0.817
1988	156	4/12 - 9.14	0.328	0.601	0.795
1989	164	3/21 - 8.31	0.327	0.644	0.712

[a] Based on 30.0/12.8°C (86/55°F) for upper/lower thresholds, respectively.
[b] Each correlation coefficient is significant at $P \leq 0.01$.

statistically significant, was not particularly high. Cumulative male moth trap catches/trap/night versus cumulative degree days ranged from r = 0.712 to 0.956. In contrast, correlation coefficients for moth trap catches/trap/night versus degree days ranged from r = 0.197 to 0.510. Exponential relationships are typical of populations of biological organisms and are described by the equation: $Y = AB^X$ (equation 1) where A and B are constants, and X is the independent variable, in this case, affecting PBW male moth trap catches (Y).

The logarithmic (base 10) transformation of equation 1 to express the data linearly is: Log Y = Log A + X Log B (equation 2). Logarithmic transformations of the mean number of male PBW moths/trap/night from early March to 31 August for each year from 1981 to 1989 in relation to cumulative degree days are shown in Fig. 3. Moth trap catch data on each sample date for each year were widely scattered. Thus, considerable variation may be expected in attempts to predict seasonal moth trap catches using cumulative degree days. The source of the variation may be a combination of overwintering diapause populations of different magnitude, weather variables, control measures taken during the cotton season, migration of moths from one area to the next, and other factors affecting population development during the season.

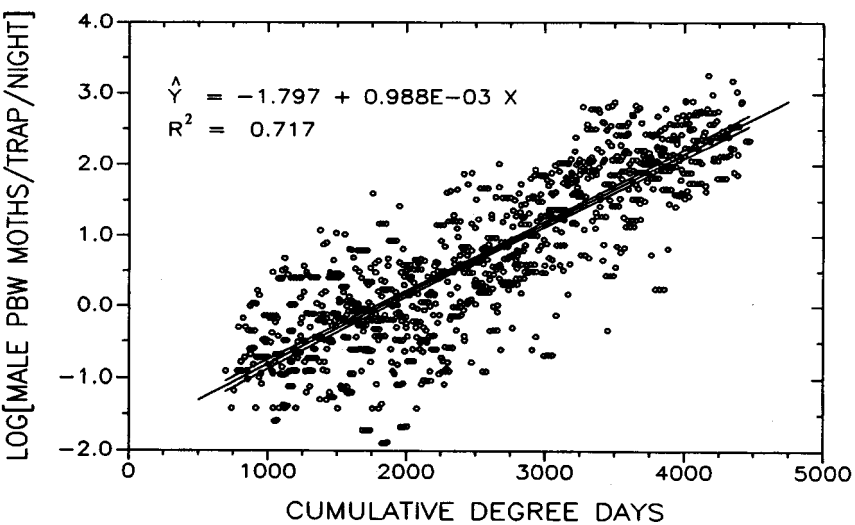

FIG. 3. Logarithms of the numbers of male pink bollworm moths caught/trap/night in relationship to the cumulative numbers of degree days during 1981 to 1989 in the Imperial Valley, CA.

Decreasing trap catches from 1 September to 12 November were described by the equation $\hat{Y}$ = -3.766 -0.398E-03 X, where $\hat{Y}$ was Log_{10} [male moths/trap/ night] and X was the number of accumulated degree days. The data fit was poor (R^2 = 0.114) probably because of decreasing temperatures beginning in September. Lingren et al. (1989) found that maximum and minimum temperatures for PBW male moth catches were 30.3 and 12.3°C, respectively, which appear to support this interpretation.

Each year of the study, cumulative moth catches/trap/night increased exponentially from late March to 31 August. The coefficient of determination for the relationship between logarithmic transformations of cumulative moth trap catches/trap/night and cumulative degree days, was 0.882 (Fig. 4), and provided the best fit for the data but the calculations were more complex since a second logarithmic transformation was involved.

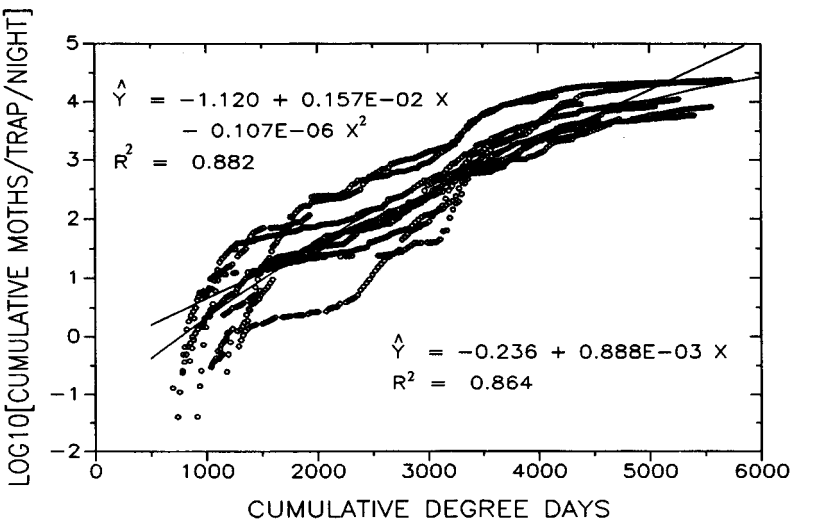

FIG. 4. Logarithms of the cumulative numbers of male pink bollworm moth catches/trap/night in relationship to the cumulative numbers of degree days from the first trapping day to 31 August from 1981 to 1989 in the Imperial Valley, CA.

The models developed using logarithm transformation of male moth catches/trap/night and logarithm transformation of cumulative male moth catches/trap in relation to cumulative degree days were developed with data obtained in 1982, 1983, 1985, 1987, and 1989. Validations of the models were attempted using data obtained in 1981, 1984, 1986, and 1988. The coefficients of determination (R^2) for linear and quadratic data fits for each year ranged from 0.743 to 0.872 using logarithmic transformations of moth catches/trap/night and 0.958 to 0.984 using logarithmic transformations of cumulative moth catches/trap/night to predict moth trap catches (Table 2). However, the regression coefficients (STRUCTR structural relations statistical program, Version 1.1, 1986, written by G. E. Dallal)[1] for each of the four

[1] B. E. Mackey (personal communication), ARS Statistical Consultant, Albany, CA. Rationale of the program was based on a theory of structural relation models, among others, in M. G. Kendall and A. Stuart, The Advanced Theory of Statistics Vol. 2, 3rd ed., 1973, New York, Hafner Pub. Co.

years for comparing observed vs. predicted values indicate that the model predictions produced regression slopes that deviated significantly from 1.000 in two of the four

TABLE 2. Validation of Derived Pink Bollworm Male Moth Catch Degree Day Models Using Male Moth Catches in 1981, 1984, 1986, and 1988.

Function	1981	1984	1986	1988
Coefficients of determination (R^2)				
	Model - Log_{10} [moths/trap/night]			
Linear	0.806	0.872	0.747	0.853
Quadratic	0.781	0.872	0.773	0.864
	Model - Log_{10} [cumulative moths/trap/night]			
Linear	0.966	0.958	0.984	0.964
Quadratic	0.970	0.960	0.980	0.958
Slopes (regression coefficients)[a]				
	Model - Log_{10} [moths/trap/night]			
Linear	1.082	1.348	1.063	1.595
Quadratic	0.941	1.226	0.996	1.567
	Model - Log_{10} [cumulative moths/trap/night]			
Linear	1.034	1.190	0.844	1.373
Quadratic	1.060	1.209	0.851	1.374

[a] The prediction is biased if the slope deviates significantly from 1.000.

years. This occurred in 1984 and 1988 using Log_{10} [moths/trap/night] transformations and cumulative degree days; and also in 1984, 1986, and 1988 using Log_{10} [cumulative moths/trap/night] and Log_{10} cumulative degree day transformations. Therefore, for practical purposes, the linear regression $\hat{Y}$ = -1.797 + 0.998E-03 X (Fig. 3), where X is the cumulative degree days and $\hat{Y}$ is Log_{10} [male moths/trap/night] caught, is just as useful for describing increasing male PBW moth catches from 28 March to 31 August with less data transformation. In any case, the variation in numbers of moths caught each year may be high, and predictions, as in our studies, accurate only 50% of the time.

Using the average values for the nine years' data (Fig. 3), our results suggest that for each increase of 10- and 100-degree days, trap catches increased 2% and 25%, respectively. An increase of 1000-degree days resulted in a 10-fold increase in PBW moth trap catches. For example, the equation estimated one PBW moth/trap/night

at an accumulated 1820-degree days from 1 January and 142 moths/trap/ night at 4000 accumulated degree days. Trapping data for nine years from 1981 to 1989 show that these accumulated degree days occurred from 25 May (1989) to 8 June (1983) and from 15 (1986) to 29 (1983) August, respectively. Actual moth trap catches on the average were 0.51 to 1.41 during 25 May to 8 June, and 162.21 to 135.39 during 15 to 29 August.

Results of these studies demonstrate the potential use of male moth trap catch data and degree days to describe increasing PBW male moth trap catches in pheromone-baited traps as an indicator of seasonal population increase. With refinement and development of the relationship between trap catches and fruiting form infestations, it may be possible to establish economic thresholds determining the need for control action. The extreme variability in the magnitude of moth trap catches between years must be considered. Variability is associated with the size of the overwintering PBW population, cotton crop management and cultural practices, initiation of the infestation in the crop, influence of weather variables, and the efficiency of control actions taken during any given growing season. However, increasing male moth catches during the season were highly correlated to day degree accumulations. Moth trap catch data and degree days may be a useful tool to identify cotton growing areas with the potential for developing economic infestation levels. Additional studies need to be conducted to verify the potential implementation and effectiveness of this approach in PBW management systems.

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The authors appreciate the help of Mr. Richard Y. Reynoso for his assistance in the collection of PBW male moth trap catch data, and Mr. Raymond Valtierra for degree day calculations. Dr. Bruce E. Mackey is also acknowledged for his assistance in the evaluation of statistical models. Partial financial support for this study was provided by the Imperial Valley Conservation Research Center Committee, Brawley, CA.

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EFFECT OF PLANTING DATE ON COTTON APHID[1] AND BANDEDWINGED WHITEFLY[2] POPULATIONS IN DRYLAND COTTON

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ABSTRACT

The influence of planting date on abundance of the cotton aphid, *Aphis gossypii* Glover, and the bandedwinged whitefly, *Trialeurodes abutilonea* (Haldeman), was studied in dryland cotton in the Texas Rolling Plains. Cotton was planted in late April, late May and late June in 1988 and 1989. Aphid and whitefly counts were taken from upper and lower leaves in untreated and insecticide-treated cotton. Percent leaf moisture and percent leaf nitrogen were determined in all treatment combinations in 1989. Cotton aphid numbers were highest in cotton that was planted in late June both years; and numbers were greater on lower leaves. High populations did not develop in treated cotton. Aphid populations increased rapidly during August each year only in June-planted cotton, which suggests that time of year interacts with plant age to influence population development. Highest numbers of bandedwinged whiteflies were found on upper leaves in cotton planted in late April and late May. Planting date affected leaf nitrogen and leaf moisture content. Nitrogen and moisture content were highest in August in the youngest cotton planted in late June. Aphids and whiteflies also reached peak abundance in August. Aphid numbers were positively associated with leaf nitrogen and leaf moisture, but the association was negative for whiteflies. There was a significant individual correlation between aphid numbers and leaf moisture content; and when leaf position was considered, there was a significant multiple correlation between aphid numbers and leaf nitrogen content. These results indicate that planting date can be used to manage cotton aphid and bandedwinged whitefly populations. An optimum planting time from mid-May to mid-June can be utilized in the Texas Rolling Plains to reduce problems imposed by the cotton aphid and the bandedwinged whitefly, as well as by the boll weevil, *Anthonomus grandis grandis* Boheman.

INTRODUCTION

The cotton aphid, *Aphis gossypii* Glover, has been present in dryland cotton in the Texas Rolling Plains for many years. Occurrence has been sporadic, and distribution has been localized in some years to widespread in other years; and very high numbers occurred throughout the production region in 1991 (Boring 1975 - 1991). Several species of whitefly occur on cotton in the U. S., but we have detected only the bandedwinged whitefly, *Trialeurodes abutilonea* (Haldeman), and this insect has not

[1]Homoptera: Aphididae

[2]Homoptera: Aleyrodidae

reached pest status in the Rolling Plains. A primary concern associated with infestations of these two pests is sticky cotton caused by honeydew excretions onto cotton lint, which interferes with manufacturing at the textile mills (Carter 1990). Another problem is yield reduction. Infestation levels of about 100 aphids per leaf have caused significant lint reductions in dryland cotton in the Rolling Plains (Price et al. 1983).

Aphid fecundity is known to be related to both total and soluble nitrogen (Klingauf 1987); and reproductive potential of the cotton aphid is affected by nitrogen availability and soil moisture, a deficiency of either reduces reproduction (Isely 1946). Slosser et al. (1989) suggested that age of plants was associated with population increase during August, with highest population levels being attained on the youngest plants. Whitefly abundance in cotton may be influenced by plant age, and it has been reported that whiteflies are most abundant in late summer when bolls are open (IPM Manual Group 1984).

As cotton plants mature and set bolls, nitrogen reserves in cotton leaves are depleted (Radin and Mauney 1986). Thus, the nitrogen balance in leaves is changing as the plant grows and matures. Planting cotton on different dates during the spring, while utilizing the same nitrogen fertility level for each planting date, provides plants of different physiological age with similar leaf nitrogen status at corresponding phenological stages of plant development during the summer. Objectives of this report are to (1) discuss the influence of planting date on the abundance of cotton aphids and bandedwinged whiteflies, (2) evaluate the effect of plant age on timing of cotton aphid population increase, and (3) relate leaf nitrogen and leaf moisture levels, as influenced by plant age, to population densities of these two pests.

MATERIALS AND METHODS

This study was conducted at the Texas Agricultural Experiment Station at Chillicothe, Texas in 1988 and 1989. In both years the cotton variety 'Paymaster 145' was grown under dryland conditions in rows spaced 40 in. apart. Normal cultural practices for dryland cotton were followed, and fertilizer (30-13-0, lbs/acre of N-P-K, respectively) was applied just prior to each planting. Furrow dikes to retain rainfall were installed in all plots during early July each year.

A split-plot experiment with four replications was employed in both years with three planting dates as whole plots and two insecticide treatments as subplots. Each subplot was 8 rows wide and 75 feet long. The three planting dates were late April (28 April 1988 and 21 April 1989), late May (23 May 1988 and 24 May 1989) and late June (22 June 1988 and 22 June 1989). The two insecticide treatment subplots within each planting date were no insecticide treatment (untreated check) or treated with an appropriate insecticide for any cotton pest that attained the damage threshold, as defined by Texas Agricultural Extension Service guidelines (Leser et al. 1986).

Aldicarb was applied at the rate of 0.3 lb. [AI]/acre at planting in all three planting dates each year, but only in the insecticide-treated plots. The western flower thrips, *Frankliniella occidentalis* (Pergande), and cotton aphid did not attain treatment thresholds in treated plots. Foliar treatments were applied in treated plots for control of boll weevils, *Anthonomus grandis grandis* Boheman, bollworms, *Helicoverpa zea* (Boddie) and cotton fleahoppers, *Pseudatomoscelis seriatus* (Reuter). Insecticides selected were azinphosmethyl at 0.25 lb. [AI]/acre for boll weevils, cypermethrin at 0.1 lb. [AI]/acre for bollworms, and dicrotophos at 0.2 lb. [AI]/acre for cotton fleahoppers. These materials were applied with a tractor-mounted sprayer that delivered about 10 gal. aqueous solution per acre through one nozzle per row.

In 1988, the following materials were applied to treated plots in each planting date. In the 28 April planting, azinphosmethyl was applied five times from 22 June to 29 July; in the 23 May planting azinphosmethyl was applied three times from 29 July to 10 August; and in the 22 June planting, one application of cypermethrin and two applications of azinphosmethyl were made between 10 - 31 August. In 1989, the following materials were applied. In the 21 April planting, azinphosmethyl was applied four times from 19 June to 13 July; in the 24 May planting two applications of azinphosmethyl and one application of dicrotophos were made between 5 July - 10 August; and in the 22 June planting four applications of azinphosmethyl were made between 10 - 29 August.

In 1988 cotton aphid density was determined on six dates at weekly intervals from 22 July to 25 August. Samples for bandedwinged whiteflies were taken on three dates at weekly intervals between 12 - 26 August. In 1989 cotton aphids were sampled 15 times at weekly intervals from 1 June to 5 September in the 21 April and 24 May planting dates, and on 10 dates from 5 July to 5 September in the 22 June planting date. Bandedwinged whiteflies were sampled on eight dates at weekly intervals from 28 July to 19 September. Five leaves from the top half and five leaves from the lower half of plants from each plot were examined visually for aphid and whitefly numbers. A leaf was picked every three to five steps down the selected row.

Ten top half and ten lower half leaves were picked in each plot for leaf nitrogen and leaf moisture content analyses. A leaf was picked every three to five steps down the selected row. Leaves from each plot were stored in individually identified plastic bags and placed on ice for transport. In the laboratory, samples were stored in a -15°F chest freezer. For moisture analysis, leaves from each bag were weighed, then dried at 122°F for 72 hours, cooled in a desiccator, and weighed. Moisture content (%) was calculated by difference. Samples were then ground to pass a 0.04 inch screen in a bench top Wiley mill. Subsequently, samples were analyzed for dry matter, organic matter, ash and Kjeldahl nitrogen by AOAC (1980) procedures.

Data were analyzed by analysis of variance for a split plot experiment, and means were separated using LSD ($\alpha = 0.05$). Analyses were performed using the FACTOR and RANGE programs of MSTAT 4.0 (MSTAT Development Team 1985). The analysis was combined over years for the aphid data since a similar test was conducted both years, but the leaf nitrogen and moisture data were from a single year. Whitefly data were analyzed separately for each year because of unequal number of samples between years. In the analyses, planting dates were treated as whole plots, insecticide treatments as subplots, and leaf location (top and bottom half of the plant) as sub subplots.

Individual and multiple correlation analyses were performed using Microstat 4.1 (Ecosoft, Inc. 1984) to determine if aphid and whitefly counts were related to leaf nitrogen, leaf moisture, and leaf position on the plant. For these correlations, there were three planting dates and two leaf positions on the plant ($n = 6$). The data were averaged over the counts taken from late July to late August in untreated cotton during 1989.

Data for cotton aphids were averaged over six sampling dates common to all three planting dates from 22 July to 25 August 1988 and from 27 July to 29 August 1989. Data for bandedwinged whitefly were averaged over three sampling dates from 12 to 26 August 1988 and over eight sampling dates from 28 July to 19 September 1989.

RESULTS AND DISCUSSION

Cotton Aphid. Planting date had a significant affect on the number of cotton aphids per leaf in 1988 and 1989 (Table 1). In both years the highest population levels

TABLE 1. Average Number of Cotton Aphids per Leaf[a/]: Year by Planting Date Interaction in Dryland Cotton. Chillicothe, TX.

Planting Date	Year[b/] 1988	1989	$\bar{X}$
Late April	0.6 a X	3.7 a X	2.2 X
Late May	1.1 a X	4.6 b X	2.8 X
Late June	17.2 b Y	11.2 a Y	14.2 Y
$\bar{X}$	6.3 a	6.5 a	

a/ Average of six sampling dates from late July to late August in both years.
b/ Values within a row followed by a different lowercase letter (a-b) and values within a column followed by a different uppercase letter (X-Y) are significantly different (LSD, $P \leq 0.05$).

occurred in cotton planted in late June. While there were no differences in aphid numbers between the late April and late May planting dates in either year, there were higher levels of aphids in the May planting in 1989 as compared to levels in 1988; and there were more aphids in the June planting in 1988 as compared to levels in 1989.

In untreated cotton during 1988, numbers of aphids (top and bottom leaves combined) remained below 5/leaf from mid-July to late August in the late April and late May planting dates; however, aphid numbers increased to about 93/leaf in the late June planting date (Fig. 1). In 1989 sampling was initiated 1 June to verify that aphid numbers were not high at any time during the growing season in cotton planted in late April and late May. Sampling was begun on 5 July in the late June planting date (Fig. 1). A peak population level of 70/leaf occurred in the late June planting on 22 August. Aphid numbers did not exceed 26/leaf in the late April or late May planting dates.

Aphid numbers were significantly reduced in insecticide treated plots as compared with numbers in untreated plots when averaged over the three planting dates (Table 2). Aphid numbers were statistically similar in plots planted in late April, but aphid numbers were significantly greater in untreated plots in both the late May and late June plantings. The year by insecticide treatment interaction was not significant ($F=0.35$; $df=1,18$; $P>0.05$), which implies that insecticide treatments effectively reduced aphids in both years. The reduction in aphids is most likely attributable to the foliar applications of azinphosmethyl and cypermethrin.

Cotton aphid abundance was affected by leaf location on the plant (Table 3). Aphid numbers were significantly greater on bottom leaves, as compared with numbers on top leaves, in cotton planted in late June. While aphid numbers were numerically

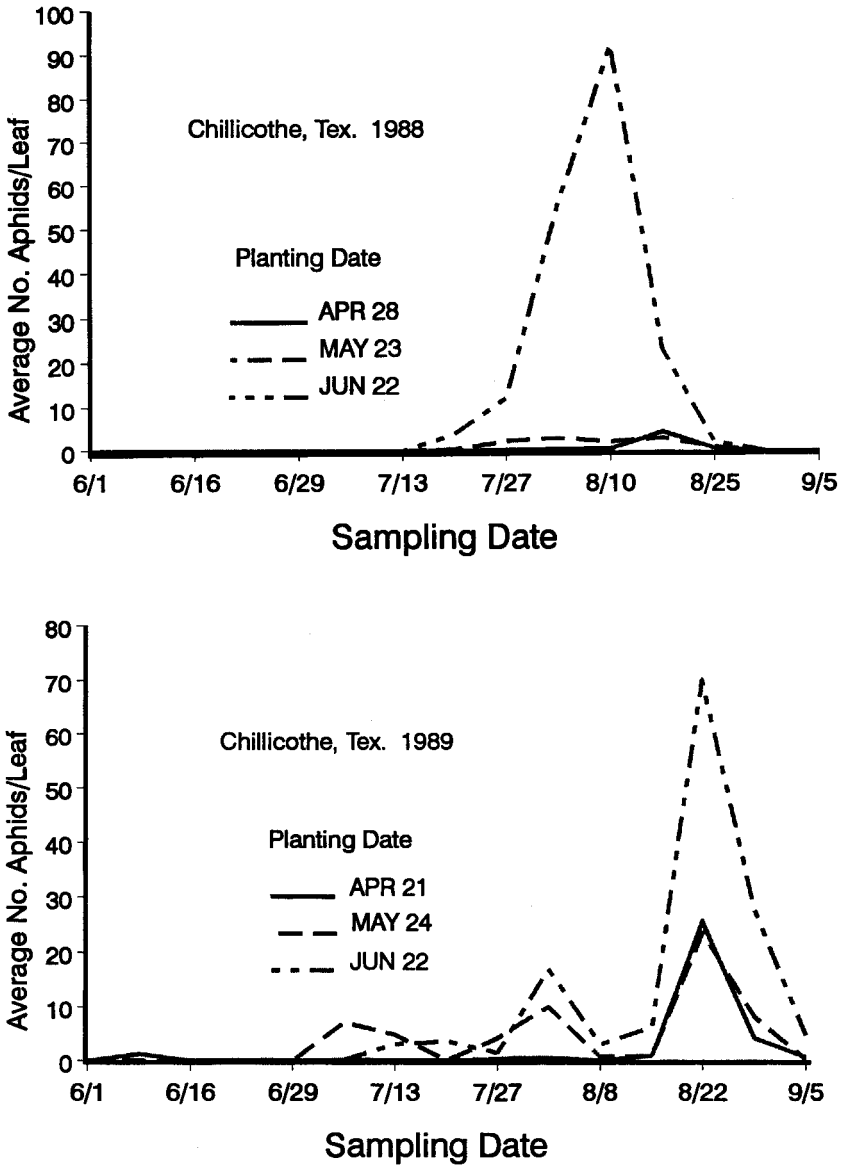

FIG. 1. Average number of cotton aphids per leaf in three planting dates in dryland cotton.

TABLE 2. Average Number of Cotton Aphids per Leaf: Insecticide Treatment by Planting Date Interaction in Dryland Cotton. Chillicothe, TX. 1988-89.

Planting Date	Insecticide Treatment[a/]		$\bar{X}$[a/]
	Treated	Untreated	
Late April	1.3 a	3.0 a	2.2 X
Late May	0.6 a	5.0 b	2.8 X
Late June	2.2 a	26.3 b	14.2 Y
$\bar{X}$[a/]	1.4 a	11.4 b	

[a/] Values within rows followed by a different lowercase letter (a-b) are significantly different, and values within columns followed by a different uppercase letter (X-Y) are significantly different (LSD, $P \leq 0.05$).

TABLE 3. Average Number of Cotton Aphids per Leaf: Planting Date by Leaf Location Interaction in Dryland Cotton. Chillicothe, TX. 1988-89.

Planting Date	Leaf Location on Plant[a/]		$\bar{X}$[a/]
	Top	Bottom	
Late April	1.5 a	2.8 a	2.2 X
Late May	2.0 a	3.6 a	2.8 X
Late June	8.5 a	19.9 b	14.2 Y
$\bar{X}$	4.0 a	8.8 b	

[a/] Values within rows followed by a different lowercase letter (a-b) are significantly different, and values within columns followed by a different uppercase letter (X-Y) are significantly different (LSD, $P \leq 0.05$).

higher on bottom leaves than on top leaves in both the late April and late May cotton, the values were not significantly different. The year by leaf location interaction was not significant ($F = 0.04$; $df = 1,36$; $P > 0.05$) which indicates that populations were influenced similarly by leaf position both years.

These data indicate that cotton aphid abundance in the Texas Rolling Plains is influenced by planting date and by leaf location on the plant. Aphid numbers were greatest in cotton that was planted in late June both years; and numbers were greater on bottom-half leaves in cotton planted in late June. Populations increased rapidly during August, which indicates that time-of-year influenced population development. Both preblooming and peak blooming cotton were available as hosts from late June

through July in cotton plots planted in late April and late May, yet high aphid populations failed to develop during June and July. Therefore, it appears that time-of-year interacts with plant age to influence population development. In this respect, Wellings et al. (1980) reported that in some aphids the numbers of ovarioles was genetically related to season (time-of-year) while the actual number of offspring was regulated by food quality; thus, rapidity of population increase and ultimate density may be determined by season and nutrition. Our observations on the consistency of the timing of cotton aphid outbreaks during August on young cotton supports their hypothesis.

Bandedwinged Whitefly. Whitefly population levels were significantly influenced by planting date in 1988 and 1989 (Table 4). In both years the highest

TABLE 4. Average Number of Whitefly Nymphs per Leaf in Relation to Planting Date and Leaf Location on the Cotton Plant. Chillicothe, TX.

Planting Date	Year and Leaf Location					
	1988[a,c]			1989[b,c]		
	Top	Bottom	$\bar{X}$	Top	Bottom	$\bar{X}$
Late April	5.3 b	0.5 a	2.9 Y	0.5	0.3	0.4 Y
Late May	5.2 b	1.1 a	3.1 Y	0.4	0.3	0.3 Y
Late June	0.7 a	1.4 a	1.0 X	0.0	0.1	0.1 X
$\bar{X}$	3.7 b	1.0 a		0.3 a	0.2 a	

a/ Average of 3 sampling dates from 12 August to 26 August.
b/ Average of 8 sampling dates from 28 July to 19 September.
c/ Values within a row, within a year, followed by a different lowercase letter (a-b) and values within a column followed by a different uppercase letter (X-Y) are significantly different (LSD, $P \leq 0.05$).

levels were recorded in cotton planted in late April and late May, and the lowest levels occurred in the late June planting. Population levels were influenced by leaf location in 1988, with significantly higher numbers recorded on top-half leaves as compared with numbers on bottom-half leaves. There were no significant differences in whitefly numbers on top and bottom leaves in 1989, but more were recorded on top leaves. In 1989 whitefly numbers peaked at 1.8/leaf on 5 September in the late April planting date, and 1.5/leaf on 29 August in the late May planting date. Whiteflies did not exceed 0.2/leaf from late July to mid-September in the late June planting date.

Whitefly numbers were significantly reduced by the insecticide treatments in 1988 ($F=65.74$; $df=1,9$; $P<0.05$), with an average of 3.9/leaf in untreated plots (averaged over all three planting dates) and 0.8/leaf in treated plots. The insecticide treatments did not affect abundance in the 1989 test ($F=0.93$; $df=1,9$; $P>0.05$); there was an average of 0.3 whiteflies/leaf in untreated plots and 0.2/leaf in treated plots.

The population levels experienced in 1988 and 1989 were below treatment threshold levels, and no insecticides were applied for control of whiteflies. However, this pest was noticeably present, and the data suggest that whitefly populations were

influenced by date-of-planting and by leaf location on the plant. Greatest numbers were found on upper leaves in cotton planted in late April and late May.

Leaf Nitrogen and Moisture. Percent leaf nitrogen from late July to late August was influenced by planting date and by leaf location on the plant (Table 5).

TABLE 5. Average Percent Nitrogen and Moisture in Cotton Leaves: Planting Date by Leaf Location Interaction in Dryland Cotton. Chillicothe, TX. 1989.

Planting Date	Leaf Location on Plant[a/b/] Top	Bottom	$\bar{X}$[a/]
	%N		
Late April	4.3	3.9	4.1 B
Late May	4.4	3.9	4.1 B
Late June	5.1	4.5	4.8 A
$\bar{X}$	4.6 a	4.1 b	
	% Moisture		
Late April	64.8	66.8	65.8 C
Late May	67.7	70.4	69.0 B
Late June	74.8	78.4	76.6 A
$\bar{X}$	69.1 b	71.9 a	

[a/] Values within rows followed by a different lowercase letter (a-b) are significantly different, and values within columns followed by a different uppercase letter (A-C) are significantly different (LSD, $P \leq 0.05$).

[b/] Average values of six sampling dates from late July to late August.

Nitrogen content was significantly higher in cotton planted in late June, the youngest cotton, as compared with nitrogen content in older cotton planted in late April and May, which had statistically equivalent nitrogen contents. Percent nitrogen content was significantly higher in the younger top leaves as compared with content in older bottom leaves. The planting date by leaf location interaction was not significant ($F=2.10$; $df=2,18$; $P>0.05$).

Percent leaf moisture content was affected by planting date and by leaf location on the plant (Table 5). Cotton planted in late June had the highest leaf moisture content, while cotton planted in late April had the lowest leaf moisture content. Moisture content was significantly higher in bottom leaves than in top leaves. The planting date by leaf location interaction was not significant ($F=3.52$; $df=2,18$; $P>0.05$).

Percent leaf nitrogen and percent leaf moisture were not influenced by insecticide treatment (Table 6), and values were statistically similar in treated and

TABLE 6. Average Percent Nitrogen and Moisture in Cotton Leaves: Insecticide Treatment by Planting Date Interaction in Dryland Cotton. Chillicothe, TX. 1989.

Planting Date	Insecticide Treatment[a/] Treated	Untreated	$\bar{X}$[a/]
	% N		
Late April	4.1	4.1	4.1 B
Late May	4.2	4.1	4.1 B
Late June	4.8	4.8	4.8 A
$\bar{X}$	4.4 a	4.3 a	
	% Moisture		
Late April	65.9	65.7	65.8 C
Late May	69.1	69.0	69.0 B
Late June	76.6	76.6	76.6 A
$\bar{X}$	70.5 a	70.4 a	

[a/] Values within rows followed by the same lowercase letter (a) are not significantly different, and values within a column followed by the same uppercase letter (A-C) are not significantly different (LSD, $P>0.05$).

untreated plots. Also, the planting date by insecticide treatment interaction was not significant (for % N, $F=0.02$; $df=2,9$; $P>0.05$; and for % moisture, $F=0.02$; $df=2,9$; $P>0.05$). Aphids were present in high numbers only in the untreated plots, which suggests that aphid feeding damage did not influence nitrogen or moisture levels in the cotton leaves. However, aphid feeding is known to alter both carbohydrate and nitrogen levels in some plants (Klingauf 1987).

Leaf nitrogen levels from late July to late August were consistently higher in cotton planted late June as compared with levels in the other two planting dates (Fig. 2; values are averages of top plus bottom leaves and of treated plus untreated cotton). Percent leaf nitrogen values averaged 4.7% during August in the late June planting while levels averaged 4.1% in the late April and late May planting dates. Leaf moisture content was higher in the late June cotton during August as compared with moisture content in the two older plantings (Fig. 2). Moisture content averaged 75.4% in the late June planting during August while moisture content averaged 66.8% in the two older planting dates. The cotton which supported the highest levels of cotton aphids during August was that planted in late June (Fig. 1), and this planting date had the highest level of leaf nitrogen content and the highest leaf moisture content during August (Fig. 2). Nitrogen and moisture content during August were a function of plant age, as it affects physiological activity of leaf tissue.

There was a significant positive correlation between aphid numbers and leaf moisture content; and when leaf position was considered, there was a significant

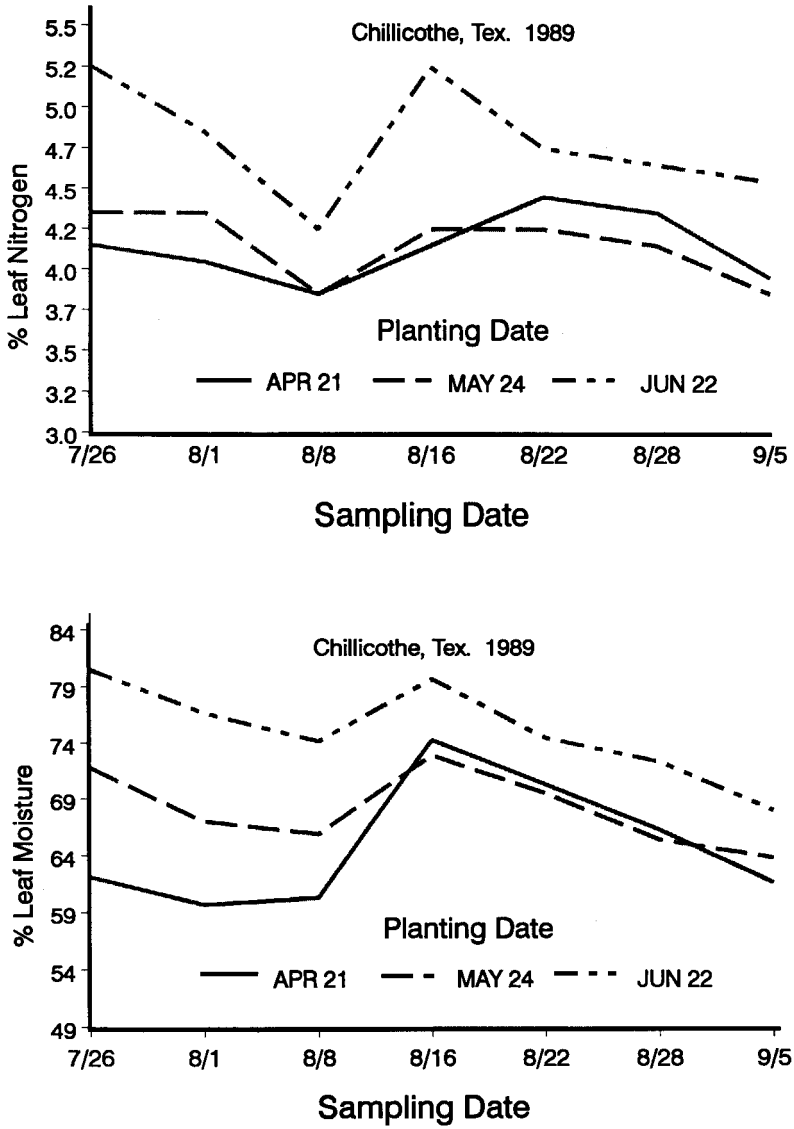

FIG. 2. Average percent leaf nitrogen (top) and average percent leaf moisture (bottom) in three planting dates in dryland cotton.

multiple correlation between aphid numbers and leaf nitrogen content (Table 7).

TABLE 7. Individual and Multiple Correlation Coefficients Between Average Number of Aphids or Whiteflies per Leaf and % Nitrogen (X_1), % Leaf Moisture (X_2), and Leaf Position (X_3) in Untreated Cotton, Chillicothe, TX. 1989.

Dependent Variable	Individual r[a/,b/]			Multiple r[a/]		
	X_1	X_2	X_3	X_1 X_2	X_1 X_3	X_2 X_3
Aphids/leaf	0.28	0.91*	0.52	0.95*	0.93*	0.95*
Whiteflies/leaf	-0.45	-0.80	-0.33	0.80	0.89	0.81

[a/] An * indicates a significant correlation with dependent variable. For a significant two-tailed test ($\alpha=0.05$), $r \geq 0.81$ and multiple $r \geq 0.93$.

[b/] Leaf position coded as 1 = top and 2 = bottom leaf for correlation analyses.

There was a negative association between whitefly counts and leaf nitrogen content and leaf moisture content. These analyses indicate that aphid populations develop better on young plants with high moisture content, but bandedwinged whiteflies do better on mature plants with a low moisture content. Aphids, but not whiteflies, developed better on plants with a high nitrogen content.

There are other factors that may influence aphid distribution on the plant in addition to nitrogen and moisture content. Shading of lower leaves by upper canopy leaves may favor aphid populations in the lower half of the plant. Also, aphid numbers may be higher on lower leaves during August because that is where populations developed first. As plants grow, a leaf that was initially a top-half leaf becomes a bottom-half leaf, so aphid numbers have had a longer time to increase on what became classified, in August, as the lower portion of the plant.

Date of planting affected both leaf nitrogen and leaf moisture content during August, the time period when cotton aphids and bandedwinged whiteflies reached peak population density. Leaf nitrogen and leaf moisture content were highest in the youngest cotton planted in late June, and cotton aphids reached their highest levels on this young cotton, particularly on leaves on the lower half of the plant. An opposite effect was recorded for bandedwinged whiteflies. Whitefly numbers were highest on upper leaves on older cotton planted in late April and late May; and cotton in these planting dates had the lowest leaf nitrogen and leaf moisture content.

These results indicate that planting date can be utilized as an effective cultural management strategy to reduce population density of aphids and whiteflies. However, choice of planting dates is dependent upon which of these two pests is most serious in a given production region. In the Texas Rolling Plains, our studies indicate that late planted cotton, that planted after 10-15 June, should be avoided to reduce aphid problems. Additionally, cotton should not be planted from late April to mid-May to avoid problems with the boll weevil. Delayed uniform planting, defined as not planting until after mid-May, has been a recommended boll weevil management strategy for many years in the Rolling Plains (Masud et al. 1985). Avoidance of early cotton for boll weevil management also reduces the threat posed by the bandedwinged whitefly. Thus, experimental evidence indicates that there is an optimum planting

window from mid-May to mid-June in the Rolling Plains. Planting during this time frame reduces problems imposed by the boll weevil and the cotton aphid. This planting period eliminates squares as a food supply for overwintered boll weevils during June and reduces leaf moisture and leaf nitrogen, which are dietary requirements of the cotton aphid, during August.

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MITOCHONDRIAL DNA RESTRICTION FRAGMENT VARIATION AND BIOSYSTEMATICS OF THE BOLL WEEVIL, *Anthonomus grandis*[1]

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ABSTRACT

Mitochondrial DNA (mtDNA) restriction fragment patterns were compared among four laboratory colonies of the boll weevil, *Anthonomus grandis* Boheman. The four colonies have diverse geographic and host plant origins; they include a southeastern USA weevil from cultivated cotton, a Mexican weevil from *Hampea nutricia* Fryxell, a weevil from wild *Gossypium thurberi* Todaro cotton in Arizona, and a weevil from cultivated cotton in El Salvador. The boll weevil mtDNA is about 19 kb. Ten of sixteen restriction enzymes employed produced polymorphic fragment patterns. Comparisons of sequence divergence among the colonies suggests that the "thurberia" and El Salvador weevils are similar and that the southeastern weevil is equally divergent from the other three colonies. The observed mtDNA variation among these lines shows that this approach may be suitable for more detailed molecular genetic analysis of the origin and dispersal of the boll weevil.

INTRODUCTION

Mitochondrial DNA (mtDNA) has proven to be an extremely powerful tool for studying systematics and evolution (Moritz et al. 1987, Avise et al. 1987). The properties of mtDNA have been reviewed in detail (Avise 1986) and are briefly summarized here. Animal mtDNA is normally a circular DNA molecule of 15-20 kilobases (kb). Its small size and sequestration outside the nucleus make it possible to separate it from nuclear DNA. The molecule seems to evolve sufficiently rapidly with respect to its primary nucleotide sequence that it often gives rise to extensive sequence heterogeneity among individuals or populations of the same species. The evolutionary rate of mtDNA in vertebrates appears to be substantially faster than that for single copy nuclear sequences. This rate disparity was not found in some insects, but it seems the explanation

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lies in faster evolution of the nuclear sequences rather than slower evolution of the mtDNAs (Caccone and Powell 1990). The overall rate of evolution in mtDNA is sufficient to produce variation present in natural populations can be used to intraspecific variation. MtDNA is maternally inherited and therefore can be used to construct maternal lineages both within species and between closely related taxa. Studies of mtDNA variation in the bollweevil, *Anthonomus grandis* Boheman, could provide valuable information on the current levels of gene flow between different populations, especially between those infesting cotton and those living on native plants.

We report here restriction fragment patterns of mtDNA from representatives of four boll weevil populations, using 16 restriction endonucleases. The detection of restriction fragment pattern variation demonstrates the potential utility of this technique for studies of the biosystematics, evolution, and dispersal of the boll weevil. These techniques may also provide molecular genetic support for hypotheses regarding boll weevil evolutionary dispersal, based primarily on host plant distribution and slight morphological differences noted in several populations of weevils (Burke et al. 1986).

MATERIALS AND METHODS

Insects. MtDNA was prepared from the following boll weevil laboratory colonies. *Eb* is a homozygous ebony (recessive, dark body) mutant originally selected from a colony reared at the Delta States Area Boll Weevil Research Laboratory, Mississippi State (Bartlett 1967); cultivated cotton, *Gossypium hirsutum*, was the host plant. MX is a strain originally collected from the host plant, *Hampea nutricia*, in Tabasco, Mexico in 1981. SM is a "thurberia" strain originally collected from the wild cotton, *Gossypium thurberi*, in the San Molino mountains near Tucson, Arizona in 1984. ES is a strain collected from cultivated cotton, *G. hirsutum*, in El Salvador in 1984. The colonies were all originally established as isofemale lines. All of the weevil strains were reared on an artificial diet at the Biosciences Research Laboratory, Fargo, ND.

mtDNA Purification. Live adults or late instar larvae were homogenized in mitochondrial isolation medium (MIM: 0.5 M mannitol, 1.0 mM ethylenediaminetetraacetic acid, 0.01 M triethanolamine, pH 7.5) on ice. Typically 10-12 g of insects homogenized at one time at a concentration of 1.0-1.25 g per 25 ml of MIM. Differential centrifugation to recover intact mitochondria was as described by Roehrdanz and Johnson (1988) except that the original slow speed pellet was resuspended and centrifuged again at 700 X g. Supernatants from the two centrifugations were combined and used for continued processing. The pellets enriched for mitochondria were resuspended, lysed, and centrifuged in two rounds of cesium chloride gradients as previously described. The mtDNA band from the second cesium chloride gradient was collected, extracted with butanol to remove ethidium bromide, and desalted and concentrated in TE buffer (0.01 M Tris, pH 8, 0.0005 M EDTA) using a Centricon microconcentrator (Amicon, Danvers, MA).

Total DNA was purified from individual frozen (liquid nitrogen) adults using the Boyce et al. (1989) modification of

the protocol of Doyle and Doyle (1990). DNA samples were stored at -80°C or at 4°C until use.

Restriction Digests. Restriction endonucleases were obtained from Bethesda Research Laboratories (Gaithersburg, MD) and New England Biolabs (Beverly, MA); digestion conditions were those recommended by the suppliers. The restriction fragments were separated and observed using 0.7-1.5% agarose gels stained with ethidium bromide. Molecular size standards on the gels were provided by the 1 kilobase-pair ladder and the 123 base-pair ladder from Bethesda Research Laboratories. All the fragment sizes were determined using digests of mass-isolated boll weevil mtDNA. Digested total DNA from single insects was used to determine if a strain was carrying more than one mt genotype. That DNA was alkaline blotted to nylon membrane (Reed and Mann 1985), hybridized with 32-P labelled boll weevil mtDNA, and detected by autoradiography. Hybridization and wash conditions were as described by Boyce et al. (1989).

Fragments that co-migrated under one or more gel conditions were considered to be the same. The fraction of identical fragments was calculated for each pair combination of genotypes using the fromula $F = 2N_{xy}/(N_x + N_y)$, where N_{xy} is the number of fragments common to the two genotypes and N_x and N_y are the number of fragments for each member of the pair (Nei and Li 1979). Estimates of the nucleotide diversity, *p*, were made using Upholt's formula (1977).

RESULTS AND DISCUSSION

The restriction fragment patterns for the 16 endonucleases in the four boll weevil lines are shown in Table 1. Restriction patterns for the first six enzymes were common to all four lines; the remaining ten restriction enzymes yielded polymorphic fragment patterns among the lines. The enzymes used were either six base-pair cutters or four base-pair cutters (including interrupted four base-pair cutters). The greatest number of fragments in the detectable size range for a single enzyme was nine with *Fnu*4HI. The six-cutter restriction enzymes *Bam*HI, *Pvu*II, *Sma*I, *Mlu*I, *Apa*I, and *Sal*I did not cleave the *Eb* mtDNA and consequently were not tested on the remaining three lines. We estimate the boll weevil mtDNA to be about 19 kilobases (kb) based on the fragments generated. Slight variation in sum of the fragments for these enzymes may be due to the existence of additional small fragments (<0.5 kb) which do not usually show up on ethidium bromide stained agarose gels (e.g., fragments of 0.47 kb and 0.30 kb were visible in *Sau*96I digests if the sample had a lot of DNA), or to imprecise measurements of the larger fragments (>12 kb) which do not separate well under the gel conditions used here. The presence of two 1.3 kb fragments in the *Msp*I digests was inferred because that band was consistently brighter than the 1.4 kb band in the *Eb* pattern and the sum of the digest products was deficient by that amount. It was later confirmed in the process of constructing a restriction site map when a double digest using *Msp*I and *Bst*UI cleaved one of the two 1.3 kb fragments into two smaller fragments and left the other intact (Roehrdanz, unpublished data).

TABLE 1. Mitochondrial DNA Restriction Fragments in Boll Weevils

Enzyme	BW Line	Fragment size in kilobase pairs
*Eco*RI	All	10.2, 5.3, 3.7
*Hind*III	All	7.6, 6.3, 3.2, 0.9, 0.6, 0.47
*Eco*RV	All	12, 3.5, 2.3
*Xho*I	All	10.4, 8.7
*Bst*UI	All	18, 1.5
*Sst*I	All	10.5, 8.8
*Kpn*I	Eb, ES	19, 0.8
	SM, MX	No site
*Hha*I	SM, Eb	19+
	MX, ES	No site
*Hpa*I	Eb	13. 5.2, 0.5
	SM	8.9, 5.2, 4.8
	MX	8.9, 5.4, 4.8
	ES	8.9, 5.0, 4.8
*Xba*I	Eb	8.0, 5.9, 2.9, 1.7
	SM, ES	8.0, 3.2, 2.9, 1.9, 1.7, 0.9
	MX	8.0, 3.2, 2.9, 2.8, 1.7
*Fnu*4HI	Eb	4.5, 3.8, 3.4, 2.9, 1.2, 0.95, 0.8, 0.75
	SM	4.5, 3.8, 2.9, 2.1, 1.22, 1.2, 0.95, 0.8, 0.75
	MX	4.5, 3.8, 3.1, 2.1, 1.22, 1.2, 0.95, 0.8, 0.75
	ES	4.5, 3.8, 2.6, 2.1, 1.22, 1.2, 0.95, 0.8, 0.75
*Cla*I	Eb, MX	9.3, 4.9, 3.5
	SM, ES	14+, 3.5
*Bgl*II	Eb	11, 8.0
	SM, ES	8.8, 8.0, 2.1
	MX	9.0, 8.0, 2.1
*Msp*I	Eb	5.1, 2.8, 2.55, 2.0, 1.9, 1.4, 1.3, 1.3
	SM	7.3, 3.7, 2.75, 1.9, 1.3, 1.3
	ES	7.3, 3.7, 2.70, 1.9, 1.3, 1.3
	MX	11, 3.0, 1.9, 1.3, 1.3
*Sau*96I	Eb	7.4, 3.5, 3.0, 2.6, 1.0, 0.6
	SM, ES	6.1, 3.5, 2.6, 1.6, 1.4, 1.0, 0.6
	MX	8.5, 3.5, 3.0, 2.6, 1.0, 0.6
*Scr*FI	Eb	7.0, 6.8, 1.8, 1.4, 0.9, 0.8
	MX, ES, SM	7.0, 5.1, 1.8, 1.4, 1.3, 0.9, 0.8, 0.4

The size of the boll weevil mtDNA, ~19 kb, is somewhat larger than the typical 16 kb molecule found in a wide variety of animals, but it is neither unique nor the largest. Some *Drosophila* species have been reported with mtDNA of this size (Fauron and Wolstenholme 1976) and a number of organisms, including some other species of weevils, have mtDNAs considerably larger (Powers et al. 1986, Snyder et al. 1987, Boyce et al. 1989). Boyce et al.(1989) have used boll weevils obtained from a colony in Mississippi as an outgroup for their studies of several species of bark weevils and have reported the boll weevil mtDNA to be 18.2 kb. It is not known if this size discrepancy represents a real difference in the size of the mtDNA in the colony they used versus the collections reported here. They document size differences and individual heteroplasmy in three species of bark weevils (*Pissodes*). Similar size differences in the boll weevil would seem equally possible. They show a restriction site map for the boll weevil, but do not list the sizes of the fragments separately. However, the approximate fragment sizes obtained from their map for *Hin*dIII, *Eco*RI, and *Xba*I appear to be about the same as those shown here. No single region of the genome exhibited consistently smaller or larger fragments as might be expected since most major mtDNA size differences are localized to the control region. (Mapping data also puts these restriction sites in the same relative orientation, Roehrdanz, unpublished data). Thus the size difference between the two sets of data may be the cumulative result of small differences in measuring the size of the fragments rather than a real difference in DNA length.

For the enzymes that produced the inter-strain polymorphism, the total size of the fragments for the SM, MX and ES lines generally exceeded 19.2 kb, often by more than 10 kb, when mass-isolated mtDNA was used. When DNA from single insects was examined from each of the three lines using blotting and hybridization with labelled boll weevil mtDNA, two different mtDNA genotypes were found in each of the three lines. Individuals contained only one of two genotypes, one of which was the *Eb* genotype and the other was unique to the geographic collection. No heteroplasmy was observed. This suggests that one (or more) females carrying the *Eb* mtDNA genotype had infiltrated the other three at some point during their culture in the laboratory. This is not an unusual occurrence when a number of collections of the same species are maintained in one laboratory. The lack of visible genetic markers for the boll weevil makes it impossible to recognize colony cross contamination until it is revealed by the molecular data. The *Eb* marker itself is an autosomal recessive and not a reliable indicator of low levels of genetic exchange between colonies. A maternal mitochondrial line could easily become established in the absence of sufficient nuclear gene mixing for a recessive marker to be scored. A similar situation was reported in laboratory reared colonies of the screwworm fly (Roehrdanz 1989). The fact that assaying single insects permitted the distinction of the mtDNA genotypes underscores the advantage of following a molecular marker that does not undergo recombination and random assortment.

The fraction of fragments common to each pairwise combination of mt genotypes and an estimate of nucleotide sequence diversity are presented in Table 2. The sixteen

restriction enzymes assayed 59-63 fragments or cleavage sites comprising about 1.6% of the mt genome (based on the total size of the genome and the number of bases in the recognition sites that produced fragments). Fragments less than 0.5 kb were not used in the calculations for the reason stated above. The fraction of common fragments (above the diagonal) ranged from about 0.70 to almost 0.93 The sequence divergence (below the diagonal) varied from 0.49 to 2.58%.

The comparisons of the sequence divergence indicate that the southeastern mtDNA sample (*Eb*) is equally divergent from the other three types. The El Salvador (ES) and "thurberia" (SM) pair are very similar. The Mexican "hampea" (MX) mtDNA sample is closer to the ES-SM pair than it is to the southeastern weevil. The observed differences in restriction fragment patterns for these mt genotypes from four different

TABLE 2. Fraction of Common Fragments and Estimated Per Cent Sequence Divergence between BW Strains[a]

Strain	Eb	MX	SM	ES
Eb	--	0.722	0.713	0.699
MX	2.35	--	0.817	0.806
SM	2.36	1.42	--	0.929
ES	2.58	1.45	0.49	--

[a] Numbers above the diagonal represent the fraction of shared fragments. Numbers below the diagonal represent the estimated per cent sequence difference. See text for description of strains.

populations of boll weevils points up the potential of this approach in assisting studies of the biosystematics and reproductive biology of the boll weevil and its close relatives.

A hypothesis of the origin and subsequent dispersal of the boll weevil has been put forward (Burke et al. 1986). This scheme has relied primarily on morphological differences and host plant associations to identify subpopulations of the boll weevil as well as a possible sibling species, *Anthonomus hunteri* (Burke and Cate 1979). Some direct genetic parameters have also been included, e.g. chromosome number polymorphisms (North and Cate, unpublished data) and isozyme variation (Terranova and North 1984). Burke et al. (1986) proposed that the boll weevil originated in tropical southern Mexico east of the central highlands. They hypothesize two major routes of evolutionary dispersal, one up the east coast of Mexico that eventually produced the southeastern weevil and a second that required crossing the central highlands to the Pacific coast followed by dispersal north as far as Arizona and south into Central America. While the data presented here may be suggestive, with only four mt genotypes sampled, no real conclusions can be drawn with regard to dispersal patterns. Thorough support for the dispersal hypothesis can come only from detailed studies of weevils in several locations, including the Arizona region, northeastern Mexico, and southern Mexico where *A. hunteri* is also found.

Mitochondrial DNA analysis has proven particularly useful in studying intraspecific differences and differences among closely related species (see reviews by Moritz et al. 1987 and Avise et al. 1987). Although the yield of DNA from various species of insects can be quite unpredictable, it appears that for many species, single insects can be used to obtain restriction fragment patterns. The ease with which unmarked stocks can become mixed makes it preferable to use field-collected samples if enough DNA can be obtained from individual insects to complete the restriction fragment survey. For an insect the size of the boll weevil, sufficient DNA for detection by Southern blotting and hybridization can be obtained from single insects. However some of these DNA preparations do not have enough DNA for a battery of 16 enzymes such as used here, and very few have enough DNA to repeat any digests. One possible solution is to collect wild, mated females and rear their F1 progeny. The F1 individuals could be frozen to provide a backup supply of the same mtDNA for later use. This would permit investigations of populations from a variety of host sources and different geographic locations.

The dispersal of the boll weevil across the Cotton Belt at the end of the ninteenth century was both abrupt and rapid. If the shift in dietary habits of the boll weevil from native plants to cotton is a recent event that occurred in a limited locale or if the cotton infestation errupted from a small resident population on wild *Gossypium*, it may have been accompanied by a genetic "bottleneck" that may have severely reduced the number of mitochondrial lineages of the southeastern weevil. If all weevils on native plants could make the shift to cotton when it became available, considerable diversity in the mtDNA of the southeastern weevil might be expected. The effect of a narrow versus a broad genetic base on various control schemes could be very different. Since there are some restricted-range boll weevil populations that have adapted to other genera of host plants (e.g., *Hibiscus* in Chiapas, Mexico) it may be possible to study the genetic consequences of several host plant shifts in the same insect species. MtDNA may also be used to study the interaction and movement of weevil populations in the southwest where a Sonoran population of the Mexican weevil has been implicated as a major source of cotton infestation and some "thurberia" populations exist in close proximity to the cotton fields. The direction and extent of movement, if any, between cotton and native plants could be investigated using mitochondrial markers.

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DIAPAUSE OF THE HORN FLY[1] IN ARKANSAS

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ABSTRACT

Flies began entering diapause at four Arkansas locations in mid-September in the north and late September in the south. The greatest percentage of diapausing individuals was observed to occur in the middle of October with 72.6, 95.8, and 88.7%, respectively, of the flies in diapause at this time from Pine Tree, Batesville, and Fayetteville. Emergence from diapause was first observed on 13 March, 14 March, 27 March, and 18 April, respectively, at Fayetteville, Pine Tree, Hope, and Batesville. These dates compare closely to data from surrounding states. Computed predicted diapause entry and emergence dates occurred later than observed entry and emergence dates.

INTRODUCTION

Knowledge of diapause dates may aid in formulating management strategies for horn fly, *Haematobia irritans*(L.), control. Horn fly diapause and the mechanisms that control it has been studied by Hoelscher et al. 1967, Hoelscher and Combs 1971, Kunz and Cunningham 1977, Thomas et al. 1987. Depner (1961, 1962) suggests that horn fly diapause is facultative and controlled by changes in temperature and photoperiod. Diapause initiation and emergence dates for the horn fly have been recorded in several states surrounding Arkansas. Horn flies commonly enter diapause in Mississippi during October with some development in late September and early November (Hoelscher and Combs 1971). Emergence was first noted in March in two out of three years and in April during the third year. Thomas et al. (1987) found that diapause was initiated during August, September, and October in Missouri while emergence occurred in May. In Texas, emergence from diapause ranged from mid-February to mid-April (Kunz and Cunningham 1977). In south-central Texas, horn flies entered diapause in October and November while first emergence was reported in late February (Thomas and Kunz 1986).

Miller (1986) developed a model that may be used to predict diapause entry and emergence dates. In this model, diapause is initiated during the fall when the two-week average temperature drops below 20°C. Emergence during the

[1]Diptera:Muscidae

spring is predicted to occur when the 14-day mean temperature is greater than 18°C.

This study was initiated to determine dates for entry into and emergence from diapause in Arkansas and to compare these dates with those observed in contiguous states. A second objective was to compare observed entry and emergence dates with the computed predicted dates of Miller (1986).

MATERIALS AND METHODS

Study sites were located at the Southwest Research and Extension Center at Hope, Pine Tree Station at Colt, Livestock and Forestry Branch Station at Batesville, and University of Arkansas Farm at Fayetteville, Arkansas. The study was conducted from 3 September 1988 to 15 May 1989. At each location, one herd of mixed-breed beef cattle that had not received any previous horn fly control was used.

Ten manure pats, ca. 12-24 hr old and a layer of soil approximately 2.54 cm thick, were collected at two-week intervals at each location on each caging date between 3 September to 16 October 1988. The pats and the soil were collected using a shovel to maintain a natural state. After collection, pats were transferred to an area near the collection site in which no cattle were present. Pats were then covered by a Saran pyramid cage 89 cm long x 81 cm wide x 81 cm deep. A piece of wood with a circle in the center was attached to the top of the cage, and an inverted jar ring was attached to it. An inverted funnel was placed inside the jar ring and a 0.9-l jar was screwed onto the jar ring. As the flies emerged, they moved through the funnel and into the jar. Jars were checked daily and flies were removed, counted, and recorded. The percentage of flies entering diapause was calculated by dividing the number of flies that emerged in the spring by the total number of flies that emerged both in the spring and fall for each caging date.

NOAA climatological data for each station were used to predict approximate diapause and emergence dates. Mean daily temperatures were calculated for entry into diapause by taking the means of the high and low temperatures for each day. Fourteen-day mean temperatures for the first day that the temperature was less than 20°C as well as the previous 13 days were calculated, creating a running 14-day mean that continued for subsequent days until the 14-day mean was less than 20°C (Miller 1986). The same method was used for emergence except that a temperature of 18°C rather than 20°C was used (Miller 1986). Predicted peak emergence coincided with the first occurrence of 14-day mean temperature of greater than 18°C with first emergence 20 days prior to this peak (Miller 1986).

Approximate dates for entry and emergence from diapause were calculated for eleven years (1978-1989). Means for yearly entry and emergence dates were calculated to show an 11-year average.

RESULTS AND DISCUSSION

At each location, the greatest percentage of flies entered diapause after the middle of October (Table 1). These data are similar to those of Hoelscher and Combs (1971), Thomas and Kunz (1986), and Thomas et al. (1987) who found that October,

November, and September, respectively, were the months when the greatest percentage of horn flies entered diapause.

Diapause began to occur in mid-September in the northern part of the state (Fayetteville and Batesville) and in late September in the southern part of the state (Hope and Colt)(Table 1).

TABLE 1. Total Number of Horn Flies Recovered from Caged Manure at Four Locations in Arkansas for Each Caging Date and the Percentage of Horn Flies Entering Diapause.

Location	Caging date	Emergence		Percent diapause
		Fall	Spring	
Hope	9/03	150	0	0.0
	9/17	450	0	0.0
	10/01	-[a]	17	-[a]
	10/15	-[a]	1	-[a]
Pine Tree	9/03	119	0	0.0
	9/17	349	0	0.0
	10/01	120	20	14.3
	10/16	20	53	72.6
Batesville	9/04	127	0	0.0
	9/18	147	3	2.0
	10/02	47	31	39.7
	10/15	1	23	95.8
Fayetteville	9/06	60	0	0.0
	9/20	8	2	20.0
	10/04	16	28	63.6
	10/18	6	47	88.7

[a]No counts were made during these collection periods.

The predicted date of entry into diapause for Fayetteville in 1988 was 4 October (Table 2). Diapause was observed to begin between 6 and 20 September. The 11-year mean date for initiation of diapause was September 27. The initiation of diapause for Batesville was predicted to be 6 October. Diapause was observed to be initiated between 4 and 18 September. The 11-year mean date for entry into diapause was predicted from this model to be October 2.

In 1988 at Pine Tree, entry into diapause was predicted to occur on 7 October (Table 2); the observed entry occurred between 17 September and 1 October. The 11-year mean date for initiation of diapause was 30 September. Entry into diapause at Hope in 1988 was predicted to occur on 7 October; also horn flies were observed to enter diapause between 17 September and 1 October. The 11-year mean date for initiation of diapause was 2 October. Predicted diapause entry dates occurred later than observed diapause entry dates for each location. The predicted dates were ca. three weeks to one month later than the earliest caging date where diapause was observed to occur.

Comparing the 11-year mean diapause entry dates with 1988 observed entry dates shows a similarity to the 1988 predicted diapause entry dates. All of the predicted dates occurred ca.

TABLE 2. Predicted Dates for Entry and Exit of Diapause of Horn Flies for the Years, 1988-1989, and the 11-Year Average for Four Locations in Arkansas.

Location	Diapause entry (1988)	Diapause exit (1989)	11-Year average entry	11-Year average exit
Fayetteville	Oct 4	Apr 7	Sept 27	Apr 21
Batesville	Oct 6	Apr 5	Oct 2	Apr 8
Pine Tree	Oct 7	Apr 6	Sept 30	Apr 8
Hope	Oct 7	Apr 7	Oct 2	Apr 3

two weeks to one month later than the earliest caging date where diapause was observed to occur.

First emergence of horn flies was recorded by the authors in Arkansas at Fayetteville and Colt on 13 and 14 March, 1989, respectively. The first records of emergence at Hope and Batesville were 27 March and 18 April, 1989, respectively. The two latter dates are not as consistent as expected by the authors because Batesville lies in the same temperature zone as Fayetteville while Hope is the furthest south. This inconsistency may have been due to the collection process at these two locations such as other species of flies being misidentified by collectors as horn flies.

Emergence dates at Fayetteville in 1989 were predicted to occur on 7 April (Table 2). Horn flies were first observed emerging 13 March. The 11-year average date for emergence was 21 April. At Batesville, the predicted date of first emergence in 1989 was 5 April; the observed date was 18 April. The 11-year average date for first emergence was 8 April.

The predicted emergence date at Pine Tree in 1989 was 6 April (Table 2). Emergence was first observed to occur on 14 March. First emergence, from the 11-year average, was 8 April. First emergence at Hope for 1989 was predicted to be 7 April. Horn flies were first observed emerging on 27 March. The 11-year average showed first emergence on 8 April.

These results indicate that the predicted dates for emergence from diapause are different than the observed dates. At three locations (Fayetteville, Hope, and Pine Tree), the observed dates were 24, 11, and 23 days, respectively, prior to the predicted dates. At Batesville, the observed date was 13 days after the predicted date.

Eleven-year averages show patterns similar to observed dates of emergence. The observed dates of emergence from the same three locations were 39, 12, and 25 days, respectively, prior to the predicted dates. The observed date occurred 10 days after the calculated date at Batesville. Thus, Miller's (1986) method of calculating dates for diapause entry and exit is not adequate for Arkansas since too much variation in predicted from observed dates were found to occur.

Arkansas is divided into two climatic regions with the northern region having lower average temperatures than the

southern region. This may explain the initiation of diapause occurring later, by ca. two weeks, in the southern region of the state. Due to location, dates of entry into and emergence from diapause in Arkansas would be expected to occur between dates recorded in Missouri, to the north, and Texas and Mississippi, to the south. These data appear to support this conclusion.

More research is needed on diapause and the mechanisms that control it for it to be effectively incorporated into new management strategies for horn fly control. Some of the newer systemic insecticides, such as ivermectin, could be used to reduce the number of horn flies entering diapause which would reduce the population that emerging the following spring. Knowledge of diapause dates would allow for properly timed application of these insecticides.

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INSECTICIDE RESISTANCE AND REPRODUCTIVE BIOLOGY OF *APHIS GOSSYPII* GLOVER[1]

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ABSTRACT

A significant level of organophosphate (OP) insecticide resistance was exhibited by a colony of the cotton aphid, *Aphis gossypii* Glover, collected following a control failure near Stoneville, Miss. Resistance was measured after 12 months in culture with no insecticide exposure, indicating that OP resistance may remain stable in the absence of OP selection pressure. OP resistant aphids exhibited significantly higher reproductive potential on days 1 and 2 of adult life compared to susceptible aphids. These data suggest that indiscriminate use of OP insecticides to control the cotton aphid and other cotton pests may not only select for stable OP resistance in the cotton aphid but also for populations of the cotton aphid with increased reproductive capability during early adulthood.

INTRODUCTION

The cotton aphid, *Aphis gossypii* Glover, has in recent years resurged as an important pest throughout the cotton belt of the United States (King and Phillips 1989). Certain insecticides, such as calcium arsenate in the 1940's (Isely 1946) and pyrethroids in recent years (King and Phillips 1989), have been shown to induce aphid outbreaks, generally believed to be through loss of early season beneficial insects. Slosser et al. (1989) discussed other factors that may induce aphid outbreaks, such as the interaction between bioclimate and plant nutritional status. Large populations also may be due in part to the development of insecticide resistance (O'Brien et al. 1990, Grafton-Cardwell 1991), particularly to the organophosphate (OP) insecticides.

Studies of insecticide resistance in aphids have demonstrated the importance of understanding the biological variables of a problem species (Sawicki et al. 1978). Rapid increases of resistant populations, sometimes after only a few insecticide applications, are a major concern in field control failure situations. Because resistant populations in laboratory colonies (O'Brien et al. in press) appear capable

[1]Homoptera: Aphididae

of rapid reproduction in the absence of insecticides, the present study was initiated to quantify OP insecticide resistance in the cotton aphid and to compare reproductive output between OP resistant and OP susceptible aphids. This information could provide a better understanding of the dynamics of OP resistant populations and also provide for the development of better recommendations for managing insecticide resistance of the cotton aphid in the Mid-South.

MATERIALS AND METHODS

Aphids. Aphids for all assays were taken from laboratory colonies that had been in continuous greenhouse culture for 12 months without insecticide exposure. The resistant clonal colony was initiated from a single viviparous female taken from a non-clonal colony collected from a field at the Delta Branch Experiment Station at Stoneville, Miss., in which insecticides failed to provide control. Positive identification of the original aphid collection as *Aphis gossypii* Glover was provided by M. B. Stoetzel (Taxonomic Services Unit, Beltsville, Md.); voucher specimens were deposited at the Louisiana State University (LSU) Entomology Museum. The clonal culture was previously found to possess resistance to the OP chlorpyrifos and to exhibit increased esterase production (O'Brien et al. in press). Because of difficulties in collecting insecticide-susceptible aphids from cotton in the Mid-South, the susceptible clonal colony was initiated from a single viviparous female taken from a non-clonal colony obtained in January 1990 from E. E. Grafton-Cardwell at the University of California at Davis, Calif. Positive identification of these susceptible aphids as *A. gossypii* Glover was also provided by M. B. Stoetzel, and voucher specimens were deposited with the LSU Entomology Museum. Both resistant and susceptible colonies were reared in the same greenhouse under artificial lights of 16:8 L:D photoperiod and ambient temperature and humidity on double-caged 'Stoneville 213' cotton.

Verification of Insecticide Resistance. Log concentration-mortality lines for the OP malathion (5EC, Red Panther Chemical Co., Clarksdale, Miss.) were estimated for both clonal colonies using a leaf dip residual assay. Susceptible aphids were tested with concentrations of 30, 50, 100, 150, and 300 ppm; resistant aphids were tested with a higher range (100, 300, 400, 500, and 600 ppm) to more precisely estimate the concentration-mortality values. Desired concentrations were prepared by weighing formulated material (mg) and diluting up to 1 liter with distilled water plus 0.1% (v:v) Tween 20 surfactant. Control solutions consisted of 0.1% Tween 20 plus distilled water. Four replicates of ten adult female aphids per replication, each female of similar size and light yellow color, were assayed at each concentration. Resulting percent mortality data were analyzed using a probit computer program (T. Sparks, DowElanco, Greenfield, IN 47140), and significance was determined by non-overlap of 95% confidence intervals.

Reproduction of OP Susceptible and OP Resistant Aphids. Daily and total reproductive output was determined by the use of a greenhouse assay. One nymph (last instar, pre-winged) from each clonal colony was placed on a cotyledon of a single

cotton plant using a camel hair brush and caged with a ventilated clip cage. Each female was checked daily for emergence as a winged adult and thereafter for the number of offspring larviposited each 24-hour period. All offspring were removed after counting. Daily output for each female was recorded until the death of the female. Females within a test were caged on 'Stoneville 213' cotton of the same age, and females were transferred to fresh cages on the same plant as sooty mold became heavy within the cage. All plants within a test were watered on the same day with a commercial 20-20-20 fertilizer solution (1-2 times per week). Overhead lights were regulated by an automatic timer to provide a 16:8 L:D photoperiod, and an evaporative cooling system was set to activate if temperatures exceeded 29°C. Actual average diurnal and nocturnal temperatures and humidities among the plants, as recorded by a hygrothermograph, were 29 ± 3°C and 16 ± 4°C, and 48 ± 11% and 95 ± 5% RH, respectively. A preliminary test using a randomized block design found no advantage to blocking on possible temperature gradients so individual infested plants in 10.2-cm pots were arranged in a completely randomized design on a single greenhouse table. A total of 6-10 individuals from each colony were assayed per test, and each test was replicated four times, resulting in a total of 30-31 individuals from each colony tested for the entire experiment. Data from all replications were pooled and peak reproductive periods and total life span reproduction of OP resistant versus OP susceptible aphids were compared using a one-tailed, two-sample unpaired *t* test for each comparison.

RESULTS AND DISCUSSION

After 12 months without insecticide exposure, the LC_{50} for malathion was significantly higher for the resistant colony than the susceptible colony, as evidenced by the non-overlap of the 95% confidence intervals at the LC_{50} estimations (Table 1). Additionally, the very high slope of the concentration-mortality line for the resistant colony suggests that resistance to malathion was quite homogenous.

TABLE 1. Response of OP Susceptible and OP Resistant Cotton Aphids to Malathion.

Colony	LC_{50}[a] (ppm)	Resistance Ratio	Slope	SE
Susceptible	53.9 (29.6-74.1)	----	1.8	0.37
Resistant	379.0 (353.3-398.6)	7.0	14.2	2.24

[a]Values in parentheses are 95% confidence limits.

Stability of OP resistance in laboratory colonies of the cotton aphid was previously reported by Takada and Murakami (1988) and suggests that OP resistance in the field may become fixed in a population with no associated disadvantages. This observation is supported by actual field experience in recent years in which OP's generally were relatively ineffective within and across seasons, and OP resistant populations did not decline in numbers as would be expected if resistant insects were reproductively disadvantaged. Experiments conducted in California also demonstrated that OP resistance was present in the cotton aphid collected from weeds prior to the cotton season, indicating that resistant populations are capable of maintaining resistance during overwintering stresses (Grafton-Cardwell, pers. comm.).

Graphic representation of total reproduction showed a trend for greater reproductive output by both susceptible and resistant aphids early in life (FIG. 1), which is considered to be characteristic of winged aphids in general (Dixon 1987). Analysis of total reproductive progeny production indicated no significant difference between susceptible and resistant aphids (Table 2). However, when the cumulative reproduction for the first two days of adulthood is considered separately, resistant aphids were found to produce significantly more offspring ($P < 0.05$) than susceptible aphids during this interval (Table 2). The susceptible colony produced significantly more ($P < 0.05$) offspring during the combined period of days 10-15 and showed slight peaks on days 17 and 23 compared with the resistant colony. However, it has been mathematically demonstrated that the more offspring an individual produces very early in life, as opposed to the total number produced over an entire lifetime, the greater its contribution to the intrinsic rate of population increase and thus to increasing numbers in succeeding generations (Dixon 1987). This is particularly true in the case of aphids, where generation time is very short (as short as 5 days for the cotton aphid). Further, the possibility of susceptible aphids realizing as long a reproductive cycle in the field as occurred in the present greenhouse study would seem unlikely, primarily due to insecticide use.

TABLE 2. Reproduction of OP Susceptible and OP Resistant Cotton Aphids Under Greenhouse Conditions.

Colony	Total Mean[a]	Initial Mean[b]
Susceptible	35.30 a (4.79)	3.13 a (0.41)
Resistant	30.47 a (4.57)	4.10 b (0.35)

[a]Total mean fecundity over the entire life span of aphids.
[b]Cumulative mean reproduction during the first two days of adulthood. Values within a column followed by different letters are significantly different ($P < 0.05$). Values in parentheses are standard error values.

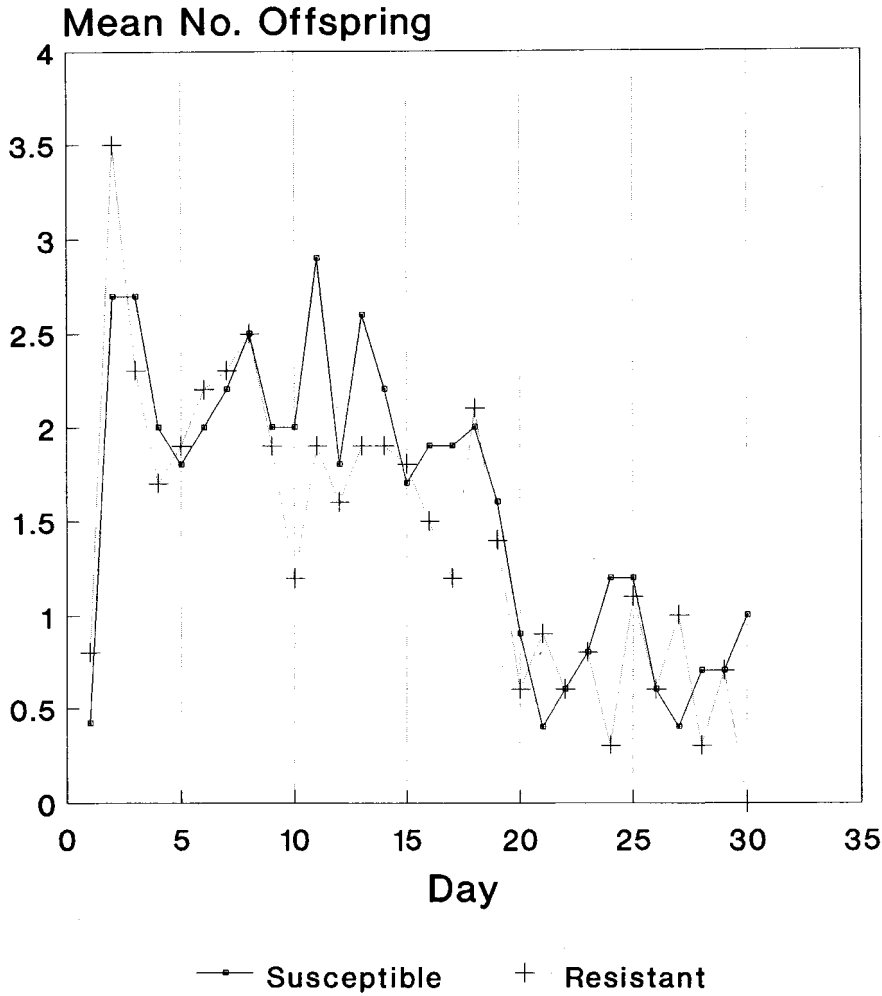

FIG. 1. Daily reproduction of OP susceptible and OP resistant cotton aphids.

Thus, OP resistant cotton aphids were under no overall reproductive disadvantage under these conditions compared to OP susceptible aphids. Since the generation time of the cotton aphid is short, this apparent biological advantage (insecticide resistance plus no reproductive disadvantage) is magnified and may explain the observed greater rate of increase in previous studies (O'Brien et al. in press).

Research conducted with the tobacco aphid (Abdel-Aal and Lampert 1991) indicated that insecticide resistance was actually associated with some degree of fitness advantage. This was evidenced by a greater multiplicative potential of resistant tobacco aphids in the laboratory compared with susceptible aphids in the absence of pesticide exposure in this species. This OP resistance in the tobacco aphid, as well as in other aphids (Devonshire 1977, Marullo et al. 1988, Wachendorff and Zoebelein 1988, O'Brien et al. in press), is due to increased esteratic metabolism, and, in the case of the OP insecticide malathion, to a malathion-specific carboxylesterase. Research with other insects such as the red flour beetle has indicated that this lack of a reproductive disadvantage is usually associated with malathion-specific carboxylesterase (Beeman and Nanis 1986). Altered sensitivity of acetylcholinesterase and increased oxidative detoxification have also been implicated in insecticide resistance in the cotton aphid (Sun et al. 1987). Studies with mites involving altered acetylcholinesterase (Smissaert 1964, Helle 1965) and oxidative activity (Roush and Hoy 1981), have found little or no reproductive disadvantages associated with these mechanisms of resistance.

The apparent stability of OP resistance and the lack of reproductive disadvantage observed in this study may be important factors in insecticide resistance management of the cotton aphid. Based on these results, an assumption of renewed OP efficacy after an interval of reduced or no OP selection within a season following a control failure may be erroneous. Further, continuous use of one compound exhibiting poor performance may select not only for greater resistance but also for higher reproduction during early adulthood, both highly undesirable outcomes. Therefore, a strategy of rotation of different classes of insecticides, careful scouting, and recognition of biotic controlling factors such as beneficial insects and diseases may prove helpful in managing populations and resistance in the cotton aphid.

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IN VITRO REARING OF *BRACON MELLITOR*[1] AND *CATOLACCUS GRANDIS*[2] WITH DIFFERENT INSECT HEMOLYMPH-BASED DIETS

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ABSTRACT

Artificial diets containing hemolymph isolated from larvae of the greater wax moth, *Galleria mellonella* (L.), the oak silkworm, *Antheraea pernyi* (GuerinMeneville), or the boll weevil, *Anthonomus grandis* Boheman; from pupae of *A. grandis* or the tobacco hornworm, *Manduca sexta* (L.); or eggs of *M. sexta* supported the in vitro development of the hymenopteran ectoparasitoids, *Bracon mellitor* Say and *Catolaccus grandis* (Burks). Developmental time (from egg to adult) for both species was ca. two weeks. Although adult yields obtained with all test diets were very low (20 to 25%), sex ratios, and fecundity and fertility were compared. The high larval and pupal mortalities observed in this study were likely attributable to a series of nondietary factors related to rearing conditions.

INTRODUCTION

The boll weevil, *Anthonomus grandis* Boheman, is one of the most destructive pests of cotton in the United States and in other parts of North, Central, and South America where this crop is grown commercially (Ridgway and Lloyd 1983). Because present chemical control strategies are expensive, destructive to beneficial insects, and contaminate our environment, there is an urgent need to develop non-chemical control alternatives. A promising method in controlling insect pests is to use augmentative releases of parasitic and predaceous insects.

At present, boll weevils in the Rio Grande Valley of Texas are parasitized by several hymenopterous ectoparasitoids in the field. One of the most abundant species is *Bracon mellitor* Say, which parasitizes third-instar boll weevil infesting fruit in the plant canopy. *Bracon mellitor* is considered the most important native parasitoid of the boll weevil in the southern United States and probably in northern Mexico (Cross and Chestnut 1971). Another ectoparasitoid, the pteromalid *Catolaccus grandis* (Burks), is also known to effectively attack third-instar boll weevil infesting fallen *Hampea* spp. and *Cienfuegosia* spp. fruit in southern Mexico and Central America (J. R. Cate, personal communication). Thus, these two ectoparasitoids are potential candidates for augmentation against the boll weevil in commercial cotton in the Rio Grande Valley of Texas (E. F. Knipling, personal communication).

One impediment to augmentation, however, is the cost of mass propagation (Thompson 1975, Leppla and Guerra 1989). Propagation of these ectoparasitoids have only been achieved in the laboratory using very costly and labor intensive in vivo rearing techniques (Cate 1987) which require the production of insect hosts. Rearing of parasitoids from host insects is impractical, and it limits the use of parasitoids in research and makes it difficult to develop and test biological control programs (Nettles 1990).

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[1]Hymenoptera: Braconidae

[2]Hymenoptera: Pteromalidae

Research efforts to develop in vitro rearing methodologies, using artificial diets, for mass propagation of some parasitic Hymenoptera (Liu et al. 1979, Nettles et al. 1985, Xie et al. 1986) have been undertaken in an effort to reduce production costs. To date, the most successful results have been obtained when these parasitoids were reared in vitro on diets containing insect hemolymph or egg fluid (Irie et al. 1987). Only 6 families and 11 species of parasitic Hymenoptera reportedly have been reared with a degree of success on artificial diets (Nettles 1990). However, neither *C. grandis* nor any species of Braconidae were included in this report.

Thus, research aimed at the development of an artificial larval diet which could be incorporated into a practical and economic in vitro rearing system to support the mass production of these ectoparasitoids has been initiated. The study reported here compared diets prepared using hemolymph from a variety of sources.

MATERIALS AND METHODS

Two ectoparasitoids, *B. mellitor* and *C. grandis*, were used in our nutritional bioassays. *Bracon mellitor* adults were obtained from cotton bolls collected near Elsa, Texas; *C. grandis* adults were obtained from Dr. J. R. Cate from colonies maintained at the Department of Entomology, Texas A&M University, College Station, Texas. Populations of these two species were maintained in the laboratory and reared in vivo on third-instar boll weevil larvae using parafilm cells as described by Cate (1987). Rearing room conditions were maintained at ca. 28°C and 60-65% relative humidity with a 14-h photophase. Experimental diets were evaluated using an open bioassay system where individual eggs were placed inside aluminum foil dishes (1.5-cm in diameter) containing circular pieces of No. 1 Whatman filter paper (0.5-cm in diameter) impregnated with the test diet. The aluminum foil dishes were then placed inside 35-mm plastic tissue culture petri dishes and placed in controlled humidity desiccators within 24-h periods. Test larvae were examined and transferred daily to new aluminum foil dishes containing fresh diet samples until pupation occurred. *Bracon mellitor* pupae were examined for cocoon formation, and the number and sex of resulting adults were recorded. Sex ratios were estimated from adults which emerged from 100 pupae for each treatment. Ectoparasitoid fecundity was estimated from the total number of eggs oviposited by 150 one-week old females on 100 third-instar boll weevil larvae for two consecutive hours . Fertility was estimated by recording egg hatch from 200 eggs (obtained from the fecundity test described above) for each treatment.

Experimental diets were modifications of an oligidic formulation used by Xie et al. (1986) for the in vitro culture of *Trichogramma pretiosum* Riley. The basic medium was composed of insect hemolymph (50%), fresh chicken egg yolk (25%), 15% dry milk reconstituted with water (25%, v:v), and gentamicin solution (1%). During these tests, we evaluated diets prepared with hemolymph isolated from seventh-instar larvae of the greater wax moth *Galleria mellonella* (L.); pupae of the tobacco hornworm, *Manduca sexta* (L.); fifth-instar of the oak silkworm, *Antheraea pernyi* (GuerinMeneville); third-instar larvae and pupae of the boll weevil *A. grandis*; or fluid from *M. sexta* eggs. The control diet consisted on third-instar boll weevil larvae reared and infested with the ectoparasitoids using the parafilm method described above. Statistical analysis of the data was conducted using the GLM procedure on PC-SAS (SAS Institute 1985); and means were compared using the Waller-Duncan K-ratio t-test.

RESULTS AND DISCUSSION

The results (Table 1) indicated that *B. mellitor* and *C. grandis* can be reared with comparable success on semisynthetic diets containing insect hemolymph from different sources. These findings are important because insect hemolymph, which is presently our most expensive ingredient of the basic diet, could be procured by researchers elsewhere from the most economic source available. Although there were significant differences in the duration of the life cycle of both ectoparasitoids when they were reared with the different hemolymph-based diets, the average duration of development times from egg to adult fluctuated between 11 and 12.5 days, and these intervals were only one or two days

longer than those of ecto-parasitoids reared in vivo (controls). Percent pupation and percent adult emergence of both ectoparasitoids were comparable when reared with all the experimental hemolymph-based diets tested; however, they were much lower when compared to those obtained with control insects. Lower yields obtained with the experimental hemolymph-based diets also reflected the extensive losses of first-instar larvae which escaped from the rearing containers. Approximately 45% of all the first-instar larvae escaped from the dishes containing the feeding pads and either dessicated, starved to death, or disappeared during bioassays. Second-instar larvae remained near the feeding pads most of the time. *Bracon mellitor*, which normally forms a cocoon to pupate (ca. 95% of the time) when reared in vivo didn't form a complete cocoon 65% of the time; larvae produced abundant silk material, which was deposited on the bottom of the rearing dishes, and pupated naked or with an incomplete cocoon. This atypical form of pupation didn't affect pupal survival or adult emergence.

TABLE 1. Effect of a Larval Diet Containing Egg Yolk (25%), Whole Dry Milk (25%), and Insect Hemolymph (50%) From Different Sources on Duration of the Life Cycle % Pupation, and % Adult Emergence of the Ectoparasitoids *Bracon mellitor* Say (B.m.) and *Catolaccus grandis* (Burks) (C.g.); 50 reps/test, 1 egg/rep, 4 tests.

Hemolymph source	Avg. duration (days) (Egg-Adult)[a]		% Pupation		%Emergence	
	B.m.	C.g.	B.m.	C.g.	B.m.	C.g.
Galleria mellonella						
(larvae)	11.5 c[b]	12.0 b	37	35	22	20
Anthonomus grandis						
(larvae)	12.0 a	11.0 c	40	34	24	25
(pupae)	11.0 d	11.5 b	42	35	25	20
Manduca sexta						
(pupae)	11.0 d	12.0 b	38	40	20	23
(eggs)	12.5 a	12.5 a	35	35	24	21
Antheraea pernyi						
(larvae)	12.0 c	11.5 b	46	32	22	20
Control[c]	10.0 e	10.0 d	91	73	85	69

[a]Calculations were made on observed insects only. Parasitoids which escaped from the feeding pads or rearing containers were not included.

[b]Means in a column followed by the same letter (a, b, c, d, e) are not significantly different at the 5% level by the Waller-Duncan K-ratio T test.

[c]Parasites grown on third-instar boll weevil larvae.

Although nearly 40% of all parasitoids completed larval development and pupated, a little more than half of these reached the adult stage. Of the total number of adults obtained from naked or semi-naked pupae, ca. 55% were females; and adults from both sexes exhibited normal physical characteristics, copulated and laid fertile eggs. No apparent behavioral differences were exhibited by parasitoids reared in vivo and those which developed on all the experimental hemolymph-based diets tested. Sex ratios obtained with both parasitoid species were close to 1:1 regardless of the test diets used. Observations also indicated that the fecundity and fertility of both ectoparasitoid species

reared on diets containing the different hemolymph sources tested were comparable with that of control adults reared in vivo on boll weevil larvae.

In any case, since the basic experimental diet tested in this work was developed to rear the egg parasitoids, *Trichogramma* spp., it will be necessary to make further changes in the formulation, as well as modifications of our rearing conditions, before we can develop and optimize a feasible system for in vitro mass production. The very low yields of adults obtained during our study were probably not due entirely to nutrition but to the overall effect of a series of factors related to the rearing process. Some of these factors could be: 1. The diet retainer used to present the liquid diet, 2. The rearing container used in our open-bioassay system, 3. The lack of optimum climatologic conditions in our rearing rooms, and 4. Manipulation injuries sustained during daily larval transfers in our bioassay.

An artificial diet containing insect hemolymph or egg fluid has potential for being incorporated into a properly optimized mass rearing program for *B. mellitor*, *C. grandis*, and perhaps other ectoparasitic species when appropriate improvements are made. This particular basic diet can also serve as a valuable tool to develop more efficient chemically defined dietary formulations completely devoid of insect components. This would make feasible the development of an in vitro mass propagation program to propagate candidate ectoparasites which could be used to control boll weevils infesting commercial cotton.

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RNA IN THE DEVELOPING OVARIES OF THE STABLE FLY, *STOMOXYS CALCITRANS* (L.)[1]

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ABSTRACT

Ribonucleic acids were analyzed from each stage of the developing ovary of *Stomoxys calcitrans* (L.). The level of total RNA increased as the ovary progressed from the previtellogenic stage to the mature oocyte. Total RNA reached a maximum level at stage IV. The banding patterns of yolk proteins synthesized by cell-free translation were the same for RNA extracted from each stage. Translation of isolated mRNA in the presence of microsomal membranes resulted in apparent changes in bands having molecular weights estimated at 49,900, 51,100 (doublet), 53,500 (doublet), and 55,900.

INTRODUCTION

The composition of the insect polytrophic follicles changes during vitellogenesis as the oocyte develops to maturity (King 1970, Telfer 1975, Khipple and King 1976, Berry 1982, Chia et al. 1982, Gutzeit 1986). One major developmental change is the accumulation of yolk proteins (YP) in the oocyte (Hagedorn and Kunkel 1979, Adams and Filipi 1983). Yolk proteins are synthesized in insect fat body and ovary (Brennan et al. 1982, Izumi and Tomino 1983, De Bianchi et al. 1985, Minoo and Postlethwait 1985) or in the ovaries exclusively (Chen et al. 1987, Handler and Shirk 1988). Maturation of YP involves translation and post-translational modifications which include proteolysis and the addition of carbohydrate, lipid and phosphate components to proteins (Minoo and Postlethwait 1985, DiMario et al. 1987, Kim et al. 1988, Purcell et al. 1988). The nucleic acid content of the ovary also changes during oogenesis. In *Drosophila melanogaster* rRNA makes up the major portion of RNA in the developing ovary (Mermod et al. 1977). Additionally, mRNA has been shown to be 1-3% of the total RNA in the ovaries of *D. melanogaster* (Lovett and Goldstein 1977) and *Calliphora erythrocephala* (Kirchhoff 1981). However, most of the mRNA in the oocyte was described by Lovett and Goldstein (1977) to be in an untranslated state (i.e., not associated with polysomes). This mRNA is in a storage form and non-translatable during the development of the oocyte.

The follicles of the polytrophic ovaries of *Stomoxys calcitrans* (L.) have been characterized as developing through six oogenic stages (Venkatesh and Morrison 1980). Vitellogenesis becomes evident as the oocytes develop from stage N (previtellogenesis) to stage I. Follicular size and YP deposition increase with successive blood meals as development proceeds from stage I to stage V, the terminal stage (Chia et al. 1982, Kunz 1982).

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[1]Mention of a commercial or proprietary product in this paper does not constitute an endorsement of this product by the U.S. Department of Agriculture.

In the present study we determined the levels of RNA in the developing ovary. In addition, we examined the RNA of developmental stages by analyzing the cell-free translation products directed by the ovarian RNA.

MATERIALS AND METHODS

Insects and reagents. Stable flies were reared according to the standard methods of this laboratory (Bridges and Spates 1983). Adult flies were fed citrated bovine blood obtained from a local slaughterhouse. Under these conditions, the first clutch of eggs are fully developed and deposited by 7 days after adult eclosion. Chemicals were of the highest grade commercially available and obtained from various sources. All solutions, glassware and plasticware were sterilized to minimize ribonuclease degradation.

Extraction of total RNA. Ovaries were dissected in 10 mM potassium phosphate buffer (pH 7.5) containing 150 mM NaCl, and immediately placed in 1.5 ml polypropylene microcentrifuge tubes on dry ice and stored in liquid nitrogen. Total ribonucleic acids were extracted from 100 stage N ovaries and 20 ovaries each of stages I-V (Venkatesh and Morrison 1980). Dissected ovaries were homogenized with Potter-Elvehjem homogenizers in RNAzol (2 ml/100 mg; CINNA/BIOTECX, Friendswood, TX) and homogenates were transferred to 4.5 ml polypropylene tubes on ice. Chloroform (0.2 ml per 2 ml of homogenate) was added and each tube was capped and shaken vigorously for 15 seconds. Samples were kept on ice for 15 min and centrifuged at 12,000 g for 15 min at 4°C. Resulting upper aqueous phases were transferred to 1.5 ml microcentrifuge tubes. RNA was precipitated by adding an equal volume of -20°C isopropanol and storing at -20°C for 45 min. RNA was pelleted by centrifugation at 12,000 g for 15 min at 4°C. Supernatants were aspirated and the pellets were washed twice by vortexing in 1 ml ice-cold 75% ethanol and centrifugation at 4°C and 12,000 g for 8 min. Washed pellets were solubilized in 1 mM ethylenediaminetetraacetic acid (EDTA) and reprecipitated by adding NaCl to a final concentration of 0.2 M and an equal volume of isopropanol (-20°C). These solutions were kept at -20°C for 2 h before the RNA was pelleted by centrifugation at 12,000 g at 4°C for 8 min. Pellets were dissolved in 1 mM EDTA and stored at -70°C. The RNA prepared in this manner from each stage of ovarian development had A_{260}/A_{280} ratios ranging from 1.82 to 2.33. A ratio of 2.0 is indicative of pure RNA (Scheif and Wensink 1981, Maniatis et al. 1982) and relative concentrations were determined as 23 A_{260} units = 1 mg RNA (Clemens 1984a).

Isolation of mRNA. Messenger RNA was separated from total RNA extracted from stage II ovaries of *S. calcitrans* by a modification of the method in Pharmacia publication 70-01-030 (Pharmacia 1985). Ovaries were homogenized in 4 M guanidinium isothiocyanate (7.5 ml/g tissue) with a Potter-Elvehjem homogenizer kept on ice. Messenger RNA was isolated by affinity chromatography on an oligo(dT)-cellulose (New England BioLabs, Beverly, MA) column (0.7 cm I.D. X 5 cm). Following reprecipitation (see above), mRNA was dissolved in water and stored at -70°C. Messenger RNA prepared in this manner had an A_{260}/A_{280} of 1.93.

Translation of RNA. Total RNA and mRNA were translated with a rabbit reticulocyte lysate translation kit (Promega, Madison, WI) in 1.5 ml polypropylene microcentrifuge tubes. Messenger RNA was estimated to be 2-3% of the total RNA extracted from the ovaries. Therefore, 33.3 μg of total RNA (estimated to be 1 μg mRNA) from stages N-V was used for *in vitro* translation. One and two micrograms of RNA were used for the analysis of translation products for RNA isolated by oligo(dT)-cellulose. L-[4,5-^{3}H]-Leucine (56 Ci/mmol; New England Nuclear, Boston, MA) was used to label nascent peptides.

Post-translational modifications were carried out by incubating the translation mixture in the presence of dog pancreatic microsomal preparations (Promega).

Immunoprecipitation. Translation products were immunoprecipitated with antiserum to *S. calcitrans* yolk protein 3 (YP3 Chen et al. 1987) by a modification of the method of Clemens (1984b). The translation mixture was diluted 5-fold at

the end of incubation with immunoprecipitation buffer (0.1 M NaCl, 1 mM EDTA, 1% Nonidet P-40 (NP-40), 1% lysozyme, 10 mM Tris-HCl, pH 7.5). A predetermined amount of antiserum was added to the mixture and antigen-antibody complexes were allowed to form for 3 h at room temperature. Protein A-Sepharose CL-4B (Sigma Chemical, St. Louis, MO) hydrated in immunoprecipitation buffer (minus lysozyme and NP-40) was added to the mixture to adsorb antigen-antibody complexes. After incubation with shaking at room temperature for 40 min, the precipitate was pelleted by centrifugation at 14,000 g for 1 min. The supernatant was removed by aspiration and the pellet was washed three times by vortexing in 1 ml of immunoprecipitation buffer (minus lysozyme) and centrifugation. Immunoprecipitation buffer (0.1 ml; minus lysozyme and NP-40) was added to the pellet followed by vortexing and centrifugation at 14,000 g for 1 min. Supernatant was discarded by aspiration and the pellet was resuspended in 20 μl of sodium dodecylsulfate polyacrylamide gel electrophoresis (SDS-PAGE) sample buffer and heated at 90°C for 3 min. Protein A-Sepharose was removed by centrifugation at 14,000 g for 1 min.

Polyacrylamide gel electrophoresis. Electrophoresis was carried out according to Laemmli (1970) using 10% or 8-10% linear gradient acrylamide. Proteins were stained with 0.125% Coomassie blue. The protein markers and the respective molecular weights were: BSA (bovine serum albumin), dimer, 132,000; phosphorylase b, 97,400; BSA, 66,000; ovalbumin, 45,000; carbonic anhydrase, 29,000, α-lactalbumin, 14,200.

Fluorography. Fluorography was conducted as described by Bonner and Laskey (1974) using a commercial scintillant (EN^{3}HANCE; New England Nuclear, Boston, MA).

RESULTS

Ovarian RNA during development. Total RNA extracted with RNAzol increased 31-fold as the ovary developed from stage N (0.6 $\pm$ 0.6 μg/ovary) to stage IV (18.7 $\pm$ 2.8 μg/ovary; Fig. 1).

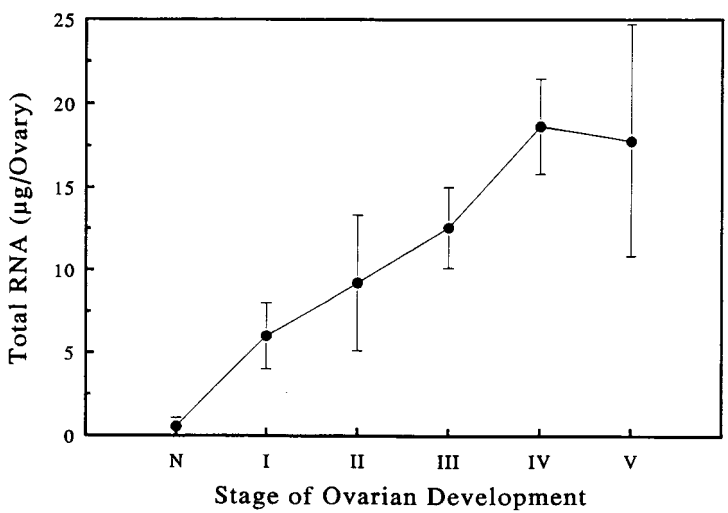

FIG. 1. Developmental profile of the stable fly ovarian RNA. Total RNA was extracted from each stage of the developing ovary of *S. calcitrans* with RNAzol. Concentrations were determined as 23 A_{260} = 1 mg RNA. Each point represents mean $\pm$ SD of 5 (stage N) or 6 (stages I-V) repetitions.

RNA obtained by CsCl from 372 stage II ovaries was 2702 μg. The recovered mRNA was 77.4 μg or 2.9% of the total RNA extracted. Therefore, it was estimated that each stage II ovary contained 0.2 μg mRNA in 7.3 μg total RNA. Total RNA extracted from stage II ovaries with RNAzol was 9.5 $\pm$ 4.5 μg per ovary. These data indicate that the quantities of total RNA obtained by CsCl centrifugation and RNAzol were similar.

Cell-free translation of mRNA. An SDS-PAGE analysis of *in vitro* translation products directed by mRNA from stage II ovaries is shown in Fig. 2. More than 40 bands were observed. The relative densities of the bands increased as mRNA was increased from 1 to 2 μg. Four prominent bands consistently appeared on the fluorographs (Fig. 2, brackets). The estimated molecular weights for these bands were 49,900, 51,100, 53,500 and 55,900, respectively.

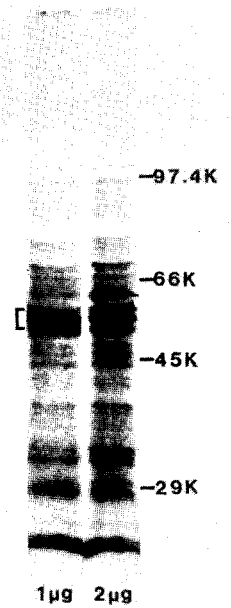

FIG. 2. Fluorograph of translation products of 1 μg and 2 μg mRNA isolated from stage II ovaries. Total RNA was isolated by CsCl centrifugation. Messenger RNA was isolated by chromatography on an oligo(dT)-cellulose column. Electrophoresis was carried out on 10% (w/v) acrylamide gel slab.

Post-translational modifications. The effects of translational modifications were examined for mRNA from stage II ovaries and total RNA from stages N-V using dog pancreatic microsomes. Translation products in the presence and absence of microsomes were precipitated with anti-YP3 (nonspecific binding did occur, but was lessened by the presence of blocking agents). SDS-PAGE analysis of immunoprecipitation products of oligo(dT)-cellulose purified RNA showed four prominent bands with molecular weights similar to YP (Fig. 3). These bands had relative molecular weights of 49,900, 51,100, 53,500, and 55,900, respectively; the 53,500 MW band was the most prominent. Translation of the isolated mRNA in the presence of microsomes resulted in the decrease in intensity of all four bands

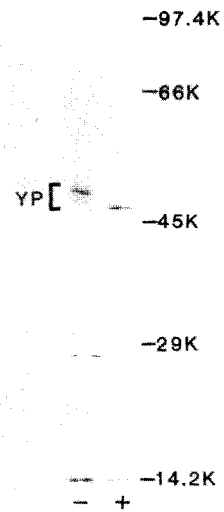

FIG. 3. Fluorograph of translation products precipitated with antibody to YP3. Translation was conducted with 1 μg of mRNA in the absence (-) and presence (+) of canine pancreatic membranes. Electrophoresis was carried out on 10% (w/v) acrylamide gel slab.

and a 47,700 MW band appeared. Careful inspection of the fluorographs indicated that the 51,100 and 53,500 bands are actually doublets. Relative molecular weights for bands of translation products other than those between 49,900 and 55,900 MW did not appear to change when translation was carried out in the presence of canine pancreatic microsomes. Although translation products of RNA from stages N, I and stage V exhibited less intense bands in areas of yolk proteins, the banding pattern was similar for all stages (Fig. 4).

DISCUSSION

The level of total RNA increased overall as the ovaries developed from stage N to V. Much of the increase of the RNA is likely due to the nurse cell and follicle cell growth. During ovarian development, follicle and nurse cells undergo massive growth, which would result in an increase in total RNA. The stable fly synthesizes YP exclusively in the ovaries (Chen et al. 1987). It is evident from data described herein that mRNA's for the yolk proteins are present in the ovaries. Increases in YP mRNA may account for some of the increase in total RNA. Investigation is currently underway to elucidate which cells contain these yolk protein mRNA's.

An unexpected observation was that the translatable RNA from all developmental stages of the ovary was qualitatively the same. *In vitro* organ culture showed that stage N and early stage I ovaries do not synthesize yolk

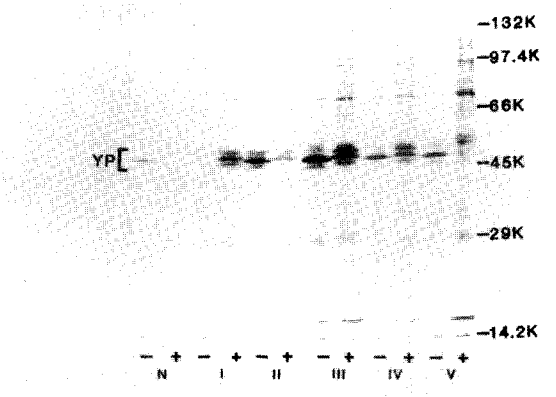

FIG. 4. Fluorograph of translation products precipitated with antibody to purified YP3. Total RNA from stages N-V was translated in the presence (+) and absence (-) of canine pancreatic microsomal preparations. Electrophoresis was carried out on 8-10% (w/v) gradient acrylamide gel slab.

proteins (Chen et al. 1987). These results indicate the presence of control mechanisms for the translation of mRNA's in the intact organ. That is, mRNA for yolk protein is present in stage N ovary, but the proteins are not synthesized. A somewhat similar situation of untranslated mRNA is found in *Drosophilia melanogaster*, although the mRNA is maternal RNA stored in the oocyte and used by the developing embryo (Lovett and Goldstein 1977).

Messenger RNA represented 2-3% of the total RNA extracted from stage II ovary. Cell-free translation of the extracted total RNA and isolated mRNA resulted in at least 40 discernible polypeptide bands on SDS-PAGE. Four of those bands were consistently prominent on fluorographs of cell-free translation products of isolated mRNA. The estimated molecular weights of the four prominent proteins were 49,900, 51,100, 53,500, and 55,900, respectively. Both the 51,100 and 53,500 MW bands appeared to be doublets. Chen et al. (1987) demonstrated that the stable fly YP have molecular weights of 41,000, 42,600, 44,100, 46,600, 48,900, and 50,600, respectively. Vitellogenins are commonly translated as large precursors which are cleaved to form smaller proteins (Purcell et al. 1988, Harnish and White 1982, Harnish et al. 1982, Pereira and De Bianchi 1983). The vitellogenins of *D. melanogaster* lose a 1,800 MW peptide during maturation (Minoo and Postlethwait 1985), but exhibit an apparent molecular weight change of only 1,000 (Brennan et al. 1982, Warren et al. 1979). This change in molecular weights was attributed to charge differences contributed by phosphorylation during post-translational modification. Likewise, vitellogenins of *Dacus oleae* are subjected to post-translational modifications by the removal of 7,000 MW peptides (Levedakou et al. 1988). It is possible that yolk protein precursors of *S. calcitrans* lose peptides within a possible molecular weight range of 5,000-15,000 during post-translational modification. This cleavage is not accomplished by the canine pancreatic microsomes. Some modification resulted in the shifting of the banding pattern in the 50,000 MW region. But, it is not yet clear what translation and post-translation modifications of YP in *S. calcitrans* entail.

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EARLY-SEASON ETHEPHON APPLICATIONS: EFFECT ON COTTON FRUITING AND INITIATION OF PINK BOLLWORM INFESTATIONS AND COTTON YIELDS[1]

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ABSTRACT

The reproductive potential of pink bollworm (PBW), *Pectinophora gossypiella* (Saunders), moths from overwintering larvae is low because significant numbers emerge when limited host material is available; mortality is induced from high soil temperature exposure and other adverse environmental factors occur. We conducted studies in Arizona and California in 1988 and 1989 to determine the potential of using a plant growth regulator (ethephon = Prep®) to reduce numbers of available early-season cotton flower buds during overwintering moth emergence to increase environmental stress and delay initiation of early-season infestations. Ethephon applied at rates of 0.38 to 1.40 kg AI/ha reduced the number of PBW-infested bolls on early-season fruiting branches. Rates of application as low as 0.56 kg AI/ha reduced cotton lint yields under short-season growing conditions but rates as high as 0.84 kg AI/ha were acceptable under full-season cotton production conditions. High rates of application (1.12 kg AI/ha and above) consistently reduced yields. In most cases, ethephon treatments delayed flowering. Treated plants compensated for removal of early-season squares and the accumulated flowers surpassed accumulated flowering of untreated control plants at some point later in the growing season. However, when accumulated flowering of treated plants was less than that of untreated plants, because of early harvests, yields were reduced.

INTRODUCTION

In Arizona and southern California, moths from overwintered pink bollworm (PBW), *Pectinophora gossypiella* (Saunders), larvae emerge from late March to early August (Wene et al. 1961, Rice and Reynolds 1971). Moth emergence may occur earlier when prepupae overwinter in free cocoons as opposed to overwintering in

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[1] This paper presents the results of research only. Mention of a proprietary product does not constitute an endorsement by the USDA.

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bolls (Fye 1979). The eggs laid by emerged moths produce first generation larvae that enter squares (flower buds) (Slosser and Watson 1972). Highest early-season infestations are produced in cotton cultivars that initiate square formation earliest and most profusely. Planting cotton later delays initiation of cotton fruiting and has been shown to delay initiation of PBW infestations and the occurrence of economic infestation levels (Henneberry et al. 1982). Moth emergence that occurs before fruiting forms of cotton are available as a source of larval food is termed suicidal (Chapman et al. 1960). Suicidal emergence ranges from 57 to 86% in the desert valley growing areas of Arizona and California and from 28 to 80% at the higher elevation of Safford, Arizona (Slosser and Watson 1972, Bariola 1978).

Ethephon (Prep®) is registered for use on cotton to accelerate mature boll opening. In Arizona, late-season applications to cotton also reduce late-season fruiting forms as a source of host material for development of diapause PBW larvae (Bariola et al. 1990). Promising results that show reduced development of late-season boll weevil, *Anthonomus grandis* Boheman populations (Henneberry et al. 1988) have also been reported. We conducted studies in 1988 and 1989 in southern California and in 1989 in Arizona to determine the potential of using ethephon to remove early-season cotton squares, thus extending the PBW suicidal emergence period and delaying initiation of early-season infestations.

MATERIALS AND METHODS

Brawley, California 1988. 'Deltapine 61' cotton fields (planted 20 March, ca. 1.6 ha each) were divided into a four replication, split-split plot experimental design. Whole plot treatments were insecticides vs. no insecticides, split plots were early-season ethephon applied 7 June to cotton plots 16 rows wide by 67.2 m long, at rates of 0.38, 0.56, 0.84 kg AI/ha and untreated controls. Split-split plots were late-season vs. no late-season ethephon applications at the rate of 1.7 kg AI/ha. Pretreatment counts of 1/3-grown squares (7 to 10 days old) were made in 4 m of row on 4 June in all plots. The effect of the treatments on squares was determined by counting abscission sites and healthy squares on all plants from 0.9 m of row randomly selected in each plot on day 6 following applications. Flowers were counted daily in 67.2 m of cotton row in each plot from day 6 following treatment to 26 August. Cotton was hand harvested from 4 m of row on 9-12 September from all plots to determine the effect of the treatments on cotton yields.

Seven gossyplure-baited PBW live traps (Lingren et al. 1980) were distributed throughout the fields to monitor PBW moth populations. Insecticide treatments (azinphosmethyl®) were applied to randomized whole plots on 27 July, 4, 13, and 19 August. Late-season applications of ethephon were made 23 August, 19 days following the last irrigation. All ethephon applications were made by ground equipment in approximately 281 liters of water/ha. Insecticide applications were made by air. The effect of ethephon applications on initiation and distribution of PBW infestations was determined by harvesting three whole cotton plants from each plot on 8-9 August. All cotton bolls (open and immature) were counted on each fruiting branch. Each boll was examined for PBW exit holes and other evidence of PBW feeding damage.

Maricopa, Arizona 1988. 'Deltapine 61' cotton plots were planted on 28 March in a randomized block design of five treatments and four replications. Each plot was 4 rows wide by 15 m long. Ethephon applications were made on 10 June with a backpack sprayer. Rates were 0.56, 0.84, 1.12 and 1.40 kg AI/ha in ca. 178

liters water/ha. Untreated plots were controls. Pretreatment counts of 1/3-grown or larger squares on all plants in 4 m of row at 9 sites randomly selected throughout the field were made on 10 June. Abscission sites and healthy squares were counted on all plants from 0.9 m of row in each plot on day 6 after treatment. Also, numbers of healthy 1/3-grown squares were counted in 4-m of row in all plots on days 3, 7 and 10 following treatment. All flowers in 15.2 m of row were counted daily in each plot beginning 7 days after treatment and continuing to 18 September. Cotton was hand harvested in 4 m of row from all plots on 26 October to determine the effects of treatments on cotton yields.

Gossyplure-baited Delta sticky traps were placed two per field quadrant to monitor PBW male moth populations. No insecticides were applied to any of the plots. Whole plants, (four) were harvested from each plot on 17 September. All open mature and immature green cotton bolls were counted on each fruiting branch and examined for PBW damage as previously described.

Maricopa, Arizona 1989. 'Deltapine 90' cotton plots were 0.035 ha each. The experiment was conducted in a randomized block design of three treatments with eight replications. Ethephon applications were made in 205.7 liters of water/ha with a high clearance ground sprayer. Rates of ethephon were 0.84 and 1.12 kg AI/ha applied at the one-third-grown stage (8 June 1989) of plant development. Untreated plots were controls. No insecticides were applied. One-third-grown squares in each plot were counted on 8, 13, 15 and 21 June in 4-m of row to determine the effect of ethephon on square abscission. All white flowers in 16-m of row were counted at 2- to 8-day intervals from 27 June to 14 September to determine the effect of treatments on cotton flowering.

Whole cotton plants (five) were picked from each plot on 18 September. Mature and immature cotton bolls and PBW-infested and damaged bolls were counted on each fruiting branch to identity initiation of PBW infestations and distribution on the plant. Also immature cotton bolls (50) were picked each week from each plot from 11 July to 2 August to determine the effect of the treatments on development of early-season PBW infestations. Boll samples were held in screen-ventilated plastic boxes for 2-3 weeks. All emerged PBW and those found in bolls after dissection were recorded. The effect of treatments on cotton yield was determined by picking and weighing seed cotton from all open bolls in 4-m of row on 7 November. Seed cotton was ginned, lint weighed and seed x-rayed to determine PBW seed damage.

Data were subjected to the analysis of variance, and means were separated using Duncan's (1955) multiple range test ($P \leq 0.05$). Additionally, multiple regression analyses were performed to examine potential relationships between healthy and abscised squares as a result of treatments on final cotton lint yields.

RESULTS

Brawley, California 1988. Mean numbers of 1/3-grown squares prior to ethephon treatment ranged from 15 to 17/4 m of cotton row (Table 1). There were no significant interactions between early- or late-season ethephon applications and insecticide vs. no insecticide applications for any of the data. Thus, means for early-season ethephon application include all insecticide, no insecticide, late-season ethephon and no late-season ethephon plots. Six days (13 June) after treatment, numbers of healthy squares/plant ranged from 20 in control plots to 12 in plots treated with 0.84 kg AI/ha of ethephon. Numbers of male PBW moths

TABLE 1. Effect[a] of Early-Season Ethephon Applications on Cotton Squares at Brawley, California and Maricopa, Arizona 1988.

Treatment	Pretreatment	Post-treatment (6 days)		
		No. squares / plant in 0.9 m of row		
Ethephon (kg AI/ha)	1/3-grown squares No./4 m of row	Present	Abscission Sites	% Abscised
Brawley, California:				
Control	15	20 a	3 b	13 b
0.38	15	19 a	4 b	18 b
0.56	17	17 ab	5 b	23 b
0.84	17	12 b	8 a	40 a
Maricopa, Arizona:				
Control	10	5 a	1 c	17 c
0.56	--	3 b	3 b	50 b
0.84	--	2 cd	2 bc	50 b
1.12	--	1 d	3 a	75 a
1.40	--	1 d	5 a	83 a

[a] Means in a column within a location not followed by the same letter are significantly different, (Duncan's [1955] multiple range test, $P \leq 0.05$).

caught/trap/night were 0.06, 0.10, 0.22 and 1.32 during the weeks of 6 June, 13 June, 20 June, and 27 June, respectively, when fewer 1/3-grown squares were available as PBW infestation sites in ethephon-treated plots compared to untreated control plots.

Total number of accumulated flowers (1000's/ha) from 13 June to 18 August were 1699, 1771, 1759 and 1828 for the control, and plots treated with 0.38, 0.56 and 0.84 kg AI/ha of ethephon, respectively (Fig. 1). Total accumulated numbers of flowers in plots treated with ethephon were significantly greater than the total numbers of accumulated flowers in control plots. Numbers of flowers in plots treated with ethephon at rates of 0.38 or 0.56 kg AI/ha were not significantly affected for 53 days following treatment, but generally, higher numbers of flowers occurred in the ethephon-treated plots than in the control plots after that date (Fig. 1). Reduced flowering occurred in plots treated with 0.84 kg AI/ha between days 13 to 38 following treatment. Accumulated numbers of flowers were similar from day 33 to day 43 following treatment, and, after day 53, total accumulated flowers exceeded numbers of flowers in the untreated control plots.

The average node number of the first fruiting branch with an open or green boll ranged from 6.7 to 7.2 in ethephon and control plots (Table 2). The number of the first fruiting branch with a PBW-infested boll varied but differences were not significant. However, numbers of PBW-infested bolls on fruiting branches 9 to 14 in control plots were significantly higher than on the same fruiting branches from ethephon-treated plots.

Cotton lint yield from plots treated with 0.38 kg AI/ha of ethephon were not significantly different from untreated cotton plots (Table 3). Cotton lint yields from plots treated with ethephon at 0.56 and 0.84 kg AI/ha were significantly lower than from untreated plots and plots treated with 0.38 kg AI/ha. Final yields were positively correlated ($r = 0.50$) to numbers of healthy squares and negatively correlated ($r = -0.57$) to the numbers of abscised squares approximately 1 week after ethephon treatment ($P \leq 0.05$).

Maricopa, Arizona 1988. Pretreatment counts showed an average of 10, 1/3-grown squares/4 m of cotton row (Table 1). Numbers of squares 6 days after application ranged from 1 to 3/plant in plots treated with ethephon at 1.40 and 0.56 kg AI/ha, compared to 5/plant in the untreated control plots. Numbers of PBW male moths caught in gossyplure-baited traps showed moth emergence from overwintering larvae increased from 29 May to 5 June decreasing thereafter to 22 June (Fig. 2). Numbers of 1/3-grown squares beginning just after the peak of moth emergence were more than 14x greater in control plots than in plots treated with 1.12 or 1.40 kg AI/ha of ethephon (Fig. 1).

Numbers of flowers were reduced in ethephon-treated plots beginning 15 to 20 days following treatment and thereafter as compared to control plots, during the season for the 1.40 kg AI/ha ethephon rate (Fig. 3). Results varied at other rates of application, but the total accumulated numbers of flowers from 13 June to 8 September were 1919, 1867, 1935, 1923 and 1623 (1000's/ha) for the control plot and plots treated with 0.56, 0.84, 1.12 and 1.40 kg AI/ha of ethephon, respectively (Fig. 3). Accumulated numbers of flowers in plots treated with 1.40 kg AI/ha of ethephon were significantly lower than in the control and all other ethephon-treated plots.

The numbers of the nodes of first fruiting branches with an open or green boll were higher in ethephon treated plots as compared to the control but differences were not significant at the 0.56 and 0.84 kg AI/ha rates (Table 2). However, the average number of the first fruiting branch with PBW-infested bolls occurred 2.9 to

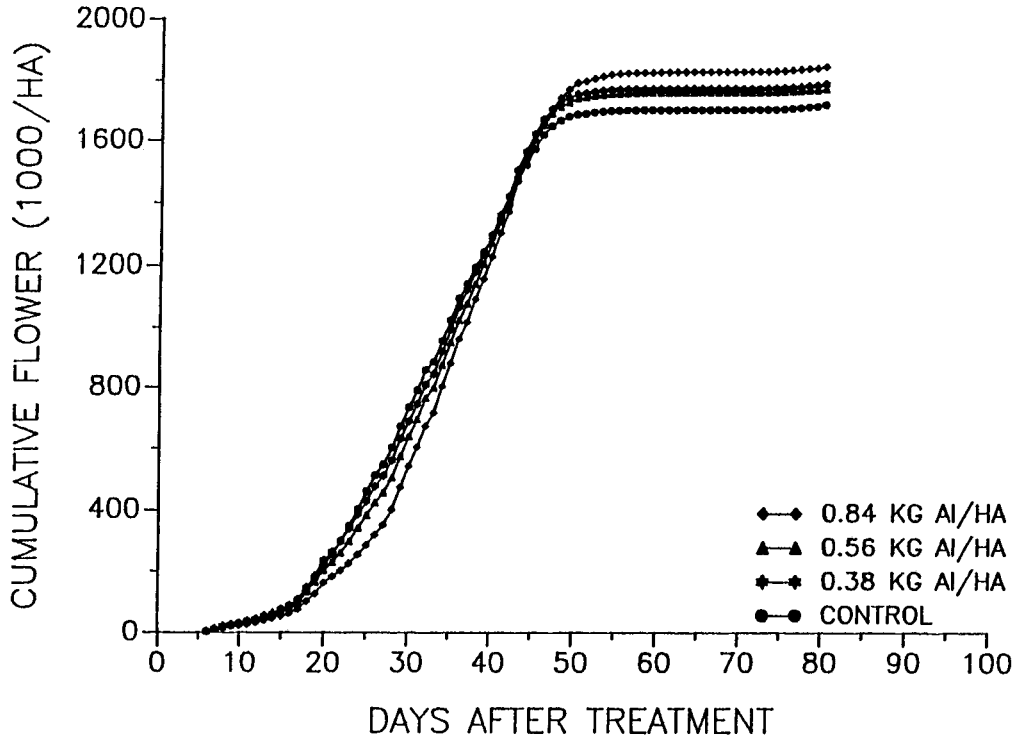

FIG. 1. Mean number of accumulated cotton flowers at Brawley, California, in ethephon treated and untreated cotton 1988.

TABLE 2. Effects[a] of Early-Season Ethephon Treatments on Number of the Node of First Cotton Fruiting Branch and the First PBW-Infested Boll and Total Number of Bolls and Number Infested on Fruiting Branches at Nodes 9 to 14 1988.

Ethephon rate (kg AI/ha)	Node Number of First Fruiting Branch		Fruiting Branches at Nodes 9-14	
	With cotton bolls	With a PBW infested boll	Total No. cotton bolls	No. bolls PBW Infested
Brawley, California:				
Control	6.7 a	9.8 a	53.8 a	4.0 a
0.38	7.0 a	11.3 a	53.3 a	2.0 b
0.56	7.2 a	8.8 a	51.5 a	1.8 b
0.84	7.0 a	11.0 a	38.5 b	1.3 c
Maricopa, Arizona:				
Control	8.9 b	10.8 b	31.0 a	13.8 a
0.56	11.8 ab	13.7 ab	15.8 b	6.0 b
0.84	10.7 ab	13.2 ab	15.8 b	6.5 b
1.12	12.5 a	14.9 a	9.0 b	4.0 b
1.40	12.7 a	14.5 a	11.0 b	5.8 b

[a] Means in a column of each location not followed by the same letter are significantly different, (Duncan's [1955] multiple range test, $P \leq 0.05$).

TABLE 3. Mean[a] Cotton Lint Yields in Ethephon-Treated and Untreated Control Cotton Plots, 1988.

Ethephon (kg AI/ha)	Cotton Lint Yield (kg/ha)	
	Brawley, California	Maricopa, Arizona
Control	756 a	966 ab
0.38	923 a	--
0.56	691 b	1003 a
0.84	628 b	1041 a
1.12	--	752 c
1.40	--	786 bc

[a] Means in a column within a location not followed by the same letter are significantly different, (Duncan's [1955] multiple range test $P \leq 0.05$).

TABLE 4. Effects[a] of Early-Season Ethephon Treatments on Cotton Fruiting, PBW Infestations and Cotton Yield Maricopa, Arizona 1989.

Ethephon Rate (kg AI/ha)	Squares 6/8 Pretrmt	Squares 6-13[b] 6/21	Flowers 6/27[c] 7/14	Flowers 7/20[d] 8/4	Fruiting branches at Nodes 9-14[e] No. Cotton Bolls	Fruiting branches at Nodes 9-14[e] No. Bolls PBW Infested	Lint Yield (kg/ha)	PBW Seed Damage (%)	PBW Larva/100 Bolls[f]
Control	59 a	299 a	77 a	13 b	114 a	7 a	1833 a	13.9 a	3.0 a
0.84	53 a	153 b	47 b	34 a	83 b	1 b	1709 ab	13.6 a	3.8 a
1.12	36 a	104 b	59 b	39 a	62 b	0 b	1491 b	11.7 a	4.8 a

[a] Means 8 replications in the same column not followed by the same letter are significantly different, (Duncan's [1955] multiple range test $P \leq 0.05$).
[b] Means of 4 replications each on 6/13, 6/15 and 6/20.
[c] Means of 4 replications each on 6/27, 6/30, 7/5, 7/7, and 7/14.
[d] Means of 4 replications each on 7/20, 7/28, and 8/4.
[e] Whole plant samples 9/18.
[f] Means 8 replications each on 11, 17, and 25 July and 2 August.

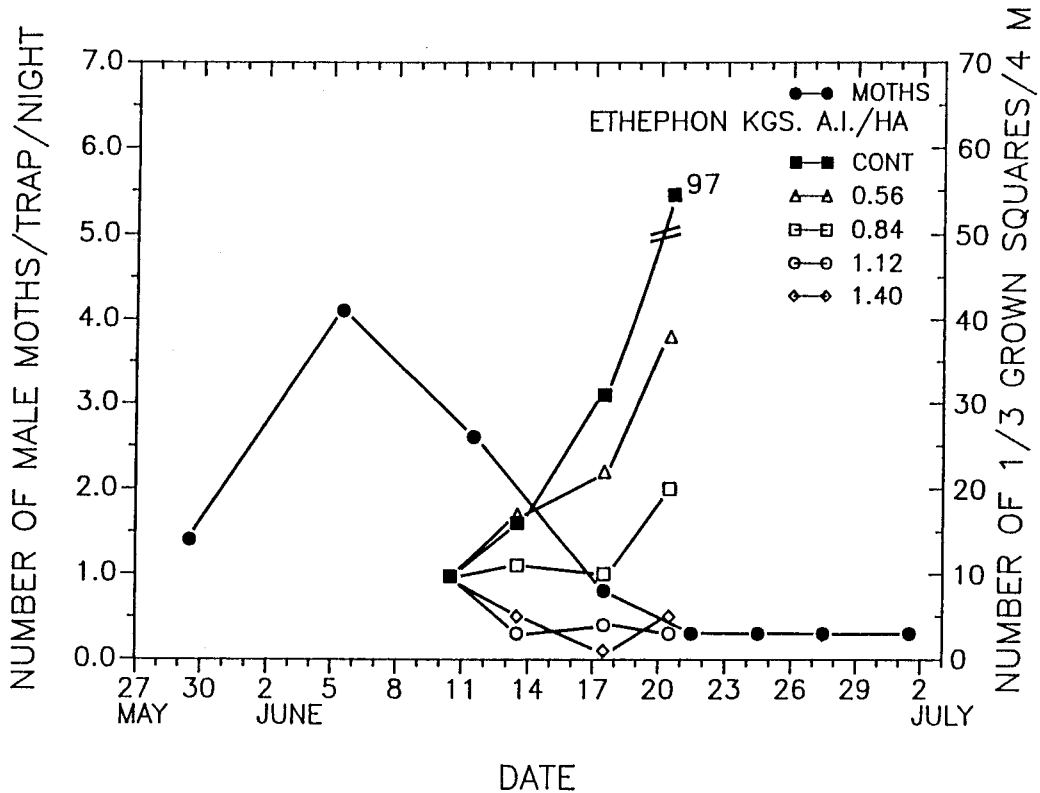

FIG. 2. Mean number of pink bollworm male moths caught per trap per night in relation to the mean number of cotton squares in ethephon treated and untreated control plots at Maricopa, Arizona 1988.

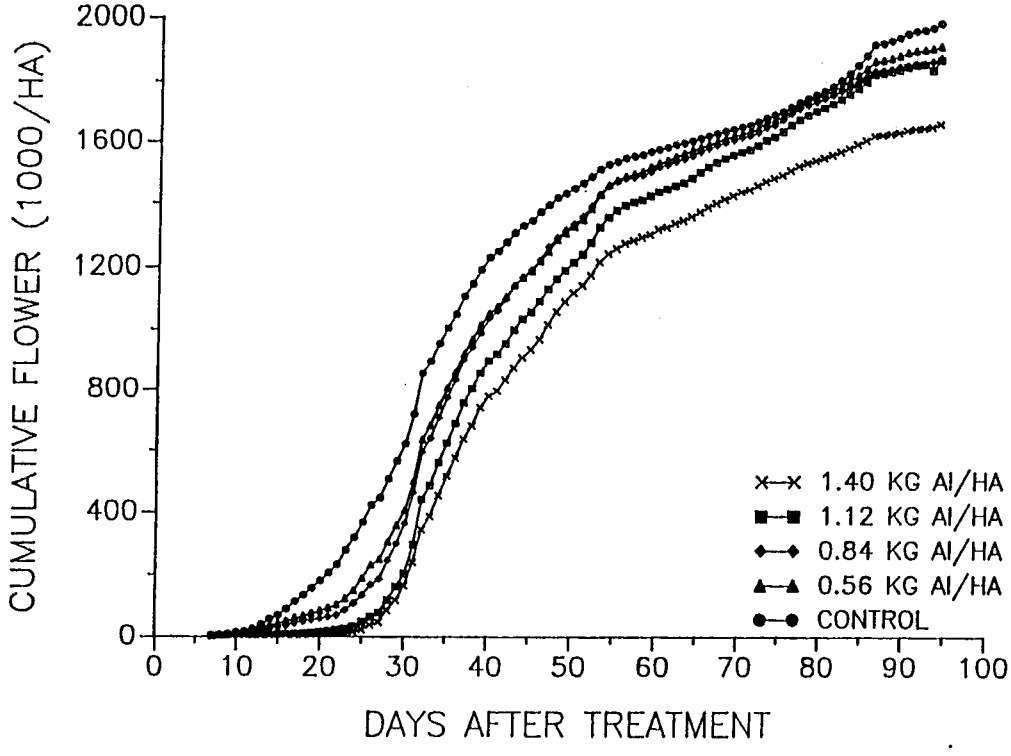

FIG. 3. Mean number of accumulated cotton flowers at Maricopa, Arizona, in ethephon treated and untreated cotton 1988.

4.1 nodes later in ethephon-treated plots (1.12 or 1.40 kg AI/ha) compared to control plots.

Cotton lint yields from plots treated 10 June with 0.56 or 0.84 kg AI/ha were not significantly different from the untreated control, but lint yields were reduced compared to the control at rates of 1.12 and 1.40 kg AI/ha (Table 3). Final yields were negatively correlated (r = -0.50), to numbers of abscised squares but were not significantly correlated to the numbers of healthy squares present (r = 0.43) 1 week following treatment ($P \leq 0.05$).

Maricopa 1989. Ethephon application reduced the numbers of 1/3-grown squares 49 to 65% during the first 8 days following treatment (Table 4). Numbers of cotton fruiting forms and numbers of PBW-infested (open and immature) cotton bolls were significantly higher on fruiting branches 9 to 14 in untreated plots compared to plots treated with 0.84 or 1.12 kg AI/ha. There were no significant differences in PBW-infested bolls sampled 11 July through 2 August nor in percentages of PBW seed damage.

Numbers of flowers were higher in untreated plots compared to ethephon treated plots on days 19 to 33 following application (Table 4). Numbers of flowers in the ethephon treated plots increased thereafter and were higher than in control plots on days 42 to 57 following treatment. Cotton lint yields were lower in ethephon treated plots than in untreated plots but were not significantly reduced except in plots treated with 1.12 kg AI/ha (Table 4).

DISCUSSION

The development of an early-season strategy to limit the reproductive potential of the surviving overwintering PBW population could result in management of the PBW at levels below economic thresholds for most of the cotton-growing season, thus reducing the need for insecticidal control and increasing the potential of beneficial insects. Such a strategy could be integrated with developed methodology for reducing the overwintering population by the use of ethephon and other plant growth regulators (Bariola et al. 1990) and cultural methods (Watson 1980). These practices remove late-season host material and prevent development of the diapause generation, and such strategies have been adopted by many growers.

The results of the present studies show that ethephon removal of squares during the overwintering moth emergence period significantly delayed the initiation of early-season PBW infestations. Reproductive branches on the cotton plant develop new nodes about every 3 days during the growing season (Deterling and El-Zik 1982). Our results showed fewer PBW infested bolls on early fruiting branches as a result of removal of squares with ethephon during the spring moth emergence. This could have a significant impact on the population dynamics of the PBW and delay the development of economic infestation levels. The adverse affect on cotton yields at rates of application above 0.38 kg AI/ha under short-season conditions and 0.84 kg AI/ha under full-season conditions is unacceptable, but may be related in our studies to early harvest, time or rate of application, or cotton cultivar. Our data show the compensatory late-season fruiting response of cotton in relation to removal of early-season squares. In most cases, flowering rates during late-season were significantly higher in plots following early-season square removal with ethephon than in untreated control plots. These results are similar to the report of Ungar et al. (1987) that manual removal of cotton squares in early season did not effect yield, but yield accumulation was delayed up to 3 weeks in some cases. King et al. (1990) also

reported that ethephon applied to problem cotton at rates of 0.34 and 0.68 kg AI/ha delayed flowering for approximately 3 weeks, after which rates of flowering were higher in ethephon treated as compared to untreated plots, and lint yields were not affected. The ability of cotton plants to compensate for loss of early-season squares is well known (Wilson 1982, 1986). Obviously, early-season square removal delays crop maturity. Competitive yields and acceptable lengths of growing season must be maintained to be practical. The potential benefits of developing early-season PBW suppression technology to integrate with cultural and other methods to reduce late-season overwintering populations justifies continued research to further investigate this concept.

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SEASONAL DYNAMICS OF SWEETPOTATO WHITEFLY IN ARIZONA

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ABSTRACT

The sweet potato whitefly (SPWF), *Bemisia tabaci* (Gennadius), has become a serious problem of a number of agricultural crops in the southern U.S. In the Southwest, SPWF seriously affects summer crops such as melons and cotton as well as fall, winter and spring vegetable crops such as lettuce, broccoli and cauliflower. Since this insect has no overwintering stage, a succession of host plants is necessary to span the gap from cotton season to cotton season. This study characterized seasonal population trends of SPWF in cotton and identified subsequent hosts which were important in the overwintering survival of this insect. The "off-season" hosts included certain weeds as well as cultivated crops; all appear to be important in the seasonal population dynamics of this species.

INTRODUCTION

In the more southerly areas of the U.S. cotton belt, the sweetpotato whitefly, *Bemisia tabaci* (Gennadius) (SPWF) has become a significant problem in cotton production and has now extended its host range to include many additional agricultural crops. Therefore, the WF problem is not merely a cotton problem or a vegetable problem, but one of the general "crop ecology". This indicates the need to attack the problem on a community or regional basis in order to satisfactorily manage this pest.

The SPWF was first collected from cotton, *Gossypium* spp. in Arizona, in 1926 and in California in 1928 (Russell 1975). Throughout the past decade, the importance of the SPWF as a pest in irrigated vegetable and fiber crops has increased dramatically in the arid regions of Arizona and California and in the adjacent state of Sonora Mexico (Brown 1991). Populations reached unprecedented levels in cotton during 1981 in Arizona and California (Duffus and Flock 1982, Butler and Wilson 1984) and in vegetable crops including carrots, *Daucus carota* L.; lettuce, *Lactuca sativa* L.; melons, *Cucumis melo* L.; squash, *Cucurbita* spp. Duch.; and tomatoes, *Lycopersicon esculentum* Mill. in the southwestern U.S. and west coastal Mexico (Brown and Nelson 1984). More recently, SPWF has proliferated to include additional crops over wider areas. The authors have found infestations and severe damage on other crops such as alfalfa, *Medicago sativa* L.; broccoli, *Brassica oleracea* L.; cauliflower, *Brassica oleracea* L.; and peanuts, *Arachis hypogaea* L. Severe infestations were reported on cotton and/or vegetables during 1991 in such diverse areas as Arkansas, Florida, Georgia, Tennessee, and Texas.

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Damage to cotton caused by whiteflies (WF) is mostly associated with sticky lint and sooty molds caused by the honeydew produced (Gerling et al. 1980) and, to a lesser extent, reduced yields (Mound 1965). The sticky cotton problem resulting from honeydew secreted by WF may seriously reduce market price and the ability to market the fiber because of problems in ginning and milling. Where heavy infestations occur, harvesting also may be hendered.

The seasonal dynamics of WF, both bandedwinged (BWWF), *Trialeurodes abutilonea* (Haldeman) and SPWF, in cotton have been intensely studied during the past 3 years at the University of Arizona, Yuma Valley Agricultural Center (YVAC). Based upon earlier observations, SPWF populations appeared to escalate markedly in later stages of the cotton production season. The characteristic explosion of SPWF in cotton seemed to occur in close coincidence with crop cut-out and the late season development of the top crop. Since SPWF has no well-defined overwintering stage, reproduction continues throughout the year. Thus, other hosts are necessary after the cotton season for SPWF to survive until the next cotton crop.

A field experiment was initiated in 1989 to document the relationship between SPWF and the growth and developmental patterns of cotton. The cotton phase of this study utilized both short-, *G. hirsutum* L., and long-staple, *G. barbadense* L., cotton varieties, two planting dates and two irrigation termination dates to determine effects of these cotton production systems on the seasonal dynamics of SPWF. An additional objective was to determine the hosts that are important for SPWF survival during winter months. These crops and selected weed hosts, alkali mallow, *Sida hederacea* (Doug.) Torr.; globemallow, *Sphaeralcea* spp.; ground cherry, *Physalis wrightii* Gray; little mallow or cheeseweed, *Malva parviflora* L.; London rocket, *Sisymbrium irio* L.; and spiny sowthistle, *Sonchus asper* (L.) Hill, were sampled during the "off-season" to gain a better understanding of their importance as alternate hosts.

METHODS

The YVAC experiment was conducted on an Indio silt loam [coarse-silty, mixed (calcareous), hyperthermic Typic Torrifluvent]. The Yuma Valley extends north from the U.S.-Mexico border approximately 25 km along the east (Arizona) side of the Colorado River. Elevations range from approximately 23 to 53m above sea level. Treatments consisted of Upland and Pima cotton types, planting dates (PD), and irrigation termination dates (IT). Cotton types (Upland var. DPL90 and Pima Var. S-6) served as mainplots, while PD and IT combinations were factorily arranged as subplots within a randomized complete block design with four replications. In this manner, each distinct PD by IT treatment combination consisted of an experimental unit with 16 rows that were 300 feet long and 40 inches apart. The PD and IT combinations are outlined in Tables 1 and 2. Planting dates were chosen with one date close to an optimum of approximately 300 to 500 heat units (UH, 86/55 F thresholds, Brown 1989) and a second PD slightly past an optimum point (Silvertooth et al. 1989, Silvertooth and Farr 1990, Silvertooth 1991, and Brown et al. 1992) due to the fact that highest yields often are obtained with more indeterminate varieties at relatively early PDs, which also respond to delays of planting past an optimum period with substantial declines in yield potential and increased vegetative tendencies (Silvertooth et al. 1989).

The dates of IT were selected with the initial date of termination imposed at a time after cut-out in the Upland crop, so that bolls set prior to cut-out could be matured with adequate soil water (Silvertooth 1990). That estimate required a projection of time, approximately 600 HUs to mature the fruit while maintaining

available soil water (Silvertooth et al. 1991). The first date of IT with the Pima crop was somewhat more subjective or arbitrary due to a more sustained flowering and fruiting phase, with often no distinct cut-out. Thus, this decision was based upon the season and the fruiting pattern of the crop. The second date of IT usually received two additional irrigation events past the early IT treatment, allowing the development of a "top crop" or second fruiting cycle as in the case of the Upland crop.

Irrigations were applied on the regular farm schedule, ca. every 14 days, throughout the season in a manner that maintained a non-stress condition for all experimental units. Plant nutrition was maintained optimally by use of University of Arizona guidelines and required only nitrogen (N) fertilization as a nutritional supplement. The N fertility for both the Upland and Pima crops was maintained by use of petiole sampling and crop monitoring (Silvertooth and Doerge 1990).

Weekly sampling of WF populations in all treatments was conducted throughout the season by use of sticky traps (adults) and leaf samples (egg and immature stages). As cotton plants continued to grow, they were subdivided into either two (1991) or three (1989 and 1990) substrata sampling sites to determine the effect of within-plant-canopy site on population growth of this insect. A 1-inch diameter leaf disc from each of five leaves per sample site comprised a sample unit. These were examined under a binocular microscope to determine numbers of eggs and immatures.

Early studies revealed that the WF in cotton was a component of a crop ecology complex involving alternate hosts that permitted the WF to bridge the gap from cotton season to cotton season. Therefore, plants that served as late fall, winter and early spring hosts were included in the year-round sampling plan to better understand the annual dynamics of SPWF populations. These involved lettuce, broccoli and cauliflower as cultivated hosts, and the weed hosts alkali mallow, little mallow, London rocket and spiny sowthistle.

Sampling sites were selected throughout the Yuma Valley to study the post-cotton season dynamics of WF populations on fall and winter vegetable crops. Data from both sticky traps and leaf samples were utilized to assess population changes. Fields were selected in close proximity to cotton fields and in relation to the sequence of planting dates of the fall crops. In some experiments, lines of sticky traps were installed to study WF dispersal away from the source host and in relation to wind flow. Sample units were similar to those for cotton described earlier.

RESULTS AND DISCUSSION

Various agronomic and production inputs associated with the cotton production system at the YVAC are presented in Table 1. Table 2 shows season-long population trends of the BWWF and SPWF in the PD and IT treatments for both Pima S-6 and DPL-90 cotton varieties. Abbreviations are made for PD and IT dates as A1 or A2 and B1 or B2, respectively. The collective description of all WF stages from each treatment for each sample date clearly demonstrates the seasonal population trends and the nature of late-season infestations on cotton.

For purposes of illustrating seasonal trends of both egg and immature stages of the SPWF for each PDxIT treatment, only those data from the top stratum are presented. To clearly show the significance of the SPWF problem in the latter part of the growing season, Figs. 1 and 2 present total SPWF stages for the first-PD and first-IT, and second-PD and second-IT treatments, respectively. Again, dates and levels at which populations peaked clearly show the influence of late-season termination of irrigation.

TABLE 1. Agronomic and Other Production Data Relative to Cotton Management for Whitefly Control, Yuma, Az 1990.

	Planting date/irrigation termination for two cotton varieties[1,2]							
	A1B1		A1B2		A2B1		A2B2	
Inputs/Yields	SS	LS	SS	LS	SS	LS	SS	LS
Total N/A (lbs.)	210	210	210	210	210	210	210	210
Total irrigations[3]	4	5	6	7	6	7	9	9
Number pesticide applications	12	12	12	12	12	12	12	12
Last application	8/20	8/20	8/20	8/20	8/20	8/20	8/20	8/20
Irrigation termination dates	7/10	7/18	8/07	8/07	8/07	8/07	9/06	9/06
Defoliation[4]	8/16	8/16	8/30	8/30	8/30	8/30	9/19	9/19
Harvest dates	8/28	8/28	9/12	9/12	9/12	9/12	10/5	10/5
Mean number of lbs. of cotton/acre	2026	1512	3327	1633	3348	2051	3478	2268
Honeydew rating[5]	Low	Low	Med.	Med.	Med.	Med.	High	High

[1] PD/IT legend: A1=Planting Date 1 (2/27/90); A2=Planting Date 2 (3/21/90; B1=Irrigation Termination 1; B2=Irrigation Termination 2.
[2] Cotton varieties: SS=DPL 90; LS=Pima S-6.
[3] Number irrigations after planting.
[4] Defoliation: Drop+Accelerate
[5] Visual rating of honeydew contamination of lint.

A review of captures of adults on sticky-traps revealed a close similarity to that of immatures on leaf samples. Adult catches were much greater late in the season in the A2B2 treatment in both DPL-90 (Fig. 3) and Pima S-6 (Fig. 4). These figures represent the latter part of the seasonal curves, when populations increased sharply. Even though SPWF populations started at very low levels in young cotton and remained low until well past mid-season, population increases occurred so rapidly in late season that severe damage resulted from honeydew contamination on lint where cotton growth was allowed to progress for the entire season.

Populations of SPWF moved from cotton to vegetables in the fall. Reproduction continued on broccoli and cauliflower. Reproduction on lettuce was observed for the first time in the fall of 1991. These crops, plus suspected weed hosts, appear to be the primary link that perpetuates the SPWF problem from one cotton season to the next.

Because of the difficulty of controlling the SPWF in cotton with insecticides, it is believed that the most desirable method of suppressing this pest in cotton is to block its development and prevent its establishment and reproduction in cotton. Therefore, a major purpose of this project was to investigate the dynamics of the SPWF during the non-cotton season on other hosts. The primary goal was to find a weak link to break the cycle between cotton seasons. The following discussion and figures illustrate results and analysis in this direction.

Table 2. Combined Total Numbers of Eggs, Crawlers, Immatures and Adults of Both Sweetpotato and Bandedwinged Whiteflies/Week/Treatment[1] in Both Pima S-6 and DPL 90.[2]

Date	TOTAL	
	PIMA S-6	DPL 90
5/09	89	100
5/14	66	75
5/22	27	31
5/29	15	14
6/05	18	11
6/11	5	3
6/19	19	10
6/25	14	5
7/05	48	59
7/10	93	53
7/16	141	61
7/24	1071	614
7/31	1757	1397
8/06	3357	2301
8/13	5440	2740
8/21	17677	10115
8/28	16783	11310
9/12	25333	29152
9/19	22896	20608

[1] Treatments: A1=Planting Date 1 (2/27/90); A2=Planting Date 2 (3/21/90; B1=Irrigation Termination 1; B2=Irrigation Termination 2.
[2] WF species ratios: From 5/9 to 5/22 population was 100% Bandedwinged; from 5/29 to 6/19 a 50-50 sweetpotato to Banded-wing ratio; from 6/25 to 9/19 population was 100% sweetpotato.

Pre-cotton season. During the spring, both BWWF and SPWF were present in the field. It appears that BWWF is more prevalent earlier in the year, gradually declines and is later replaced in summer field crops by SPWF. Figures 5 and 6 illustrate this in cotton and okra, *Hibiscus esculentus* L., respectively. Other spring crop hosts which support season-long populations of SPWF are squash, watermelons, *Citrullus lanatus* (Thumb.) Mansf., and cantaloupes (Fig. 7). These are important in cotton production since higher populations in these crops were correlated with higher populations in nearby cotton.

Post-cotton season. Studies in the late summer and fall were conducted to determine relationships of fall populations in cotton to subsequent WF population buildup in alternate hosts such as fall and winter vegetables (lettuce, broccoli, and cauliflower) and the sequencing of hosts as it relates to the re-infestation of the next year's cotton.

The location of fall host plants relative to sites of infested cotton fields, in terms of both distance and direction relative to wind flow, appears to be extremely important in vulnerability to SPWF infestations. Fig. 8 illustrates the effect of distance, and Fig. 9 shows trends of adult, egg, and immature populations in fall lettuce in fields bordered on the north or south by infested cotton. Prevailing winds are commonly from the north during this part of the year in the Yuma Valley, and Fig. 9 shows the effect of these northerly winds. Fig. 10 shows similar population trend comparisons with cotton fields located east and north of fall cauliflower fields. In summary, these figures show: 1) the farther removed a new host is from the SPWF source, the less the likelihood of becoming infested, and 2) newly-planted hosts located downwind from the SPWF source are more likely to become infested.

Date of alternate host availability relative to the end of the cotton season appears to be an important factor in perpetuating fall populations of SPWF. For example, Fig. 11 shows declining SPWF egg population levels with each delay of plantings of lettuce and cauliflower. Another illustration (Fig. 12) shows relative abundance of SPWF adults on lettuce, broccoli and cauliflower during the same time period and in the same general location, indicating that some preference may be involved in host selection. Fig. 13 shows population trends of the different SPWF stages on the same host, cauliflower.

Winter Season. Monitoring data from the winter vegetable season of 1990-91 show that SPWF of adults, eggs, and immatures decreased drastically when winter temperatures dropped, but populations did not disappear completely from vegetables such as broccoli, cauliflower, and lettuce. Reproduction did not cease, although it was substantially lower due to the cold weather. It took intensive sampling to find eggs or immatures in their late instar stages on broccoli and cauliflower. Adults were found on lettuce but no immature stages past the second instar were found. This indicated that the SPWF did not complete its life cycle on the winter crop of lettuce. During December 1991, however, empty pupal cases were found on lettuce, indicating that at least a small portion of a population can complete its life on lettuce. The SPWF was also more abundant in and near vegetable fields in December 1991, compared with past infestations, and were also found reproducing in alfalfa. This trend may be attributed to a change in the basic biological requirements of the SPWF. A new biotype (B) which is distinctly different from an earlier SPWF biotype (A), based upon biological characteristics and differences in genetic (esterase) markers, has recently been identified (Brown et al. 1991, Costa and Brown, 1991).

In early February 1991, sampling procedures were initiated on the most abundant weeds throughout the Yuma Valley: alkali mallow, *Sida hederacea* (Doug.) Torr.; London rocket, *Sisymbrium irio* L.; and spiny sowthistle, *Sonchus asper* (L.) Hill, were sampled. Usually these weeds grow undisturbed and are commonly found on ditchbanks or at the edge of cultivated fields. It is important to mention that the weeds sampled had 10-20 times more WF (BWWF and SPWF) than did the vegetables in mid-winter and early February. BWWF and SPWF adults and eggs were present on London rocket, but no immatures were found; adults, eggs, and immatures of both species were found on spiny sowthistle. Cast skins of immatures were found on this weed, thus complete reproduction was indicated. More immatures than eggs were found on alkali mallow, and the ratio of BWWF:SPWF adults was ca. 3:1. The number of whitefly adults on weeds diminished after early March due to cool rainy weather.

WF populations declined during March compared with February levels, primarily because of unseasonably cool March weather. During late March, some BWWF and SPWF adults were found on cantaloupes and early cotton between San Luis and Somerton, Arizona. We believe that WF came from alkali mallow on nearby ditch-

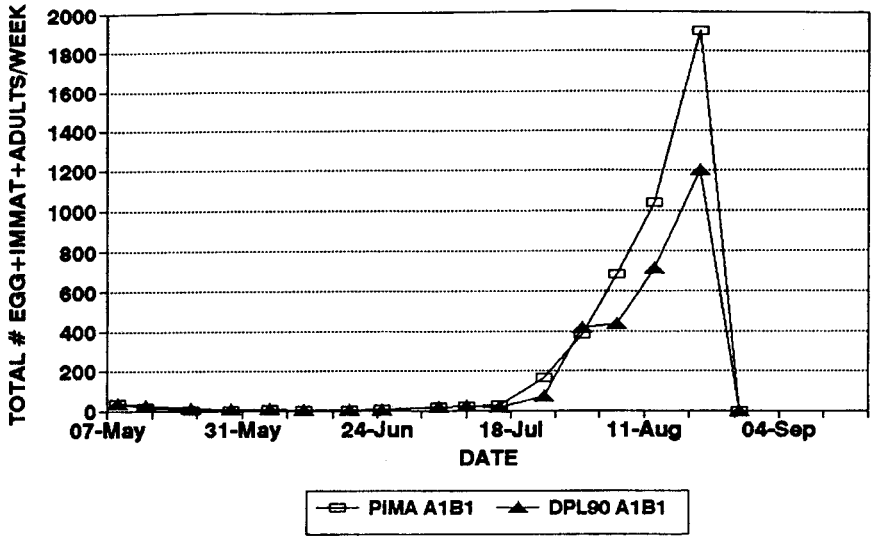


FIG. 1. Comparative seasonal sweetpotato whitefly population trends in Pima S-6 and DPL-90 cotton for the first planting (A1) and first irrigation termination (B1) treatment.

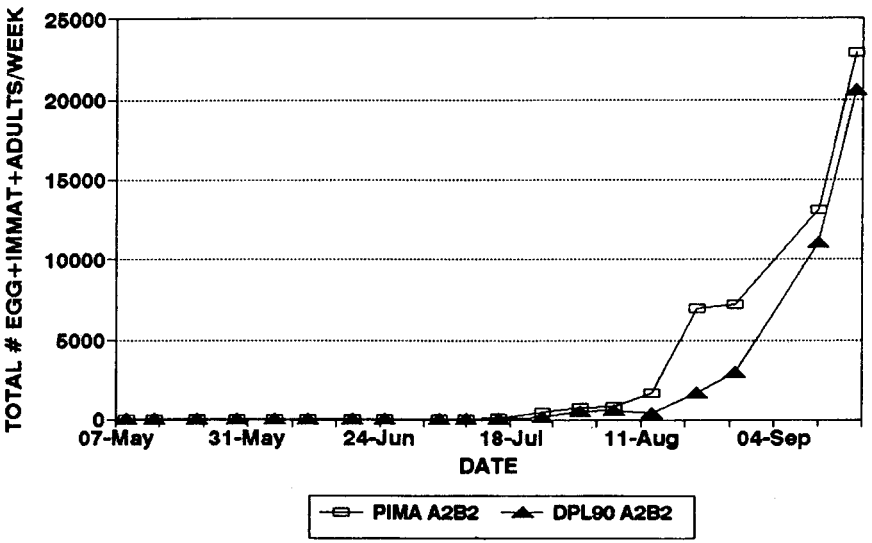


FIG. 2. Comparative seasonal sweetpotato whitefly population trends in Pima S-6 and DPL-90 cotton for the second planting (A2) and second irrigation termination (B2) treatment.

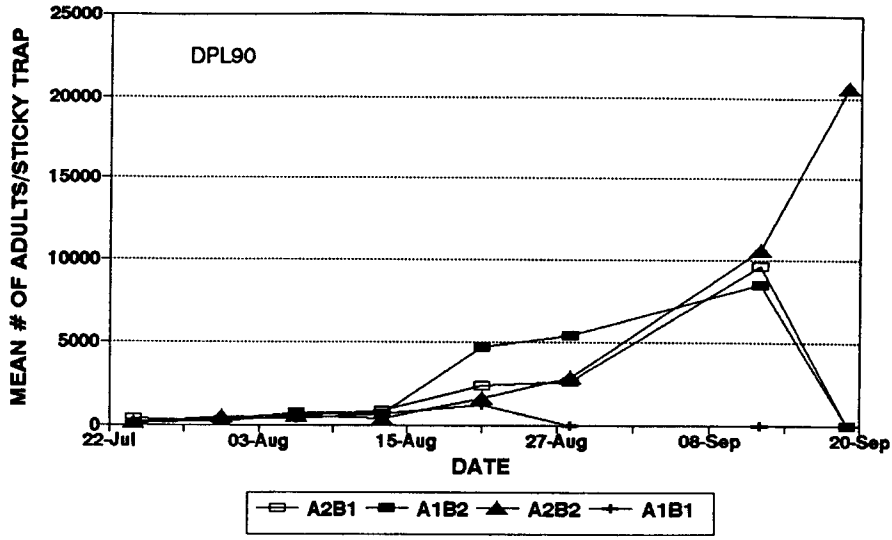


FIG. 3. Comparative seasonal adult sweetpotato whitefly population trends in DPL-90 for all PD and IT treatments.

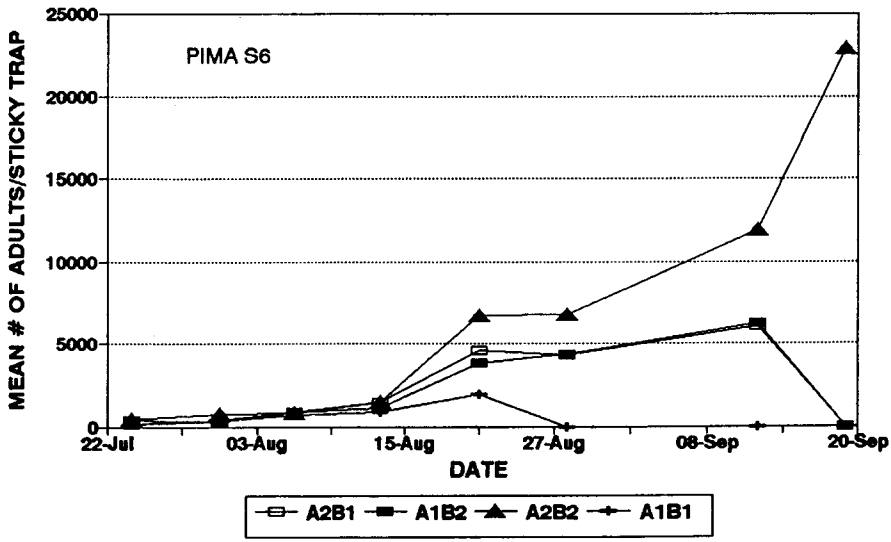


FIG. 4. Comparative seasonal adult sweetpotato whitefly population trends in Pima S-6 for all PD and IT treatments.

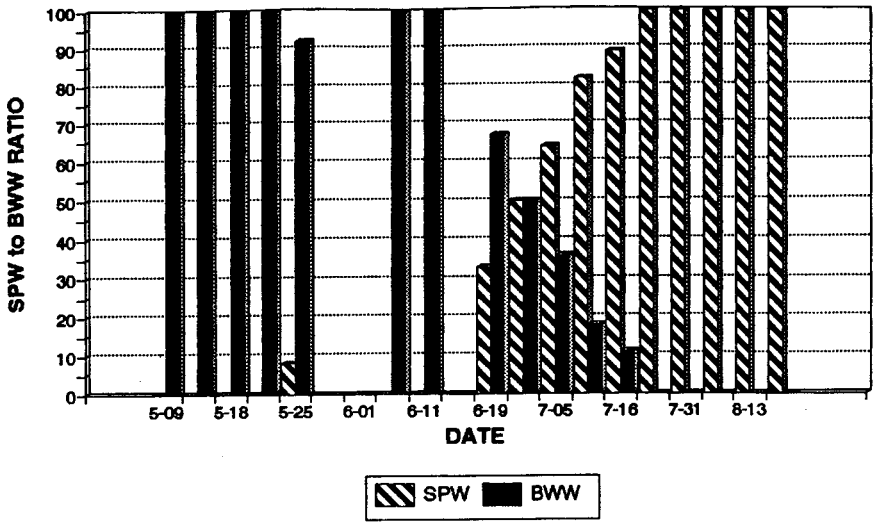


FIG. 5. Seasonal ratio of bandedwinged to sweetpotato whitefly in DPL-90 in the PD1 (planting date 1) treatment.

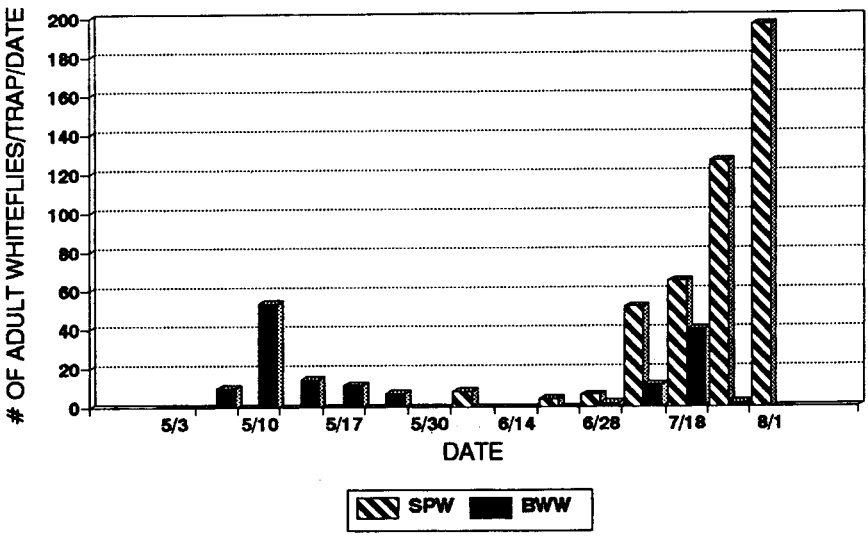


FIG. 6. Seasonal occurrence of bandedwinged and sweetpotato whitefly on okra.

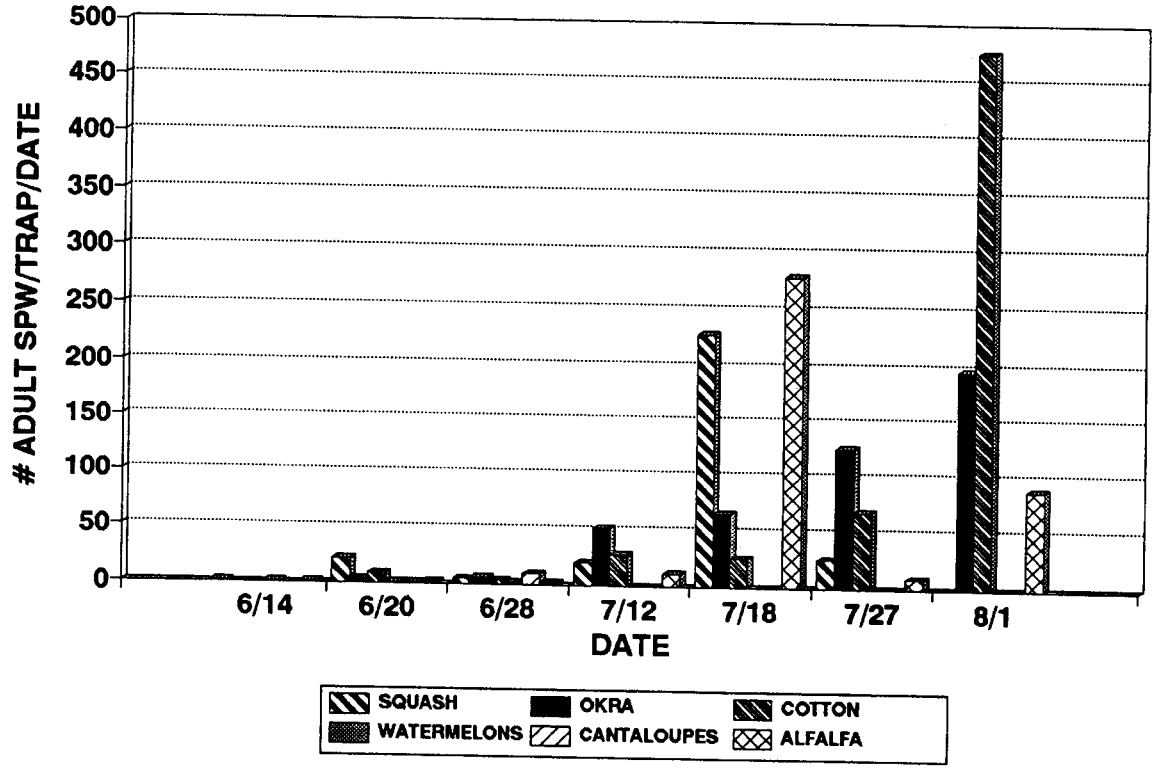


FIG. 7. Seasonal sticky trap catches of adult sweetpotato whitefly in various summer crops.

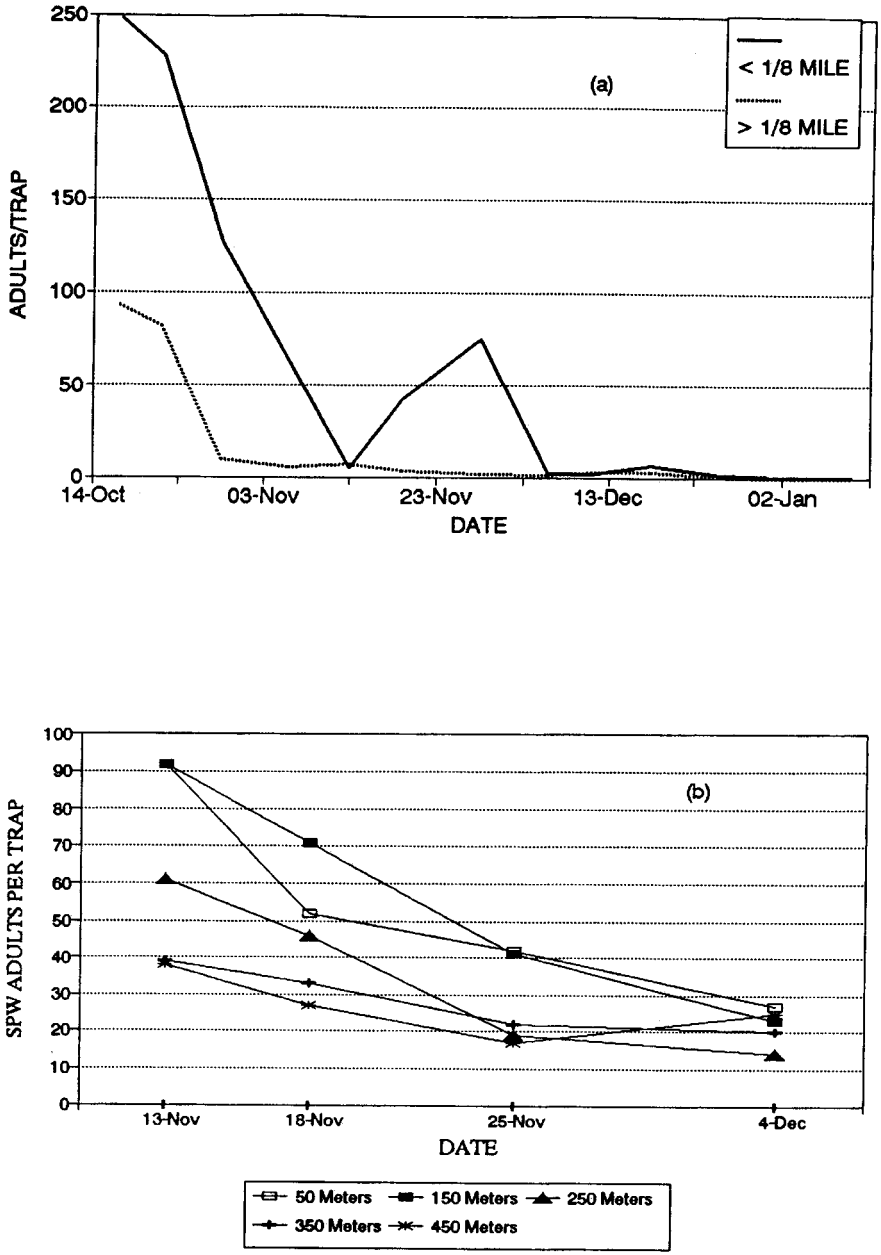
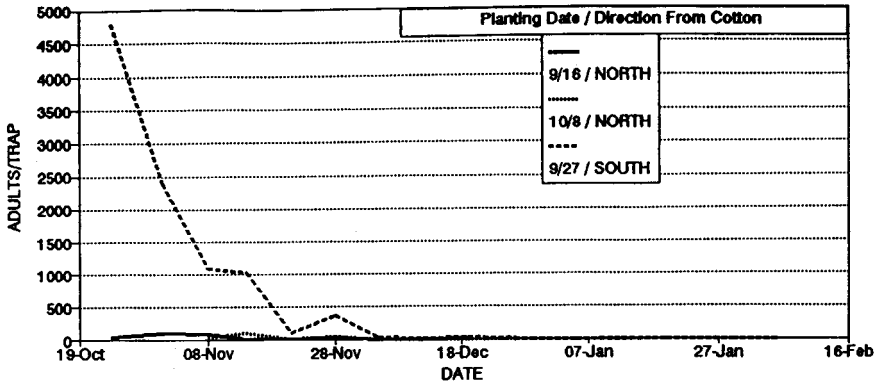
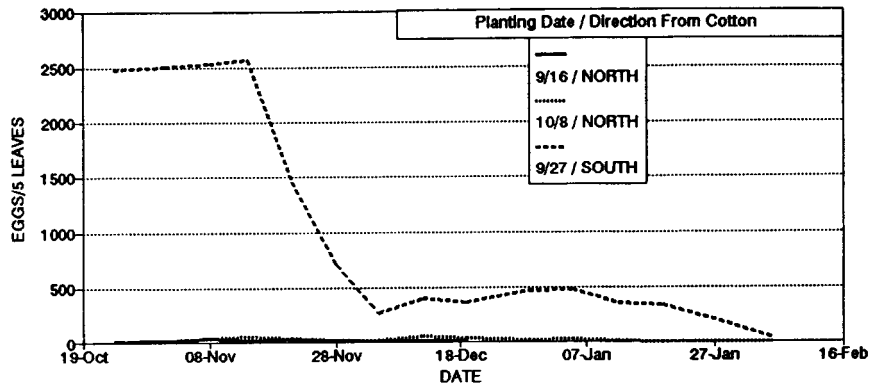


FIG. 8. Comparative adult sweetpotato whitefly population trends in lettuce trapped less than or greater than 1/8 mile from adjacent cotton (a) or at specified distances from the cotton source (b).





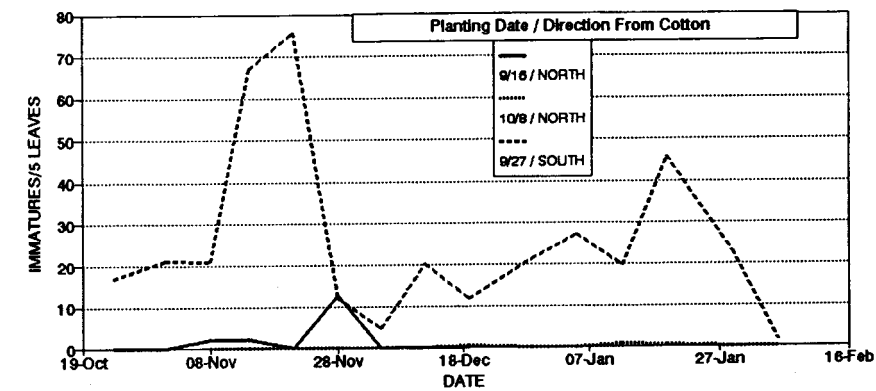
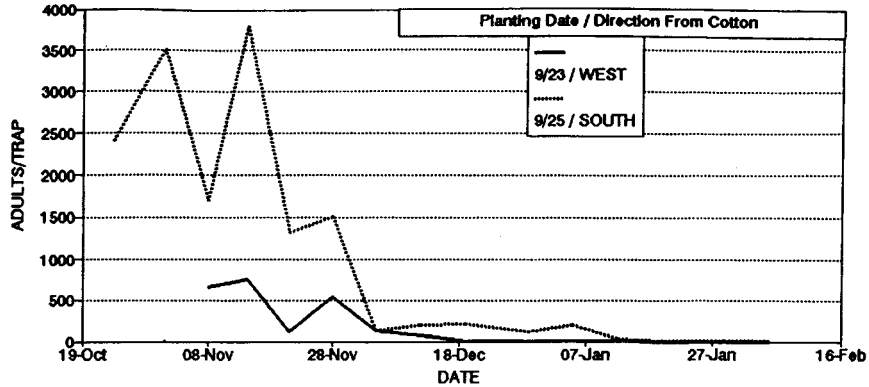
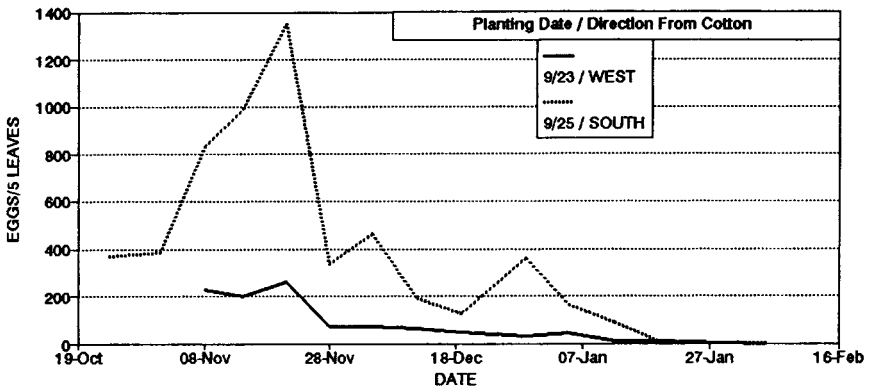


FIG. 9. Fall population trends of sweetpotato whitefly in lettuce with cotton located either to the south or north.





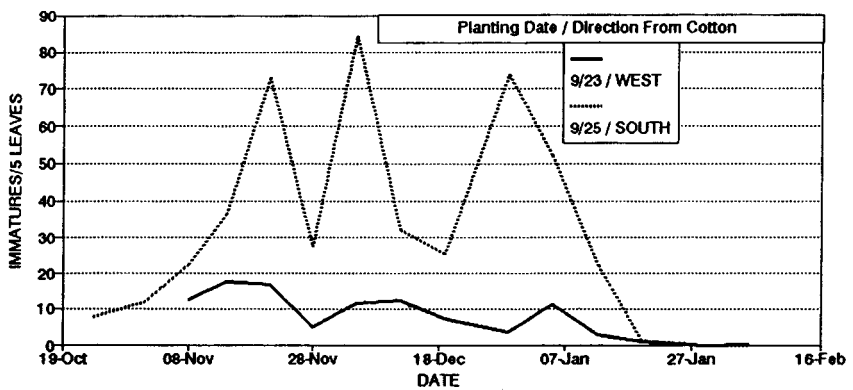
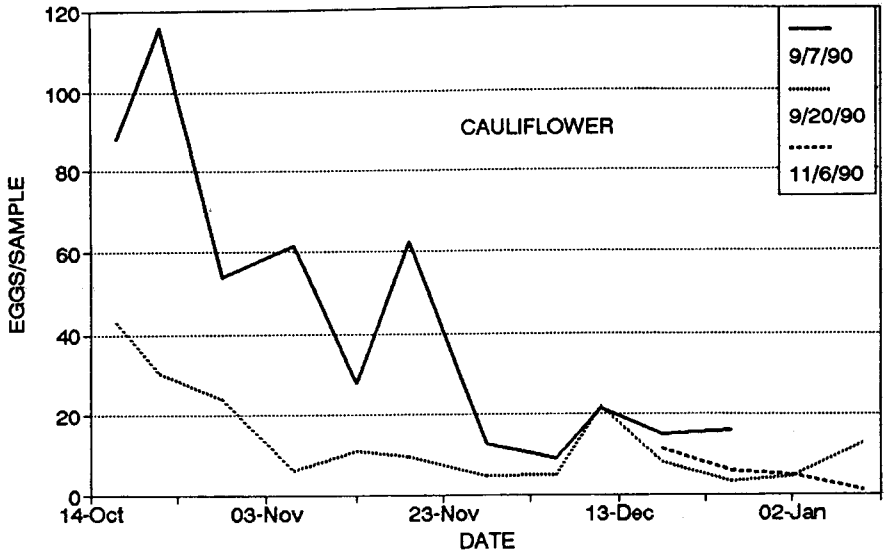


FIG. 10. Seasonal population trends of sweetpotato whitefly in cauliflower with cotton located either to the east or north.



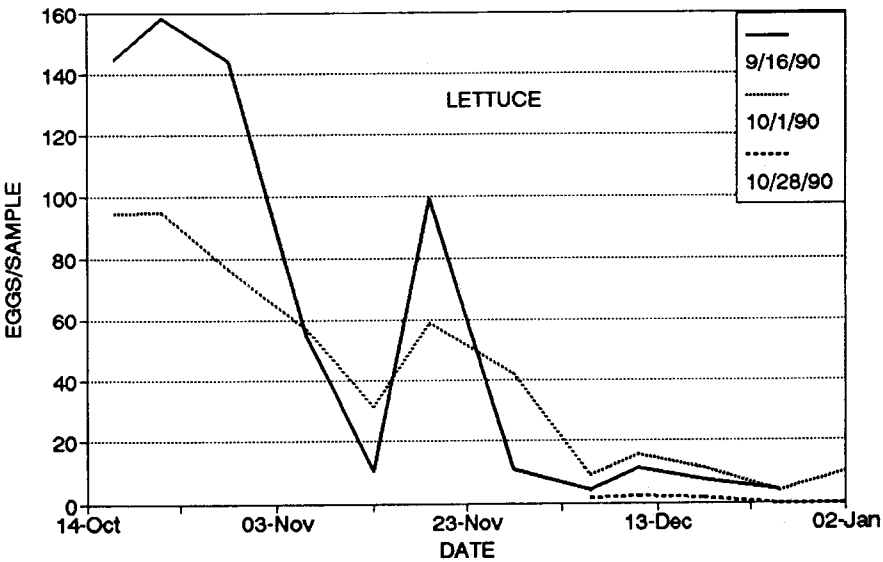


FIG. 11. Influence of planting date on sweetpotato whitefly egg populations in Fall cauliflower and lettuce.

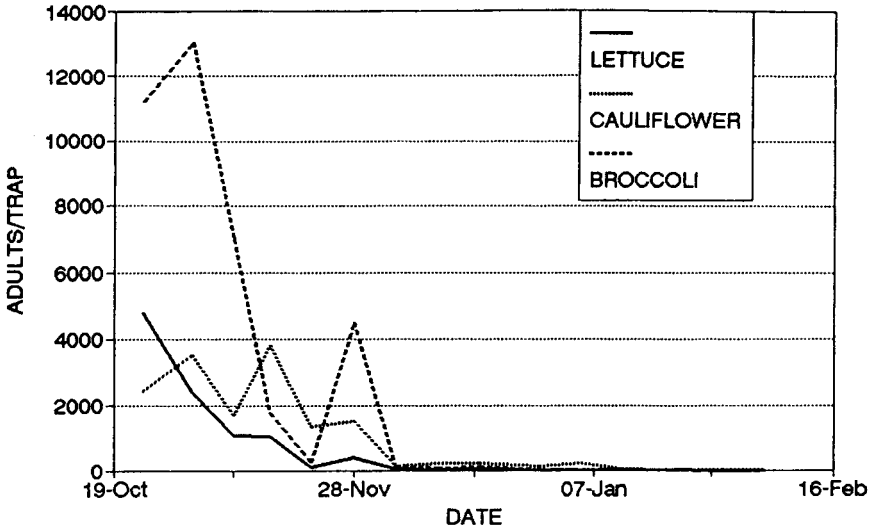


FIG. 12. Comparative adult sweetpotato whitefly populations in lettuce, cauliflower and broccoli with cotton located to the north.

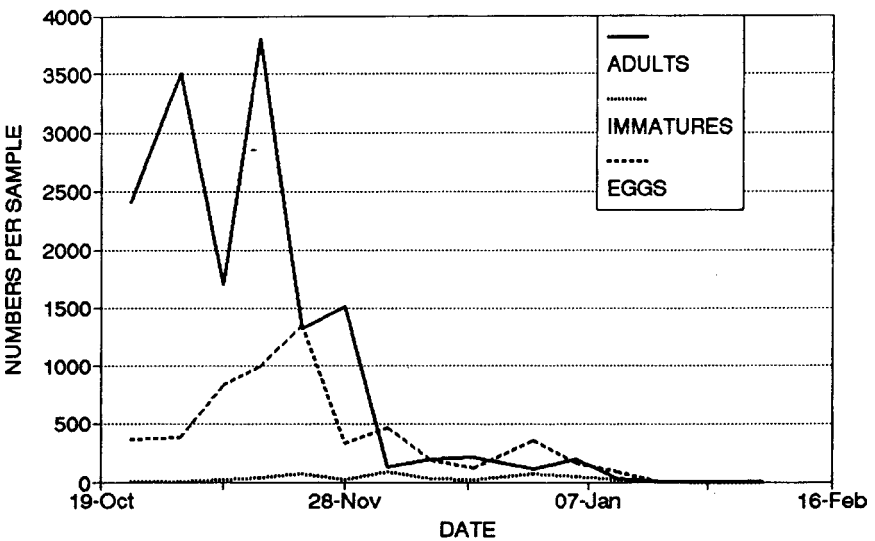


FIG. 13. Population trends of adult, egg and immature stages of SPWF in cauliflower with cotton located to the north.

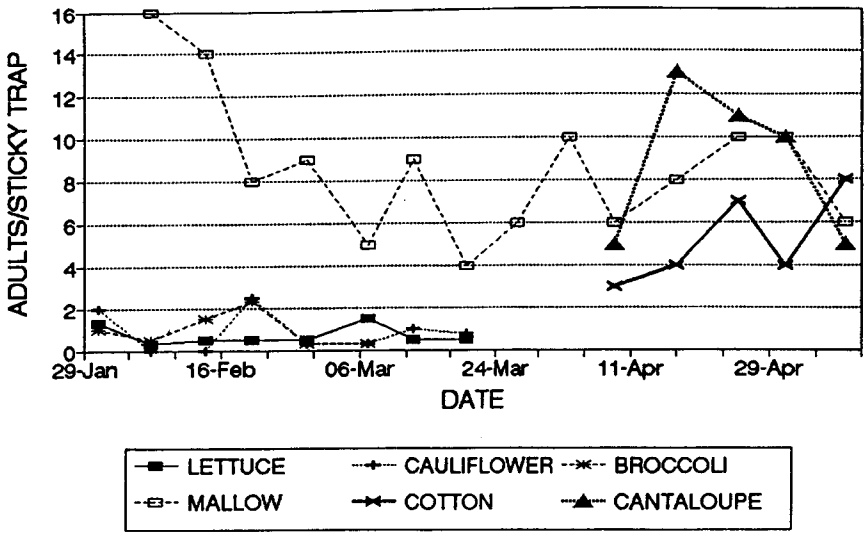


FIG. 14. Host sequence of SPWF populations in late winter and spring relative to subsequent infestations in cantaloupe and cotton (1991).

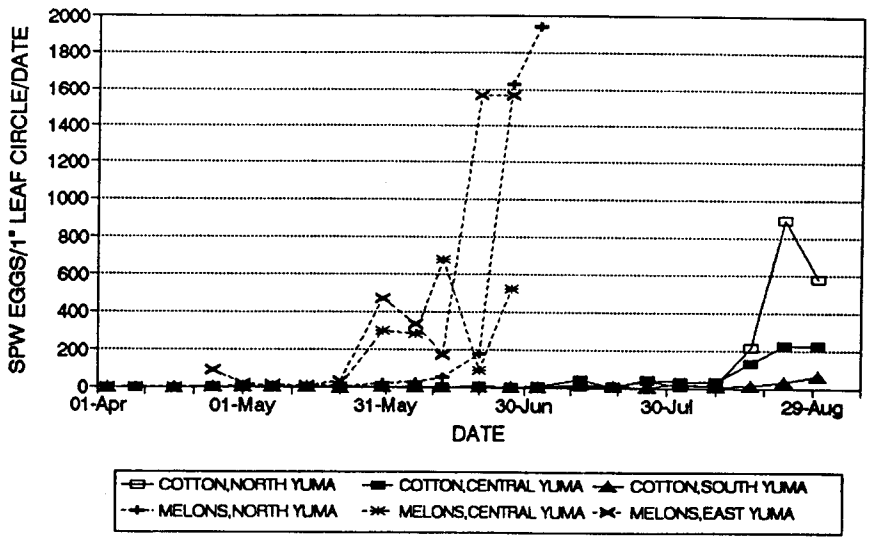


FIG. 15. Correlation of SPWF egg populations in spring cantaloupe with later development in cotton.

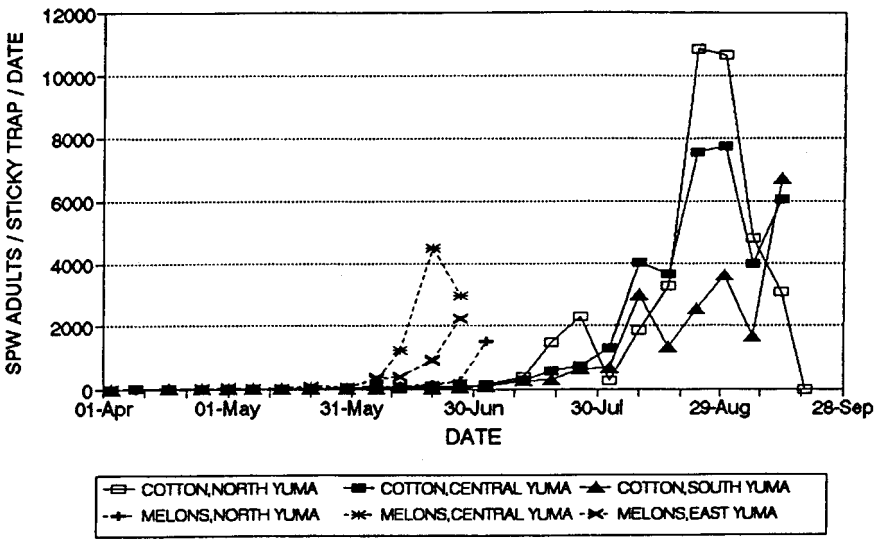


FIG. 16. Correlation of SPWF adult populations in spring cantaloupes with later development in cotton.

banks. Intensive monitoring of the population dynamics of the SPWF indicated that WF moved in mid-winter to weeds from vegetable fields and from weeds to cotton and curcurbits in the late-winter months. Dispersal of WF appears to be greatly dependant upon wind direction.

Fig. 14 indicates that the infestation in mallow had already occurred by early February and appears to be the prime source of WF that move to cantaloupes and cotton in early April. The importance of WF egg and adult populations in spring cantaloupes to those subsequently occurring in cotton are illustrated by Figs. 15 and 16, respectively. These graphs depict WF populations in three areas of the Yuma Valley, designated North, Central and South Yuma. Relative levels of WF obtained on cantaloupes in each area correspond directly with levels subsequently obtained on cotton.

Our research to date indicates that WF populations in cotton increase dramatically from mid-to late-season. Following the cotton season, WF disperse, usually downwind, to fall and winter melon and vegetable crops. Winter weather greatly slows WF development and reduces overwintering populations, nevertheless, populations do persist and are present to re-invade the subsequent cotton crop. The most vulnerable time of the year for the WF is late winter when populations are at their lowest level. At this time a combination of several practices on an area-wide basis might result in breaking the cycle and preclude a subsequent problem in melons or cotton. These practices would include: residue disposal (shredding and tillage) as soon as the last harvest is completed on winter vegetables; treatment of weeds along irrigation ditches with herbicides and/or insecticides; planting of melons and cotton as far away as possible, and upwind, from winter vegetable fields; delayed planting of cotton to coincide with optimum heat unit accumulation; and, use of effective

insecticides to treat seedling cotton adjacent to WF sources. In order to be successful, a carefully coordinated community-wide effort would be required, with all practices deployed on a timely basis.

An earlier IT date provides an alternative to a long-season cotton production system which results in development of a top-crop and thus, creates a favorable habitat for accelerated growth of SPWF populations. The earlier IT needs to be timed to provide adequate soil moisture to complete maturation of the first fruit set up to the cut-out period. These data indicated that such a system would provide a useful management tool for optimal production of cotton, including both quantity and quality. This is essentially an "avoidance" strategy for a cotton production system. In considering this option, differences in yield potential and declining quality associated with increasing numbers of SPWF must be carefully and realistically considered. It would also be advantageous to collectively manage the IT of cotton fields in a given area so that populations of SPWF do not pose an overwhelming threat to the few fields being maintained (irrigated) for late season production. Delays in the planting of early lettuce or other vegetable crops would also by detrimental to SPWF populations if cotton crops were terminated in a synchronous, community-based level.

ACKNOWLEDGMENT

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LA COMPOSICION DE PRESAS DE LA AVISPA LODERA *SCELIPHRON JAMAICENSE LUCAE* [1] EN LA REGION DEL CABO, MEXICO.

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ABSTRACT

Nests with prey of the mud-dauber wasp *Sceliphron jamaicense lucae* (Saussure) (Hymenoptera: Sphecidae) from five localities in Baja California Sur, México, were identified and analyzed. The prey were 36 species of spiders from 14 families. The most frequently captured spiders were *Misumenops sierrensis* (Schick) (Thomisidae), *Cheiracanthium inclusum* (Hentz) (Clubionidae), *Olios peninsulanus* Banks (Heteropodidae), *Isaloides* sp. (Thomisidae) and *Aysha incursa* (Chamberlin) (Anyphaenidae). We describe some ecological aspects of the wasp, including its relationship with other solitary wasps.

RESUMEN

En este trabajo, se da a conocer la composición de presas de los nidos de la avispa lodera *Sceliphron jamaicense lucae* (Saussure) (Hymenoptera: Sphecidae) de cinco localidades de Baja California Sur, México. Las presas fueron exclusivamente arañas pertenecientes a 36 especies de 14 familias. Las especies de arañas capturadas con más frecuencia fueron *Misumenops sierrensis* (Schick) (Thomisidae), *Cheiracanthium inclusum* (Hentz) (Clubionidae), *Olios peninsulanus* Banks (Heteropodidae), *Isaloides* sp. (Thomisidae) y *Aysha incursa* (Chamberlin) (Anyphaenidae). Se describen algunos aspectos ecológicos de la avispa, incluyendo sus relaciones con otras avispas solitarias.

INTRODUCCION

El género *Sceliphron* (Hymenoptera: Sphecidae) agrupa 30 especies ampliamente distribuidas en el mundo, de las cuales tres se encuentran en México: *S. assimile* (Dalhbom), *S. fistularium* (Dalhbom) y *S. jamaicense lucae* (Saussure) (Bohart y Menke 1976). Esta última especie se encuentra ampliamente distribuida en la región del Cabo, al extremo sur de la península de Baja California, aunque Van der Vech y Van Breugel (1968) la citan también para el estado de Jalisco.

Las avispas del género *Sceliphron* se caracterizan por construir nidos de lodo, que son provistos con arañas que sirven de alimento a sus larvas. Cada nido consiste de varias celdas en forma de tubo, que las hembras modelan con pequeñas esferas de lodo (Bohart y Menke 1976). Estos nidos son colocados en grietas de paredes rocosas, bajo raíces de árboles que crecen en los riscos (Iwata 1976), bajo puentes ó cualquier otro sitio protegido (Dorris 1969). Generalmente varias celdas de un nido están cubiertas con una capa de lodo, pero White (citado por Bohart y Menke 1976) menciona que el 40% de los nidos de *S. spirifex* no presentan esta capa y argumenta que esta conducta se ha ido perdiendo, debido a que la avispa está cada vez más asociada con las habitaciones humanas, ya que en ellas encuentran mayor protección que en los sitios naturales de anidación.

Existen varios estudios sobre la relación entre las avispas solitarias del género *Sceliphron* y las arañas que sirven de alimento a su progenie (Rau y Rau 1916, Rau 1928 y Dorris 1969, 1970). Algunos de

[1] Hymenoptera: Sphecidae

estos estudios han revelado aspectos acerca del contenido de sus nidos (Muma y Jeffers 1945, Horner y Klein 1979) y la conducta depredadora de dichas avispas (Peckham y Peckham 1898), algunas de las cuales como *S. caementarium*, son muy selectivas con sus presas (Rau 1935 a, b; Eberhard 1979), actuando como huéspedes de otras avispas como *Chalybion* y *Trypoxylon* (Landes *et al.* 1987, Dean *et al.* 1988, Coville y Griswold 1984, Iwata 1976). En este trabajo se da a conocer por primera vez el contenido de los nidos de las avispas loderas *S. jamaicense lucae*, algunos aspectos de su ecología como preferencia de presas, pautas de comportamiento sobre la construcción de nidos y su relación con otros artrópodos.

MATERIALES Y METODOS

Este estudio se realizó en cinco localidades de la región del Cabo, Baja California Sur, México, localizadas aproximadamente entre los 23° 13' y los 23° 29' N y 109° 43' y 110° 20' O (Fig. 1). La vegetación de las zonas bajas es del tipo matorral sarcocaule con dominancia de cactus (*Pachycereus pringlei*) y asociaciones de chollas (*Opuntia cholla*), lomboy blanco (*Jathropha cinerea*) y mezquites (*Prosopis glandulosa*), entre otros. En las partes medias de la Sierra de la Laguna predomina la selva baja caducifolia con dominancia de palo blanco (*Lysiloma candida*), mauto (*L. divaricata*), palo zorrillo (*Cassia emarginata*), lomboy blanco(*Jatropha cinerea*), lomboy rojo (*J.vernicosa*) y el cardón barbón (*Pachycereus pecten-aboriginum*) (León de la Luz *et al.* 1988). El clima es muy árido, cálido y extremoso, con lluvias en verano, y temperaturas medias que van de 29 a 35° C (García 1981).

La colecta de nidos se realizó desde julio hasta octubre de 1989 y de abril a julio de 1990. Se obtuvieron muestras en los ejidos El Comitán y El Pescadero , en los Poblados de San Bartolo y Santiago y a 540 m de altura en la selva baja caducifolia del Cañón de la Zorra en la Sierra de la Laguna. Los nidos se removieron de los interiores y exteriores de casas-habitación, así como de tuberías de asbesto fuera de servicio y debajo de rocas, estos fueron transportados en bolsas de papel para su análisis en el laboratorio. El contenido de los nidos se extrajo y se conservó en alcohol al 70%. Posteriormente se identificó y cuantificó con la ayuda de un microscopio estereoscópico e instrumentos de disección. El material aracnológico fué identificado por el primer autor, consultándose las siguientes referencias: Berman y Levi 1971, Brady 1964, Chamberlin 1924, Dondale y Redner 1982, Gertsch 1939, Gertsch y Ennik 1983, Jiménez 1989 b, Levi 1956, 1957, 1968, 1970, 1973, 1975, 1976, 1977 a , b y 1978, Platnick 1974, Platnick y Shadab 1974, Roth 1985, Sauer y Platnick 1972.

RESULTADOS

Preferencia de presas.

Se colectaron 45 nidos, de los cuales 30 contenían presas con larvas y/o pupas de avispas, mientras que el resto se encontraron vacíos. Las presas de las avispas loderas fueron exclusivamente 36 especies de arañas. Las arañas correspondieron a 14 familias, de las cuales las más abundantes fueron: Thomisidae con un 31.6%, Araneidae con un 14.4%, Heteropodidae con un 13.9%, Clubionidae con un 11.7% y Anyphaenidae con 9.3% (Cuadro 1).

Las especies capturadas con más frecuencia por las avispas fueron: *Misumenops sierrensis* (Schick), *Olios peninsulanus* Banks, *Isaloides* sp., *Cheiracanthium inclusum* (Hentz),y *Aysha incursa* (Chamberlin) (Cuadro 2).

Estas especies están ampliamente distribuidas en las localidades de estudio , excepto *Isaloides* sp. que se restringe a la selva baja caducifolia de la Sierra de la Laguna (Jiménez 1988, 1989a y 1990).

El número de presas varió según el tamaño de las mismas y el rango fué de 3 a 29 individuos por nido; excepcionalmente se registraron 103 arañas en un nido. El tamaño de las arañas osciló entre 3.2 a 7.8 mm en promedio (Cuadro 1). Las presas indicaron diferentes estados de desarrollo; sin embargo, los juveniles fueron los más abundantes (44.2%), así como las hembras adultas (44.2%); el resto (11.5%) correspondió a los machos adultos.

Construcción de nidos.

Los nidos son construidos únicamente por las avispas hembras, en lugares preferentemente oscuros y protegidos, como cuevas, bajo rocas grandes y troncos, cerca de arroyos ó charcas, y en viviendas sobre paredes y objetos que no son movidos frecuentemente por el hombre, como techos, muebles, etc. Para la construcción de las celdas, utilizan generalmente arcilla, pero suelen usar cualquier otro material

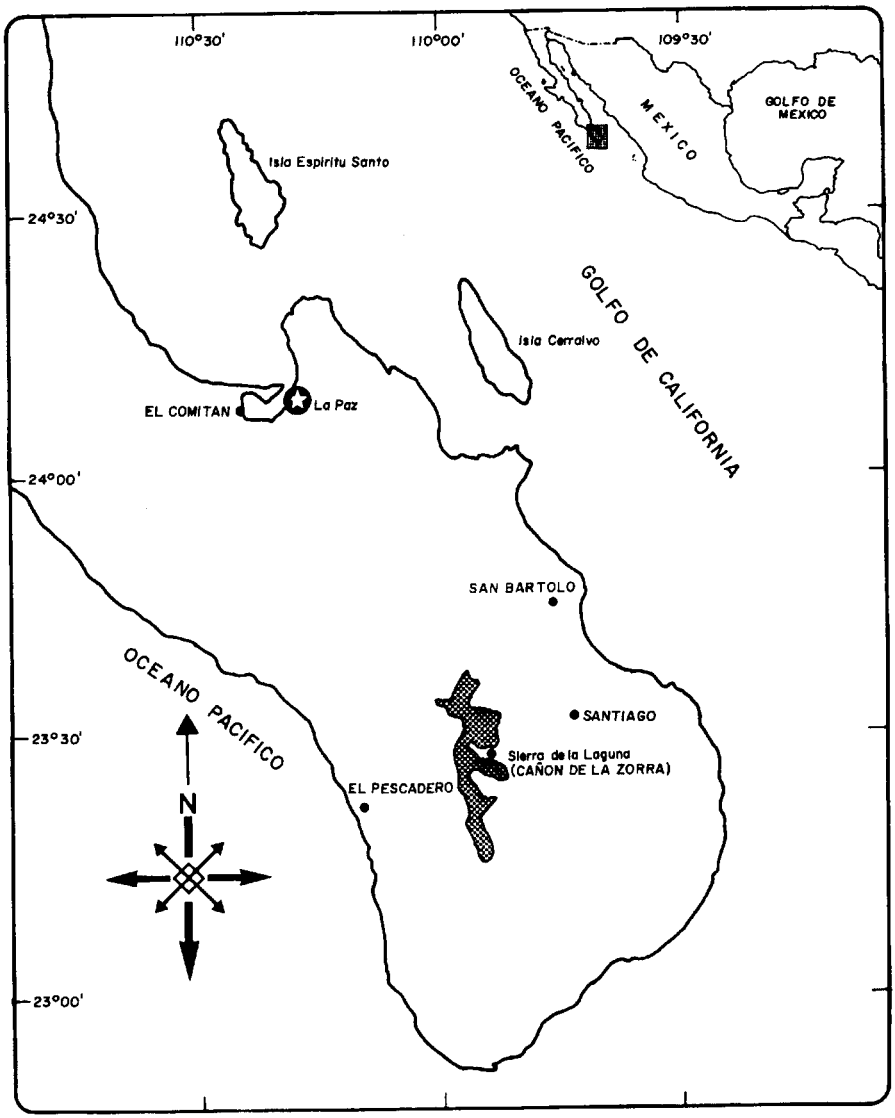

Fig. 1. Localización de las áreas de estudio.

maleable disponible. La construcción de un nido se inicia con la selección de un sitio hacia el cual las avispas acarrean pequeñas esferas de lodo con los apéndices bucales. Cada esfera es modelada con las mandíbulas y patas anteriores, moviendo la cabeza de atrás hacia adelante. La primera esfera es adherida al sustrato y con ayuda de las mandíbulas la van alargando hasta obtener una pequeña saliente que funciona como soporte, donde a su vez, son colocadas las siguientes esferas dándoles forma de medios anillos a uno y otro lado de la saliente, de manera simultánea y de tal forma que sobreponen uno de sus extremos, dando la apariencia de un trenzado, (Fig. 2a). La celda terminada tiene forma cilíndrica y su extremo distal reforzado funciona como entrada. Para colocar la siguiente celda, la avispa engrosa un área lateral o dorsal construída con anterioridad, para iniciar otra vez la colocación de los medios anillos. Un nido terminado puede estar formado de 1-23 celdas, casi siempre con una celda vacía, posiblemente para aereación, para distraer o engañar a los depredadores o porque la hembra murió, dejando una celda vacía; o bien, cerrada con lodo y las hormigas se encargaron de vaciarla (Fig. 2b). Generalmente los nidos se colocan en posición horizontal en relación al sustrato y siempre con la entrada de cada celda por un lado.Cada celda mide en la entrada unos 9 mm de diámetro, por una longitud total de 30-38 mm donde caben unos l4 a 25 anillos.

Cuando el nido es despegado de una superficie plana, el contenido puede a veces exponerse, como en el caso de los nidos construídos por *S. madaspatanum* en Japón (Iwata l976); en otros se observa una capa de lodo gruesa que protege el contenido. Las celdas dañadas son reparadas por las avispas, quienes les colocan esferas de lodo en la superficie. Cuando los nidos son abandonados, pueden ser habitados nuevamente por avispas que recubren el exterior con lodo, dándole la apariencia de una masa amorfa.

Las hembras trabajan durante las horas más cálidas del día en la construcción de los nidos, y en las primeras horas de la mañana en la captura de presas, actividad que puede prolongarse durante varios

Cuadro 1. ABUNDANCIA RELATIVA Y TALLAS PROMEDIO DE PRESAS DE ARAÑAS DE *Sceliphron jamaicense lucae.*

FAMILIAS	%	TALLAS PROMEDIO (mm)
THOMISIDAE	31.48	5.25
ARANEIDAE	14.43	7.78
HETEROPODIDAE	13.86	7.57
CLUBIONIDAE	11.65	5.72
ANYPHAENIDAE	9.30	5.82
THERIDIIDAE	8.74	4.12
OXYOPIDAE	6.80	7.02
SALTICIDAE	2.09	6.39
MIMETIDAE	0.68	4.74
SCYTODIDAE	0.28	8.13
PHILODROMIDAE	0.27	3.24
LOXOSCELIDAE	0.14	6.90*
PHOLCIDAE	0.14	5.00*
SELENOPIDAE	0.14	4.00*
TOTAL	100.00	

* Sólo se midió un ejemplar.

Cuadro 2. ANALISIS DEL CONTENIDO DE PRESAS DE 30 NIDOS DE LA AVISPA LODERA *Sceliphron jamaicense lucae* EN LA REGION DEL CABO B.C.S.

ESPECIES PRESA	Nidos #	Frecuencia de estadios J	M	H	Total	%
ANYPHAENIDAE						
Aysha incursa (Chamberlin)	13	20	25	22	67	9.29
ARANEIDAE						
Metepeira sp.	1	1				0.14
Araneus pegnia (Walckenaer)	2	1		3	4	0.55
Araneus sp.	5	1	2	5	8	1.10
Neoscona oaxacensis (Keyserling)	1	1		1	2	0.28
Larinia directa (Hentz)	4	13	4	1	18	2.50
Acacesia hamata (Hentz)	1			1	1	0.14
Eustala californiensis Keyserling	6	16	3	2	21	2.93
Gasteracantha cancriformis (Linnaeus)	1	7			7	0.98
Cyclosa caroli (Hentz)	1	5		2	7	0.98
Cyclosa bifurcata (McCook)	1			1	1	0.14
Gea heptagon (Hentz)	1	1		2	3	0.42
Eriophora edax (Blackwall)	4	16		1	17	2.35
Ocrepeira redempta (G y M)	2	1	1		2	0.28
Metepeira crassipes Chamberlin e Ivie	5	6		5	11	1.52
Argiope argentata (Fabricius)	1			1	1	0.14
CLUBIONIDAE						
Cheiracanthium inclusum (Hentz)	9	29	30	12	71	9.84
Trachelas speciosus Banks	3	1	8	4	13	1.80
HETEROPODIDAE						
Olios peninsulanus Banks	17	57	30	13	100	13.87
LOXOSCELIDAE						
Loxosceles baja Gertsch y Ennik	1		1		1	0.14
MIMETIDAE						
Mimetus sp.	5	1		4	5	0.69
OXYOPIDAE						
Hamataliwa grisea Keyserling	6	11	12	2	25	3.46
Peucetia longipalpis F.O.P. Cambridge	7	22		2	24	3.32

Cuadro 2 (continúa)

ESPECIES PRESA	Nidos #	Frecuencia de estadios J	M	H	Total	%
PHILODROMIDAE						
Ebo mexicanus Banks	1			1	1	0.14
Philodromus jimredneri Jiménez	1			1	1	0.14
PHOLCIDAE						
Physocyclus sp.	1	1			1	0.14
SELENOPIDAE						
Selenops sp.	1	1			1	0.14
THERIDIIDAE						
Steatoda fulva Keyserling	1	4			4	0.55
Anelosimus studiosus (Hentz)	3	1	51	7	59	8.19
SALTICIDAE						
Thiodina sylvana (Hentz)	5	5		5	10	1.38
Plexippus paykulli (Sav. y Aud.)	1			5	5	0.69
THOMISIDAE						
Misumenops sp.	4	5		2	7	0.98
Misumenops sierrensis Schick	10	35	107		141	19.70
Isaloides sp.	6	69	4	3	76	10.55
Misumenoides sp.	1			2	2	0.28
SCYTODIDAE						
Scytodes sp.	1	2			2	0.28
TOTAL					721	100.00

J = juveniles, M = machos, H = hembras

días. Se desconoce la forma de cómo *S. jamaicense lucae* captura a sus presas para alimentar a su progenie.

Hemos observado que la primera presa de las avispas loderas, es generalmente una araña hembra adulta o preadulta (Fig 3), la que es introducida hasta el fondo de la celda, en la cual la avispa deposita un huevecillo de 5 mm de longitud. Una vez colocada la primera araña, la avispa obtiene más presas que va acomodando cuidadosamente una tras de otra hasta llenar la celda (Fig 4). Si no termina esta actividad en el transcurso del día, al atardecer sella la entrada con una cubierta delgada de lodo, la que remueve al día siguiente, cortando por la orilla con sus apéndices bucales y terminando el llenado de la celda con más presas. Finalmente, la avispa sella herméticamente la entrada con una cubierta de lodo de 1.01-2.77 mm de espesor. El reposo se lleva a cabo en las horas del crepúsculo sobre el follaje; esta actividad coincide con la observada por Rau y Rau (1918) en *S. caementarium* que reposa también sobre las flores.

a b

Fig. 2. a) - Nido de la avispa lodera mostrando las entradas de las celdas cubiertas con cal, por *Chalybion sp.* y celda donde se aprecia la disposición de los anillos de lodo. b). - Vista lateral de un nido de lodo con su celda vacia.

Fig. 3. Araña hembra *Cheiracanthium inclusum* (Hentz) con una larva de la avispa *Sceliphron jamaicense lucae*, para mostrar la posición caracteristica del huevo y de la larva joven sobre la presa.

Fig. 4. Celda de un nido de lodo mostrando la disposición de las arañas presa, y como la celda es densamente llenada.

Fig. 5. Larvas y pupas de la avispa parasitoide *Melittobia sp.* dentro de una pupa de la avispa *Chrysis sp.*

Asociacion de *S. jamaicense lucae* con otros artrópodos.

Existen ciertas asociaciones entre las avispas loderas y otros artrópodos como se expresa en la figura 6. Se ha observado que las avispas *Chalybion* sp. habitan los nidos desocupados de las avispas loderas y cubre la entrada de cada celda, y algunas partes externas, con cal y algunas veces con resina vegetal (Fig. 2a), como lo menciona Iwata (1976). De acuerdo con este autor, es probable que esta especie utilice el excremento de aves y reptiles, así como de algunos mamíferos para cubrir las entradas de las celdas, cuando no dispone de los materiales mencionados al principio. Las avispas *Trypoxylon (Trypargilum) dubium* Menke, aprovechan las celdas abandonadas de las avispas loderas para criar a su progenie (Muma y Jeffers 1945, Coville 1982, Rau 1928). Hemos encontrado que esta especie divide cada celda en dos o tres cámaras, con paredes delgadas de lodo, y los orificios de emergencia se observan en la pared lateral de cada cámara. De los 30 nidos que se analizaron sólo 8 estuvieron compartidos por pupas de *T. dubium*, y sólo uno de ellos contuvo arañas pequeñas de la especie *Oecobius annulipes* Lucae; aunque *T. dubium* también hace nidos de lodo, no se obtuvo ninguno.

Pudimos observar varias larvas de *Chrysis* sp. alimentándose de las larvas de las avispas loderas, pero se desconoce cómo introduce su progenie al nido. De acuerdo con Rau y Rau (1916) y Gauld *et al.* (1988), es probable de que se trate de una especie que sólo se alimenta de estadios larvales en desarrollo avanzado, por lo que su ciclo de vida puede ser más complejo y debe ser estudiado con más detalle. Las larvas de *Chrysis* sp. fueron a su vez devoradas por ácaros de la familia Trombidiidae.

Las avispas calcídidas del género *Melittobia* sp. (Fig. 5), fueron encontradas como parasitoides de las larvas de *S. jamaicense lucae* y *Chrysis* sp. Las avispas del género *Melittobia* sp. pueden penetrar a los nidos de lodo por pequeños orificios, o bien, hacen pequeñas perforaciones con sus mandíbulas hasta introducirse dentro de la celda (Rau y Rau 1916).

Con menos frecuencia, las larvas de las avispas loderas son consumidas por larvas de *Sarcophaga* sp. (Diptera: Sarcophagidae), y por larvas de coleópteros. En la mayoría de las celdas desocupadas por las avispas estuvieron presentes los psocópteros consumiendo los restos de arañas, excremento o cubiertas de pupas. Cabe señalar que un solo nido puede ser compartido, al mismo tiempo por larvas de las avispas loderas, *Trypoxylon dubium, Chrysys* sp., *Chalybion* sp. y de dípteros.

DISCUSION

Aparentemente *S. jamaicense lucae*, es un depredador generalista, por la variedad de especies de arañas que captura. Nuestros resultados muestran que las avispas tienen preferencia por las arañas que se encuentran en la vegetación arbustiva o sobre flores. Su actividad depredadora no sólo se restringe a un sustrato, sino que también capturan arañas que se encuentran en el suelo ocultas entre hendiduras o bajo piedras, como *Loxosceles baja* Gertsch y Ennik, *Scytodes* sp. y *Steatoda fulva* (Keyserling). Asimismo, capturan arañas que se distribuyen en el estrato arbóreo como *Hamataliwa grisea* Keyserling y *Ebo mexicanus* Banks, se piensa que estas arañas fueron capturadas oportunamente o bien porque las avispas las localizaron en abundancia, como en el caso de un nido provisto exclusivamente con *H. grisea*, o bien, porque una avispa en particular haya aprendido a encontrar una especie y después especializarse en ella. Observamos que *S. jamaicense lucae* tiene mayor preferencia por las arañas de la familia Thomisidae, resultados que son similares a los obtenidos por Muma y Jeffers (1945), Dean *et al.* (1988) y Horner y Klein (1979), que observaron a *S. caementarium* especializada en capturar arañas de las familias Thomisidae y Araneidae.

Respecto a los estados de desarrollo, es probable que los resultados coinciden con los cambios poblacionales, debido a que las arañas juveniles y las hembras siempre son más abundantes que los machos, y que éstos últimos, de corta vida, están ausentes fuera de la época de reproducción.

De acuerdo con Orbin (en Landes *et al.* 1987), se puede inferir que las avispas seleccionan a sus presas principalmente por el tamaño. Esto puede deberse a que las arañas capturadas deben tener un tamaño óptimo para poder ser introducidas dentro de las celdas. Se ha observado que cuando capturan especies grandes como *O. peninsulanus*, el número se reduce a pocos ejemplares. El color y la forma puede ser un factor de selección y en este caso las avispas tienen mayor preferencia por arañas de color claro como *M. sierrensis, A. incursa, C. inclusum* y *O. peninsulanus*. Sin embargo, la disponibilidad y abundancia de otras especies poco frecuentes en los nidos como *Cyclosa caroli* y *C. bifurcata*, entre otras, influyen para que sean también seleccionadas como presas, aunque en menor proporcíon. Estas especies de arañas decoran sus telarañas con restos de las presas que captura, alineándolas en una fila, dando una apariencia similar en color, forma y tamaño de la araña misma, la cual llega a ser imperceptible, al permanecer en la parte media de la telaraña. De acuerdo con Nyffeler *et al.* (1986), es probable que al igual que *Cyclosa turbinata*, las especies *C. caroli* y *C. bifurcata* sean excepcionalmente capturadas por las avispas loderas, debido a que la disposición de las presas en las redes de estas arañas, funciona como un mecanismo de defensa contra los depredadores voladores.

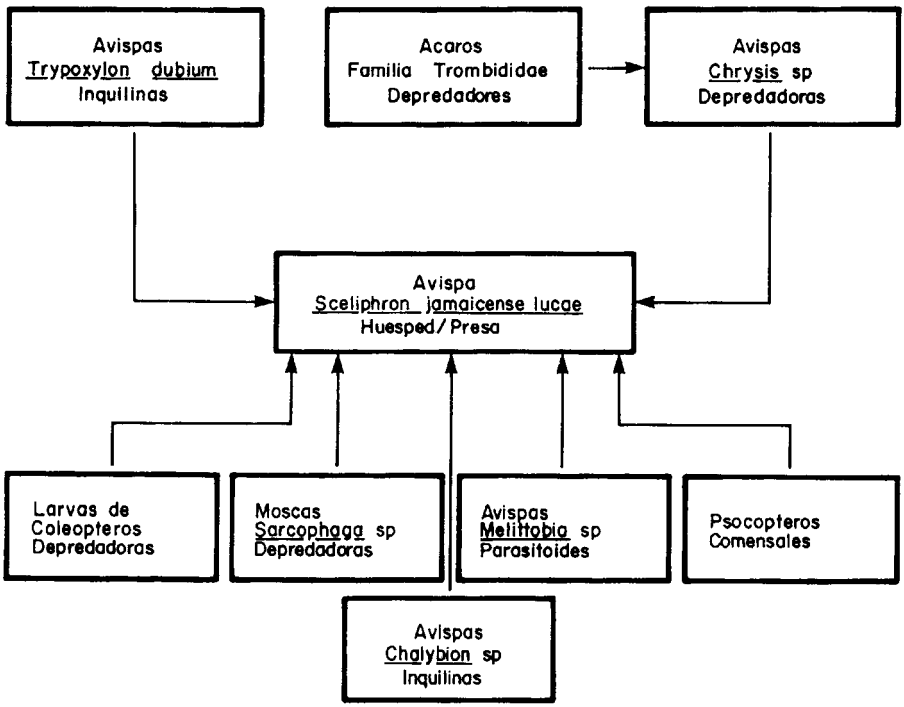

Fig. 6. Algunas asociaciones en los nidos de la avispa lodera *Sceliphron jamaicense lucae*.

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SCIENTIFIC NOTE

TWO FUNGI INFECTING RED IMPORTED FIRE ANT[1] FOUNDING QUEENS FROM TEXAS

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The red imported fire ant (RIFA), *Solenopsis invicta* Buren, is native to the state of Mato Grosso in Brazil, South America (Jouvenaz 1986). Since its introduction into the United States, relatively few natural enemies of this ant have been identified in this country (Jouvenaz 1986). The microsporidians *Telohania solenopsae* Knell, Allen and Hazard and *Vairimorpha invictae* Jouvenaz and Ellis, and the fungus *Metarhizium anisopliae* (Metchnikoff) Sorokin infect *S. invicta* in Brazil (Allen and Buren 1974, Jouvenaz 1986, Jouvenaz and Ellis 1986). Also, mildly pathogenic or symbiotic yeasts have been detected in the haemolymph of RIFA workers in the USA (Jouvenaz 1986). We are attempting to develop strategies for use of entomopathogenic fungi for *S. invicta* population management and are therefore interested in detecting RIFA pathogens in the USA. We collected approximately 1000 RIFA founding queens after a nuptial flight 4-5 August 1989 in College Station, Texas. The ants were maintained in artificial plaster nests (Banks et al. 1981) and were observed for mortality and pathogen development.

Two different fungi were observed on some of the collected queens. A fungus of the genus *Conidiobolus* (probably *C. macrosporus* Dreschler) (Zygomycetes: Entomophthorales) was identified from three dead RIFA queens collected in the field. These ants showed fungal growth at the intersegmentary membranes. Also, four queens maintained in the laboratory for 72 h died of green muscardine (*Metarhizium anisopliae* var. *anisopliae* (Johnston) Tulloch) infection. Both fungi were isolated and cultured on Sabouraud dextrose agar plus 1% yeast extract (SDAY) (Difco Laboratories, Detroit, Michigan). To test the pathogenicity of these fungi we conducted two separate bioassays in the laboratory.

In the first bioassay RIFA's were exposed to *Conidiobolus* conidia. Because *Conidiobolus* spp. produce conidia that are forcibily ejected from conidiophores, laboratory bioassays were performed by placing fire ants under inverted, sporulating cultures of *Conidiobolus* on SDAY. Ants were exposed to conidial showers for 48 h in moist chambers (Petri dishes lined with moistened filter paper). Twenty adult queens, three groups of 30 adult workers, and three groups of 30 worker larvae (2nd to 4th instars) were separately exposed. Worker larvae subsequently were confined to moist chambers without adult nurses. Additionally, conidia were harvested from lids of several cultures and suspended in sterile distilled water, and adult worker ants were dipped into the solution and incubated in moist chambers. All treatments and controls were incubated at room temperature (25°C).

No *Conidiobolus*-induced mortality was observed in adult workers or alate females. However, all of the larvae were rapidly killed (48h) by *Conidiobolus*. No mortality in control larvae was observed. Adult ants were engaged in active grooming and cleaning behaviors and were probably able to remove the relatively large (30-65 μm diam.) *Conidiobolus* conidia before infection occurred. This *Conidiobolus* strain is now on deposit in the USDA-ARS Plant Protection Research Unit, Entomopathogenic Fungi Culture Collection, Ithaca, NY (Accession no. 2819).

[1]Hymenoptera: Formicidae

For the second bioassay, 15 alate RIFA females were dusted with conidia from *Metarhizium*-infected cadavers and were placed in humid plaster nests at 25°C. All challenged ants died (100% mortality) within five days. Control groups showed no mortality. This strain is in our entomopathogenic fungi collection (Texas Tech) (Accession Ma-89-2).

The two fungi discussed herein seem to be the only reported native fungal pathogens from RIFA in the USA. Presumably, both of these species are adapted to the environmental conditions of Texas; in particular, *M. anisopliae* may show promise as a biological agent for fire ant population management. These findings are especially important because these native pathogens may be free of many technical restrictions concerning the introduction and release of exotic natural enemies into the USA to combat the RIFA.

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SCIENTIFIC NOTE

PECAN WEEVIL ERADICATION - TULAROSA, NEW MEXICO

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Pecan weevil (PW) distribution is widespread on pecan in the U.S. from Texas to Georgia and absent in Mexico (Harris 1979). Larvae of the pecan weevil, *Curculio caryae* (Horn), (Coleoptera: Curculionidae), destroy nuts of hickories, *Carya*, by feeding on nearly mature kernels (Harris 1983). Fully grown, legless larvae (typically 3/nut) exit through emergence holes chewed in the shell and enter the soil beneath infested trees during October-November; most emerge as adults in August-September two years later, while a few take three years. Control of this key pest on pecan requires killing the adult female after emergence from the soil but before oviposition into developing kernels has occurred, typically a 3-5 day interval.

The PW was collected for the first time in Otero County, New Mexico, at Tularosa during the winter of 1969-70. On 8 January 1970, Douglas Bryant brought pecan nuts provided by Walter Wade, Otero County Extension Agent, to the senior author's office. Nuts appeared to have been infested by pecan weevil due to their distinctive exit holes. On 9 January, Wade and Nielsen went to the Kirk property at Tularosa; numerous nuts with exit holes were found beneath the trees and in nuts stored in the garage. Forty-three larvae were collected from ca. 500 stored nuts. Larval specimens were sent to the U.S. National Museum on 10 January; larvae subsequently were identified as *Curculio*, probably *caryae*. On 24 March an adult PW was collected from soil on the adjacent Brusuelas property beneath the large tree belonging to Mr. Smith. This weevil was sent to the U.S. National Museum and was identified as the PW, *Curculio caryae*.

A delimiting survey conducted in January and February 1970, indicated the PW infestation was confined to three adjoining urban properties, Kirk, Brusuelas and Smith, within about a 100 m radius. On 27 January NMDA inspectors collected and burned debris. During February and March 1970, eight of the largest pecan trees were topped so they could be effectively treated with a pesticide in the fall. Smaller trees were not topped or pruned, since these trees served as "trap trees" for emerging weevils. The cooperation of the homeowners was unconditional -- they willingly agreed to remove the trees to eradicate the PW. However, trees were retained in hope of reducing dispersal of emerging adult PW to previously uninfested trees. Trees were pruned again during the winter of 1972 to facilitate treatment in 1973 and in subsequent years. On 20 July 1970, and in subsequent years through 1976, modified pink bollworm traps (pyramid traps, 1.5 x 1.5 m at base tapering to a cone with a hole at the apex, covered by a bottle) were placed beneath the infested trees to collect adult PW emerging from the soil. On 24 October 1970, Otero County growers and

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NMDA personnel harvested immature nuts from trees suspected of being infested and placed them in screened boxes to dry. Early removal was to prevent any larvae that may have been in the nuts from reaching the soil. Although soil treatment had not been proven an effective control method, research results in other areas indicated carbofuran had given control of larvae and adults in the soil. Therefore, on 8 and 12 October 1973, soil beneath suspect trees was treated with carbofuran 4 EC (2 pounds actual per 100 gallons water). As an added precaution, sheets of 4 mil polyfilm were placed beneath trees on 19 October 1973 to collect larvae that might fall from infested nuts (no larvae were noted). Trees within an approximately 100 m radius were treated to the point of runoff at approximate weekly intervals each year beginning from August 1970 through 1975 with carbaryl 4 pounds actual per 100 gallons water. The number of applications applied varied from 7 in 1970 to 10 in 1974 depending on maturity of crop and the first killing frost. Nuts were harvested and shelled each fall from 1970 through 1974. Since no larvae were ever found in cracked nuts, nuts harvested in 1975 were not cracked but were checked at the time of harvest and on several occasions until they were used by the homeowners.

Specimens collected since discovery of the PW infestation in 1969-70 included: 1970 - 20 adults (fall); 1971 - 3 adults + 2 larvae (spring--soil beneath infested trees), 4 adults (fall--traps); 1972 - 5 adults (fall--traps, foliage, beneath infested trees) 4 nuts with exit holes (fall--beneath infested trees); 1973 to 1976 - No adults, no larvae, no nuts with exit holes. The formal eradication program was deemed a success after 1976 and less intensive monitoring through 1990 has not shown any pecan weevils or any other evidence of infestation in Otero County.

Several factors contributed to the eradication of PW at Tularosa, New Mexico: 1) Cooperative Extension Service (CES) and New Mexico Department of Agriculture (NMDA) personnel cooperated in collecting and identifying specimens; 2) Research personnel in Georgia, Mississippi, Oklahoma and Texas promptly provided information on the life cycle and control of PW in their areas; 3) CES personnel prepared and disseminated material with photographs of the adult, larvae, damaged nuts, etc., to newspapers and to growers in the area to make people in Otero County aware of the problem and to request their assistance in finding other trees that might be infested; 4) Excellent cooperation was obtained from the affected homeowners; and 5) a PW Control Committee was organized in Otero County on 17 March 1970.

The source of the initial infestation remains obscure, but one resident recalled a grocery sack full of "bad" pecan nuts obtained from the indigenous range of the PW that she discarded in her backyard at Tularosa during the early or mid 1960's. The annual infestations observed from 1969-1972 of this predominantly biennial pest indicate more than one generation occurred before it was detected. The pecan is a popular yard tree in Tularosa, yet the infestation did not spread far (<200 meters) prior to its detection. This indicates the adult PW primarily infests nuts in the immediate vicinity of where they emerge. This apparent low tendency to disperse compensated for the long life cycle (2-3 years) so that intensive eradication practices could be concentrated for an extended period of time in a small area to achieve eradication.

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PREDATORS AND PARASITOIDS OF RUSSIAN WHEAT APHID[1] IN CENTRAL MEXICO

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The Russian wheat aphid, RWA, [*Diuraphis noxia* (Mordvilko)] is a serious pest of small-grain cereals, especially wheat, *Triticum aestivum* L., and barley, *Hordeum vulgare* L., in many parts of the world. The aphid was first detected in Mexico in 1980 (Gilchrist *et al.* 1984). Chemical management of RWA is expensive (Anon. 1990) and is made difficult by the aphids' habit of living in tightly rolled leaves. Biocontrol of RWA represents a possible alternative or supplementary means of management. This article represents a record of the predators and parasitoids of RWA collected during winter/spring 1990 from wheat and barley plants, artificially infested with RWA, in screening nurseries at El Batan in central Mexico (19° 31'N, 98° 50'W, 2249 masl). Notes were taken on the predators and parasitoids from February to May, before the onset of the rainy season.

The most abundant coccinellid RWA predators were *Hippodamia convergens* Guerin and *Coccinella nugatoria* Mulsant; others included *Olla v-nigrum* Mulsant, *Cycloneda sanguinea* (L.), *Adalia bipunctata* (L.) and *Paranaemia vittigera* (Mannerheim). Larvae and adults of these species were observed feeding on RWA but their impact on RWA numbers appeared slight as only younger larvae were small enough to be able to feed within rolled leaves. *Paranaemia vittigera* is presumed to be an aphid predator but no aphid host data have been reported (Gordon 1985). A colony of this beetle has been maintained at CIMMYT for one year on a diet of RWA. Larvae and adults of *Scymnus* spp., including *Scymnus* (*Pullus*) *loewii* Mulsant, and *Diomus* spp. were abundant, and were able to feed within rolled leaves thereby representing more feasible candidates for use in controlling RWA. Naranjo *et al.* (1990), however, indicated that *Scymnus frontalis* (F.), an imported predator of RWA, required higher temperatures than RWA to complete development and reproduce although it was able to feed within rolled leaves.

The anthocorid *Orius tristicolor* (White) was frequently observed feeding on RWA inside rolled leaves.

Larvae of four syrphid species; *Eupeodes volucris* Osten Sacken, *Allograpta obliqua* (Say), *A. exotica* (Wiedemann) and *Platycheirus* (*Carposcalis*) sp.; were collected from within rolled leaves infested with RWA. All were common but were heavily parasitized by *Pachyneuron albutius* Walker and *Syrphophagus* sp., which probably had a negative effect on the abundance of subsequent generations.

Larvae of four species of Neuroptera; *Chrysopa carnea* Steph., *Hemerobius pacificus* Banks, *Micromus variolosus* Hagen and *Sympherobius angustus* (Banks); were also collected. It is unlikely that these had much effect on RWA numbers as they appeared in April/May when RWA numbers were very high and were unable to feed effectively in rolled leaves.

[1] Homoptera: Aphididae.

The parasitoids of RWA and their hyperparasitoids were counted by removing RWA mummies from plants to the laboratory and collecting the adults that emerged. These numbered 286 in total, as follows: Braconidae; *Diaeretiella rapae* (M'Intosh), 266; *Aphidius ervi* Haliday, 3; Pteromalidae; *Asaphes* sp., 5; *Pachyneuron siphonophorae* (Ashmead), 6; Eulophidae; *Aprostocetus* sp., 1; Megaspilidae; *Dendrocerus* sp., 4; Charipidae; *Alloxysta fuscicornis* (Hartig), 1.

Diaeretiella rapae was the most abundant parasitoid, and preliminary work has been carried out on its suitability as a RWA biocontrol agent (Stary and Gonzalez 1991). *Aphidius ervi* is a common parasitoid of the pea aphid *Acyrthosiphon pisum* (Harris) in several countries, including Mexico. Large numbers of this aphid were present on vetch, *Vicia sativa* L., in a field adjacent to the RWA screening material; presumably, they transferred to RWA. The hyperparasitoids *Dendrocerus* sp. and *A. fuscicornis* appear to have had little impact on the abundance of RWA primary parasitoids.

This note does not present an exhaustive account of the predators/parasitoids of RWA in central Mexico. An attempt will be made in the future to sample predators and parasitoids of RWA during the whole year.

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EFFECTS OF LEAFTIERS ON BROOM SNAKEWEED IN CENTRAL NEW MEXICO

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Perennial snakeweeds, *Gutierrezia* spp., infest much of the rangeland of the western United States and northern Mexico (Huddleston and Pieper 1989). Snakeweeds are known to be poisonous to livestock (Smith and Flores-Rodriguez 1989) and are hardy competitors with perennial grass species. Grass production can be increased two to five fold by removing or controlling dense stands (McDaniel et al. 1982). Over 300 species of native insects have been collected on snakeweed in west Texas and New Mexico (Foster et al. 1981, Wangberg 1982, Thompson and Richman 1989). The leaftying moths, *Synnoma lynosyrana* Walsingham and *Synlocha gutierreziae* Powell (Tortricidae), can, under certain circumstances, become quite numerous, and many ranchers believe the insects can kill snakeweed. A heavy infestation of snakeweed leaftier larvae was reported from the Youngblood ranch, ca. 27 km east of San Antonio, Socorro Co., NM, during the summer of 1987.

Six transects, 30m long and 0.6m wide, were destructively sampled in late summer and early fall in both 1987 and 1988. Canopy width, presence and number of leaftier ties, presence and identity of root borers, and plant condition (percent aboveground dead biomass) were recorded for every plant in each transect (746 plants in 1987 and 450 plants in 1988). Multiple linear regression analysis [PROC REG (SAS Institute, 1985)] was used to determine the best predictors of snakeweed plant condition. Twenty sets containing equal numbers of tied and untied stems were collected in November, 1987. The number of paired stems varied between samples. Stems were placed in envelopes, dried at 43°C for 12 days, and weighed ($\pm$0.0001 g). Differences between number of seed heads per stem sample and average seed weight were determined by analysis of variance. Means $\pm$ SEM are reported.

The percent of dead or unhealthy (at least 20% dead) plants increased markedly in transects between 1987 and 1988. The number of dead or unhealthy plants rose from a mean of 19.3 $\pm$ 9.14% per transect in 1987 to 50.0 $\pm$ 12.27% per transect in 1988. The total number of plants also dropped from 7.91 plants/m^2 in 1987 to 4.17 plants/m^2 in 1988. This does not seem to be a result of drought as rainfall records show that summer, fall, and winter rains and snowfall for 1987 at the ranch were above normal.

Seed production from stems tied together by leaftiers (348.9 $\pm$ 111.85 seedheads per stem) was less (ANOVA P=0.009 df=39) than stems from the same plant not tied together (623.8 $\pm$ 118.86 seedheads per stem); however, the average weight per seedhead

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was not different (0.0015 and 0.0016 g, respectively). The number of leaftiers dropped from 2.41 per plant in 1987 to 0.02 per plant in 1988. Several parasitoids probably contributed to the leaftier decline. Of 1,891 pupae collected from the six transects in 1987, 23.3 ± 9.80% were parasitized. The most abundant parasitoid (ca. 90% of all the emerging parasitoids) was *Glypta albitibia* Dasch (identified by V. Gupta, American Entomological Institute, Gainesville, FL) which attacked the late larva or pupa. This ichneumonid was described from British Columbia and Montana (Dasch 1988), with no known host. Several tiny chalcidoid wasps also emerged as did one specimen of *Macrocentrus* sp. (undescribed Hymenoptera: Braconidae) (identified by Paul Marsh, U. S. D. A. Systematic Entomology Laboratory, Beltsville, MD). A total of 482 parasitoids emerged. Of the adult leaftiers that emerged (n=1,413), 52.6% were males and 47.4% were females.

Leaftiers were *Synnoma lynosyrana* (identified by Jerry Powell, University of California at Berkeley and John Hepner, Florida State Collection of Arthropods, Gainesville, Florida). No adult moths were seen flying until the first week of November when this species normally flies (Powell 1976). We cannot rule out the presence of the related multivoltine leaftier, *Synlocha gutierreziae*, but they seemed to be a minor component of the infestation if they were present. Wisdom et al. (1989) found both species at their study site in Sevilleta National Wildlife Refuge in northern Socorro Co., NM.

Wisdom et al. (1989) suggest that *Gutierrezia sarothrae* senescence is related to the abundance of leaftiers; however, because plants were not destructively sampled, the influence of other herbivores, specifically root borers, was unknown. The regression model of plant condition as a function of sample date, presence of root borers, number of leaftiers and canopy width was highly significant ($F = 173.7$; $df = 4, 1190$; $P < 0.0001$; $R^2 = 0.37$). All four regressors were significant ($P < 0.0001$). The activity of root boring insects had the highest relative importance on predicting plant condition (partial $r^2 = 0.21$). In fact, 65.2 ± 20.91% of the dead or unhealthy plants and 72.0 ± 12.88% of the dead or unhealthy plants collected in 1987 and 1988, respectively, were bored. Leaftiers had the lowest relative importance on predicting plant condition (partial $r^2 = 0.02$) and the partial regression coefficient is negative suggesting that leaftiers are associated with healthier plants. The two most common borers were the cerambycid *Crossidius pulchellus* and the tortricid moth *Eucosma ridingsana* (Robinson). Usually only one *C. pulchellus* was found per plant, but there were often two or more *E. ridingsana* larvae.

Results of this study suggest that the leaftiers have little direct effect on plant mortality. Mortality was more closely correlated to the presence of the cerambycid *C. pulchellus*. This beetle is known to kill stands of mature snakeweed plants under certain circumstances (Richman and Huddleston 1981). There appears to be a relationship between the presence of leaftiers and root borers (83% of bored plants collected in 1987 had leaftiers), but it is not certain whether this is due to interactions between these two herbivores or is a function of plant size or some other physical characteristics. It is possible that plants were already senescing and root borers simply hastened their decline.

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ADDENDUM

In "The Influence of ULV Malathion, Applied for Boll Weevil Control, on Other Pest and Beneficial Species in Arizona Cotton Fields 1989-90", *Southwest. Entomol.* 17(1), 49-61 (1992) by J.E. Leggett, a page was omitted. The succeeding page (172) should follow page 54 in the aforementioned publication.

numbers of adult whiteflies were low for six weeks with no significant difference between treatments from 21 May through 25 June. On the week of 2 July, adult whitefly numbers began to increase in one treated field and in three untreated fields with up to 695 adults per 200 sweeps on one sample date. The samples in weeks 7-9 following treatment had higher numbers of adults but there were no differences between treatments and it was too late in the season for the counts to have been influenced by the malathion treatments (Fig. 4). Nymphal populations in untreated fields increased slightly during week 7 but increased drastically by week 10 following treatment (Fig. 5).

Orius *(Orius tristicolor)* (White). In 1989, populations were similar in the treated and untreated fields on the week of the first malathion treatment. By three weeks after treatment, the number per 200 sweeps increased significantly from three to 19 in treated fields as judged by t-test at $P = 0.05$ with 25 df and from three to six in untreated fields. In 1990, *Orius* populations were significantly greater as judged by t-test at $P = 0.05$ with 19 df in the treated fields for weeks 3-5 after the malathion treatments. The numbers in untreated fields increased gradually throughout the test (Fig. 6). *Orius* sp. and thrips were the only two species that occurred in greater numbers in the treated fields.

Other Predators. This group of insect species consisted of assassin bugs, *Sinea confusa* Caudell and *Zelus renardii* Kolenati; *Geocoris pallens* Stål and *G. punctipes* (Say); nabids, *Nabis alternatus* Parshley; lacewings, *Chrysopa carnea* Stephens; lady beetles, *Hippodamia convergens* Guérin-Méneville and *Scymnus* spp.; and *Collops* spp. The most abundant insects in this group were *Geocoris* spp. This group of insect species was reduced for two weeks after malathion treatments, but the numbers in treated fields were not different by week 3 in 1989 and from week 3-9 in 1990. An unexplained reduction occurred in the untreated fields on week 6 (Fig. 7). The percent of individual predator species collected in 1989 and 1990 and summarized in Fig. 6 and 7 is shown in Table 2.

TABLE 2. Percent of Individual Predator Species in Sweep Samples (Total Shown in Fig. 6 and 7) 1989 and 1990, Arizona.

	1989		1990	
	Untreated	Treated	Untreated	Treated
Orius	26.6	66.6	42.5	73.1
Assassin Bug	2.1	1.8	12.7	3.3
Geocoris	37.2	9.7	14.2	5.4
Nabid	14.9	8.4	7.6	3.2
Green Lacewing	11.4	6.6	16.1	8.0
Lady Beetles	3.4	2.4	4.9	3.6
Collops	4.5	4.5	2.1	3.4

Parasitoids. Parasitoids included the families Braconidae, Ichneumonidae, Chalcidoidae, and Trichogrammatidae. Malathion treatments had no affect on these groups in 1989 or 1990. Numbers increased from one per 200 sweeps on 21 May 1990 to six per sample on 11 June 1990, then declined to two per sample on 16 July 1990 (Fig. 8).

MINUTES OF THE 16TH ANNUAL MEETING OF THE SOUTHWESTERN ENTOMOLOGICAL SOCIETY

The 16th Annual Meeting of the Southwestern Entomological Society was called to order by President Marvin Harris at 3:30 p.m., February 10, 1992 in the Magnolia Room of the Sheraton-Kensington Hotel, Tulsa, Oklahoma.

A motion was made, seconded, and passed to dispose with the reading of the minutes of the 15th Annual Meeting of the Society since they were published in the June 1991 (Vol. 16, No. 2) issue of the *Southwestern Entomologist.*

President Harris discussed several steps planned to increase membership in the Society. Secretary-Treasurer Allen Knutson distributed the Treasurer's report and expressed his appreciation to past Secretary-Treasurer Don Nordlund for his assistance in transferring those responsibilities.

MINUTES OF THE FEBRUARY 10, 1992 MEETING OF THE EXECUTIVE COMMITTEE OF THE SOUTHWESTERN ENTOMOLOGICAL SOCIETY

The Executive Committee met at 3:00 p.m. in the Cypress Room of the Sheraton-Kensington Hotel. Those attending were: President M.K. Harris, Past-President R.E. Wright, Secretary-Treasurer A.E. Knutson, President-Elect J.E. Slosser, Committee Members J. Michels and W.P. Morrison. Charles Ward and D. Rummel were also present.

Reports from Editor D.E. Bay and Secretary-Treasurer A.E. Knutson were distributed, reviewed and approved. W.P. Morrison reviewed the process by which he or D. Rummel proof the blue-line copy of each Journal for PrintTech. The need for a person fluent in Spanish to format and prepare camera-ready copies of Spanish manuscripts was discussed but no decision was made. The following steps to increase membership were approved: revise the current brochure which describes the Society, prepare a poster/display about the Society, and insert a card inviting membership to the Society with the reprints provided authors. Those requesting reprints of papers published in the *Southwestern Entomologist* are prospective members and would receive information and an invitation to become a member via the card inserted with their reprint. A letter of appreciation will be sent to FMC Corporation for their interest and funding of Supplement No. 15. There being no further business, the meeting was adjourned at 3:30 p.m.

AUDIT COMMITTEE REPORT:

On February 12, 1992, I inspected the 1991 fiscal transaction records of the Southwestern Entomological Society prepared by Secretary-Treasurer Allen E. Knutson. I examined the records of income and expenses and associated pertinent documents and found these records to be in order.

Respectfully Submitted,
Don Rummel, Chairman

SOUTHWESTERN ENTOMOLOGICAL SOCIETY
SECRETARY-TREASURER'S REPORT 1991:

Balance on hand as of December 31, 1990	$14,229.79

Income January 1, 1991 - December 31, 1991

Dues	$ 1,600.00
Subscriptions	1,642.70
Page Charges	25,475.12
Interest Earned	167.99
Miscellaneous Income	162.66
Total Income	$29,048.47

Expenses January 1, 1991 - December 31, 1991

Journal:	
Printing	$23,201.78
Secretary	1,827.60
Supplies	46.40
Postage & Handling	2,291.42
Editors	2,075.00
Society Operations:	
Secretary	900.00
Supplies	460.00
Postage	266.74
Miscellaneous Expense	76.44
Secretary-Treasurer	992.96
Total Expenses	$32,138.58

Balance on hand as of December 31, 1991	$11,139.68

As of December 31, 1991 there were 368 members and 103 institutional subscribers in the Southwestern Entomological Society. There were 24 unpaid page charges totaling $5,251.08.

Respectfully Submitted,
Allen Knutson
Secretary-Treasurer

EDITOR'S REPORT:

There were 47 manuscripts and a total of 384 pages in the four regular issues in 1991. This is a decrease from 57 manuscripts and a total of 510 pages in 1990.

Supplements Numbers 14 and 15, edited by J.E. Slosser and D.R. Rummel, respectively, were also completed during 1991.

I received 56 manuscripts for consideration of which 11 (19.6%) were rejected. This 19.6% rejection rate represents a considerable increase from the 9.7% level in 1990.

Editor's Financial Report
(January 1 - December 31, 1991)

Date	Description	Receipts/Expenditures	Balance
12/11/90	Balance Forward		$6.03
12/30/90	From Treasurer: $200.00		206.03
01/14/91	File Folder Labels	$5.23	200.80
01/14/91	Stamps	90.00	110.80
03/04/91	Airborne Express: Vol. 16 #1	10.00	100.80
04/29/91	Stamps	41.00	59.80
05/16/91	Airborne Express: Vol. 16 #2	10.00	49.80
06/24/91	Stamps	25.00	24.80
08/05/91	Airborne Express: Vol. 16 #3	10.00	14.80
08/27/91	From Treasurer: $46.40		61.20
09/11/91	Stamps	50.40	10.80
10/14/91	Airborne Express: Vol. 16 #4	10.00	0.80

Cash Summary

Balance Forward 01/01/91	$6.03
Receipts	246.40
Expenditures	251.63
12/31/91	$0.80 Ending Balance

Respectfully Submitted,
Darrell E. Bay, Editor

NOMINATION COMMITTEE REPORT:

The Nomination Committee recommended to the Executive Committee that John E. George and Charles Ward be placed in nomination for the position of President-Elect of the Southwestern Entomological Society.

Charles Ward was elected to the office by mailed ballot.

Respectfully Submitted,
R.E. Wright
Chairman

NEW BUSINESS:

There being no new business, President Harris asked John Thomas to escort President-Elect Jeffrey Slosser to the podium and passed the gavel to him.

President Slosser presented Past-President Harris with a plaque in appreciation for his services to the Southwestern Entomological Society.

President Slosser discussed the need to increase membership and review editorial procedures for Supplements.

The meeting was adjourned at 4:20 p.m.

Respectfully Submitted,
Allen E. Knutson
Secretary-Treasurer

THE ROLE OF VELVETLEAF GROWING IN OR NEAR COTTON FIELDS AS A HOST FOR TOBACCO BUDWORMS[1] AND BOLLWORMS[1] IN THE MISSISSIPPI RIVER DELTA[2]

Don E. Hendricks

Southern Insect Management Laboratory
USDA, ARS, P. O. Box 346, Stoneville, Miss. 38776

ABSTRACT

Velvetleaf, *Abutilon theophrasti* Medikus, adequately sustained the progeny of bollworm, *Helicoverpa zea* (Boddie), and tobacco budworm, *Heliothis virescens* (F.), moths that emerged from local overwintered pupae or immigrated into the area in the early spring. Velvetleaf also supported major peaks of eggs of both species during the cotton-growing season and substantial tobacco budworm larval populations from late September to November that could be a major source of pupae overwintering near cotton fields. Blooms and buds of this weed provided attractive feeding and oviposition sites for females of both species from about 30 days before to 40 days after peak blooming of cotton. Velvetleaf plants were host to about 18 times the number of tobacco budworm eggs and 28 times the number of bollworm eggs found on cotton during the cotton growing season from 29 May to 5 October 1991. Sex ratios determined from eggs and larvae collected from velvetleaf and cotton were about 1:1 for both species.

INTRODUCTION

Velvetleaf, *Abutilon theophrasti* Medikus, is a prevalent perennial weed in the Mississippi River deltas of Mississippi, Arkansas, and Louisiana (Elmore 1988). Each year about 150,000 acres of this region are used to produce cotton, *Gossypium hirsutum* L.; velvetleaf commonly occurs intermixed with cultivated cotton, in contiguous stands, along ditchbanks and in borders of cotton fields. Velvetleaf has been reported as an early-season host for bollworms, *Helicoverpa zea* (Boddie), and tobacco budworms, *Heliothis virescens* (F.) (Stadelbacher et al. 1986), and nutrients of this host adequately supported larval stages during that time of year. Dense moth populations could therefore be generated and subsequently oviposit on cultivated crops during the summer growing season. However, the abundance of eggs and larvae of these two insect species on velvetleaf and the importance of this weed as a reservoir for these pests have not been investigated on a season-long basis.

One purpose of this study was to determine the relative density of tobacco budworms and bollworms on velvetleaf in the springtime, on both velvetleaf and cotton during the summer cotton-growing season, and on velvetleaf in the fall. Moreover, the temporal role of velvetleaf as a host sustaining larval populations before and after the cotton-growing season was studied and characterized.

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[1] Lepidoptera: Noctuidae (subf., Heliothinae); *Heliothis virescens* F. and *Helicoverpa zea* (Boddie), respectively.

[2] Mention of a proprietary or commercially marketed product does not constitute endorsement by the U. S. Department of Agriculture.

MATERIALS AND METHODS

Abundance of bollworms and tobacco budworms was estimated from analyses of numbers of eggs and larvae collected from velvetleaf from 2 May to 17 November 1991, and from cotton during the growing season. Three 150-ha or larger cotton fields located in Washington Co., Miss. were used for survey sites throughout this study. Stands of velvetleaf occurring in these fields, or along their borders, were selected for inspection in the spring before cotton was planted. The cotton-growing season began about 20 May with planting and ended by 5 October after picking. During this period, inspections were made of velvetleaf and cotton plants as pairs of plants, where they grew adjacent to each other along the immediate border of cotton fields or within fields. Numbers of insects found were collated as paired data, and relative numbers of eggs found on either plant were analyzed as an indication of some type of selective behavior by ovipositing females. Most cotton was picked in the surveyed fields by 5 October; after this time, only velvetleaf plants that persisted as regrowth in picked cotton fields or that grew along immediate borders of fallow cotton fields were inspected.

The cotton planted in the inspected fields was an early-maturing, smooth-leafed (glabrous) variety, 'Deltapine 50', commercially marketed by Delta and Pine Land Co., Scott, MS. Velvetleaf buds, stems, and foliage were highly pubescent and physically larger than the analogous parts of the cotton plants inspected; they had germinated at least 20 days before cotton was planted.

Collection of insects began on 2 May with visual inspection of velvetleaf plants (3-leafed) and, on 29 May, with inspection of cotton plants (4-leafed). Collections were made two days each week, usually on Monday and Thursday, from 2 May until 17 November. Inspections were made of 100 velvetleaf plants and, after 29 May, of 100 cotton plants on each sample date. Entire plants were inspected until the first bud or cotton "square" appeared. Thereafter and until the first "killing" freeze, only foliage, blooms, and buds of the top 1/3 of the plants were inspected. Eggs and larvae found were reared to the adult stage in a controlled environment chamber (Model 71039, Sherer-Gillet Co., Marshall, MI.) at 27°C, 75% RH, and 11:13 LD photoperiod. Species and gender were determined using the adult stage, and sex ratios were calculated.

Numbers of eggs and larvae of either insect species that were found on either plant species were collated on a daily sample basis, and these data were graphically plotted in both daily and weekly forms. Differences between numbers of eggs or larvae, collected from either 100 velvetleaf plants per sample day or from 100 cotton plants per sample day, were statistically analyzed by a single-tailed t-test for related pairs of data (Huntsberger 1967). Numbers of tobacco budworm or bollworm larvae found on velvetleaf per six successive sample days were averaged and plotted. These 6-point averages progressed in increments of single sample days throughout the growing season and were plotted to clarify the importance of velvet-leaf as a host of larvae that ultimately overwinter as pupae (Peairs and Davidson 1956). The possibility of a preference for either plant species by ovipositing moths was investigated. Analyses were based on statistical comparisons of numbers of eggs found and on the frequency that at least one egg or larvae was found per sample day on either plant species throughout the cotton-growing season.

Inverted-cone traps (70-cm diam), baited with appropriate pheromone, were used to monitor moth populations throughout the year. Moth capture data were used to confirm oviposition sequences on cotton or velvetleaf determined by plant inspection throughout the growing season.

RESULTS AND DISCUSSION

Most of the Mississippi River delta region was inundated by flood waters from 19 to 28 February and again from 28 April until about 5 May; many of the early-season wild host plants that support springtime insect populations, such as wild geraniums (*Geranium carolinianum* L. and *G. dissectum* L.), were diminished or destroyed. Germination of velvetleaf, however, was not delayed, and seedlings appeared in typical habitats in late April. Cotton planting was completed by 25 May, and cotton squares and blooms had developed by 20 June.

Velvetleaf plants flourished from 2 May until the first freeze on 6 November. Buds and blooms were available as feeding and oviposition sites for female moths about 30 days before to 40 days after peak blooming in cotton which occurred about 15-24 July. Velvetleaf plants that germinated in late April were taller than cotton plants during the cotton-growing season.

Based on numbers of moths caught in traps baited with pheromone, populations of both tobacco budworms and bollworms in this region were relatively sparse in 1991 compared with populations in 1988 - 1990. The highest peaks in numbers of moths caught in traps were only 155 moths per trap-night during September for both species, whereas records from three previous years showed peaks of at least 300 moths per trap-night for both species.

Plots of the numbers of eggs or larvae per 100 plants indicated that velvetleaf provided nutrients and habitats for development of both tobacco budworms and bollworms throughout the summer season. Two major peaks in tobacco budworm oviposition on velvetleaf occurred: one with 198 eggs per 100 plants on 29-31 May, and another with 146 eggs per 100 plants on 27 August-12 September (Fig. 1). Eggs deposited between 29-31 May could have generated a major portion of subsequent generations of tobacco budworms during the 1991 growing season on both velvetleaf and cotton. Peaks in numbers of tobacco budworm larvae on velvetleaf followed the oviposition peaks with a 2- to 3-day lag from early June to mid-September. However, by early autumn, a relatively large peak (47 larvae per 100 plants) of tobacco budworm larvae appeared just before the first freeze and spanned the 34-d period from 27 September to 1 November.

The largest peak in the numbers of tobacco budworm eggs on cotton (Fig. 2) occurred 27 August-5 September with 13 eggs per 100 plants; this peak corresponded to the second largest peak of eggs on velvetleaf. Prior to this period, there were four other minor peaks in tobacco budworm ovipostion on cotton; however, none were more than 3 eggs per 100 plants.

Two major peaks of bollworm ovipostion on velvetleaf occurred relatively early in the season (Fig. 3); one appeared on 25-28 June with 98 eggs per 100 plants and another appeared on 9-11 July with 36 eggs per 100 plants. Peaks in numbers of bollworm larvae on velvetleaf followed the oviposition peaks with a 2- to 3-day lag. The peaks of bollworm eggs and larvae found on velvetleaf in early May (Fig. 3) apparently were the progeny of moths that emerged from overwintering pupae prior to flooding between 28 April and 5 May or were from moths that had immigrated into the region from southern latitudes. Possible immigration of bollworm moths was implicated by the capture of unseasonably high numbers of bollworms (85 moths per trap-night) in traps baited with pheromone between 28 March and 10 April before heavy rains that caused the second flooding. Captures of tobacco budworm moths during this period were minimal (<0.01 per trap night). There were substantially fewer bollworm larvae (<5 per 100 plants) on velvetleaf (Fig. 3) during autumn (27 September to 1 November) compared with tobacco budworms (Fig. 1) for the same period. Bollworm eggs peaked four times on cotton (Fig. 4); the highest number was three eggs per 100 plants on 1 August.

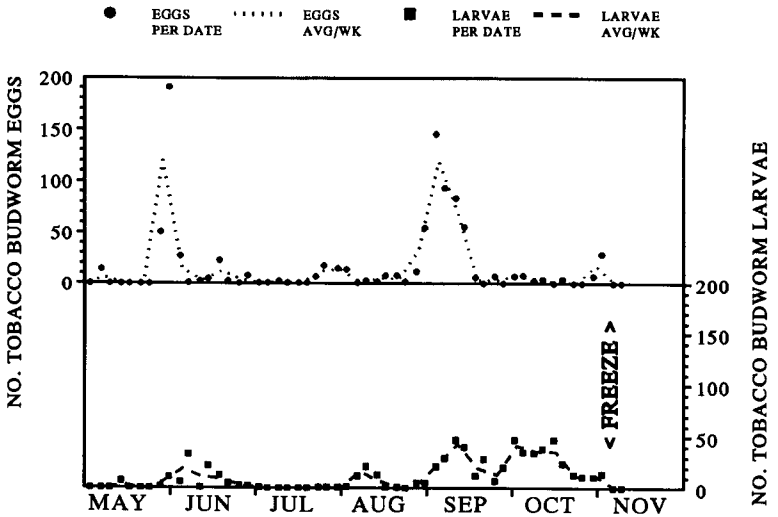

FIG. 1. Numbers of tobacco budworm eggs or larvae found per day on 100 velvetleaf plants inspected twice each week and average numbers per week from 2 May to 17 November, 1991.

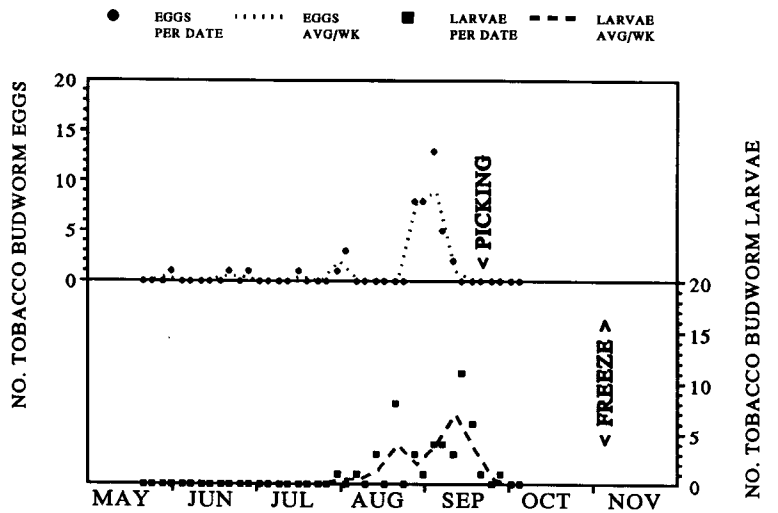

FIG. 2. Numbers of tobacco budworm eggs or larvae found per day on 100 cotton plants inspected twice each week and average numbers per week from 29 May to 5 October, 1991.

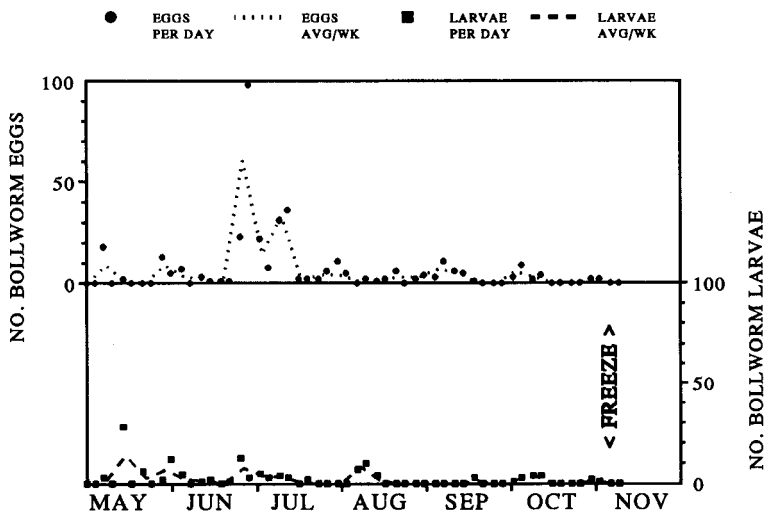

FIG. 3. Numbers of bollworm eggs or larvae found per day on 100 velvetleaf plants inspected twice each week and average numbers per week from 2 May to 17 November, 1991.

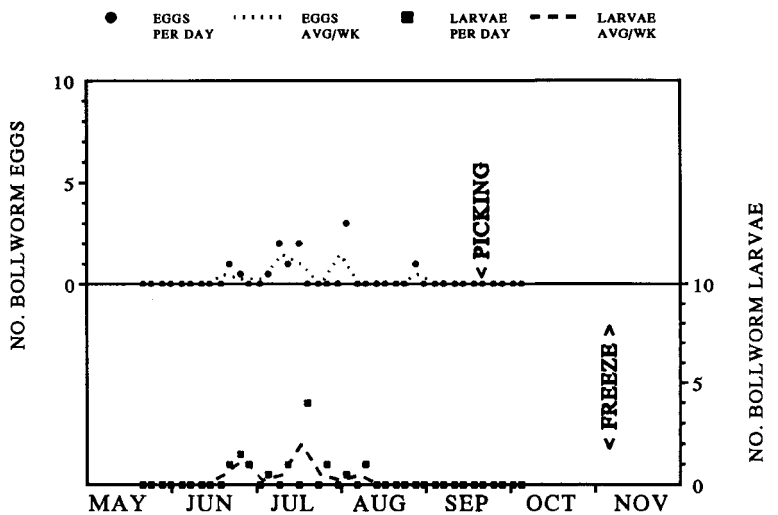

FIG. 4. Numbers of bollworm eggs or larvae found per day on 100 cotton plants inspected twice each week and average numbers per week from 29 May to 5 October, 1991.

No eggs or larvae were found on cotton after picking was completed on 5 October. From the total insects collected, sex ratios (male:female) were estimated as 50.7:49.3 for tobacco budworms and 50.9:49.1 for bollworms.

Average numbers of eggs and larvae of both insects found per 100 cotton or 100 velvetleaf plants per sample day were partitioned into three categories: before cotton-growing season, cotton-growing season, and after cotton season (Table 1). T-tests for related data pairs indicated, for both insect species, that numbers of eggs or larvae found on 100-plant samples of velvetleaf were significantly higher than numbers found on cotton. Frequency analyses indicated that at least one egg or larvae was found in a significantly greater percentage of the 37 daily (100-plant) inspections from velvetleaf than from those of cotton. At least one tobacco budworm egg was collected from velvetleaf on each of 35 (94.6%) of the 37 sample days, and at least one bollworm egg was collected on each of 31 (83.8%) of the 37 sample days. Conversely, frequency of sample days when at least one egg of either insect was found on cotton was significantly lower: 29.7% for tobacco budworms and 21.6% for bollworms. During the cotton-growing season, velvetleaf plants were host to about 18 times the number of tobacco budworm eggs and 28 times the number of bollworm eggs found on cotton. As derived from data plotted in Figs. 1-4, actual numbers of eggs of either insect species collected from velvetleaf plants exceeded or equalled numbers of eggs on cotton plants on each of the 37 sample days throughout the cotton-growing season. It therefore seems apparent that some active selection for velvetleaf, over cotton, was made by ovipositing moths of both species. The earlier presence, taller height (Fig. 5), and relatively large size of velvetleaf foliage and blooms, as well as chemical emissions from glands or flowers, may have cued this selection. Many noctuid moths feed from blooms of wild and cultivated plants at dusk, and if these plants are suitable, females typically oviposit on them 2 to 3 h after feeding. The availability and sequence of velvetleaf blooms compared with those of cotton may have influenced the selection of velvetleaf by female tobacco budworms and bollworms in the inspected fields since velvetleaf blooms appeared earlier, higher, more consistently and later than cotton blooms.

Velvetleaf supported substantial numbers of tobacco budworm larvae during a 34-d period just before the first freeze on 6 November. Presumably, these larvae pupated in soil along the borders and in ditch banks adjacent to cotton fields where their velvetleaf host plants grew. The 6-point progressive average of numbers of larvae per 100 plants plotted for the entire season (Fig. 6) depicted the largest increase of tobacco budworm larvae for a period after the cotton-growing season and before the first freeze. This graph elucidates that, in late September and October, velvetleaf is an important reservoir for tobacco budworm larvae that subsequently overwinter as pupae. The 6-point average plotted for bollworm larvae on velvetleaf indicated three minor increases from May to July and one just before the first freeze, but no major buildup was indicated.

Velvetleaf was an important plant host for oviposition by both tobacco budworms and bollworms and for nutrition by developing larvae. Both insects were found on velvetleaf in the spring as early as May, during the cotton-growing season, and after the the cotton season until the first freeze. By autumn, there was a substantial increase of tobacco budworm larvae on velvetleaf; these larvae presumably pupated and overwintered in the soil inside of and at the borders of cotton fields where their host plant had been growing. Moths emerging from pupae at these sites could easily contribute to an early buildup of egg and larval populations on both velvetleaf and cotton during the spring months of the following year.

TABLE 1. Numbers of Tobacco Budworm and Bollworm Eggs or Larvae Collected from Velvetleaf or Cotton in Washington Co., Miss., 1991.

Insect and plant species	Avg. No.±SEM/100 plants/day[a,b]		Frequency[c] that >1 insect was found/sample day (%)	
	Eggs	Larvae	Eggs	Larvae
BEFORE COTTON SEASON, 2 MAY to 28 MAY, 5 SAMPLE DAYS				
TOBACCO BUDWORM				
VELVETLEAF	2.8±2.0	1.4±1.2	20.0	20.0
BOLLWORM				
VELVETLEAF	4.0±3.5	7.4±5.3	40.0	60.0
COTTON-GROWING SEASON, 29 MAY to 5 OCTOBER, 37 SAMPLE DAYS				
TOBACCO BUDWORM				
VELVETLEAF	22.2±6.9A	12.1±2.3E	94.6L	86.5R
COTTON	1.2±0.4B	1.3±0.4F	29.7K	35.1TW
BOLLWORM				
VELVETLEAF	8.6±2.8C	2.2±0.5G	83.8L	51.4W
COTTON	0.3±0.1B	0.3±0.1F	21.6K	24.3T
AFTER COTTON SEASON, 6 OCTOBER to 17 NOVEMBER, 8 SAMPLE DAYS				
TOBACCO BUDWORM				
VELVETLEAF	5.9±3.4	24.0±4.9	62.5	100.0
BOLLWORM				
VELVETLEAF	1.2±0.5	1.4±0.6	50.0	50.0

[a] One hundred plants of both kinds, when present, were inspected on each sample day. Velvetleaf was present from 2 May to 17 November. Cotton plants larger than 4-leafed seedlings were available after 29 May and "chemically-burned," picked, and terminated by 5 October.

[b] Numbers followed by the same letter, in columns, were not significantly different as indicated by t-tests for differences between related data pairs (numbers of insects per 100 plants per day) where $P<0.05$, df=36, and $t_{tabl.}=1.688$. For numbers of eggs found on 100 velvetleaf vs 100 cotton plants during the cotton-growing season, $t_{calc.}$ was 3.142 for tobacco budworms and 2.927 for bollworms. For larvae found on velvetleaf vs cotton during this season, $t_{calc.}$ was 4.818 for tobacco budworms and 3.556 for bollworms. All four of these $t_{calc.}$ values exceeded $t_{tabl.}=1.688$, indicating significant differences.

[c] Frequency=percentage of the sample days (per category) that at least one egg or one larvae was found per 100-plant sample. Numbers, in columns, followed by the same letters were not significantly different as indicated by t-tests of the disparity between frequencies of occurrence; $t_{tabl.}=2.439$, df=36, and $P<0.01$. Four t values calculated for occurrence of eggs or larvae on either plant exceeded $t_{tabl.}$ indicating significant differences.

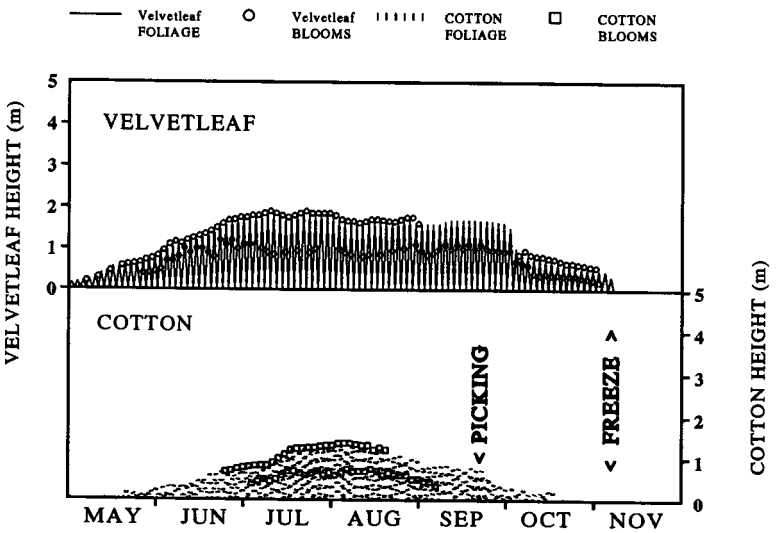

FIG. 5. Sequence of foliage and bloom development and relative height of velvetleaf and cotton plants in Washington Co., Miss. from 2 May to 17 November, 1991.

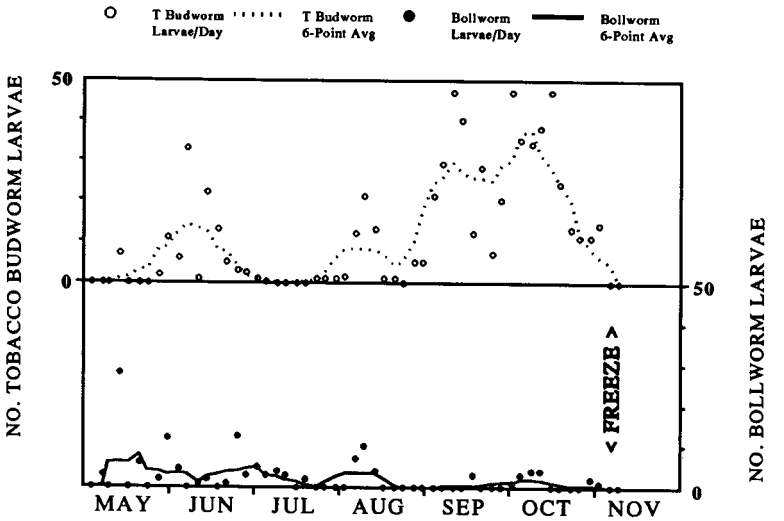

FIG. 6. Numbers of tobacco budworm (O) or bollworm (●) larvae found on 100 velvetleaf plants two days per week and the 6-point progressive averages (--, —) of the numbers of larvae found from 2 May to 17 November, 1991.

The relatively high frequency and numbers of eggs that were found on velvetleaf during the cotton-growing season indicated that some active selection in favor of velvetleaf was exhibited by ovipositing tobacco budworms and bollworms. It is not likely that this selection was biased by the vast number of cotton plants or blooms that occurred in each field surveyed since fewer insects were found on cotton. The greater size of velvetleaf and the early and late presence of its blooms and pubescent foliage were more likely key factors influencing the selection of velvetleaf by ovipositing moths. Velvetleaf is a perennial weed that perpetuates multiple generations of these insects each year in the delta region of the Mississippi River. Destruction of this weed along the borders of cotton fields and on ditchbanks, as a annual agronomic practice in September or immediately after cotton is picked, could significantly reduce numbers of larvae that ultimately overwinter as pupae.

ACKNOWLEDGMENT

Standardized collection of insects from velvetleaf and cotton plants and species identification were conducted by Don W. Hubbard and Larry C. Adams, ARS, Southern Insect Management Laboratory, Stoneville, Miss., and their efforts are gratefully appreciated.

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CORN INSECT PESTS AND PATHOGENIC DISEASES IN RELATIONSHIP TO SHATTERCANE POPULATIONS

L. A. Rodríguez-del-Bosque and E. Rosales-Robles

Campo Experimental Río Bravo, SARH-INIFAP, Apartado Postal 172, Río Bravo, Tamaulipas, México 88900

ABSTRACT

The influence of shattercane, *Sorghum bicolor* L. (Moench), on the incidence of insects and pathogens of corn was studied in northern Tamaulipas, Mexico. Insects and pathogens studied included *Helicoverpa zea* (Boddie), *Spodoptera frugiperda* (J.E. Smith), *Diatraea lineolata* (Walker), *Eoreuma loftini* (Dyar), *Phyllophaga crinita* (Burmeister) *Fusarium* spp., *Ustilago maydis* (DC.) Cda., and *Macrophomina phaseolina* (Tassi) G. Goid. Except for *P. crinita*, all insects and pathogens occurred independently of shattercane. Populations of *P. crinita* increased with increasing shattercane densities, following a curvilinear response. This is probably a result of adults preferring shattercane over corn and/or denser vegetations.

RESUMEN

Se determinó el efecto de la cañita, *Sorghum bicolor* L. (Moench), sobre la incidencia de insectos y enfermedades del maíz en el norte de Tamaulipas, México. Los insectos y enfermedades estudiados fueron *Helicoverpa zea* (Boddie), *Spodoptera frugiperda* (J.E. Smith), *Diatraea lineolata* (Walker), *Eoreuma loftini* (Dyar), *Phyllophaga crinita* (Burmeister) *Fusarium* spp., *Ustilago maydis* (DC.) Cda., y *Macrophomina phaseolina* (Tassi) G. Goid. A excepción de *P. crinita*, la incidencia de todos los insectos y enfermedades ocurrió independientemente de la presencia de cañita. Las poblaciones de *P. crinita* fueron más altas al incrementarse las densidades de cañita, siguiendo una respuesta curvilínea. Lo anterior se debe probablemente a que los adultos prefirieron la cañita en lugar del maíz y/o las vegetaciones más densas.

INTRODUCTION

Shattercane, *Sorghum bicolor* (L.) Moench, an annual, tall forage-type sorghum, was recently detected in northern Tamaulipas, Mexico. This new weed, known locally as "cañita", is thought to be introduced in contaminated grain sorghum seed. It has spread rapidly across this region as a consequence of its great reproductive ability. In a short time, shattercane has shown the potential of becoming one of the most important weed species in this area (Rosales-Robles 1988). In addition, the presence of shattercane in northern Tamaulipas has affected the sorghum seed industry; hybrid production has decreased greatly during the past few years because of the high risk of contamination with shattercane pollen.

Once introduced to an area, shattercane spreads readily by run-off water, surface irrigation water, machinery, manure, or by passing through livestock. Shattercane matures before most row crops, including corn. Shattercane seeds fall to the ground, and remain viable in soil for as long as 13 years (Hayden and Burnside 1987).

Significant yield reductions in corn caused by shattercane competition are well documented (Burnside 1970, Hayden and Burnside 1987, Beckett et al. 1988, Camacho et al. 1990). Yield loss is the result of competition for water, nutrients, and light. A survey in northern Tamaulipas demonstrated that corn fields infested with shattercane at densities averaging 12 plants/m of row sustained an average loss of 34% (Rosales-Robles 1988). Yield losses >90% have been reported for grain sorghum (Vesecky et al. 1973) and soybeans (Burnside 1968).

In addition to yield losses, weeds can also affect crops by harboring and augmenting pests, particularly when both crop and weed(s) are closely related. For instance, McWhorter (1989) listed a large number of insects, bacteria, viruses, fungi, and nematodes common to sorghum and corn that also attack johnsongrass, all of the family Poaceae. The objective of this study was to determine whether shattercane, associated with corn, increases incidence of the most common corn insect pests and pathogens in northern Tamaulipas.

MATERIALS AND METHODS

An experiment was conducted at the Campo Experimental Río Bravo, SARH-INIFAP, near Río Bravo, Tamaulipas, Mexico, during the spring season of 1990. The experiment was arranged in a split-plot design with four replications. Main plots were shattercane densities (0, 1, 2, 4, 8, 16, and 32 plants/m of row); subplots were durations of shattercane competition with corn from time of planting (20, 40, 60 d, and season-long). Subplot size was four rows (80 cm each) by 5 m. Data were obtained from the two center rows. Corn and shattercane were planted on 5 March 1990. Corn hybrid H-422 was planted at 55,000 plants/ha. Shattercane seeds were collected near Río Bravo the previous year, and a preliminary test one month before planting the experiment showed a germination of 90%. Shattercane densities were established in corn rows at different intervals using marked chains. To maximize emergence of shattercane, 2-3 seeds were planted per interval; extra plants were removed where more than one plant emerged. To avoid interference from other weeds, 1 Kg (A.I.)/ha atrazine was applied at planting.

Corn pests were monitored at vegetative and reproductive corn growth stages. However, since no important corn insects or pathogens occurred during the vegetative stage, only ear and stalk pests during the reproductive stage are reported here. Insects and pathogens were evaluated at corn physiological maturation. Ear pests were evaluated visually as percent of ear damaged (10 ears/subplot) and included the corn earworm, *Helicoverpa zea* (Boddie); fall armyworm, *Spodoptera frugiperda* (J.E. Smith); ear rot, *Fusarium* spp.; and common smut, *Ustilago maydis* (DC.) Cda. Stalk pests, evaluated by dissecting 10 stalks/subplot and counting the internodes damaged, included the neotropical cornstalk borer, *Diatraea lineolata* (Walker); Mexican rice borer, *Eoreuma loftini* (Dyar); and charcoal rot, *Macrophomina phaseolina* (Tassi) G. Goid. When ears were damaged, but insects were not found, damage in tips and sides of the ear were attributed to either *H. zea* or *S. frugiperda*, respectively; this differential damage was observed to be typical in preliminary observations. Similarly, damage in the stalk either by *D. lineolata* or *E. loftini* is characteristically different. Tunnels of *D. lineolata* are longitudinal and clear of frass, with stalks showing conspicuous exit holes. Tunnels of *E. loftini* are both

transverse and longitudinal, packed with frass, and located mostly near the nodes (Rodriguez-del-Bosque et al. 1988).

Attractiveness of shattercane densities to adults of the white grub, *Phyllophaga crinita* (Burmeister), was tested by sampling larvae, which indirectly indicated activity by their progenitors (Rodriguez-del-Bosque 1984), and revealed any preference for plots with varying shattercane populations. *P. crinita* was sampled only in subplots with season-long shattercane; shattercane in other competitive periods (20, 40 and 60 d) had already been removed from the plots by the time *P. crinita* adults were active (May). During 5-10 July, 10 soil samples/replication (40 samples total) of 30 x 30 x 30 cm were taken for each shattercane density, and white grub larvae (most at 2nd-instar) counted.

Ear and stalk pest data were subjected to analysis of variance (ANOVA) and means separated by Tukey's studentized range test (p= 0.05) (SAS Institute 1988). Numbers of white grubs (y) were fit to the exponential model $y = a - e^{(-bx)}$, where a and b are constants and x the shattercane density. Parameter estimates of a and b were obtained by using PCNONLIN (Statistical Consultants 1989). To test the association among ear and stalk insects and pathogens, a correlation matrix with all variables was obtained (SAS Institute 1988).

RESULTS AND DISCUSSION

Incidence of all ear and stalk insects and pathogens was independent of shattercane density and competition period. In all cases, the analysis of variance indicated no significant difference (p> 0.05) for either main plots (shattercane densities), subplots (shattercane competition period), or the interaction of both factors (Table 1). No differences were found even when organisms were combined according to their resource exploited (*H. zea* + *S. frugiperda* or *D. lineolata* + *E. loftini*) (Table 1) or when all pest (ear and stalk insects and pathogens) damages were pooled (data not shown). Shattercane evidently did not influence the occurrence of these pests on corn.

The correlation matrix of all ear and stalk pest variables indicated significant associations (p<0.01) only between *Fusarium* and the ear insects: r= 0.3588 with *H. zea*; r= 0.3749 with *S. frugiperda*; and r= 0.4406 with *H. zea* + *S. frugiperda*. Apparently, ear insects promoted infection by *Fusarium* spp., as reported by Shurtleff (1980).

White grub numbers were related to shattercane density following a curvilinear response (Fig. 1). White grubs (y) fit the exponential model y= 3.196 $-e^{-0.1976x}$ closely (R^2= 0.867). Apparently, adults of *P. crinita* oviposited more frequently or were more attracted to plots with higher shattercane densities. This is probably a result of several interacting factors. First, there may be a biological response of *P. crinita* to oviposit preferentialy in denser vegetations to maximize resources for its progeny. Also, shattercane may be preferred over corn as a host. Rodríguez-del-Bosque (1984) demonstrated sorghum to be the preferred host among several host plants for *P. crinita*. When given a choice, *P. crinita* is apparently attracted to oviposit on a specific host plant at an optimal host density, in addition to an optimal soil humidity (Gaylor and Frankie 1979).

In conclusion, this study showed no evidence that shattercane was associated with incidence of corn ear and stalk insect pests and pathogenic diseases. However, it did demonstrate that white grubs populations increased with an increase in shattercane densities.

TABLE 1. Source of Variation and Average Incidence of Corn Pests at Different Shattercane Densities and Periods of Competition.

Source of variation[a]	*F* value	Pr > *F*	Average (± SEM)[b]
Helicoverpa zea			1.42 (0.13)
A	1.14	0.35	
B	1.84	0.15	
A*B	1.06	0.41	
Spodoptera frugiperda			0.26 (0.06)
A	1.32	0.26	
B	1.36	0.26	
A*B	0.74	0.75	
H. zea + S. frugiperda			1.68 (0.16)
A	1.64	0.15	
B	1.90	0.14	
A*B	1.04	0.44	
Diatraea lineolata			0.20 (0.02)
A	0.81	0.57	
B	0.20	0.89	
A*B	0.91	0.57	
Eoreuma loftini			0.06 (0.01)
A	1.39	0.23	
B	0.07	0.97	
A*B	1.34	0.20	
D. lineolata + E. loftini			0.27 (0.03)
A	0.49	0.81	
B	0.20	0.90	
A*B	1.18	0.30	
Fusarium spp.			5.98 (0.69)
A	0.65	0.69	
B	0.06	0.98	
A*B	1.46	0.14	
Ustilago maydis			0.36 (0.01)
A	1.06	0.39	
B	1.84	0.15	
A*B	0.83	0.66	
Macrophomina phaseolina			2.71 (0.06)
A	0.75	0.62	
B	1.24	0.26	
A*B	1.51	0.12	

[a] A= Shattercane density; B= Shattercane competition period.
[b] *n*= 112. Averages of *H. zea, S. frugiperda, Fusarium* spp. and *U. maydis* represent % ear damaged, whereas *D. lineolata, E. loftini,* and *M. phaseolina,* number of internodes damaged.

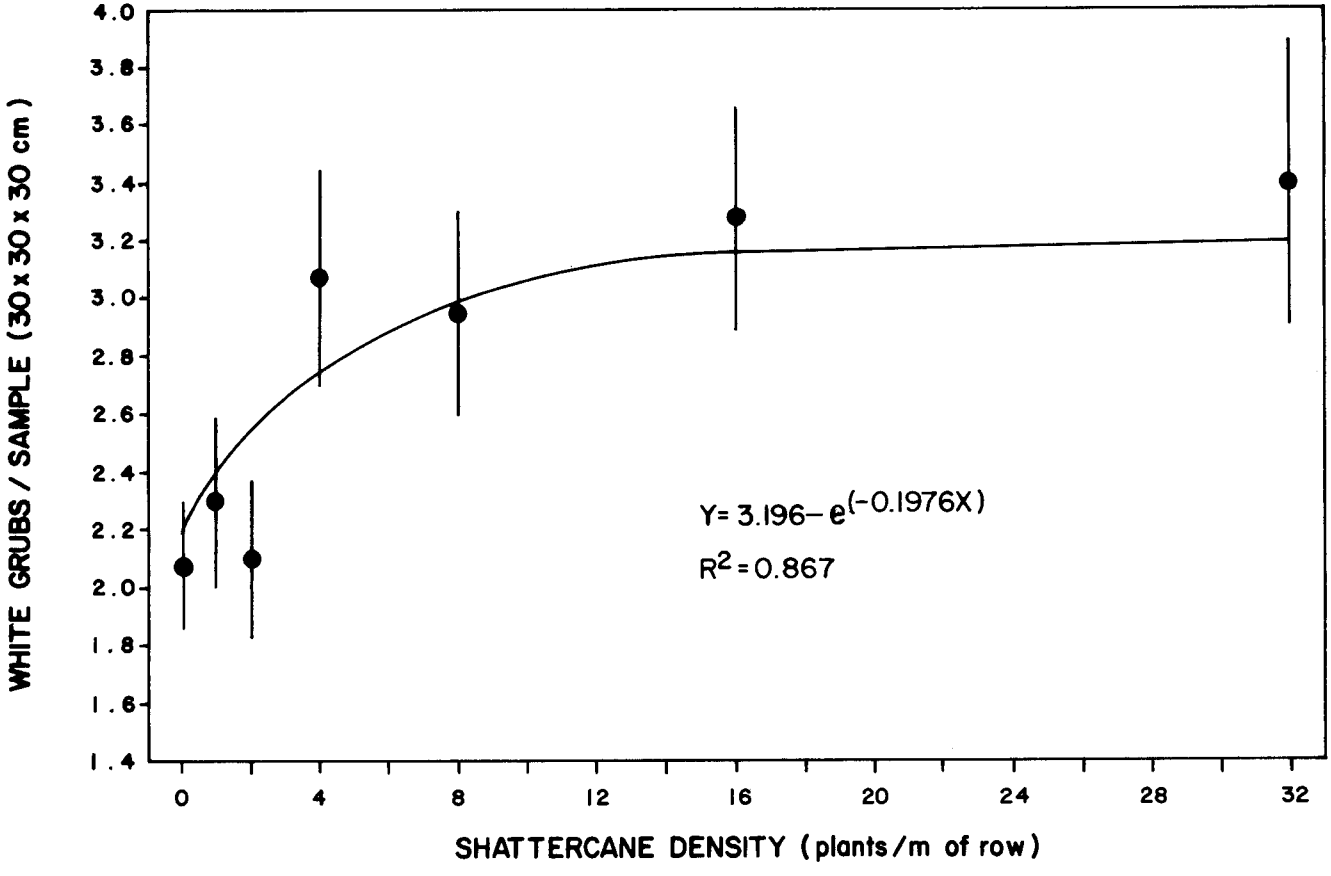

FIG. 1. Relation of white grub density to shattercane population. Bars indicate ± SEM (n= 40).

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FULLER ROSE BEETLE[1] EMBRYONIC DEVELOPMENT AND OBSERVATIONS ON OVIPOSITION

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ABSTRACT

Eggs weigh an average of 95μg and are 0.85mm in length by 0.46mm in diameter and are secured to the substrate with an adhesive that becomes relatively water insoluble 1 h after deposition. Egg development at 27°C was first observed on day two, having mitotic cells visible. On day three, the germ band migrates into the yolk and the germ band elongates then shortens during the next three days. By the seventh day, the cerebrum is visible and ganglia appear segmented by day eight. Longitudinal rotation of the embryo occurs on day nine and ten. Mandibles and eyes become visible on day twelve and thirteen with mandible sclerotization on day fifteen. Larval emergence starts on the seventeenth day.

INTRODUCTION

Fuller rose beetle, *Asynonychus godmani* Crotch, is a pest of citrus. The larvae are root feeders that pupate in the soil. After emergence, adults (incapable of flight) climb into trees and cause minor foliar damage. The parthenogenic adults deposit eggs mostly under the calyx of fruits (Coats and McCoy 1990). Infested citrus fruits destined for Japan must be fumigated with methyl bromide which can result in up to 60% loss of lemons due to phytotoxicity (Griffiths et al. 1986). A fruit inspection and phytosanitation certification program also has been allowed by the Japanese to avoid shipment of infested fruit into Japan. Fruits are inspected for eggs at the port of Long Beach, California.

Investigation into the relative susceptibility of Fuller rose beetle eggs to various fumigants has shown that only methyl bromide was effective, and that a greater concentration than currently used would be necessary to effect an acceptable level of control (Soderstrom et al. 1991). Apparently insecticides are prevented from reaching the embryo or are somehow detoxified. Chauvin and Barbier (1979) noted that several envelopes (the chorion, vitelline membrane, serosa, and

[1]Coleoptera: Curculionidae

embryonic membrane) protect the insect embryo. Additionally, eggs are deposited in masses of up to ca. 30 eggs that are covered with an adhesive that may also protect the embryo. Determination of insecticidal effects on Fuller rose beetle eggs by observing egg hatch is time-consuming due to a long developmental time of about 17 days at 27°C.

To date there have been no embryological studies reported for this species. This research describes the egg, identifies embryological characteristics useful for determining developmental status of eggs, and reports on the beetle's behavior during the ovipositional process. In addition, the duration of egg development was determined under our test conditions and compared to two previously published degree-day models for this insect.

MATERIALS AND METHODS

Fuller rose beetle adults were collected from citrus orchards in the San Joaquin Valley, CA. Adults were maintained at 12°C with 70-80% humidity and 10:14 h L:D photoperiod in 3.8-l glass jars with screen closures. Fresh citrus shoots with young leaves were provided for food. To obtain eggs for testing, adults were exposed to 27°C for 24 h, then allowed to oviposit in laminated wax paper egg collection devices (Soderstrom et al. 1991). Eggs were collected daily to obtain eggs of known age over the entire developmental period. Eggs were removed from the collection devices, placed in petri dishes and held at 27°C and 85% relative humidity.

Eggs, in clusters consisting of ca. 30 eggs, of known age were placed in 0.5% sodium hypochlorite solution (10% chlorine bleach) for approximately 9 min to partially dissolve the adhesive and disrupt the chorion. After rinsing in tap water, eggs were held in Bouin's fixative (Galigher and Kozloff 1971) for 3 days at room temperature (ca. 24°C), dehydrated in an increasing series of 30, 50 and 70% ethanol for 10-20 min each, then washed continuously in 70% ethanol for 1-2 days until cleared of picric acid. Eggs were then stained for 1-1.5 h in leucobasic fuchsin after acid hydrolysis according to a technique used as a chromosome stain by von Borstel and Lindsley (1958) and mounted in Coverbond (Harleco, Gibbstown N.J.) under coverslips on glass microscope slides. Eggs were photographed through a Leitz Dialux 22 compound microscope (Ernest Leitz Wetzler GMBH, West Germany) using Kodak Tmax 100 film at magnifications of 62.5 or 250X. Photographs were examined for developmental, time-related embryonic characteristics.

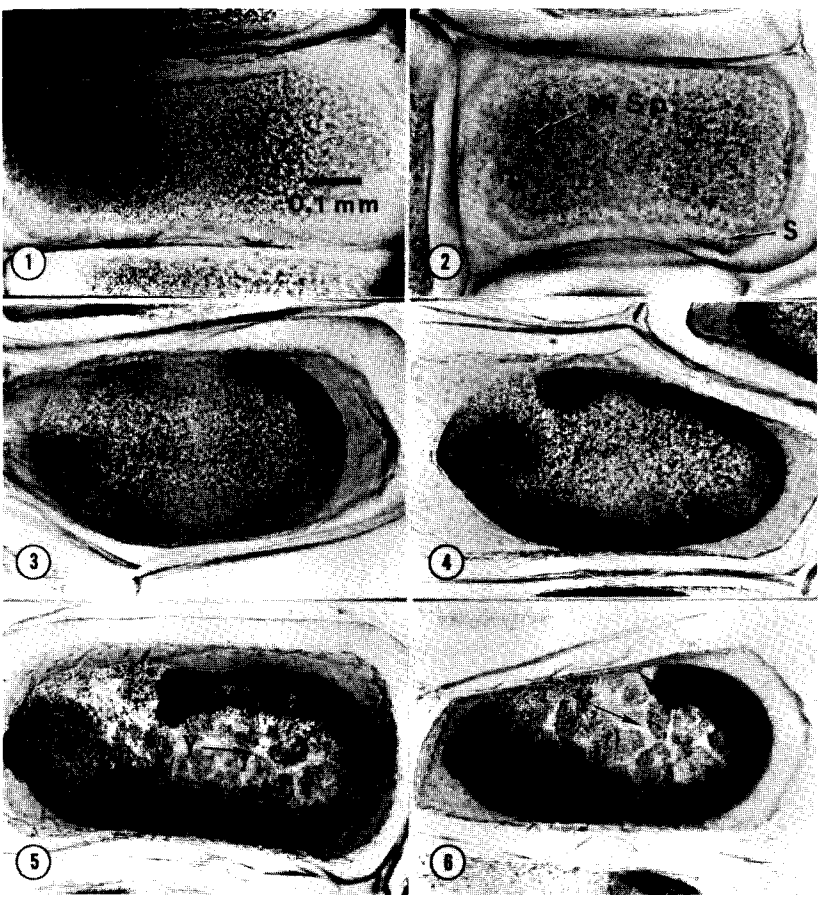
Adult behavior was observed (27°C) while they oviposited: (1) under coverslips mounted on glass slides, (2) into crevices in layered wax paper egg collection devices, or (3) into crevices between the calyx and rind of lemon fruit. Duration of egg development from oviposition through larval emergence was determined at 27°C and 85% RH. Ten egg clusters were observed and the time required for first larval emergence and average time of emergence were recorded.

RESULTS AND DISCUSSION

Egg Description. Eggs are oval in shape, averaging 0.85 mm (SD=0.03) in length by 0.46 mm (SD=0.03) in diameter.

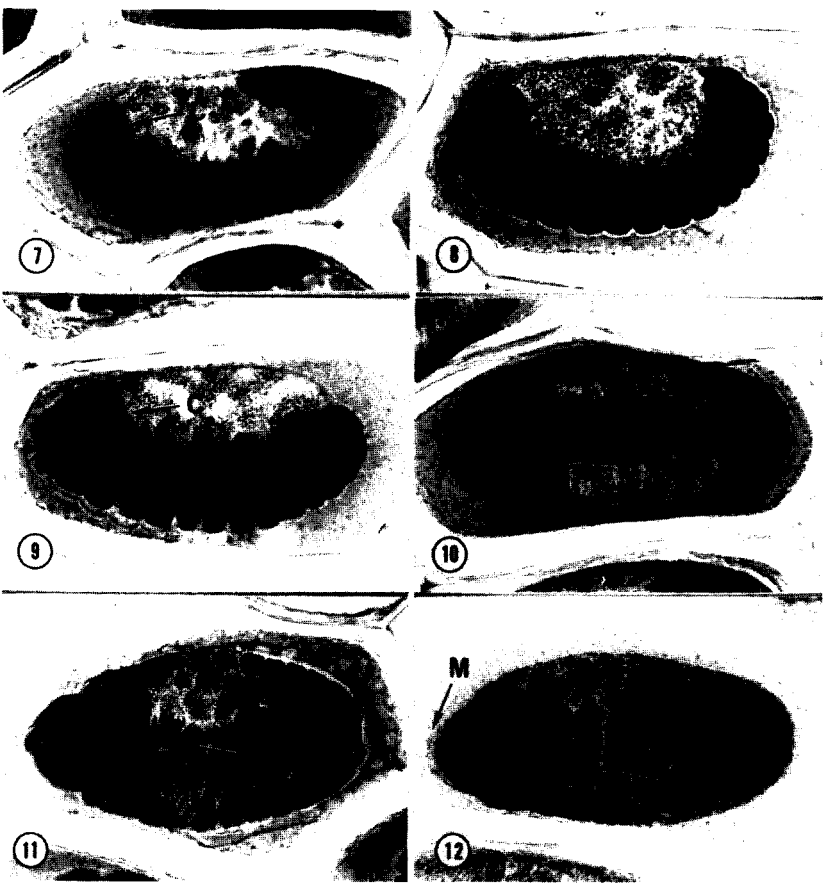
Forney et al. (1991) showed that eggs have an average weight of 95 ± 8μg. They are uniformly pale yellow when first deposited. The chorion surface is lightly patterned when viewed without the adhesive covering, but this pattern is normally obscured by the covering adhesive. The yolk area develops a deeper yellow color after 48 h while the ends of the egg become lighter in color.

Embryological Development. Tiegs and Murray (1938) described the embryological development of another curculionid, the rice weevil, *Sitophilus oryzae* L. Fuller rose beetle development resembles that of the rice weevil except that the rice weevil development takes ca. 4 days while Fuller rose beetle development requires ca. 2 weeks. Development of the Fuller rose beetle embryo is shown in Figs. 1-15. The newly deposited egg is uniformly pale yellow and has no discernible



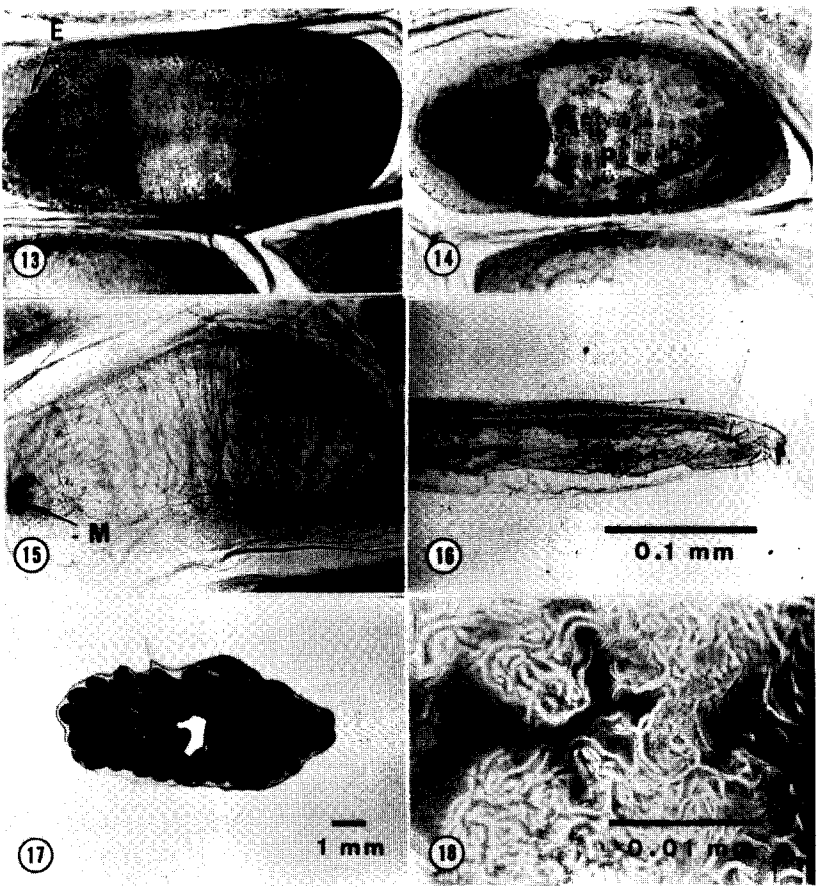
FIGS. 1-6. Daily development of Fuller rose beetle embryo; figure numbers also represent developmental age in days. Legend: mitosis (Mi); serosa (S); yolk sacs (Y).

cellular change during the first 24 h (Fig. 1). During the second day, mitosis (Mi) is evident as indicated by the presence of spindle systems, and nuclei are visible in the serosa (S) that envelops the developing egg (Fig. 2). On the third day the germ band forms and sinks into the yolk, appearing as a "C" shaped darkening (Fig. 3). The germ band elongates and appears oval shaped with the ends slightly enlarged and separated from each other by a small gap on day 4 (Fig. 4). Day 5 development is marked by a shortening of the germ band with the posterior portion becoming more knobbed in appearance. A division of the yolk into yolk sacs (Y) occurs and the head area of the embryo shows a pronounced frontal bulge (Fig. 5). The embryo continues to decrease in length on day 6 (Fig. 6) as well as on day 7 when a cerebrum (C) begins to develop (Fig. 7). The cerebrum increases in size, the embryo appears "C"



FIGS. 7-12. Daily development of Fuller rose beetle embryo; figure numbers also represent developmental age in days. Legend: cerebrum (C); mandible (M); proctodeum (P).

shaped, and its ganglia are strongly segmented on day 8 (Fig 8). On day 9 the cerebrum continues to enlarge (Fig. 9) and an axial rotation of the embryo begins. On day 10 the axial rotation of the body is complete, the head capsule is distinguishable, the cerebrum (C) appears as two lobes, and proctodeum (P) formation (darker area) is initiated (Fig. 10). Proctodeum development continues, visible as a dark lateral band on day 11 (Fig. 11), becoming more apparent as a lighter tube-like structure on day 12 (Fig. 12), and becomes liquid-filled on day 14 (Fig. 14) (liquid was observed in live specimens). Mandibles (M) are visible on day 12 (Fig. 12) and compound eyes (E) become evident on day 13 (Fig. 13). Sclerotization of the mandibles is apparent on day 15 at which time



FIGS. 13-18. 13-15. Daily development of Fuller rose beetle embryo; figure numbers also represent developmental age in days. Legend: compound eye (E); mandible (M); proctodeum (P). 16. Ovipositor showing hairs. 17. Orientation of eggs in a cluster. 18. Fibrous adhesive surrounding eggs.

stain uptake of the embryo decreases (Fig. 15), probably due to the formation of chitin which becomes more prevalent throughout the head and body. The embryo appears fully formed and larval emergence starts around the 17th day.

Oviposition. Adult weevils prefer to oviposit in narrow protected places (cracks and crevices) such as between the sepals and peel of citrus fruits. Eggs are placed with the ovipositor that extends 4-5 mm and searches for suitable locations far under the fruit calyx. Observation of the oviposition process has shown the ovipositor to move into a crevice and when an apparently suitable tactile response is received (note hairs on the ovipositor (Fig. 16), a drop of fluid (adhesive) about half the size of the egg, precedes the egg down the ovipositor and may assist in the orientation and sticking of the egg to the substrate. Eggs subsequently deposited are oriented parallel in regard to the previously deposited eggs as shown in Fig. 17. In large clusters, the outer eggs are usually oriented perpendicular to the central eggs. The accessory fluid deposited with the eggs functions as an adhesive that secures the eggs to a substrate. During normal oviposition (under fruit buttons or on the surface of fruit), eggs may be piled on top of and around each other, whereas in thin cracks such as with our wax paper collection method, eggs are deposited in a single layer. The adhesive that surrounds the eggs during oviposition is a clear fluid that hardens within 1 h and has an opaque, intermeshing fibrous appearance (Fig 18). This observation differs from "a white spongy material" reported for this species by Dickson (1950). Prior to hardening, the adhesive is water soluble and readily stains to a cherry red color with acid fuchsin. After hardening, the adhesive is insoluble in water, and treatment with acid fuchsin results in only a light pink color. The adhesive may be dissolved with a 10% chlorine bleach solution but is not readily affected by ethanol, detergents, or hot water. Papain or trypsin enzymes also do not readily digest the adhesive; however, exposure to these enzymes caused increased surface wetting, increased flexibility, and increased stain uptake.

Egg Developmental Time. Degree-day models for Fuller rose beetle have been developed by Tarrant and McCoy (1989) and by Lakin and Morse (1989). Tarrant and McCoy reported 267 degree-days above a developmental threshold of 10.8°C. Lakin and Morse reported 251 degree-days above a developmental threshold of 11.7°C for 50% egg hatch. Either model calculates to 16.4 days at our rearing temperature of 27°C. Our observations averaged 17 days for 50% hatch which is in close agreement with the previously published models.

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WESTERN GRAPELEAF SKELETONIZER[1] PHEROMONE TRAP CATCH SEASONAL PATTERNS IN CENTRAL CALIFORNIA

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ABSTRACT

Male moth flights of the western grapeleaf skeletonizer (WGLS), (*Harrisina brillians* Barnes and McDunnough) were monitored for three or more years (1986 to 1991) in several vineyards in Fresno County, California, using traps baited with synthetic sex pheromone lures. Several trapping devices were compared for relative efficiency. WGLS moths began to emerge from overwintering pupae in individual vineyards from mid March to early April. First occurrence trap catch usually occurred between 16 and 25 March. Moth flights were similar among vineyards within a year, but showed greater variation among years. Compared to calendar time, WGLS seasonal occurrence correlated closest with degree-days (i.e., physiological time) accumulated from the first day moths were caught each spring. Comparison of two bucket trap styles demonstrated a moderate difference in relative efficiency.

INTRODUCTION

The use of pheromone systems as a monitoring tool has been of particular interest to practitioners of integrated pest management. Pheromone traps can be very useful for monitoring a species' presence, and they offer the potential to greatly reduce the frequency of more time consuming and labor intensive monitoring techniques. Along with monitoring the overall seasonal occurrence of a species, pheromone traps may be useful in identifying important events during the season. Thus, the approximate time of first moth appearance may often be used as a "biofix" for phenology model initialization.

While sex pheromone trap catch data can be valuable in describing the general seasonal occurrence pattern of a species, particular care must be taken when attempting to use

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such data to reflect relative population abundance on a daily basis. Such information can only be interpreted within the context of a detailed knowledge of the biology of the organism. The field dynamics of competition involving synthetic pheromone and female moth pheromone attractiveness to male moths is a complex subject. For example, pheromone trap catch of the western grapeleaf skeletonizer (WGLS), (*Harrisina brillians* Barnes and McDunnough), frequently declines during the middle of a generation curve, while at the same time visual count data of flying moths signify peak male and female moth abundance (Carr et al. 1992).

Originating in southwestern United States and Mexico, the western grapeleaf skeletonizer was first detected in San Diego County, California during 1941 (Lange 1944). It is now established in the southern and central regions of California and may ultimately become established in the northern viticultural regions of the state. WGLS larvae are primarily grapevine defoliators. However, they will feed on fruit clusters on extensively defoliated vines, often leading to *Botrytis* bunch rot. Research on WGLS biology, biological control, chemical control, and pheromone attractants has been conducted intermittently for more than forty years since Lange's (1944) report on this insect as a new California grape pest (Steinhaus and Hughes 1952; Barnes et al. 1954 a,b; Hall 1955; Smith et al. 1956; Clausen 1961; Myerson et al. 1982; Stern et al. 1983; Soderstrom et al. 1985; Curtis et al. 1989; Stern and Federici 1990; Mayse and Carr 1990; Federici and Stern 1990; Roltsch et al. 1990; Carr et al. 1992).

The primary objective of this project was to investigate WGLS moth seasonal activity patterns within and between years in vineyards in the Fresno County region of California's San Joaquin Valley. Furthermore, several commonly used moth traps were compared for their relative efficiency. Results would be useful in understanding the manner in which patterns of WGLS activity may vary or coincide among vineyards and years in the central San Joaquin Valley. This research is part of a broader research program directed at understanding WGLS ecology, with the ultimate goal of helping growers improve ways to manage WGLS populations.

MATERIALS AND METHODS

Studies during 1986-87 were conducted on the California State University, Fresno (CSUF) farm vineyard. Counts were made of male moths collected from wing and delta style pheromone traps paired within each division of a 1 ha, six-quadrat grid as outlined by Carr et al. (1992). Each trap contained the same type and amount of the 1 cm x 0.5 cm laminated lure (Hercon Environmental Corp., Emigsville, Pa.). The WGLS lure contained the synthetic analog of female pheromone (sec-butyl-(Z)-7-tetradecenoate)(Soderstrom et al. 1985, Curtis et al. 1989). Both trap designs received new lures every six weeks. The sticky bottom inserts were replaced on an individual basis depending on each trap's condition. Each day when the 12 traps were checked, WGLS moths were counted and removed from the inserts so that the sticky surface would remain effective in capturing moths attracted the next day. Pheromone traps

were monitored in the early afternoon on a daily basis. In 1986, monitoring began on 1 June, prior to the start of the second moth flight of the season. In 1987, monitoring began during the first week of March, well before the spring emergence from the overwintering population of pupae. For all years, monitoring continued into November, by which time moths were rarely caught.

During 1988, a bucket trap (Multipher I, Bio-control Services, Canada) was placed in the CSUF vineyard, along with traps (1 per vineyard) in six commercial or abandoned Thompson Seedless vineyards in the Fresno vicinity. While five vineyards were located within 13 km east, west or south of CSUF, one vineyard was approximately 29 km south of CSUF. During 1989-91, trapping was continued in three Fresno area vineyards, including the CSUF vineyard. Relative to the CSUF vineyard, the other sites were situated 12 to 29 km southeast or west. A trap was placed in each vineyard prior to spring emergence of WGLS in March, and checked at three-to-four day intervals during each flight cycle and weekly between cycles. During 1991, each Multipher I trap was paired with a second bucket trap design [Funnel Trap (unitrap), AgriSense, Fresno, CA] to compare their relative efficiency. The two traps were hung from the trellis in the same row, 20 meters apart at an approximate height of 1.5 m. Bucket traps were used during 1988-91 because of their ability to trap large numbers of moths without the diminished effectiveness expected from a sticky type trap. Sticky traps required daily removal of moths during peak flight periods when an accurate assessment of moth flight activity was necessary. The collection unit of each bucket trap contained a small piece of insecticidal strip material (a.i.= dichlorvos) that remained effective throughout the entire season. The collection unit capacity for moths was very large. On several occasions, traps were noted as being approximately one-third full, while they each contained nearly 500 moths.

WGLS trap catch data for 1986-87 are presented as daily trap catches. Those collected during 1988-91 are presented as the estimated daily trap catch, which equals the number of moths caught between check dates divided by the number of days between check dates. These data were plotted against the Julian date or accumulated degree-days accrued midway between dates on which traps were checked.

Degree-day accumulations began on the first day that moths were caught during spring emergence within each vineyard. Degree-days were calculated using the Baskerville and Emin (1969) single-sine approximation method with a lower (9.0°C) and upper (28.2°C) threshold (Roltsch et al. 1990). Temperature data were accessed through the University of California IMPACT program (Anonymous 1987). Temperature data recorded by a California Irrigation Management Information System (CIMIS) station located on the CSUF campus were used for all vineyards, with two exceptions. Data from a second CIMIS station were used in relation to the two distant vineyards monitored during 1988 and 1991. This station was located within 6.5 km of both sites.

Differences in wing versus delta trap catch data collected in the CSUF vineyard during 1986 and 1987 were found to be

relatively minor (Carr et al. 1992). As a result, data for these two sticky trap designs were pooled for presentation. For each vineyard during 1991, season-long, daily trap count differences between the two bucket trap styles were compared using the Students t statistic for paired comparisons [PROC MEANS (SAS Institute 1985)].

RESULTS AND DISCUSSION

Occurrence of four distinct moth flights was demonstrated in 1986, 1987 and 1989, suggesting that as many as four generations can occur in the San Joaquin Valley in a given year (Figs. 1,2). Relatively low trap counts during the fourth moth flight indicated that the majority of moths emerging in the spring were probably from pupae that entered diapause during the third and perhaps earlier generations. In comparing WGLS degree-day requirements for development with the 1986-90 degree-day accumulations during late summer and fall, it is suggested that larvae which are not third- to fifth-instars by mid-October are likely to encounter insufficient cumulative degree-days to complete development. For example, during the period from 15 October to 30 November of 1986 to 1990, the mean number of degree-days ($\bar{x}$ $\pm$SD) accrued at the CSUF site was 271 $\pm$15.7 DD. In comparison, WGLS larvae (i.e., five instars) require 384 DD to develop from egg hatch to pupation (Roltsch et al. 1990). The slow accumulation of

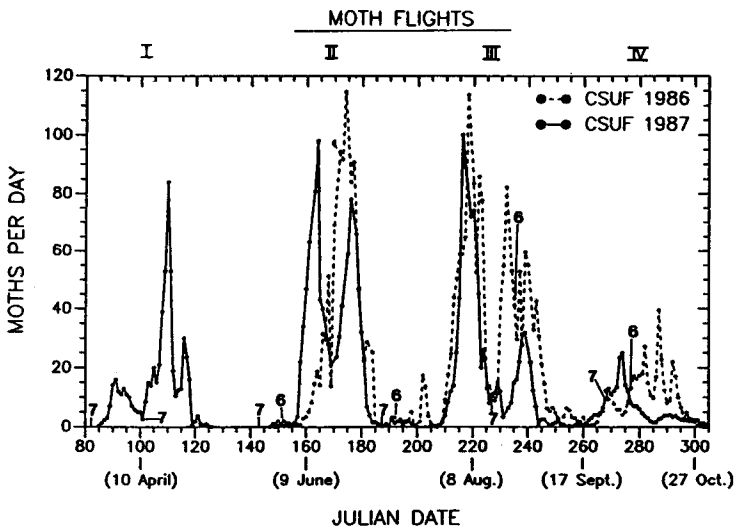

FIG. 1. Average daily pheromone trap catch counts for male moths during 1986-87 in CSUF vineyard. Averages calculated daily from 12 traps for all dates except from 26 March to 10 April during 1987, at which time four traps were used. Number values designate dates of pheromone lure replacement for each year (6=1986, 7=1987).

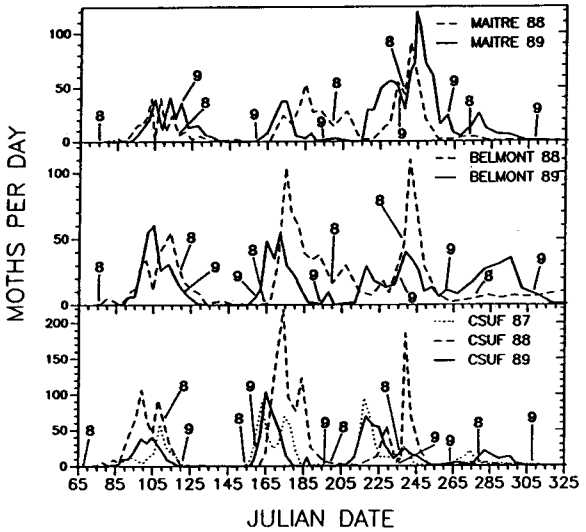

FIG. 2. Estimated daily trap catch counts for male moths in the CSUF vineyard during 1987-89, and in two additional vineyards during 1988-89. Counts plotted against Julian dates. Number values designate dates of pheromone lure replacement for each year (8=1988, 9=1989).

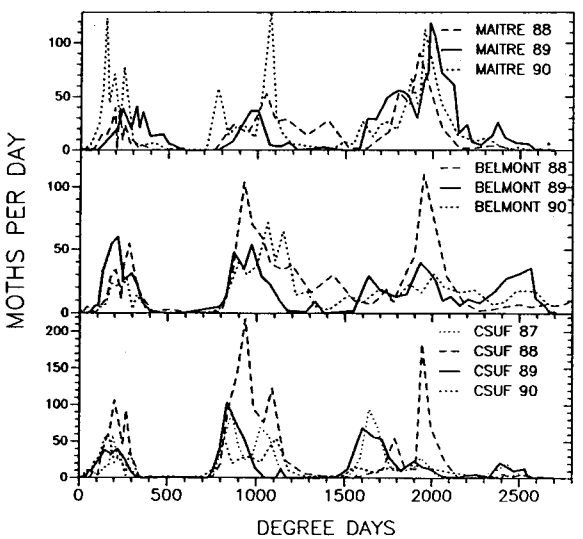

FIG. 3. Estimated daily trap catch counts for male moths in the CSUF vineyard during 1987-90, and in two additional vineyards during 1988-90. Counts plotted against degree-days.

degree-days coupled with high leaf loss as vines enter dormancy are likely to have a marked impact on completion of development of late season larvae.

Based on 14 records of first trap catch from 1987 to 1990, the earliest date that moths were caught in a trap was on 13 March, 1988. The latest date during this time period that the first moth of the season was caught in a given vineyard was 3 April 1990. The majority of first seasonal trap catches (i.e., 10 of 14) occurred between 16 and 25 March. Periods of moth flight activity during each generation commonly lasted as long as 20-30 days.

During the unusually cool spring of 1991, moth emergence was delayed in all three vineyards monitored (i.e., 28 March, 8 and 9 April). In comparing degree-days among years, the mean cumulative degree-days ($\bar{x}$ $\pm$SD) during March of 1986 to 1990 was 164 $\pm$21.8 DD, compared to 82 DD during March of 1991.

Generational trap catch curves commonly exhibited several peaks (Figs. 1,3,4). The decline in trap catch resulting in bimodal curves did not reflect a decline in moth abundance. Studies of WGLS moth flight behavior have shown that both male and female flight activity is often very high during this time (Carr et al. 1992). We suggest that an increased proportion of male moths may be attracted to actual pheromone-emitting females rather than to the synthetic lure traps at these times. However, it is unknown to what extent moth population density influences competition and in turn alters trap catch.

Relative to the remainder of the season during 1987, spring emergence trap data may have been artificially high until 11 April (Fig. 1). This probably resulted from having only four traps in the vineyard until 11 April, compared with 12 traps through the remainder of the 1987 season.

Trap catch data plotted against degree-days improved the correspondence of seasonal flights among years within vineyards (Fig. 3). This was most evident by comparing second moth flight overlap between the 1988 and 1989 seasons (Figs. 2,3). Data in Fig. 3 illustrate the degree of variation observed between years for each of the three vineyards monitored for three or four consecutive years. In contrast to the second flight trap catch in 1988 and 1990, the 1989 flight differed in that the duration of trap catch was shorter, and the curve shifted to the left. Overall, moth flight occurrence within a year was fairly consistent from vineyard to vineyard (Fig. 4). This is of particular significance because vineyard management differed greatly. Four of the seven vineyards monitored in 1988 had been abandoned for one or more years.

Wing and delta trap catch data collected in 1986 and 1987 indicated that slightly greater numbers of moths were caught in wing traps (Carr et al. 1992). In comparing trap catch between the two kinds of bucket traps, significantly more moths ($\bar{x} \pm$ SE) were caught in the Funnel Trap in two of three vineyards (Fig. 5) (Maitre: Funnel trap, 30.9 $\pm$ 8.4; Multipher I, 17.4 $\pm$ 3.6; $t=2.44$, $df=37$; CSUF: Funnel trap, 9.5 $\pm$ 2.0; Multipher I, 4.8 $\pm$ 0.9; $t=4.13$, $df=41$, $P<0.05$). It was also noted that first trap catch occurred in the Funnel Trap in all three vineyards. This may be considered very important when attempting to establish an early season biofix (capturing the

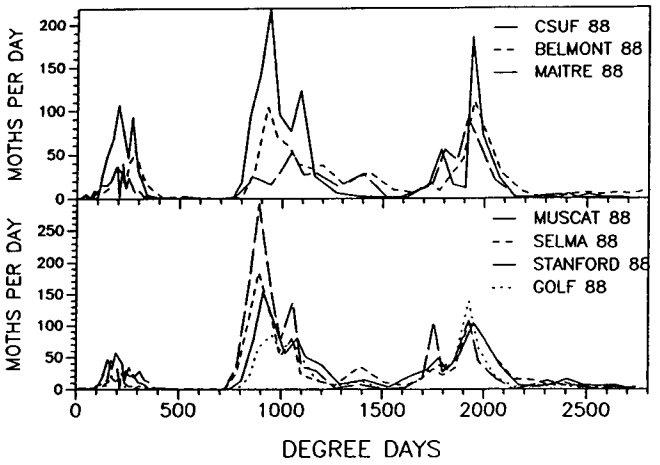

FIG. 4. Estimated daily trap catch counts for male moths in seven vineyards in 1988. Counts plotted against degree-days.

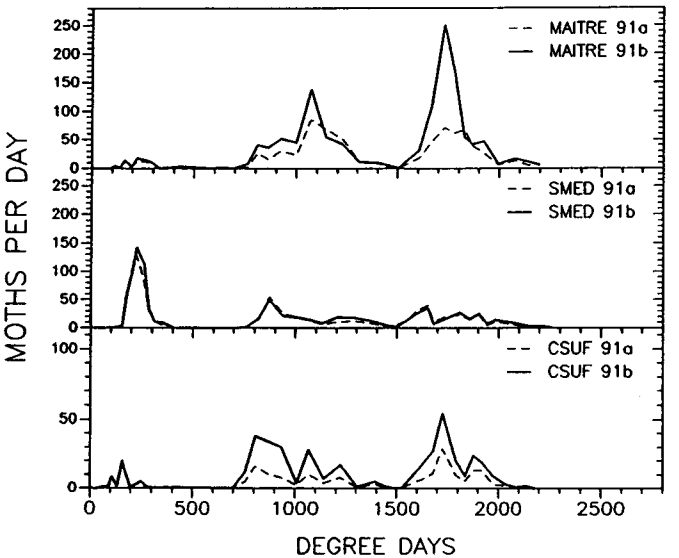

FIG. 5. Estimated daily counts of male moths in two bucket trap designs located (1 ea.) in three vineyards in 1991. Counts plotted against degree-days. a: Multipher I, b: Funnel Trap.

first moths). However, while a slightly more effective trap is more likely to capture those few initial moths when paired with another trap, each is likely to perform equivalently when operating alone. This is supported by Multipher I trap records (1988 to 1990) indicating that only single moths were collected during first trap catch, and that first trap catch occurred well before moth flight became common. These Multipher I traps were situated alone in each vineyard.

In conclusion, first, second and third seasonal moth flights demonstrated similar patterns of occurrence among vineyards within a year. However, greater differences occurred in moth flight duration among different years. Results clearly indicate that WGLS has three distinct generations per year, and moth trap counts suggest that a small fourth generation may occur. However, positive confirmation of a distinct fourth generation of WGLS will require retaining pupae from the second and third generations, and observing their fate under ambient conditions throughout the remainder of the season. Using pheromone traps to determine the approximate time that WGLS moths first occur in a vineyard during the spring seems reasonable. In every vineyard for all years, traps initially caught one or two moths. Thereafter, each trap count gradually increased. This would be expected if traps were catching moths at levels representative of a gradually increasing moth population emerging from an overwintering population of pupae.

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LARVAL DEVELOPMENT, APHID CONSUMPTION AND OVIPOSITION FOR FIVE IMPORTED COCCINELLIDS[1] AT CONSTANT TEMPERATURE ON RUSSIAN WHEAT APHIDS[2] AND GREENBUGS[2]

G. J. Michels[3] and R. V. Flanders[4]

ABSTRACT

The effects of two aphid prey species on larval development, aphid consumption, and oviposition for eleven exotic species or strains of imported coccinellid beetles were studied at 20°C constant temperature. Complete development was observed with all species and strains regardless of the prey species. The predator species with the most rapid larval development and greatest daily consumption of Russian wheat aphid, *Diuraphis noxia* (Mordvilko), were *Coccinella septempunctata* L. at 13.8 days and 43.1 aphids per day; a *Hippodamia variegata* (Goeze) strain from Chile at 11.6 days and 28.7 aphids; and a *Propyleae quatuordecimpunctata* (L.) strain from the Ukraine at 10.1 days and 25.6 aphids. When greenbugs, *Schizaphis graminum* (Rondani), were utilized as a prey species, the most efficacous predator species were *Hippodamia tredecimpunctata* (L.) strains from Kirghizia at 12.6 days and 26.3 aphids and Moldavia at 12.0 days and 25.8 aphids, a strain of *H. variegata* from Moldavia at 12.9 days and 31.9 aphids, and a Moldavian strain of *P. quatuordecimpunctata* at 10.4 days and 21.1 aphids. Oviposition rates were not consistent with regard to prey. Most species produced greater numbers of eggs when fed greenbugs, but a greater percent of eggs eclosed when the parent female was fed Russian wheat aphids.

INTRODUCTION

The Russian wheat aphid, *Diuraphis noxia* (Mordvilko), is a substantial pest of wheat and other small grains in a large part of the western United States (Kindler and Springer 1989). Through cooperative efforts between federal and state agencies, a national program was initiated to import natural enemies into the United States for control of the Russian wheat aphid (Anon. 1991). As a part of this national effort, the entomology project at the Texas A&M Research and Extension Center at Amarillo/Bushland, in cooperation with the United States Department of Agriculture (USDA) Animal and Plant Health Inspection Service (APHIS) National Biological Control Laboratory at Niles, Michigan, evaluated developmental rates and aphid consumption for the following selected exotic coccinellid beetles. The research reported here summarizes the ability of these predators to feed and develop on Russian wheat aphids and greenbugs in the laboratory.

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[2] Homoptera: Aphididae
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Coccinella septempunctata (7-punctata) L. is a large (6.50-7.80 mm) coccinellid of Palearctic origin that is well established in much of the U.S. (Obrycki 1989). In its Old World distribution, it is a dominant species in many localities, similar to the ecological position of *Hippodamia convergens* (Guerin-Meneville) in the United States. It had been introduced into the United States several times from 1956 to 1971 without success, but was reported to be established in 1973 when a colony was found in Bergen Co, New Jersey. It was first recorded in Texas from Deaf Smith Co. in 1986 (Michels unpublished data). Although now considered well established in the United States, it was included in this research because of its relatively new status as an aphid predator.

Hippodamia tredecimpunctata (L.) is a medium-sized beetle (4.50-6.40 mm). The Old World subspecies, (*H. t. tredecimpunctata*) which was imported for this research, is Holarctic from Europe to Western Asia. In the northern Ukraine region of the U.S.S.R., *H. tredecimpunctata (13-punctata)* is often found in wheat (Dyadechko 1954). A subspecies, *H. t. tibialis* (Say), occurs naturally in the United States with a northern distribution. Specimens for this research were imported from the U.S.S.R. republics of Kirghizia and Moldavia.

Hippodamia variegata (Goeze), referred to as *Adonia variegata* Goeze in older U.S. and some current European literature, is a small to medium-sized (3.75-5.60 mm) coccinellid of Paelarctic origin that feeds on aphids in a number of habitats in the Old World. It is found as far north as Siberia, ranging through Europe and the Middle East to South Africa. It is a very temperature-tolerant species, found in regions where the yearly average temperature ranges from 30°C in Ethiopia to -10°C in the Yakutsk region of the U.S.S.R. (Honek 1979). It is also frequently found in temperate climates (Hodek 1973). The Chilean specimens were originally introduced into Chile in 1975 from South Africa, as part of a program to establish predators and parasites of small grain aphids in Argentina, Brazil, and Chile (Zuniga et al. 1986). The species is well established in Chile, but apparently not in Argentina or Brazil (Pike and Reed 1991). Gordon (1985) reports that *H. variegata* had previously been imported into the United States from India (1957-1958, 1981), South Africa (1983), and the U.S.S.R. (1981). Releases have been made in Arizona, California, Delaware, Florida, Georgia, Louisiana, Maryland, Maine, Nebraska, New Jersey, North Carolina, Pennsylvania, South Carolina, and Texas. Although establishment is not reported in the United States, this predator has recently become established in Quebec, Canada. Specimens studied were imported from Canada, Chile, Moldavia, and Morocco.

Propyleae quatuordecimpunctata (L), as is *Coccinella 7-punctata*, is a widespread Old World species. It is a small beetle (3.50-5.20 mm) that is found in habitats ranging from orchards to potato crops to small grains. According to Hodek (1973), *P. quatuordecimpunctata (14-punctata)* prefers humid over arid sites. Feran et al. (1984) noted that *P. 14-punctata* is well adapted to a Mediterranean climate, and can consume many aphids in a short time span. It ranges from the central Ukraine, through Europe, to the Middle East. It was previously imported into the U.S. for control of greenbugs, but has not been established (Rogers et al. 1972). Specimens studied were imported from Moldavia, Turkey, and the Ukraine.

Semiadalia undecimnotata (Schneider) is a medium-sized (4.00-5.00 mm), South-Palearctic beetle that is common in Europe and the U.S.S.R (Hodek 1973). Hodek (1973) noted that *S. undecimnatata (11-notata)* prefers dry and warm sites where it often replaces *P. 14-punctata*. Specimens studied were imported from the Ukraine. Additional details for all imported species are contained in Table 1.

TABLE 1. Origin, Year of Importation, and Importation Codes for Eleven Species/Strains of Exotic Coccinellid Predators.

Species	Origin	Location	Prey[a] species	Year of importation	Code[b]
Coccinella 7-punctata	U.S.A.	Texas (Bushland)	RWA	1989	Native
Hippodamia 13-punctata	U.S.S.R.	Kirghizia	RWA	1989	EPL89-90
Hippodamia 13-punctata	U.S.S.R.	Moldavia	RWA	1989	EPL89-42
Hippodamia variegata	Canada	Quebec	Misc.	1989	BIRL89-113/162
Hippodamia variegata	Canada	Quebec	Misc.	1990	BIRL90-96/106/113
Hippodamia variegata	Chile	Los Andes	RWA	1990	PSRF90-02
Hippodamia variegata	Morocco	Meknes	RWA	1990	T90-015
Hippodamia variegata	U.S.S.R.	Moldavia	RWA	1989	EPL89-41
Propyleae 14-punctata	U.S.S.R.	Ukraine	RWA	1989	EPL89-107
Propyleae 14-punctata	Turkey	Beypazari	RWA	1988	EPL88-51
Propyleae 14-punctata	U.S.S.R.	Moldavia	RWA	1989	EPL89-40
Semiadalia 11-notata	U.S.S.R.	Ukraine	RWA	1989	EPL-89-103

[a]RWA = Russian wheat aphid.
[b]Quarantine identification code for insects processed by:
BIRL - Beneficial Insect Research Laboratory, Newark, DE.
EPL - European Parasite Laboratory, Paris, France.
T - Texas A&M University Biological Control Laboratory, College Station, TX.
PSRF - BIRL code for this shipment from Chile.

MATERIALS AND METHODS

The studied coccinellids were reared from specimens that were field collected by various state and federal researchers in Canada, Chile, Kirghizia (U.S.S.R.), Moldavia (U.S.S.R.), Morocco, Turkey, and Ukraine (U.S.S.R.). All beetles arrived in the United States at the USDA/ARS Quarantine Laboratory at Newark, DE for quarantine and initial rearing. European and Asian species were first sent to the USDA/Agricultural Research Service (ARS) European Parasite Laboratory in Paris, France for identification. Once found to be free from contamination (parasites and pathogens), beetles were shipped to the USDA/APHIS/Plant Protection and Quarantine (PPQ) National Biological Control Laboratory at Niles, MI for increase and distribution. Fifty to 100 founder adults of each species were sent from the Niles laboratory to the Texas A&M Research and Extension Center at Bushland, TX.

Upon arrival at Bushland, adults were separated by gender; paired males and females were placed in plastic petri dishes, and provided an overabundance of greenbug or Russian wheat aphid prey on a daily basis. Previous research (Michels and Behle 1988) has shown that, at constant temperatures, Russian wheat aphids produce the largest number of nymphs at 20°C. Since these beetles were imported as additional predators of Russian wheat aphid in the Western United States, our studies were conducted at 20°C, the temperature that results in maximum Russian wheat aphid reproduction. Adults were held for mating in Percival® I30BLL incubators at

20°C (±0.5°C) constant temperature. After 48 h, males were removed from petri dishes in order to preclude egg cannibalism. Once a sufficient number of eggs were available to begin a study, the female was removed and the eggs were monitored daily for eclosion.

Twenty-five neonate larvae of each species were sequestered individually into 30-ml plastic Dixie® condiment cups that were secured with paper lids. Each larva was considered a replication. A known number of greenbugs or Russian wheat aphids, depending on the experiment, were added to each cup on a daily basis. To guarantee an overabundance of prey, the number of prey added daily was increased as the larvae progressed through the various instars. This number ranged from as few as five aphids for each first-instar larvae to as many as 50 aphids for each fourth instar larvae. Each day, aphids remaining were counted and removed, and fresh aphids were added. Larvae were examined daily for molting and exuviae were removed. This process was repeated until all surviving individuals molted to the adult stage.

Newly emerged adult beetles were sexed; females were paired for mating with males of the same species from the founder colonies for 48 h, and then isolated and held for 90 days to determine oviposition rates. A 90-day oviposition period was established in order to complete the studies in a workable time frame. Although additional eggs could be oviposited after 90 days, we determined that roughly three months would represent a typical period when a prey source would be available to the beetles in the Texas Panhandle.

Results were statistically analyzed using the general linear model procedures for unequal replication available in the Statistical Analysis System for Personal Computers (SAS Institute 1988). Only larvae that completed development to the adult stage were included in the analyses. Significant means were separated using the Student-Newman-Keuls test at the $P > 0.05$ level.

For purposes of this study, each species from a geographically different origin was designated as a strain. Voucher specimens of each species and strain were retained in the entomology collection at the Texas A&M Research and Extension Center at Bushland, TX.

RESULTS AND DISCUSSION

Mean total development time from egg to adult, mean larval development time, mean total aphids consumed, and the average number of aphids consumed per day by each species and strain are listed in Table 2. Data in Table 2 are arranged by prey species and by the average number of aphids consumed per day. The average number of aphids consumed per day is based on the total number of aphids consumed over the number of days in the larval period.

Greenbug studies. Mean total days to develop from egg to adult and the mean total days in the larval stage (excluding egg, prepupal and pupal stages) generally followed trends in the size of the species, with the notable exception of *S. 11-notata*. The most rapid development was recorded for *P. 14-punctata* from Moldavia, although *P. 14-punctata* strains were not significantly different from each other. The longest development time was associated with *C. 7-punctata*. This beetle had a significantly longer development time, both in total days and days in the larval stage, than any other species or strain. Total aphids consumed also generally followed the trend in predator size, with larger individuals (*C. 7-punctata*, *S. 11-notata*) consuming more total aphids than the medium (*H. 13-punctata*, *H. variegata*) and small-sized (*P. 14-punctata*) individuals.

TABLE 2. Development and Aphid Consumption for 11 Species and Strains of Exotic Coccinellids Fed on Greenbugs or Russian Wheat Aphids at 20°C Constant Temperature.

Predator species	Origin	n[a]	Total days		Aphids consumed	
			Egg to adult	Larval period	Total	Per day
			Greenbug			
Coccinella 7-punctata	Texas	19	35.4 a[b] A[c]	19.5 a A	713.6 a A	36.1 a B
Hippodamia 13-punctata	Kirghizia	21	26.9 c A	12.6 cd B	334.1 de A	26.3 d A
Hippodamia 13-punctata	Moldavia	12	24.6 d B	12.0 de B	319.0 de A	25.8 d A
Hippodamia variegata	Canada	13	30.3 b A	15.5 b A	358.8 d A	23.1 e A
Hippodamia variegata	Chile	14	24.4 d A	13.1 cd A	290.0 e A	21.9 ef B
Hippodamia variegata	Moldavia	20	26.1 cd B	12.9 cd B	414.4 c A	31.9 b A
Hippodamia variegata	Morocco	13	24.8 d B	11.3 ef B	326.9 de B	28.7 c B
Propyleae 14-punctata	Moldavia	22	21.4 d B	10.4 f B	220.4 f A	21.1 f A
Propyleae 14-punctata	Turkey	22	24.0 d A	10.2 f B	128.9 g B	12.6 g B
Propyleae 14-punctata	Ukraine	23	22.4 d A	11.3 ef A	282.2 e A	24.9 d A
Semiadalia 11-notata	Ukraine	18	25.1 d B	13.6 c B	482.7 b B	35.3 a B
			Russian wheat aphid			
Coccinella 7-punctata	Texas	24	26.1 cd B	13.8 b B	595.5 b B	43.1 a A
Hippodamia 13-punctata	Kirghizia	20	27.7 bc A	15.7 a A	309.0 d A	19.7 d B
Hippodamia 13-punctata	Moldavia	20	31.1 a A	16.4 a A	298.6 d A	18.1 e B
Hippodamia variegata	Canada	18	27.7 bc B	14.2 b B	192.7 g B	13.6 g B
Hippodamia variegata	Chile	16	23.9 e B	11.6 d B	332.9 d A	28.7 c A
Hippodamia variegata	Moldavia	16	28.1 b A	14.4 b A	219.0 fg B	15.3 f B
Hippodamia variegata	Morocco	20	26.8 bcd A	13.5 bc A	505.1 c A	37.2 b A
Propyleae 14-punctata	Moldavia	19	25.9 d A	12.7 c A	186.1 g B	14.5 fg B
Propyleae 14-punctata	Turkey	20	23.9 e A	11.8 d A	233.9 ef A	19.8 d A
Propyleae 14-punctata	Ukraine	20	21.0 f B	10.1 e B	258.8 e A	25.6 c A
Semiadalia 11-notata	Ukraine	17	30.3 a A	16.4 a A	707.5 a A	43.2 a A

[a]n = number of predator species in study out of 25 surviving to adult stage.
[b]Means followed by the same lower case letter are not significantly different within a prey species (Alpha = 0.05, SNK).
[c]Means followed by the same upper case letter are not significantly different between prey species for the same coccinellid species or strain (Alpha = 0.05, SNK).

Means for the average numbers of greenbugs consumed per day by the 11 predators examined in this study separated statistically into seven significant groups. *Coccinella 7-punctata* and *Semiadalia 11-notata* comprised the first group and consumed significantly more greenbugs per day than any other species or strain in the study (36.1 and 35.3 aphids per day respectively). This result may be expected since

both *C. 7-punctata* and *S. 11-notata* were the largest species in the studies. Although *C. 7-punctata* consumed significantly more aphids in total than *S. 11-notata* (713.6 vs 482.7), it also took significantly more days to complete total development (35.4 vs 25.1 days) and larval development (19.5 vs 13.6 days) compared to *S. 11-notata.*

Between the two *H. 13-punctata* strains there were no significant differences, except in total days to develop where the Kirghizian strain took significantly longer to develop than the Moldavian strain.

Among the four *H. variegata* strains, the Canadian strain took significantly longer to complete total development than all other *H. variegata* strains, which did not differ significantly from one another. The Canadian strain also had a significantly longer larval period than did all other strains in this group, and the Moroccan strain exhibited a significantly shorter larval period than the other strains. The Moldavian strain consumed significantly more aphids than the other strains, and therefore, also consumed significantly more aphids per day than the rest of the *H. variegata* strains.

Among the three *P. 14-punctata* strains, there were no significant differences in total or larval development days. In total aphids consumed and aphids consumed per day, the Ukrainian strain consumed significantly more aphids than the Moldavian strain, which consumed significantly more than the Turkish strain.

Russian Wheat Aphid Studies. As with the greenbug studies, development times and aphid consumption generally fell along the lines of predator size, with larger beetles usually taking longer to develop and consuming more aphids than smaller beetles. The means for the average number of aphids consumed per day separated into seven statistically significant groups. The most rapid development time among all species and strains was found with *P. 14-punctata* from the Ukraine, while the longest development time was found with *H. 13-punctata* from Moldavia and *S. 11-notata. Semiadalia 11-notata* consumed the most aphids in total and per day, while *P. 14-punctata* from Moldavia consumed the least total aphids and *H. variegata* from Canada had the lowest daily consumption rate.

Coccinella 7-punctata and *S. 11-notata* consistently ranked at the top of the group in all four categories. *Semiadalia 11-notata* ranked higher than *C. 7-punctata* in terms of total days to develop, larval development period, and total aphids consumed. There was not a significant difference between these two species in the number of aphids consumed per day.

The two *H. 13-punctata* strains differed significantly in total days to develop and aphids consumed per day. The Moldavian strain took significantly longer to develop, but consumed significantly fewer aphids per day than the Kirghizian strain. There was no significant difference in larval period or total aphids consumed between these two strains.

The *H. variegata* strains presented a mixture of results. In terms of development, the Canadian, Moldavian, and Moroccan strains were generally very similar and always had significantly longer developmental periods than the Chilean strain. In terms of aphid consumption, the Moroccan strain consumed significantly more aphids in total and per day than the other three strains. The Chilean strain ranked second in aphid consumption. The Moldavian strain consumed significantly fewer total aphids than the Chilean strain, but was not significantly different from the Canadian strain. The Moldavian strain did consume significantly more aphids per day than the Canadian strain.

Propyleae 14-punctata fed Russian wheat aphids did not follow the trends found when fed greenbugs. Where there were no significant differences among the three strains in terms of total and larval development periods when fed greenbugs, the Moldavian strain had a significantly shorter development period than the Turkish

strain, which developed significantly faster than the Ukrainian strain, when fed Russian wheat aphids. There was no difference between the Turkish and Ukrainian strains in total aphids consumed, while the Moldavian strain consumed significantly fewer Russian wheat aphids than the other two strains. The Moldavian strain also consumed significantly fewer Russian wheat aphids on a daily basis than the Turkish strain, which consumed significantly fewer aphids than the Ukrainian strain.

Comparisons Between Prey by Species/Strain. Of the 11 species/strains examined in these studies, four broad groups can be discerned based on larval development period and aphids consumed per day (Table 3). The first group consisted of *H. variegata* from Canada. These beetles developed significantly faster on Russian wheat aphids than on greenbugs, but consumed significantly more greenbugs per day than Russian wheat aphids. *Coccinella 7-punctata, H. variegata* from Chile, and *P. 14-punctata* from the Ukraine developed significantly faster when fed on Russian wheat aphids, and generally consumed significantly more Russian wheat aphids per day than greenbugs. The third group, composed of Moroccan *H. variegata*, Turkish *P. 14-punctata*, and *S. 11-notata* developed significantly faster on greenbug, but consumed significantly more Russian wheat aphids per day than greenbugs. In the fourth group, Kirghizian and Moldavian *H. 13-punctata, H. variegata,* and *P. 14-punctata* developed significantly faster when fed greenbugs, and consumed significantly more greenbugs than Russian wheat aphids on a daily basis.

TABLE 3. Comparison of Eleven Exotic Coccinellids for Best Aphid Prey in Terms of Larval Development and Aphid Consumption at 20°C Constant Temperature.

Predator species	Origin	Shortest[a] development period	Highest aphid[b] consumption
Hippodamia variegata	Canada	RWA[c]	GB
Coccinella 7-punctata	Texas	RWA	RWA
Hippodamia variegata	Chile	RWA	RWA
Propyleae 14-punctata	Ukraine	RWA	RWA[d]
Hippodamia variegata	Morocco	GB	RWA
Propyleae 14-punctata	Turkey	GB	RWA
Semiadalia 11-notata	Ukraine	GB	RWA
Hippodamia 13-punctata	Kirghizia	GB	GB
Hippodamia 13-punctata	Moldavia	GB	GB
Hippodamia variegata	Moldavia	GB	GB
Propyleae 14-punctata	Moldavia	GB	GB

[a]Species/strain developed significantly faster on indicated prey.
[b]Species/strain consumed significantly more of indicated prey.
[c]GB = greenbugs; RWA = Russian wheat aphid.
[d]More but not significantly more.

TABLE 4. Sex ratios and egg production for exotic coccinellid fed greenbugs or Russian wheat aphids at 20°C constant temperature.

Predator species	Origin	Prey[a] species	n[b]	M[c]	F[d]	Sex ratio M to F	Total eggs	Total Hatch	Percent hatch	Average eggs per F	Average percent hatch per F
Coccinella 7-punctata	Texas	RWA	24	12	12	1.00	627	318	50.72	52.25	26.50
Coccinella 7-punctata	Texas	GB	19	11	8	1.38	no records kept				
Hippodamia 13-punctata	Kirghizia	GB	21	9	12	0.75	658	360	54.71	54.83	30.00
Hippodamia 13-punctata	Kirghizia	RWA	20	11	9	1.22	275	68	24.73	30.56	7.56
Hippodamia 13-punctata	Moldavia	GB	12	6	6	1.00	452	230	50.88	75.33	38.33
Hippodamia 13-punctata	Moldavia	RWA	20	12	8	1.50	23	21	91.30	2.88	2.63
Hippodamia variegata	Canada	GB	13	8	5	1.60	749	301	40.19	149.80	60.20
Hippodamia variegata	Canada	RWA	18	6	12	0.50	342	158	46.20	28.50	13.17
Hippodamia variegata	Chile	GB	14	7	7	1.00	2273	891	39.20	324.71	127.29
Hippodamia variegata	Chile	RWA	16	11	5	2.20	no records kept				
Hippodamia variegata	Moldavia	GB	20	12	8	1.50	572	213	37.24	71.50	26.63
Hippodamia variegata	Moldavia	RWA	16	8	8	1.00	232	70	30.17	29.00	8.75
Hippodamia variegata	Morocco	RWA	20	10	10	1.00	1701	644	37.86	170.10	64.40
Hippodamia variegata	Morocco	GB	13	4	9	0.44	439	129	29.38	48.78	14.33

Propyleae 14-punctata	Moldavia	GB	22	15	7	2.14	2540	1121	44.13	362.86	160.14
Propyleae 14-punctata	Moldavia	RWA	19	11	8	1.38	87	55	63.22	10.88	6.88
Propyleae 14-punctata	Turkey	GB	22	13	9	1.44	737	275	37.31	81.89	30.56
Propyleae 14-punctata	Turkey	RWA	20	17	3	5.67	84	33	39.29	28.00	11.00
Propyleae 14-punctata	Ukraine	RWA	20	12	8	1.50	1657	400	24.14	207.13	50.00
Propyleae 14-punctata	Ukraine	GB	23	17	6	2.83	605	202	33.39	100.83	33.67
Semiadalia 11-notata	Ukraine	GB	18	12	6	2.00	eggs infertile				
Semiadalia 11-notata	Ukraine	RWA	17	14	3	4.67	eggs infertile				

[a]GB = greenbug; RWA = Russian wheat aphid.
[b]n = number surviving out of original 25 larvae.
[c]M = male.
[d]F = female.

Oviposition/Fecundity Studies. Results of the 90-day oviposition and egg hatch studies are found in Table 4. In general, most species/strains had higher total eggs and eggs per female when reared on greenbugs than on Russian wheat aphids. Notable exceptions were *H. variegata* from Morocco and *P. 14-punctata* from the Ukraine, which laid more eggs when reared on Russian wheat aphids. More viable larvae hatched from egg masses produced by adults fed Russian wheat aphid than those fed greenbugs. Frequently, total egg production by Russian wheat aphid-fed adults was much lower than those fed greenbugs, skewing the percent hatch to artificial highs. *Semiadalia 11-notata* did not produce any viable eggs in this study. This problem has been encountered before with *S. 11-notata*, and is primarily due to the need for pollen or some other protein source in the diet (Michels, unpublished data) which these beetles did not receive in this study. Additional information presented in Table 4 includes the number of male and female beetles resulting from each study, along with sex ratios.

Since rapid development, high aphid consumption, and high oviposition rates are desirable characteristics in natural enemies of aphids, *Coccinella 7-punctata H. variegata* from Chile and *P. 14-punctata* from the Ukraine seem to be the best suited predators in terms of utilizing Russian wheat aphids as a prey species at this temperature. These three predators ranked significantly higher than the other species and strains in rapidity of larval development and aphids consumed per day. Unfortunately, oviposition records are not available for *C. 7-punctata* fed greenbugs and *H. variegata* fed Russian wheat aphids. With greenbugs as a prey species, *H. 13-punctata* from Kirghizia and Moldavia, *H. variegata* from Moldavia, and *P. 14-punctata* from Moldavia ranked as the best overall candidates. In terms of oviposition and fecundity, all of these four species/strains produced high numbers of eggs when fed greenbugs, although percent hatch varied as explained above.

The conclusions from this research are somewhat empirical and should not invalidate the use of any of the predators as natural enemies. Other factors, such as searching efficiency, prey availability, and interspecific competition also play major roles in potential aphid control. This research restricted the predators to one aphid species. If free choice were given (i.e., the predators were presented with a mixture of greenbugs and Russian wheat aphids), different results could be expected. In addition, although the point of this research was to address predator efficiency at peak Russian wheat aphid reproduction, different temperature regimes would have a significant effect on all factors examined here. Additional research is needed to develop a broad range of temperature profiles for each species.

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SUBLETHAL EFFECTS OF INSECTICIDES ON COTTON APHID[1] REPRODUCTION AND COLOR MORPH DEVELOPMENT

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ABSTRACT

Cotton aphids *Aphis gossypii* Glover residually exposed to LC_{10} doses of sulprofos or cypermethrin on leaf discs had significantly lower intrinsic rates of increase and longer prereproductive development times than untreated aphids or those treated with dicrotophos. Because aphid reproduction was not stimulated by the insecticides, hormoligosis is probably not involved in cotton aphid outbreaks following applications of cypermethrin or sulprofos applied at LC_{10} dosages. Untreated aphids and aphids treated with dicrotophos did not differ significantly in intrinsic rates of increase, development times, or nymph production. There were no significant differences in cotton aphid longevity among the treatments. Significantly more cotton aphids exposed to LC_{10} doses of sulprofos developed into dark color morphs than did aphids in the other treatments. Sulprofos-treated aphids developed significantly fewer intermediate color morphs than dicrotophos-treated aphids. There were no significant differences among treatments in frequency of light-colored morphs.

INTRODUCTION

The cotton aphid, *Aphis gossypii* Glover, has recently become an increasingly destructive pest in cotton throughout many cotton growing regions of the United States (King et al. 1987, 1988). Cotton aphid outbreaks have been reported most often following insecticide application aimed at Heliothinae control (Edelson 1989, Kerns and Gaylor 1991). These outbreaks have been attributed to destruction of natural enemies, hormoligosis (direct stimulation of reproduction) or trophobiosis (indirect stimulation of reproduction) (Slosser et al. 1989). However, cotton aphid outbreaks following applications of sulprofos were not due entirely to destruction of natural enemies (Kerns and Gaylor 1991). Hormoligotic and trophobiotic effects of sublethal doses of pesticides have been demonstrated with other aphid species (Maxwell and Harwood 1960, Lowery and Sears 1986). Sublethal doses of insecticides can affect insect behavior and gene expression (Plapp 1984, Hayes 1988). Altered gene expression induced by sublethal doses of insecticides could convey insecticide insecticide resistance and may be expressed phenotypically. Variations in color morphs has

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been correlated to insecticide resistance in *Myzus nicotianae* Blackman (Harlow and Lampert 1990). This is a report of the impact of sublethal doses of two organophosphate and one pyrethroid insecticide on reproduction and color morph development of the cotton aphid.

MATERIALS AND METHODS

Cotton aphids were from a colony established 4 months before the initiation of this study from a single apterous, parthenogenically reproducing female. The colony was maintained on 'Deltapine 90' (Delta Pineland & Seed Co., Scott, MS) cotton in an environmentally-controlled cabinet at 20 $\pm$ 3°C and a photoperiod of 14:10 (L:D). The cabinet was lighted with cool white fluorescent and 60-W incandescent lamps producing 650 μmol quanta m^{-2} s^{-1} at 40-cm height. During the experiment, aphids were maintained on 'Deltapine 90' cotton leaf discs (3.14 cm^2) in environmental cabinets under the same conditions as the parent colony. Plants used for leaf discs were grown in a insect-free environment and maintained at 26 $\pm$ 5°C on a table lighted with plant grow lights (General Electric, Grow & Show, Model FL40PL) with a 14:10 (L:D) photoperiod. One leaf disc per plant was cut, using a cork borer, from a fully expanded leaf located near the plant terminal. The disc was taken from an area approximately one-half the distance between the petiole and the tip, centered on the mid-vein. To prevent desiccation, leaf discs were excised while submerged in distilled water.

Insecticides were prepared by dissolving technical grade insecticide in acetone and diluting with an equal portion of distilled water containing 0.02% v/v Triton X-100 wetting agent. The control solution consisted of equal portions of acetone and wetting agent solution. Leaf discs were pooled, arbitrarily selected, and treated by dipping in an insecticide solution containing cypermethrin (3 ppm), dicrotophos (17 ppm), or sulprofos (840 ppm). These insecticide concentrations represent LC_{10} dosages (Kerns and Gaylor 1992). Untreated leaf discs were dipped in the control solution. All insecticides used in this study are recommended for use in cotton by the Alabama Cooperative Extension Service (Smith et al. 1990). Insecticides were obtained from ICI Americas Inc., Goldsboro, N.C (cypermethrin); E. I. DuPont De Nemours & Co., Wilmington Del. (dicrotophos); and Miles Corp., Kansas City, Mo. (sulprofos).

After leaf disc treatment, two light-colored, last-instar nymphs without wing pads were collected from the same leaf of the parent colony and placed, using a camel's haired brush, on the ventral side of the disc. Leaf discs with aphids were then floated ventral side up on 15 ml of a nutrient solution (Hoagland and Arnon 1950) in covered 60 x 15-mm petri dishes. Ten replicates of each treatment were arranged on trays in a randomized complete block design and placed in an environmental cabinet. However, because of lack of establishment, data from only six to nine replicates were used. Aphids were monitored at 6-h intervals until at least two F1 progeny were produced. Adults and all but two newborn nymphs were removed from the discs. F1 nymphs were monitored for the first 5 d at 24-h intervals and, thereafter, at 6-h intervals for survival and production of the F2 generation. Aphids were transferred, using a camel's hair brush, to freshly cut and treated leaf discs every 7 d for the duration of the experiment.

Color morph development and longevity of the F1 generation, and the number of F2 nymphs produced were recorded. Aphid color morph was described as light, intermediate, or dark (Wall 1933). F2 nymphs were removed from the leaf discs and discarded after being counted.

Intrinsic rate of increase (r_m) for each replicate was calculated using the following formula developed for aphids by Wyatt and White (1977):

$$r_m = 0.738(\log_e M_d)/d$$

The d represents the time required for an aphid to reach reproductive maturity, and M_d represents the number of nymphs, beginning with the first nymph born, produced in a time period equal to d. We estimated d and longevity in hours ($\leq$ 6 h accuracy) but transformed these data to days for mathematical computations and presentation. Raw data for d, M_d, r_m, and longevity were subjected to analysis of variance (ANOVA) using general linear model (GLM) procedures (SAS Institute Inc. 1988). Means were separated ($P < 0.05$) using pairwise t tests protected by an overall F test ($P < 0.05$) (SAS Institute Inc. 1988). Statistical differences in frequency of color morph development were based on multivariant X^2 contingency table comparisons ($P < 0.05$) (SAS Institute Inc. 1988).

RESULTS AND DISCUSSION

Reproduction Potential. As indicated by the r_m values, more offspring were produced per day on the dicrotophos-treated and untreated leaf discs; M_d values however, did not differ among treatments (Table 1) because they are a function of d, and the d values of cotton aphids reared on leaf discs treated with cypermethrin or sulprofos were significantly longer compared to those reared on dicrotophos-treated or untreated leaf discs.

TABLE 1. Prereproductive Development Time (d), Nymphs Produced in a Time Equal to d (M_d), Intrinsic Rate of Increase (r_m) and Longevity (L) of Cotton Aphids Reared on Leaf Discs Treated with Insecticides.

Insecticide	d (Days[a] ± SE)	M_d (Nymphs ± SE)	L (Days ± SE)	r_m (Rate ± SE)
Cypermethrin	9.04 ± 0.70a	15.17 ± 2.14a	21.96 ± 2.97a	0.22 ± 0.02a
Dicrotophos	6.92 ± 0.65b	19.17 ± 1.40a	19.46 ± 0.99a	0.32 ± 0.02b
Sulprofos	8.84 ± 0.33a	16.38 ± 0.76a	20.47 ± 0.87a	0.23 ± 0.01a
Untreated	6.56 ± 1.57b	16.56 ± 1.56a	21.19 ± 2.41a	0.32 ± 0.02b

[a]Means in a column followed by the same letter are not significantly different based on paired t tests protected by overall F tests ($P \geq 0.05$; SAS Institute Inc. [1988]).

In an environment devoid of natural enemies, cotton aphids often survive lengthy periods beyond cessation of reproduction (Paddock 1919). Although longevity is not a reliable indicator of reproductive duration or potential of an individual aphid, it may influence the survival and growth potential of the colony. The presence of non-reproducing adults in an aphid colony may be beneficial through host conditioning, promotion of dispersal, and reduction of predation losses (Dixon 1985, Krebs 1985, Klingaulf 1987). If sublethal doses of insecticides alter aphid longevity, colony survival may be affected; however, we could not detect differences in aphid longevity among our treatments.

The r_m values are a function of both d and M_d and followed the same pattern of significant differences as d, thus indicating that the differences among r_m values are mostly attributed to differences in d. Thelytokous, viviparous aphids maximize reproduction by

coupling a short development time and telescoping of generations (nymphs are born containing embryos) with a rapid reproductive period (Dixon 1987). Aphids reared on dicrotophos-treated or untreated leaf discs were able to take advantage of their innate ability to increase, but aphids reared on cypermethrin- or sulprofos-treated leaf discs had expanded prereproductive developmental times, thus resulting in a need for expanded reproductive periods to obtain similar M_d values.

The r_m values indicate that cotton aphids exposed to sublethal residual doses of cypermethrin or sulprofos may have their population growth potential reduced. However, cotton aphid outbreaks have been reportedly induced by cypermethrin (Edelson 1989). Also, field studies indicate that cotton aphid outbreaks can be greater in cotton treated with sublethal doses of sulprofos than in untreated cotton or in cotton treated with sublethal doses of cypermethrin (Kerns and Gaylor 1991). It therefore appears that cotton aphid outbreaks induced by sulprofos or cypermethrin may not be the result of hormoligosis. Although we detected no evidence of trophobiosis involving leaf discs, it is possible that trophobiosis could be involved *in situ*.

Color Morph Frequency. Sublethal doses of insecticides affected color morph frequency of cotton aphids (Table 2). Aphids treated with sulprofos produced a significantly greater frequency of dark individuals than any other treatment, and fewer intermediate individuals than the dicrotophos-treated aphids. Color morph frequency was not significantly different among cypermethrin-treated, dicrotophos-treated, or untreated aphids. Color morph development in cotton aphid has been attributed to a cyclic process involving color and presence or absence of alate forms (Wall 1933), and more recently variations in temperature (Miyazaki 1987). However, these factors do not explain the increased frequency of dark morphs and decreased frequency of intermediate morphs (compared to dicrotophos-treated aphids) by the sulprofos-treated aphids because all of our parent test aphids were of a similar age and color, all were apterous, and the experiment

TABLE 2. Frequency of Color Morph Occurrence in Cotton Aphids Reared on Insecticide Treated or Untreated Cotton Leaf Discs.

		Color morph		
Treatment comparison[a]	n	%Light	%Intermediate	%Dark
Cypermethrin vs.	6	83.3	16/7	0.0
Dicrotophos	6	66.7	33.3	0.0
Cypermethrin vs.	6	83.3	16.7	0.0**
Sulprofos	8	37.5	0.0	62.5
Cypermethrin vs.	6	83.3	16.7	0.0
Untreated	9	33.3	55.6	11.1
Dicrotophos vs.	6	66/7	33.3**	0.0*
Sulprofos	8	37.5	0.0	62.5
Dicrotophos vs.	6	67.5	33.3	0.0
Untreated	9	33.3	55.6	11.1
Sulprofos vs.	8	37.5	0.0	62.5*
Untreated	9	33.3	55.6	11.1

[a]Paired frequencies in columns followed by * and ** are significantly different ($P < 0.05$ and $P < 0.025$, respectively) based on multivariant X^2 contingency table comparisons (SAS Institute Inc. 1988).

was performed at a constant temperature regimen. Sulprofos may have altered gene expression. Sublethal doses of insecticides have been shown to alter gene expression in several insects, thus conferring insecticide resistance (Agosin 1984). Variations in color morph development in *M. nicotianae* have been linked with insecticide resistance (Harlow and Lampert 1990). Whether or not variations in color morph development in cotton aphid implies insecticide resistance should be investigated; otherwise, the consequences of altered gene expression in cotton aphid by sulprofos is not certain.

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INGESTION OF ^{14}C-LABELED DIET BY *LYGUS HESPERUS* KNIGHT [1]

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ABSTRACT

Immature and adult plant bugs, *Lygus hesperus* Knight, were fed ^{14}C inulin-labeled diet through a Parafilm "M" membrane. The amount of diet ingested increased rapidly as the insects developed from first to third instars. Third instars and older stages ingested similar amounts of meridic diet in 24 h. Generally, early instars made more punctures through the feeding membrane. Thus, older bugs ingested more meridic diet per feeding bout than younger bugs, but they fed less frequently.

INTRODUCTION

Lygus hesperus Knight is a pest of many crops, including cotton, in the western U.S. (Strong 1970). A criterion of one nymph equaling two adults has been used in economic thresholds for *L. hesperus* (Falcon et al. 1971); however, this criterion is apparently not based on empirical evidence. Feeding rates are important in furthering our understanding of how *Lygus* spp. damage plants. The goal of this study was to determine the relative feeding rates of *L. hesperus* nymphs and adults using an artificial, liquid diet (Patana and Debolt 1985) radiolabeled with ^{14}C inulin. Most insects are unable to utilize inulin, and it passes through the digestive tract without significant digestion (Wiesenborn and Morse 1985). Because we examined only ingestion, and not egestion, this property was not essential to this experiment.

MATERIALS AND METHODS

We maintained a laboratory colony of *L. hesperus* (27 ± 1°C, 14:10 L:D photoperiod) adapted to feed on meridic diet. Diet was prepared as described by Patana and Debolt (1985) and centrifuged at 4000 rpm for 15 minutes. The supernatant was decanted and centrifuged for an additional 15 minutes and used as a liquid diet. About 0.04 mg/ml and 0.05 mg/ml of ^{14}C-labeled inulin (2.75 μCi/mg) was dissolved into the diet for the first and second trial, respectively. Diet packets (4 X 4 cm) were made by heat sealing two layers of Parafilm "M" membrane on three sides (Patana 1982). About 1 ml of ^{14}C-labeled diet was dispensed into each packet, and the remaining side was sealed.

[1] Heteroptera: Miridae

Feeding rates of nymphs and adults were determined by measuring the quantity of ingested, ^{14}C-labeled diet. Diet packets were placed on top of 20-ml plastic, liquid scintillation vials that contained the bugs. These vials were then maintained at 27°C for 24 h under constant light. All vials contained a small (≈ 1 X 5 cm) piece of xerox paper (white, 4200 DP 20 lb.) to reduce cannibalism and allow better access to the diet packets. Packets were removed after 24 h. Replicates with dead bugs were omitted.

In the first trial, two *L. hesperus* were placed into each vial. There were five replicates for each nymphal instar and the adult stage (5-10 d old). In the second trial, nine replicates were used for each developmental stage, and only one bug was used per replicate. The sex of the adults in the second trial was recorded.

Three control replicates, consisting of diet-filled packets on vials without bugs, were made for each trial. Control replicates were used to test the integrity of the feeding membrane to leakage and dehydration. Standard replicates were made by adding 5 μl of C^{14}-labeled diet to each of three vials containing 10 ml of Beckman Ready Safe cocktail. Additional standard replicates were made by adding 5 μl of ^{14}C-labeled diet from the control packets, after 24 h, to vials containing 10 ml cocktail. Eight and six background replicates, containing 10 ml of cocktail and 5 μl of unlabeled diet, were made for the first and second trial, respectively.

We used a glass rod to crush *L. hesperus* within each vial, and 10 ml of cocktail was added. Radioactivity (disintegrations per minute [DPM]) was determined with a liquid scintillation counter (Beckman LS5000 TD). Each diet packet was examined under a dissecting scope, and the number of feeding punctures, identifiable by the small amount of diet that accumulated and dried around each puncture, was recorded.

The dry weight of each nymphal instar and adults (5-10 d old) was determined. Specimens were removed from the existing colony, sacrificed with carbon dioxide, and placed in a oven at 80°C for 24 h. The total weight of all individuals from each nymphal instar was subsequently recorded. Male and female adults were separated, and pairs of the same sex were weighed.

Background and control treatments from both trials were combined because there were no significant differences in mean DPM values (t-tests, $P > 0.50$). The resulting mean value (59.3 ± 1.6 DPM) was subtracted from all replicates. DPM values in the first trial were then divided by two because each vial contained two bugs. Data were also corrected to account for differences in inulin concentration between trials. We considered a replicate unmarked, and thus the bugs did not feed, if its corrected DPM value was < 5.

A maxima function ($Y = a*X * \exp^{[b*X]}$; where Y is μl of diet ingested and X is dry weight in mg) was used to regress mean ingestion on dry weight (SAS Institute 1988, non-linear regression). Adult dry weight was calculated as the average mass of one male and one female. Fourth and fifth instars were combined during regression using their average dry weight. Fisher's protected LSD mean separation was used to detect differences among developmental stages in the number of feeding punctures (SAS Institute 1988).

RESULTS AND DISCUSSION

The dry weight of *L. hesperus* approximately doubled as bugs passed from one developmental stage to the next (Table 1). Adult females were ≈ 1.5 times heavier than males. Developmental stage significantly influenced the number of feeding punctures ($F = 5.10$; $df = 5, 57$; $P < 0.001$). Early instars made more feeding punctures than did older nymphs and adults (Table 1).

TABLE 1. Mean Weight of *L. hesperus* and the Number of Feeding Punctures per Bug, in 24 h, from Packets Containing 14C-Labeled Diet (± Standard Error, n).

Developmental Stage		Weight in mg[a]	Feeding Punctures[b]
Instar:	1	0.041 (---, 17)	4.55 (0.98, 10) a
	2	0.114 (---, 17)	5.45 (1.06, 11) a
	3	0.258 (---, 12)	5.41 (1.38, 11) a
	4	0.572 (---, 10)	3.21 (0.93, 12) ab
	5	1.172 (---, 13)	0.89 (0.20, 9) b
Adults:	♂♂ & ♀♀	----	0.55 (0.16, 9) b
	♂♂	2.401 (0.123, 5) a	
	♀♀	3.530 (0.102, 8) b	

[a] Adult means not followed by a common letter are significantly different ($t = 6.97$, $df = 11$, $P < 0.001$, [SAS Institute 1988]). Means for nymphal instars were determined from total weights, and thus, standard errors for these values could not be calculated.
[b] Means not followed by a common letter are significantly different ($P < 0.05$, Fisher's protected LSD, [SAS Institute 1988]).

The quantity of diet ingested increased rapidly as the insects developed from first to third instars (Fig. 1). Third instars and older stages ingested similar amounts of diet. There was a significant linear increase of variance of ingestion verses dry weight ($F = 65.1$; $df = 1, 4$; $P < 0.01$; $r^2 = 0.94$). This is reflected by the standard error bars (Fig. 1). A greater proportion of fourth instar and older bug replicates (≈ 23%) did not feed during the 24 h period than did younger bugs (≈ 3%)($X^2 = 5.795$; $df = 1$; $P < 0.02$). Thus, older bugs ingested more diet per feeding bout, but they fed less frequently than did less mature bugs.

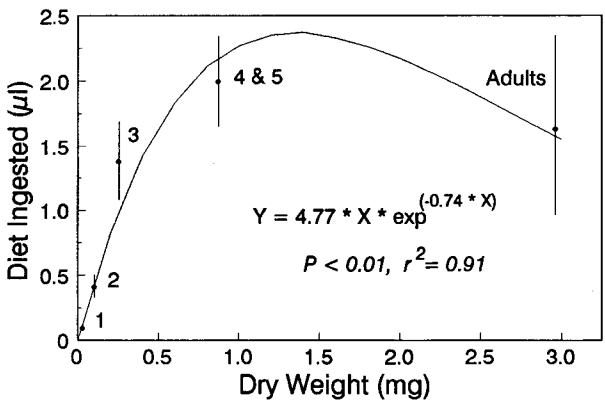

FIG. 1. The amount of ^{14}C-labeled diet ingested by *L. hesperus*, in 24 h, versus dry weight. Data points are labeled to indicate the corresponding nymphal instar and adult stage. Means ± standard errors are plotted.

Gutierrez et al. (1977) found that feeding by first- and second-instar *L. hesperus* caused negligible damage to cotton. Thus, the amount of ingestion, rather than the number of feeding punctures, appears to be more critical in causing plant damage. Gutierrez et al. (1977) also reported that females caused ≈ 2.1 times more damage than males. During the second trial, adult females ingested 1.10 ± 0.58 μl of diet, and the males consumed 0.43 ± 0.07 μl. However, two male replicates were omitted from analysis because they had died, and this difference was not significant ($t = 0.58$; $df = 5$; $P < 0.05$).

There was no difference in DPM values between standard replicates made from control packets, after 24 h, and those made before ($t = 1.40$; $df = 11$; $P > 0.18$). Thus, dehydration of diet, which would have caused an increase in ^{14}C-inulin concentration within the packets, was negligible over the 24-h period. Leakage of diet through feeding punctures did not occur in similar studies on thrips (Wiesenborn and Morse 1985). If leakage was a major problem in this study, we might have expected early instars to have had greater values of ingestion because these individuals made more feeding punctures than did older nymphs.

A criterion of one nymph equaling two adults has been used in economic thresholds for *L. hesperus* (Falcon et al. 1971). The origin of this threshold is not clear and may reflect the opinion that the presence of immature bugs confirms an established population, and thus, is more serious. Nevertheless, these thresholds are not consistent with the relative feeding rates of nymphal instars compared with adults.

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COMPORTAMIENTO SEXUAL DE *TOXORHYNCHITES THEOBALDI*[1] BAJO CONDICIONES DE LABORATORIO

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ABSTRACT

The daily pattern of male swarming activity of *Toxorhynchites theobaldi* (Dyar & Knab) is bimodal, with the smaller peak between 0700 and 1100 h and the larger peak from 1700 to 1830 h, respectively. The daily pattern of sexual encounters is unimodal, with the peak at 1700 h. The greater number of sexual encounters in relation to the insect age occurs during the first 10 days of adult life. Finally, some adaptative advantages and evolutionary aspects of the swarming behavior in mosquitoes are discussed.

RESUMEN

El patrón diario de la actividad de enjambre de machos de *Toxorhynchites theobaldi* (Dyar & Knab) es bimodal, con el pico menor de las 0700 a las 1100 h y el mayor de las 1700 a las 1830 h, respectivamente. El patrón diario de encuentros sexuales resultó ser unimodal, con el pico ubicado a las 1700 h. El mayor número de encuentros sexuales en función de la edad del insecto, ocurrió durante los primeros 10 días de vida como adulto. Finalmente, se discuten algunas ventajas adaptativas y aspectos evolutivos del comportamiento de enjambre en mosquitos.

INTRODUCCION

El mosquito vector del dengue y la fiebre amarilla, el *Aedes aegypti* (L.), es una especie cosmopolita y un problema importante en salud pública, que se combate con la aplicación de Abate® (Temephos) a la concentración de 1 mg/L, sobre todos aquellos recipientes artificiales que funcionan como criaderos larvales, tales como llantas, tinacos, latas, etc.; sin embargo ya se ha reportado resistencia del insecto para diferentas partes de Asia y del Caribe (Georghiou et al. 1987), por lo que se requiere investigar nuevas alternativas de control. En lo que se refiere al control biológico, algunas especies del género *Toxorhynchites* han mostrado potencialidad para usarse como depredadores larvales de *A. aegypti* (Focks 1985), y en ese sentido, *T. theobaldi* se encuentra muy abundante en el noreste de México, donde prefiere áreas sombreadas para buscar y ovipositar sobre criaderos como son los floreros de los panteones (Reyes-Villanueva et al. 1987). En un estudio posterior se encontró que la especie exhibe un patrón diurno de oviposición bimodal con el pico menor a las 1100 h y el mayor a las 1900 h (Arredondo-Bernal y Reyes-Villanueva 1989). Los mismos autores reportaron que las hembras ovipositan más en la medida que aumenta la superficie de exposición del criadero.

Dada la potencialidad de la especie para utilizarla como agente de biocontrol del vector del dengue, y la necesidad de mayor información que permita su cría óptima en el laboratorio,

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aquí se presenta un estudio sobre el comportamiento reproductivo de *T. theobaldi*, que incluye el patrón diario de formación de enjambre, de encuentros sexuales y la variación en la incidencia de los encuentros sexuales durante los primeros 15 días de edad del insecto, bajo condiciones de laboratorio.

MATERIALES Y METODOS

El material biológico utilizado, fue obtenido de una colonia de *T. theobaldi*, establecida entre Agosto y Septiembre de 1987, a partir de 600 huevos colectados en los floreros del panteón de Ciudad Valles, S.L.P. México, siguiendo los métodos de colonización y mantenimiento de Focks et al. (1977). Los adultos fueron confinados en una jaula de 1.20 x 1.20 x 1.20 m forrada de plástico transparente para que permitiera la visibilidad de afuera hacia adentro y con la luz natural incidiendo por el lado norte. En la esquina noroeste fue colocada una planta de ornato de 70 cm de altura, como sustrato para que se posaran los insectos. En el centro se colocó un recipiente de plástico de 30 x 30 x 30 cm, lleno de agua, como fuente de humedad, y en la esquina sureste se colocaron dos latas de 1 litro de capacidad y de color negro, con agua potable hasta el nivel de dos terceras partes, las cuales sirvieron como lugares de oviposición para las hembras. Las condiciones de humedad relativa y de temperatura dentro de la jaula, fueron de 70±5% y de 28±5°C, durante los días en que duró el estudio. La humedad se mantuvo mediante dos riegos que se llevaron a cabo por medio de una jeringa hipodérmica, aplicando en cada uno 100 ml de agua potable, de manera uniforme en todo el interior de la jaula. El primer riego se aplicó a las 0700 h y el segundo a las 1300 h. Tanto la humedad como la temperatura se registraron cada hora con un psicrómetro Taylor® de pared, colgado sobre el lado sur de la jaula.

Para determinar el patrón diario de formación de enjambre, se confinaron 32 individuos (10 hembras y 22 machos) con un rango de 1-20 días de edad; las observaciones se iniciaron un día después de confinar a los insectos y se continuaron por 6 días (del 23 al 28 de noviembre de 1987). Se hicieron en forma directa y cada 15 minutos, registrándose el total de individuos presentes en el sitio de enjambre, y el patrón diario se determinó mediante el porcentaje promedio de la población de machos presentes en el enjambre, cada 15 minutos durante los seis días de observaciones, efectuándose estas de las 0700 a las 1100 h y de las 1400 a las 1830 h.

Para obtener el patrón diario de encuentros sexuales, se realizó un segundo ensayo en una jaula de 60 x 60 x 60 cm y también cubierta de plástico, manteniendo la humedad de manera similar que en el primer ensayo, e igualmente ubicada de manera tal que la luz natural penetrase por el lado norte. Pero en este caso no se colocó dentro ni planta ni depósito de plástico con agua. Fueron confinados 35 individuos (15 hembras y 20 machos) con un rango de edad de 1-3 días. Las observaciones se registraron durante 20 días (del 29 de marzo al 17 de abril de 1988). Se realizaron de las 0600 a las 1100 h y de las 1400 a las 1900 h, registrándose en forma directa la hora para cada encuentro sexual, la duración de la cópula con un cronómetro Steelco® y la modalidad del encuentro, es decir la forma de abordar el macho a la hembra. El patrón diario de encuentros sexuales se determinó mediante el promedio de eventos observados por hora, de un total de 20 días para cada hora.

Finalmente, en lo que toca a la variación de la incidencia de encuentros sexuales durante los primeros 15 días de edad, tanto de machos como de hembras, se utilizó una jaula de 60 x 60 x 60 cm, pero en este caso solo con plástico en un lado, para el registro de observaciones, y tela mosquitero de nylon para el resto de los lados, a excepción de un lado forrado de manta, que llevaba la manga de tela por donde se introducían y sacaban los insectos. Este ensayo se llevó a cabo bajo condiciones de insectario de 80±5% H.R., 25 ±3°C y un fotoperíodo de 14:10 h de luz-oscuridad. La fotofase abarcó desde las 0700 a las 2100 h y las observaciones solo se tomaron entre las 1730 y las 1900 h. Debido al fotoperíodo artificial y a la ausencia de un período crepuscular, observaciones diarias solo se registraron durante un intervalo de 30 minutos, cambiando éste conforme pasaron los 15 días. Así, a la edad de 1, 4, 7, 10 y 13 días, se tomaron datos de 1730 a 1800 h; a la edad de 2, 5, 8, 11 y 14 días, las observaciones fueron de las 1800 a las 1830 h, y por último, a la edad de 3, 6, 9, 12 y 15 días, las observaciones se tomaron de las 1830 a las 1900 h. En este experimento se confinaron 30 individuos (10 hembras y 20 machos), todos emergidos el mismo día. Aquí, en el intervalo de 30 minutos se anotaron el número de encuentros sexuales y de cópulas.

RESULTADOS Y DISCUSION

En lo que se refiere al enjambre, éste se formó en la parte inferior sureste de la jaula, sobre las dos latas negras. Estuvo formado por un grupo de machos, los cuales se posaban en la pared sur de la jaula y sobre los dos botes, después de efectuar un característico vuelo de enjambre en forma de un "ocho" horizontal, en dirección norte-sur y sobre las latas negras. En este caso las dos latas negras con agua funcionaron como un "marcador" de enjambre, el cual puede ser cualquier elemento conspicuo de la superficie terrestre, o bien artificiales, como marcadores visuales de ropa o papel; también pueden ser ciertas características de marcadores naturales como olor, calor convectivo o vapor de agua (Downes 1969), ésto último explica el funcionamiento de las dos latas con agua como marcador en este estudio. Por otro lado, de acuerdo a Johnson (1969), los machos de Culicidae vuelan en enjambres estacionarios para las cópulas, y menciona que el hábito de enjambrar en vuelos muy locales, previene la dispersión de las hembras, donde es más importante que los huevos sean puestos rápidamente, a que el proceso sea retenido y los huevos sean colocados en cualquier lugar. Varias especies de *Psorophora* producen enjambres en los lugares de emergencia, usan las charcas como marcador y copulan en los primeros días de vida como adulto (Provost 1958). Esta explicación es lógica para el caso de *T. theobaldi* , ya que como mosquito asociado a criaderos temporales de corta duración, la formacion de enjambres de machos sobre uno o varios criaderos, facilita la cópula y que las hembras ovipositen en los mismos criaderos del sitio de enjambre. Por otra parte, estos resultados también coinciden con una serie de observaciones que nosotros registramos en el campo. Durante tres días se estuvo observando la formación de un enjambre de *T. theobaldi* a la sombra de un árbol de la especie *Ficus elastica*, en el panteón de Ciudad Valles, S.L.P. México. El área sombreada era aproximadamente de 3 x 3 m y en ella había ocho floreros con agua que funcionaban como criaderos. Se pudo apreciar que el enjambre se formaba entre las 0900 y las 1030 h y de las 1600 a las 1900 h aproximadamente. En el laboratorio se observó una marcada tendencia en los vuelos individuales de aterrizaje, de dirijirse exactamente al punto donde ya se encontraba posado otro macho, al cual desplazaban mediante movimientos de las patas medias, logrando que iniciara el vuelo. En el patrón diario que exhibió este comportamiento (Fig. 1), se detectó la mayor actividad por la tarde, con el 41% de la población

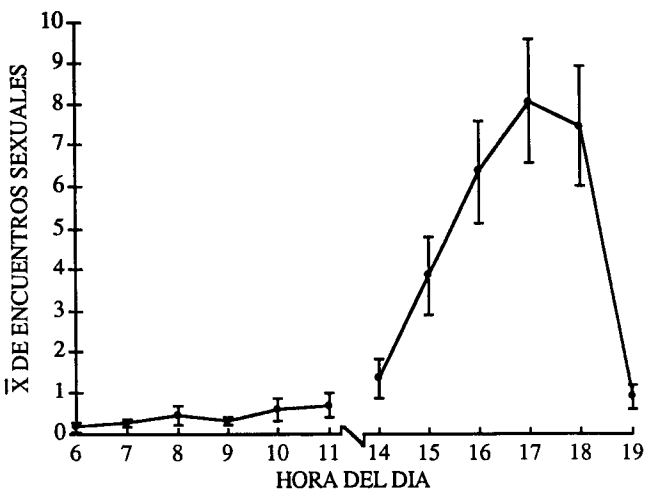


FIG. 2. Patrón diario de la actividad sexual de *Toxorhynchites theobaldi* . Las líneas verticales representan el error estándard para cada media en 20 días.

presente en el sitio de enjambre a las 1700 horas como pico máximo, bajando drásticamente hasta un 8% a las 1830 h. Por la mañana la presencia de individuos osciló de un 2 a un 9% de las 0700 a las 1100 h respectivamente, con los vuelos de enjambre menos periódicos. En este sentido Downes (1969) cita que son insectos "eucrepusculares", aquellos cuya actividad de enjambre esta determinada por las intensidades intermedias de luz de la mañana y del atardecer, con el enjambre matutino más pequeño o ausente; *T. theobaldi* cae en esta categoría.

En el segundo ensayo, que se llevó a cabo en una jaula más pequeña, se determinó el patrón diario de encuentros sexuales, el cual resultó ser unimodal con el pico definido a las 1700 h, con un promedio de ocho encuentros sexuales (Fig. 2). Las frecuencias y las formas en que ocurrieron los encuentros sexuales, de un total de 592 observados, fueron las siguientes: en el 42% de los encuentros, se observó que el macho posado detecta a la hembra en vuelo y se dirige hacia ella; en la mayoría de los encuentros (53%) tanto hembra como macho se encontraban en vuelo, y para el resto de los encuentros se observó un 3.7% para dos machos que se encuentraron en vuelo, 0.7% para dos machos posados que se dirigen hacia la misma hembra en vuelo, y solo en un caso (0.2%) para dos machos que en vuelo respondieron al vuelo de la misma hembra.

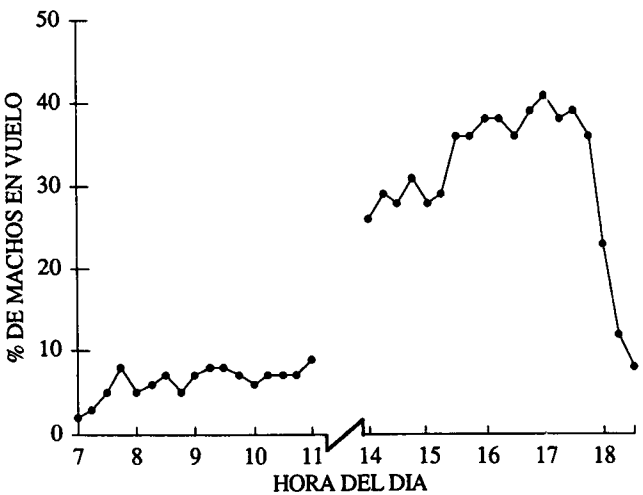
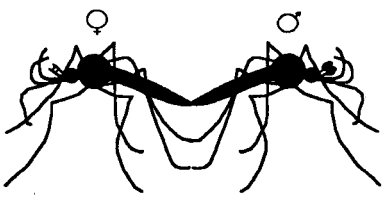


FIG. 1. Patrón diario del comportamiento de enjambre de machos de *Toxorhynchites theobaldi* a intervalos de 15 minutos. Cada punto es el % promedio de seis días.

El hecho de que en un porcentaje significativo de los encuentros (42%), un macho posado abordara a una hembra en vuelo, implica un cambio en el comportamiento reproductivo del mosquito debido al confinamiento en un espacio más pequeño. Al respecto, Downes (1969) menciona que la adaptabilidad del proceso de enjambre tiene dos líneas de desarrollo: para la primera, se trunca el sistema por la eliminación del vuelo y el inicio de cópulas en el suelo; para la segunda, se desarrolla lo que él llama "estación de espera", un sitio donde se concentran los machos en reposo, a la espera de que pase una hembra para abordarla en vuelo. Este comportamiento se considera más evolucionado, y por otra parte, Guy (1977) menciona que las poblaciones de mosquitos pueden ser eurigámicas y estenogámicas, con las primeras copulando en espacios abiertos mediante el proceso de enjambre, y las segundas copulando en espacios limitados y con tendencia a la desaparición del enjambre.

En relación a las cópulas, fueron observadas solamente ocho y ocurrieron entre las 1600 y las 1800 h, con el encuentro de la pareja en vuelo, haciendo contacto genital antes de caer al suelo de la jaula, en donde se observaron dos modalidades en el contacto genital: en uno, ambos sexos quedaron unidos linealmente y con la cabeza en direcciones opuestas (Fig. 3A) y en el segundo, hembra y macho se unieron uno frente a otro, en posicion lateral y formando un ángulo de 90° aproximadamente (Fig. 3B). La duración promedio del contacto genital fue de 12 segundos.



A

B

FIG. 3. Posiciones de cópula observadas para *Toxorhynchites theobaldi* (ver texto).

Finalmente, en lo que toca a la variación de la incidencia de encuentros sexuales durante los primeros 15 días de edad de *T. theobaldi* , el mayor número de encuentros sexuales se presentó a partir del dia 10, ya que al graficar los encuentros diarios acumulados, se puede observar una tendencia descrita por una curva sigmoidea, cuya asíntota se ubica al nivel de los 10 días aproximadamente (Fig. 4).

Lo anterior es importante desde el punto de vista práctico, porque si se cría *T. theobaldi* con el objeto de liberarlo para el control de *A. aegypti* a nivel urbano, 10 días de confinamiento de machos y hembras, es un período suficiente para que la mayoría de las hembras puedan ser copuladas.

Aunque no se hicieron disecciones de espermatecas para registrar el número de hembras inseminadas, este período como quiera es recomendable, porque se observó que en un confinamiento mayor de 10 días, las hembras empiezan a dañarse las alas por el constante contacto con la tela de la jaula y entre ellas mismas y con los machos, lo que puede afectar su vigor y longevidad en el campo y por lo mismo, la tasa de oviposición sobre criaderos de *A. aegypti* .

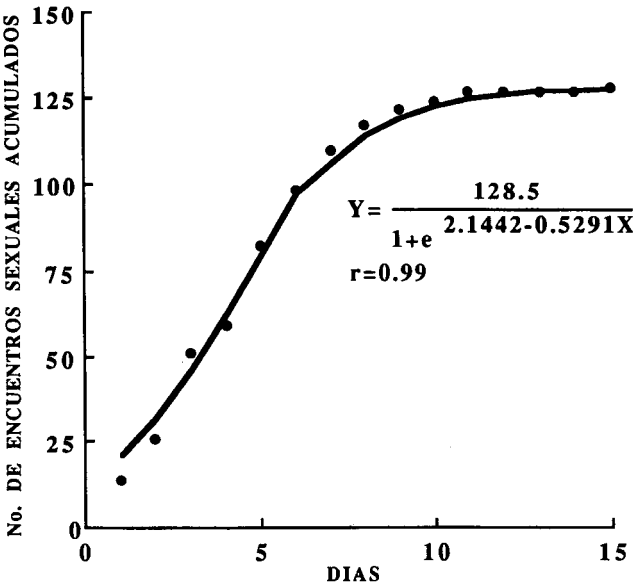


FIG.4. Curva logística que describe la actividad sexual de *Toxorhynchites theobaldi* en los primeros 15 días de edad. Cada punto es el número de encuentros sexuales acumulados diariamente.

AGRADECIMIENTOS

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DISTRIBUCION DE LOS TRIATOMINOS ASOCIADOS AL DOMICILIO HUMANO EN EL MUNICIPIO DE GENERAL TERAN, NUEVO LEON, MEXICO.

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ABSTRACT

The purpose of this work was to determine the species of triatomids present in ten villages of General Terán, N.L., México as well as their entomological indexes, monthly distributions, sex ratios and relationships with human dwellings. Captured species were *Triatoma gerstaeckeri* (192) and *T. lecticularia* (9). *Triatoma gerstaeckeri* was found in ten villages (IDD=80) and 15 houses (II=24); *T. lecticularia* on the other hand was found in only four villages (IDD=40) and five houses (II=8.08). A positive reaction to the presence of *Trypanosoma cruzi* was similar with both species (IIN=28 and 33 respectively). The greatest number of individuals were trapped during the month of June (90 *T. gerstaeckeri* and two *T. lecticularia*). Of 192 *T. gerstaeckeri* trapped during this time, 124 (64.58%) were females and 68 (35.42%) were males; only 8 females (88.89%) and 1 male (11.11%) of *T. lecticularia* were captured. Both species are considered to be peridomiciliar triatomids attempting to adapt to human dwellings because most of the *T. gerstaeckeri* (181/97.27%) and the *T. lecticularia* (6/66.67%) were captured around human dwellings.

RESUMEN

Este estudio se realizó para determinar: las especies de triatominos presentes en diez localidades del municipio de General Terán, N.L., México, los indicadores entomológicos, distribución mensual de captura, proporción sexual de los ejemplares y su relación con el domicilio humano. Las especies capturadas fueron *Triatoma gerstaeckeri* (192) y *T. lecticularia (9). T. gerstaeckeri* fué encontrada en ocho comunidades (IDD=80) y 15 viviendas (II=24); *T. lecticularia* por otra parte, fué encontada sólo en cuatro comunidades (IDD=40) y cinco viviendas (II=8.08). La positividad a *Trypanosoma cruzi* fué similar en ambas especies (IIN=28 y 33 respectivamente). El mes de mayor captura fue junio (90 *T. gerstaeckeri* y 2 *T. lecticularia*). De 192 *T. gerstaeckeri* capturadas 124 (64.58%) fueron hembras y 68 (35.42%) machos; en tanto que de *T. lecticularia* solamente fueron capturados 8 hembras (88.89%) y 1 macho (11.11%). Ambas especies son consideradas esencialmente peridomiciliarias en proceso de adaptación al domicilio humano, pues la mayoría de *T. gerstaeckeri* (181/97.27%) y *T. lecticularia* (6/66.67%) fueron capturadas en el peridomicilio.

INTRODUCCION

El conocimiento de la incidencia de la tripanosomiasis americana o enfermedad de Chagas es de gran trascendencia, debido a que en México se calculan en 3.8 millones los casos autóctonos (Rojas *et al.* 1989) y una incidencia estimada de 206 casos por 100,00 habitantes por año (Lozano *et al.* 1991). Para contribuir a la ampliación de este conocimiento se determinaron las especies de triatominos y sus respectivos indicadores entomológicos (O.P.S.1984) en 10 localidades del municipio de General Terán, ubicado al

sureste del estado de Nuevo León, México. Los indicadores mencionados permiten cuantificar el riesgo de los habitantes a adquirir la infección por *Trypanosoma cruzi* Chagas (Protozoa: Kinetoplastida) de acuerdo al grado de colonización, dispersión, hacinamiento, densidad, infección natural e infestación.

Se observó también la proporción sexual en las especies capturadas, para inferir sobre el potencial reproductivo de las mismas; su distribución intra o peridomiciliaria, lo que aunado al punto anterior permitió conocer la relación de las especies con el domicilio humano (Zeledón,1974) y la distribución anual de captura, para determinar el período en que se deben aplicar algunas medidas de control.

En México se han realizado múltiples estudios sobre la incidencia de la infección por *Trypanosoma cruzi* en triatominos y su relación con el domicilio humano (Salazar *et al.* 1988; Bautista, *et al.* 1990; Galavíz *et al.* 1991; Magallón y Katthain, 1991 y Montes *et al.* 1991), pero en pocos se han determinado los indicadores entomológicos (Galavíz *et al.* 1990a, Galavíz *et al.* 1991 y Guzmán-Marín *et al* 1991), la distribución anual de captura (Montes *et al.* 1991) y la proporción sexual en las especies colectadas (Salazar *et al.* 1988).

MATERIAL Y METODOS

Se muestrearon 10 localidades del ejido San Juan de Vaquerías en el municipio de General Terán, N.L., durante 9 meses de 1991 (marzo-noviembre). Localizadas entre los 99°6'0"-100°8'1" L.O. y los 25°2'6"-25°32'3" L.N.: La Primavera, Los Mimbres, Emiliano Zapata, El Panalito, La Libertad, San Pedro, La Escondida, La Guadiana, Buenavista y San Juan de Vaquerías. Se capturaron triatominos mediante trampas de luz negra y rastreo con lámpara-colecta manual (Beltrán y Carcavallo, 1985). Se registró la zona del domicilio donde fueron detectados y el sexo de los ejemplares colectados.

En el laboratorio se realizó el examen parasitológico de los triatominos, mediante el método de obtención de heces por expresión y revisión al microscopio (Neri *et al.* 1985). Después de lo anterior se calcularon los indicadores entomológicos por especie (O.P.S., 1984). Indice de dispersión (IDD), índice de infestación (II), índice de colonización (IC) e índice de infección natural (IIN).

RESULTADOS Y DISCUSION

En la zona muestreada se colectaron 201 ejemplares de 2 especies de triatominos, de los cuales 192 fueron de *Triatoma gerstaeckeri* Stal (Hemiptera: Reduviidae) y 9 de *T. lecticularia,* Stal. El resultado es similar al obtenido por Galavíz *et al.* (1991) quienes detectaron *T. gerstaeckeri* 126 de 140 ejemplares capturados en una zona aledaña a la de nuestro estudio y superior al obtenido por Galavíz *et al* (1990a) los cuales observaron 413 *T. gerstaeckeri* de 858 ejemplares colectados en el norte del estado de Nuevo León. Estos 3 estudios confirman la importancia de *T. gerstaeckeri* como vector en la zona noreste del país, dada su abundancia.

La especie *T. gerstaeckeri* se encontró distribuida en 15 viviendas (II=24) de 8 localidades (IDD=80) de la zona de estudio y *T. lecticularia,* en 5 viviendas (II=8.08) de 4 localidades (IDD=40) de la misma zona (TABLA 1), lo cual refleja su tendencia a invadir domicilios humanos, pero con preferencia menos marcada que otras especies de mayor importancia epidemiológica como *T. pallidipennis* reportada en 8 de 23 comunidades en Morelos (Bautista *et al.* 1990); *T. dimidiata* colectada en 21 de 22 localidades (IDD=95) y 71 de 116 viviendas muestreadas (II=61) en el estado de Yucatán (Guzmán-Marín *et al.* 1991). *T. barberi, T. picturata* y *T. longipennis* capturadas en el 26.5% de 64 viviendas muestreadas en el estado de Jalisco (Montes *et al.* 1991).

Tanto *T. gerstaeckeri* como *T. lecticularia* mostraron un IC=0 (TABLA 1) dada la ausencia de ninfas, esto difirió de lo reportado por Guzmán-Marín *et al.* (1991) que informan un IC=25 para *T. dimidiata* en Yucatán, es decir encontraron ninfas en 1 de cada 4 casas muestreadas. El resultado para *T. lecticularia* de IIN=33 (2 positivos de 6 examinados) y para *T. gerstaeckeri* de IIN=28 (21 triatominos positivos de 75 examinados) (TABLA 1), confirma lo detectado por (Galavíz *et al.* 1990a) para *T. gerstaeckeri* con una positividad de 26% , en una zona contigua a la de nuestra investigación. Estos estudios comparados a lo reportado para *T. longipennis, T. barberi* y

T. picturata (vectores de importancia epidemiológica en el centro y occidente de México) con un positividad conjunta de 35% (Montes *et al.* 1991) confirman la trascendencia sobre todo de *T. gerstaeckeri* como importante vector de la enfermedad de Chagas en la zona noreste de México.

TABLA 1. Indicadores entomológicos de las especies de triatominos capturados en 10 localidades del municipio de General Terán, Nuevo León.

INDICADOR	*T. gerstaeckeri*	*T. lecticularia*
INFESTACION	24.00	8.08
DISPERSION	80.00	40.00
COLONIZACION	0.00	0.00
INFECCION NATURAL	28.00	33.33

De los 192 ejemplares de *T. gerstaeckeri* capturados, 124 eran hembras y 68 machos (TABLA 2). Lo que indica un alto potencial reproductivo de esta especie dado que tantas hembras podrían originar un incremento poblacional impactante en corto tiempo.

TABLA 2. Distribución de *T. gerstaeckeri* por sexo e infección por *Trypanosoma cruzi* en las localidades de estudio del municipio de General Terán, Nuevo León, de marzo a noviembre de 1991.

LOCALIDAD*	HEMBRAS	(+)	MACHOS	(+)
LOS MIMBRES	15	0	0	0
EL PANALITO	9	0	6	0
SAN PEDRO	31	1	15	13
SAN JUAN VAQUERIAS	23	5	30	0
EMILIANO ZAPATA	35	1	9	0
LA ESCONDIDA	3	0	2	0
LA GUADIANA	4	0	3	0
BUENAVISTA	4	1	3	0
TOTAL	124	8	68	13

(+) Positivos

* En el resto de las localidades no se encontró esta especie.

De los 9 *T. lecticularia* capturados, 8 eran hembras y solo 1 macho. (TABLA 3). El potencial reproductivo de esta especie es alto. La alta proporción de hembras podría generar altos índices poblacionales y por tanto incremento del contacto vector-hombre, en la zona.

TABLA 3. Distribución de *Triatoma lecticularia* por sexo e infección por *Trypanosoma cruzi* en las localidades de estudio del municipio de General Terán, Nuevo León de marzo a noviembre de 1991.

LOCALIDAD*	HEMBRAS	(+)	MACHOS	(+)
SAN PEDRO	2	1	0	0
SAN JUAN VAQUERIAS	4	1	0	0
EMILIANO ZAPATA	0	0	1	0
BUENAVISTA	2	0	0	0
TOTAL	8	2	1	0

(+) Positivos

* En el resto de las localidades no se encontró esta especie.

El mes en que más triatominos se capturaron fue junio con 94 ejemplares (92 de *T. gerstaeckeri* y 2 de *T. lecticularia*), resultado similar al obtenido por otros investigadores para *T. barberi, T. longipennis* y *T. picturata* en el estado de Jalisco (Montes *et al.* 1991)

(TABLA 4). Lo cual indica la sincronización del ciclo biológico de éstas especies de triatominos con las condiciones medioambientales más favorables para su desarollo.

TABLA 4. Distribución mensual de captura de triatominos en 10 localidades del municipio de General Terán, Nuevo León de marzo a noviembre de 1991.

ESPECIE	MAR	ABR	MAY	JUN	JUL	AGO	SEP	OCT	NOV	TOT
Triatoma gerstaeckeri	3	33	32	92	8	10	8	5	1	192
Triatoma lecticularia	0	5	2	2	0	0	0	0	0	9
TOTAL	3	38	34	94	8	10	8	5	1	201

Ambas especies son consideradas esencialmente peridomiciliarias, pues del total de *T. gerstaeckeri* capturadas (124) el 94.27% lo fueron en el peridomicilio. Sucedió lo mismo con *T. lecticularia,* ya que de un total de 9 ejemplares capturados, el 66.67% eran peridomiciliarios; ello significa que las 2 especies están en proceso de adaptación al domicilio humano (Zeledon, 1974). Esto se explica en razón de que, en el peridomicilio de las casas se localizan reservorios adecuados como fuente de alimentación para ambas especies de vectores, como: didélfidos, roedores, bovinos, caninos, primates, aves y reptiles (Galavíz *et al.* 1990b). Se sugiere un proceso de adaptación al domicilio porque los triatominos capturados en el peridomicilio presentan una positividad a *Trypanosoma cruzi* (*T. gerstaeckeri* 28% y *T. lecticularia* 33%) similar a la presentada por ambas especies en ecotopos silvestres (*T. gerstaeckeri* 26.5% y *T. lecticularia* 30.7%) (Galavíz *et al.* 1990b).

AGRADECIMIENTO

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PREDATION BY RED IMPORTED FIRE ANT[1] ON *TYTA LUCTUOSA*[2], RELEASED FOR CONTROL OF FIELD BINDWEED.

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Field bindweed (*Convolvulus arvensis* L.), native to the eastern Mediterranean area of Eurasia, is a prostrate or climbing perennial weed of cultivated crops, fallow fields, and other non-cultivated land (Holm et al. 1977). It occurs throughout the continental U.S. but it is a more serious weed in the arid West (Meyer 1978), principally in corn, sugarbeets, wheat, vineyards, and a serious weed in soybeans (Chandler et al. 1984). Field bindweed is reported to be the fourteenth most important weed in the U.S. (Jansen et al. 1972) and the twelfth most important weed in the world (Holm et al. 1977). *Tyta luctuosa* (Dennis and Schiffermüller) is a common noctuid defoliator of field bindweed in Europe, completing its development only on species of *Convolvulus* and *Calystegia* (Convolvulaceae)(Rosenthal 1978, Clement et al. 1984). *Tyta luctuosa* has been cleared for release as a biological control agent for field bindweed in the U.S. (Rosenthal 1983). But, it has not been approved for release in the Pacific coastal states because of it's potential attack on native *Calystegia* spp. (Rosenthal 1983).

The red imported fire ant, *Solenopsis invicta* Buren, has been reported as an aggressive predator of boll weevil and bollworm (Jones and Sterling 1979), sugarcane borer (Adams et al. 1981), and horn fly (Summerlin et al. 1984). However, little information exists concerning effects of this ant on beneficial insects or biological control agents. Vinson and Scarborough (1991) recently reported that *S. invicta* interfered with *Lysiphlebus testaceipes* Cresson parasitizing corn leaf aphid, *Rhopalosiphum maidis* (Fitch), by removing or destroying aphids that had been parasitized. The objective of this study was to determine the effects of *S. invicta* on biological control of field bindweed by *T. luctuosa*.

Two field insectary plots, ca. 75m apart, were established on naturally occurring stands of field bindweed located on the C. L. Maedgen farm, 4 Km east of Troy, Texas. Four release cages (0.6 x 0.6 x 0.6 m) covered with plastic screen (52 x 52 mesh) were placed in each plot over the field bindweed. Three separate *T. luctuosa* releases were made on field bindweed during the summer of 1991. The first release on 10 May was preceded by treating one plot with Amdro® (0.73%) at 4 lbs per acre to chemically exclude *S. invicta*; the second plot served as an untreated control. Twenty, second- and third-instar *T. luctuosa* larvae were released on bindweed plants in each cage. The Amdro® treatment failed to control the ants, and predation of *T.*

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luctuosa larvae totaled 100% in both plots. A second release on 13 June was preceded by broadcasting granular Lorsban® (15G) at 2 lbs per acre on the treated plot, while the control plot was untreated. Thirty, third- and fourth-instar *T. luctuosa* larvae were placed on bindweed plants in each cage. After 48 hours, *S. invicta* predation of *T. luctuosa* larvae in the untreated plot totaled 100%; little or no defoliation of the field bindweed was noted. Twenty percent of the larvae survived in the treated plot, and 90-99% defoliation of the bindweed was observed. After four weeks, two adult *T. luctuosa* moths were recovered from two separate cages in the treated plot, while none were found in the untreated plot. Additional chemical treatments were not needed prior to the third release attempt because no active fire ant mounds or worker ants were found within the previously treated plot. Thirty, third- and fourth-instar *T. luctuosa* larvae were released on 11 July into each cage. After one week, larvae in the treated plot had defoliated or killed many of the field bindweed plants (Figure 1a). The untreated plot showed little or no defoliation at this time (Figure 1b).

In our study, *T. luctuosa* completed larval development, pupation, and adult emergence in field cages only when *S. invicta* were chemically excluded. These field trials suggest that *T. luctuosa* may control field bindweed; however, it's potential is limited by larval predation by the red imported fire ant.

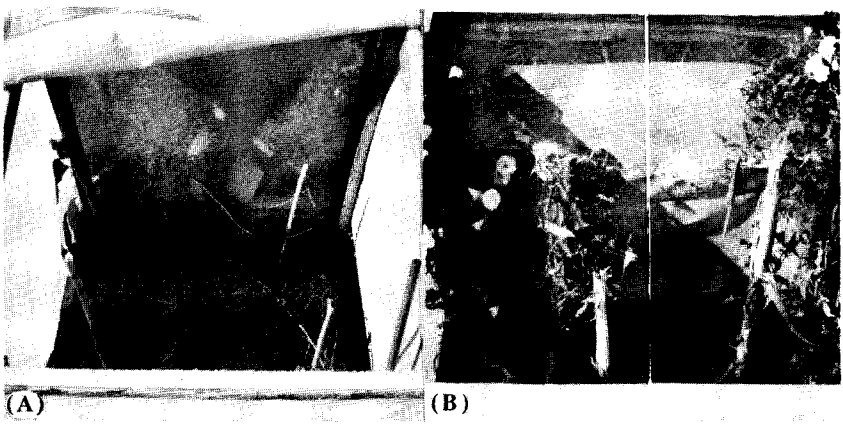

Fig. 1. *Tyta luctosa*'s effect on Field bindweed in (A) plot treated for fireants and in (B) untreated plot.

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ECOLOGY AND POTENTIAL IMPACT OF *CATOLACCUS GRANDIS* (BURKS)[1] ON BOLL WEEVIL[2] INFESTATIONS IN THE LOWER RIO GRANDE VALLEY

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ABSTRACT

Research conducted in the Lower Rio Grande Valley of Texas during 1991 provided significant insight into the ecology and potential impact of *Catolaccus grandis* (Burks), an exotic parasite of boll weevil, *Anthonomus grandis* Boheman. An evaluation of augmentative releases conducted in a 0.6-ha release site near Weslaco (11,455 female parasites between 21 May and 12 July) revealed two important trends. First, parasitism by *C. grandis* was concentrated among third-stage host larvae, the majority of which (98.7%) occurred in abcised cotton squares. Second, apparent parasitism of third-instar hosts increased from an initial incidence of 0.0% on 23 May to levels approaching 100% by 15 July, at which time the host infestation had declined appreciably. These results demonstrated the ability of *C. grandis* to search and reproduce within the release environment, and to effectively parasitize a distinct segment of the host infestation. The attributes, limitations and potential impact of *C. grandis* in the south Texas cotton environment are discussed.

INTRODUCTION

Catolaccus grandis (Burks) is one of at least 15 exotic parasite species associated with boll weevil, *Anthonomus grandis* Boheman, in its aboriginal home of southern Mexico (Cross and Mitchell 1969, Cate 1985, Cate et al. 1990). Although several attempts to establish *C. grandis* in the United States have been unsuccessful (Cate et al. 1990), field evaluations conducted in Mississippi and several areas of Texas documented significant increases in host mortality following release of the parasite (Johnson et al. 1973, Cate et al. 1990). Such results implicate *C. grandis* as a promising candidate for augmentation, an approach that is not predicated on the ability of the parasite to establish in the proposed target region.

Research was conducted in the Lower Rio Grande Valley during 1991 to evaluate the technical feasibility of parasite augmentation as a means to suppress boll weevil infestations on south Texas cotton. Procedures involved the mass-

[1] Hymenoptera: Pteromalidae
[2] Coleoptera: Curculionidae

propagation and periodic release of *C. grandis*, and included an evaluation designed to elucidate 1) the ability of the parasite to search and reproduce within the release environment, 2) the distribution of parasitism among the available host stages present, and 3) the impact of parasitism on the dynamics of the host infestation.

MATERIALS AND METHODS

Parasite releases were conducted in a 44 x 135 m (0.6-ha) plot of DPL-50 (nectaried) cotton located on ARS-USDA facilities in Weslaco, TX. A control plot of similar dimensions was located approximately 600 m to the west of the release site. Release and control plots were planted during 10-12 March and were irrigated on two occasions: at the time of planting and during 10-11 June. No insecticides, herbicides or defoliants were applied to either plot during the study.

Parasite stock used in the study were originally imported by J. R. Cate (Texas A&M University) from southern Mexico and had been maintained in continuous laboratory culture for a period of approximately four years. Parasites were reared *in vivo* using the Parafilm technique described by Cate (1987). Parafilm sheets each containing 120 third-instar boll weevil larvae (obtained from the R. T. Gast Insect Rearing Laboratory, ARS-USDA, Mississippi State, MS) were placed within 40 x 40 x 40-cm plexiglas cages and exposed to 5-to 20-day old *C. grandis* for a period of approximately 4 hours. Such cages were situated beneath a flourescent light source (14L:10D photoperiod) and maintained under relatively constant conditions (27.8 $\pm$ 2°C; 70 $\pm$ 5% RH). Exposed sheets were removed from cages and transferred to ventilated emergence cages for a period of approximately 14 days. Emerged parasites were fed honey for a period of 4 to 5 days to allow sufficient time for females to attain sexual maturity and mate, and were then collected into 1.0-liter cardboard cannisters for transport to release sites. Parasites were released at each of 10 to 15 selected points during the early-to mid-morning hours (0800 to 1000 CDST) in an attempt to minimize dispersal of parasites. At each release point, containers were opened beneath plant canopies and left *in situ*.

Boll weevil infestations in the release and control plots were monitored at weekly intervals between 23 May and 29 July. Samples collected from each of 15 to 25 randomly-selected points in each plot (locations determined by a random numbers table) consisted of 1) all floral structures (squares and bolls) attached to a single randomly-selected plant, and 2) all abcised floral structures within a 1.0 m^2 sample area centered at each point. Samples were examined under a dissecting microscope and numbers of live and dead weevils of each life stage were recorded. Parasitized host material was held in labelled tissue-culture dishes to verify identities of developing parasites.

RESULTS AND DISCUSSION

Evaluation of Augmentative Releases. The initial release of *C. grandis* in the Weslaco site was delayed considerably and involved relatively few parasites (200 females on 21 May) due to production problems encountered during the late-winter and early-spring period. As a result, the boll weevil infestation in the release site occurred at damaging levels at the time sampling activities were initiated, i.e., on 23 May (6.9/m^2; 10.3% punctured squares), and increased to a peak density of 134.0/m^2 by 18 June (73.2% punctured squares; 63.1% damaged bolls) (Table 1). An increase of this magnitude is typical of trends reported previously in the Lower Rio Grande Valley (Summy et al. 1988) and precluded a meaningful comparison of the release and control sites with respect to host densities and degree of plant damage. Nevertheless, an evaluation of augmentative releases conducted in the Weslaco site between 21 May and 15 July (11,455 female parasites) provided significant insight into the ecology and potential impact of *C. grandis* in the south Texas cotton environment.

Following the initial release of 200 *C. grandis* on 21 May (0.03/m^2), parasite densities increased to 0.7/m^2 by 29 May, i.e., prior to the second release (a 23-fold increase, compared with a 2-fold increase in host densities), and attained a peak density of 19.8/m^2 by 26 June, at which time a total of 6,055 parasites (1.0/m^2) had been released (Table 2). Since development of *C. grandis* requires a period of 12-14 days (Morales-Ramos and Cate 1992a), the increase in parasite densities during the first interval was clearly the result of reproduction by adult parasites released on 21 May. During subsequent intervals, a distinction between parasitism caused by released insects and that caused by their progeny was obscured by the release procedure employed in the experiment (sequential releases, rather than a single innoculation). However, the relatively low incidence of mortality among immature parasite stages evident in samples

TABLE 1. Densities and Age Structure of a Boll Weevil Infestation near Weslaco, TX, 1991.

Date	Boll weevils[a] ($\bar{X}/m^2 \pm$ SE)	Age distribution (%)					
		Eggs	1	2	3	Pupae	Adults
5/23	6.9 ± 2.8	33.3	8.0	28.7	29.2	1.0	0.0
5/29	12.7 ± 3.4	35.9	17.5	14.6	25.9	4.7	1.6
6/05	100.4 ± 10.6	24.9	21.8	28.3	22.7	2.3	0.0
6/12	104.1 ± 8.2	37.3	19.8	14.8	16.1	11.3	0.7
6/18	134.0 ± 14.7	34.7	29.3	17.6	9.6	7.4	1.5
6/26	67.4 ± 12.3	11.6	9.0	9.0	36.5	19.5	14.6
7/02	32.6 ± 4.7	5.2	2.5	1.4	48.8	29.7	12.3
7/10	28.9 ± 5.4	3.8	0.0	1.9	27.9	18.4	48.0
7/15	12.3 ± 3.4	9.5	0.0	0.0	26.9	27.3	36.3
7/22	2.9 ± 1.3	0.0	4.4	0.0	0.0	37.2	58.1
7/29	1.2 ± 0.8	0.0	0.0	0.0	0.0	47.0	53.0

a Includes parasitized forms.
a The original release of 200 adult parasites.

TABLE 2. Augmentative Releases and Densities of *Catolaccus grandis* in a Boll Weevil Infestation near Weslaco, TX, 1991.

Sample date	Adult parasites released in field[a]				Immature parasites ($\bar{X} \pm SE$)
	During sample interval		Cumulative		
	Total	No./m^2	Total	No./m^2	
5/23	200	0.03	200	0.03	0.0
5/29	0	0	200	0.03	0.7 ± 0.3
6/05	950	0.16	1,150	0.19	1.5 ± 0.5
6/12	1,000	0.17	2,150	0.36	5.2 ± 1.0
6/18	1,375	0.23	3,525	0.59	8.4 ± 2.1
6/26	2,530	0.43	6,055	1.02	19.9 ± 2.4
7/02	2,000	0.34	8,055	1.36	7.3 ± 0.7
7/10	2,000	0.34	10,055	1.69	0.9 ± 0.5
7/15	1,400	0.24	11,455	1.93	0.1 ± 0.1
7/22	0	0.0	11,455	1.93	0.0
7/29	0	0.0	11,455	1.93	0.0

[a] Dimensions of release site: 44 x 135 m (0.6-ha).

collected throughout the release period (0-2.7%) indicated a considerable degree of adaptability to the release environment and suggested that much of the observed parasitism was effected by progeny of released parasites. Previous research demonstrated the ability of *C. grandis* to reproduce for at least 6 weeks (a period equivalent to approximately 3 parasite generations) following a single release of 1,200 parasites in central Texas (Cate et al. 1990). Regardless, the significant increase in parasite densities clearly demonstrated the ability of *C. grandis* to effectively search and reproduce within the release environment, and was consistent with results of previous studies that suggest a rate of increase equivalent to or greater than that of the boll weevil host (Johnson et al. 1973, Morales-Ramos and Cate 1992a, 1992b).

An analysis of the distribution of parasitism among the available host stages revealed pronounced host and habitat preferences by the parasite (Table 3). Parasitism by *C. grandis* was largely confined to third-stage weevil larvae, the majority of which (98.7%) occurred in abcised cotton squares. This trend exemplified an important aspect of parasite searching behavior, i.e., the ability to effectively search the soil surface for preferred (third-instar) hosts, which almost invariably occur in abcised squares during the critical prebloom period. The absence or relatively low incidence of parasitism among the remaining host stages suggested that such individuals were either immune to attack or nonpreferred as a result of age (the first two larval instars and, to a lesser extent, pupae) or were protected in a relatively inaccessible or nonpreferred microhabitat (all stages infesting bolls, including the preferred third larval stage). In the latter case, nonpreference appears to be the most plausible explanation since studies conducted in tropical regions of Mexico have demonstrated the ability of

TABLE 3. Distribution of Parasitism by *Catolaccus grandis* Among Developmental Stages of Boll Weevil in an Infestation near Weslaco, TX, 1991.

Host stage	Percent of observed parasitism[a]			
	Squares		Bolls	
	Attached	Abcised	Attached	Abcised
Larval stages 1-2	0.0	0.0	0.0	0.0
Larval stage 3	0.0	98.7	0.2	0.0
Pupae	0.0	1.1	0.0	0.0

[a] Based on analysis of 544 parasitized boll weevils collected in random samples from the release site during 18 June to 15 July.

C. grandis to effectively parasitize third-stage weevil larvae infesting cotton bolls (deCoss F. et al. 1981).

Although attacks by *C. grandis* were concentrated within a distinct segment of the host infestation, the incidence of mortality caused by parasitism was appreciable (Table 4). Apparent parasitism of third-stage weevil larvae infesting squares increased from an initial level of 0.0% on 23 May to 79.0% by 26 June (the sample date on which peak parasite densities were evident) and approached a level of 100% by 15 July, at which time the host infestation had declined appreciably. The relatively low incidence of third-instar boll weevils dead from "unexplained" causes (0.0-0.8/m^2) suggested a negligible impact of mortality factors other than parasitism within this age category. Considering the relatively low densities of third-instar hosts during 10-15 July (0.2-1.0/m^2), the intensity of parasitism evident during this period (50-100%) may be considered *a priori* evidence of a high searching capacity (Doutt and DeBach 1964). The ability of *C. grandis* to detect and parasitize suitable hosts occurring at low densities has been alluded to in previous research and appears to be a requisite for effective suppression of the boll weevil host during the critical prebloom period (Morales-Ramos and King 1991).

An experimental comparison of the boll weevil infestations occurring in the release and control sites was complicated by two factors. In addition to the substantial damage that occurred in the release site prior to the time that parasite releases were initiated, immature *C. grandis* were detected in the control site on 5 of 6 sample dates between 5 June and 10 July. Since elaborate precautions were taken to prevent accidental contamination of the control (e.g., release and control sites were sampled by different personnel, and parasite releases were scheduled for days other than those allocated to sampling), this trend

TABLE 4. Parasitism of Third-Instar Boll Weevil Larvae Infesting Cotton Squares, Weslaco, Texas, 1991.

Date	$\bar{X}/m^2 \pm SE$ Live	Dead[a]	Parasitized	% Parasitism[b]
5/23	2.0 ± 0.6	0.5 ± 0.3	0.0 ± 0.0	0.0
5/29	3.3 ± 0.7	0.1 ± 0.1	0.7 ± 0.3	18.3
6/05	10.7 ± 1.8	0.0 ± 0.0	1.5 ± 0.5	12.6
6/12	14.0 ± 1.8	0.4 ± 0.2	5.2 ± 1.0	27.1
6/18	11.1 ± 1.9	0.1 ± 0.1	8.5 ± 2.1	43.2
6/26	5.3 ± 1.1	0.8 ± 0.4	19.8 ± 2.6	79.0
7/02	3.2 ± 0.5	0.3 ± 0.2	7.5 ± 0.7	70.2
7/10	0.3 ± 0.2	0.4 ± 0.3	0.3 ± 0.2	50.0
7/15	0.0 ± 0.0	0.1 ± 0.1	0.1 ± 0.1	100.0
7/22	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	———
7/29	0.0 ± 0.0	0.2 ± 0.1	0.0 ± 0.0	———

[a] Death due to causes other than parasitism.
[b] Calculated as the ratio of total numbers parasitized to the total of live and parasitized forms.

presumably reflected dispersal of parasites from the release site located approximately 600 m to the east. Previous research conducted in the Lower Rio Grande Valley demonstrated the ability of *C. grandis* to disperse distances up to 120 m in search of suitable hosts in low-density infestations (Morales-Ramos and King 1991). The apparent persistence of *C. grandis* in the control was more difficult to explain, but presumably reflected a combination of continuous dispersal from the release site and reproduction by progeny of released parasites (see previous discussion). Regardless, the presence of *C. grandis* in the Weslaco control site was largely a technicality since parasite densities failed to increase to the relatively high levels observed in the release site.

A comparison of the boll weevil infestations occurring in the release and control sites revealed distinct differences in host mortality patterns and clarified the potential role of *C. grandis* in the south Texas cotton environment (Table 5). Similar and relatively low rates of mortality were evident among three groups of weevils not exploited efficiently by the parasite: 1) first-and second-stage larvae infesting either squares or bolls, which averaged 4.5% mortality in both infestations ($P>0.05$), 2) pupae infesting both types of floral structure, which averaged 1.9% mortality in the release site and 2.9% in the control ($P>0.05$), and 3) third-stage larvae infesting bolls, which averaged 0.0% mortality in both plots ($P>0.05$). The principal difference between the two infestations involved differential mortality among host stages exploited most efficiently by the parasite, i.e., third-stage larvae infesting squares, which averaged 66.3% in the release site and 8.5% in the control ($t=6.695$; $df=6$; $P<0.05$). The latter difference was largely explained by differential rates of parasitism, which accounted for 96.8% of the observed mortality occurring among third-instar hosts in the release site, but only 37.8% of the significantly

TABLE 5. Average Mortality Among Developmental Stages of Boll Weevil in the Weslaco Release and Control Sites, and the Contribution Due to Parasitism by *Catolaccus grandis*, 16 June – 2 July 1991.

Host[a] stage	% Mortality[b]		% of the observed mortality due to parasitism	
	Release	Control	Release	Control
Larval stages 1-2 (aggregate)	4.5 a	4.5 a	0.0	0.0
Third-instar larvae (squares)	66.3 a	8.5 b	96.8	37.8
Third-instar larvae (bolls)	0.0 a	0.0 a	——	——
Pupae (aggregate)	1.9 a	2.9 a	11.4	0.0

a Floral structure inhabited indicated in parentheses; 'aggregate' includes both squares and bolls.
b Means followed by same letter not significantly different at 5% level (Student's-t test, using arcsine transformation).

lower mortality occurring in the control.

Summary. Despite its apparent inability to establish in subtropical and temperate regions of the United States (Cate et al. 1990), *C. grandis* appears to be a promising candidate for augmentation, an approach that is not predicated on the ability of the natural enemy candidate species to establish in the target region. The parasite exhibits several traits that have traditionally been associated with effectiveness: 1) a high rate of increase relative to the host (Johnson et al. 1973, Morales-Ramos and Cate 1992a, 1992b), 2) competitive superiority with respect to predominant native parasites such as *Bracon mellitor* Say (O'Neil and Cate 1985), and 3) the ability to disperse significant distances in search of suitable hosts, and to detect and parasitize the latter at relatively low densities (Morales-Ramos and King 1991). Moreover, *C. grandis* is clearly amenable to mass-propagation using *in vivo* rearing techniques (Cate 1987, Morales-Ramos et al. 1992), and appears to be amenable to *in vitro* rearing (Guerra 1992). Results of the present study clearly demonstrated the ability of *C. grandis* to search and reproduce within the release environment, and to effectively parasitize a distinct segment of the host infestation (i.e., third-stage host larvae infesting cotton squares). These trends were consistent with results of previous studies that

demonstrated an appreciable increase in host mortality caused by parasitism following the release of *C. grandis* in both Mississippi (Johnson et al. 1973) and central Texas (Cate et al. 1990).

One aspect of the present study was somewhat paradoxical (i.e., augmentative releases resulted in a rapid increase in parasitism, while providing little or no suppression of host densities or damage to cotton plants), but nevertheless provided insight into the type of release strategy that will probably be necessary to effectively suppress boll weevil infestations on cotton. As indicated in Table 1, parasite releases in this particular study were initiated against a host infestation characterized by relatively high densities and an advanced stage of development. Emergence of first-generation adults (evident as early as 29 May) resulted in an explosive increase in host densities and the intensity of plant damage, and tended to maintain an age structure consisting primarily of host stages not yet susceptible to attack by the parasite (i.e., eggs and the first two larval stages). Regardless of levels of parasitism achieved by augmentation, an extended emergence of relatively long-lived and highly-fecund adult boll weevils would appear to ensure destruction of the crop in the absence of some form of conventional suppression tactic (e.g., insecticidal treatment). Thus, boll weevil infestations in such an advanced stage of development do not appear to be readily amenable to suppression by *C. grandis* and other third-instar parasites. Nevertheless, the parasite could conceivably provide highly effective control if released in sufficient quantities earlier in the season. Augmentative releases timed to maximize rates of parasitism among immature boll weevils developing to the susceptible third larval stage during the first generation would necessarily reduce the incidence of surviving first-generation adults and hence, would tend to reduce the host rate of increase during subsequent generations (an effect analogous to that of preemptive insecticidal treatments). The parasite exhibits certain traits that suggest the feasibility of this approach (particularly a high searching capacity and tolerance to environmental conditions prevalent during the prebloom period) although verification will require extensive testing under normal field conditions.

To summarize, suppression of boll weevil infestations on cotton through augmentative releases of *C. grandis* appears to be a biologically and logistically feasible concept. However, the development of an effective parasite augmentation strategy will require extensive additional research in certain critical areas: 1) the development and refinement of efficient rearing technologies suitable for use on a large scale, 2) the development of efficient delivery systems based on knowledge of optimal release rates, proper timing and other considerations (e.g., the relative merits of a single innoculation versus a sequence of releases), and 3) detailed scientific evaluations of parasite efficacy under a variety of conditions likely to be encountered in the target region (e.g., parasite performance on nectaried and nectariless cultivars in irrigated and dryland plantings). Since the adoption of biological control strategies by producers will ultimately depend on economic feasibility, a

detailed cost:benefit analysis of parasite augmentation in relation to conventional control strategies will be essential. These research goals will require a substantial amount of effort, but should be pursued since parasites such as *C. grandis* provide the means by which to substantially increase mortality among immature stages of boll weevil, an effect that has not been realized with any of the conventional control strategies currently available.

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MICROALGAE *SPIRULINA MAXIMA* (OSCILLATORIACEAE) IN THE LARVAL DIET OF THE SCREWWORM (DIPTERA: CALLIPHORIDAE)

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ABSTRACT

The effect of substitution of spray dried *Spirulina maxima* (Setchell and Gardiner) Geitler powder for nonfat dry milk in the diet of the larval screwworm, *Cochliomyia hominivorax* (Coquerel), was evaluated. Pupal weight and total number of pupae produced were significantly affected by concentrations of the algal powder in the formulations tested. Percent adult emergence, sex ratio, oviposition and percent eclosion were similar between the treatments. Diet formulations containing higher concentrations of *S. maxima* powder showed the most promise as possible substitutes for the nonfat dry milk component used in the larval diet in the screwworm production facility. Use of *Spirulina* spp. could lead to a savings in the dietary ingredient costs of the larval screwworm diet.

INTRODUCTION

The sterile insect technique (SIT) being utilized to eradicate the screwworm, *Cochliomyia hominivorax* (Coquerel), in Central America and Libya depends on the weekly production of hundreds of millions of flies for sterilization and release. A gelled dietary formulation (Harris et al. 1985, Taylor and Mangan 1987), representing a modification of the liquid hydroponic larval diet originally developed by Gingrich et al. (1971) and modified by Brown and Snow (1979), is currently being used by the Mexican-American Screwworm Commission in the production plant near Tuxtla Gutiérrez, Chiapas, México. This diet contains ca. 6% whole dried bovine blood, 3% calf milk replacer (Super Fly Starter, Land O' Lakes, Inc., St Paul, MN)[1], 3% whole dried chicken egg, 0.1% formol and 1.2% of a sodium polyacrylamide polyacrylate gelling agent (Water Lock G-400, Grain Processing Corp., Muscatine, IA)[1].

Diet ingredient costs in insect mass production in general (Guerra and Garza 1978, Hou and Chen 1981, Kunz 1989, Economopoulos et al. 1990) and the screwworm eradication effort in particular (Gingrich 1972, Harris et al. 1985, Taylor 1988, Friese 1992) have historically been an important consideration. At weekly production levels of ca. 200 million flies, dietary ingredients cost ca. $30,000 (T.R. Ashley, pers. comm.). In an effort to economize screwworm production, plant managers recently replaced the original milk component of the diet with the same percentage of calf milk replacer (Friese 1992). Other less expensive alternate protein sources, either animal- or plant-derived, could be a solution to reducing overall dietary costs. Historically, plant-derived proteins are less expensive.

The blue green algae, *Spirulina* spp. (Oscillatoriaceae), are multicellular, filamentous cyanobacteria found in most aquatic environments; however, they appear to reach maximal growth potentials in alkaline lakes,

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

preferring salt concentrations of 20-70 g/liter (The Protein Advisory Group 1973, Ciferri 1983). These algae are an important food source for the micro- and macro-fauna of these lakes and represent the major food source for avifauna inhabiting them (Ciferri 1983). *Spirulina* spp. have formed part of the diet of certain tribes of native peoples in México and Africa for many centuries (Ciferri 1983, Vonshak and Richmond 1988). *Spirulina* spp. are prime candidates as an inexpensive alternative protein source to animal-derived protein due to their high protein content (60-70% of dry weight), low fat, and high vitamin content. They are also attractive because they can be produced under local conditions, using marginal land and saline water unsuitable for conventional agriculture, as well as animal and human wastes for nutrients (Vonshak and Richmond 1988).

At present, *Spirulina* spp. are cultivated on a commercial basis in several countries. Total world annual production is in the range of several hundred tons. México produces ca. 50 tons of *Spirulina* spp. powder per year. Use of *Spirulina* spp. in the diet of the screwworm could lead to a reduction in dietary costs by replacing more expensive animal-derived proteins and by reducing transportation costs due to the screwworm production plant's proximity to a local source of *Spirulina* spp.

MATERIALS AND METHODS

Methods of media preparation were similar to those used by Taylor and Mangan (1987). The gelled control diet was prepared by combining 70 g of dried whole bovine blood, 30 g dried whole chicken egg, 30 g dried nonfat milk and 12 g of Water Lock G-400. One liter of warm water (35° C) containing 1.2 ml of formol was combined with the dry ingredients. Treatment diets were prepared in the same manner. All diets were permitted to gel for 5 min prior to use. Spray dried *Spirulina maxima* (Setchell and Gardiner) Geitler powder (Earthrise Farms, Carson, CA)[1] was substituted for a portion of the nonfat dry milk in the following ratios (g of *S. maxima* : g of milk): 0:30 (control), 5:5, 10:5, 15:5, 20:5, 25:5 and 30:5. Because a milk component had been considered necessary for normal larval growth and development, no attempt was made to completely eliminate milk.

All experiments utilized the USDA-ARS VF-84 fly strain which originated from native adult egg masses collected in 1984 in Villa Flores, Chiapas, México and which had been in laboratory culture for approximately four years. Larval rearing was initiated on day 0 by placing 50 mg of freshly oviposited screwworm eggs on a 2.5 g patty of ground horse meat. Horse meat and eggs were placed in petri dishes (9-cm dia.) lined with moistened absorbent paper, covered, and placed on a metal rack 3 cm above the surface of a heated water bath (39° C). On day 1, the meat patty with eclosed first-instar larvae was placed in a 2-l plastic rearing pan (15X18X8 cm : LXWXD) with 0.5-l diet. An additional 0.5 and 1.0 l of diet were added on days 3 and 4, respectively. Larval rearing pans were maintained in a heated water bath at 37° C and 50-70% RH. When crawl-off commenced, usually on day 5, larval rearing pans were placed inside larger pans with a 5-cm layer of sawdust covering the bottom. Five days after commencement of crawl-off, larval rearing pans were discarded. Pupae in the crawl-off pans were recovered by sifting to remove sawdust the day after crawl-off was completed. Total volume of pupae per pan was recorded, and a 10-ml sample was weighed, counted, and held until all adults had emerged. Each sample therefore provided data on pupal weight, percent adult emergence, number of pupae/ml, and sex ratio. Total number of pupae produced was estimated by multiplying the number of pupae/ml by the total volume of pupae produced. A second sample of up to, but not exceeding, 50 ml of pupae was placed in a sleeve cage (15X30X12 cm). After emergence, adults were provided honey and water *ad libitum* and induced to oviposit 8 days post emergence. Total weight of eggs oviposited per cage and percentage eclosion were recorded. Oviposition (mg eggs/female) was estimated using the following formula:

$$\frac{\text{EGGWT}}{(\text{PUP}/10) \times \text{VOLCAGE} \times \text{FEMPER}}$$

where EGGWT = Total weight of eggs oviposited per cage; PUP = Number of pupae in 10-ml sample; VOLCAGE = Volume of sample placed in sleeve cage; FEMPER = Percentage of females in 10-ml sample.

Each treatment was replicated four times. All replicates of each treatment were performed simultaneously; however, due to space and time limitations, the 5:5, 10:5 and 15:5 treatments and the 20:5, 25:5 and 30:5 treatments were performed, each with a separate control, on two different dates. The effects of treatment diets were assessed on the following parameters: pupal weight (mg) at 5 days of age, total number of pupae produced (survival), % adult emergence, sex ratio (% male), % egg eclosion and female oviposition (mg eggs/female) at 8 days of age. Data were analyzed with the PROC ANOVA and PROC MEANS procedures of the SAS statistical package. Tukey's studentized range test was used to determine significant differences between the treatment means at P=0.05 (SAS Institute 1988).

Since an ANOVA performed on the two different control groups indicated no significant differences in any of the parameters, data from both experiments were combined into the same analysis of variance. The control treatment therefore represented eight replicates while each *S. maxima* treatment was comprised of four replicates.

RESULTS AND DISCUSSION

Mean pupal weights, ranging from 43.9 mg to 50.9 mg, of all *S. maxima* treatments were statistically similar (Table 1); however, pupal weight for the 15:5 treatment was significantly smaller than that of the control. Mean number of pupae

TABLE 1. Mean Pupal Weight and Number of Pupae Produced by Larvae Reared on Diets Containing Nonfat Dry Milk and *Spirulina maxima* Powder.

	$\bar{X}$ ± S.E.[a]	
g *Spirulina maxima* : g NFDM[b]	Pupal Weight (mg)	Number
0:30 (Control)	51.84 ± 1.05a	680.78 ± 86.71ab
30:5	49.98 ± 0.27ab	699.00 ± 64.76ab
25:5	49.45 ± 1.28ab	885.83 ± 36.57a
20:5	50.88 ± 0.61ab	951.50 ± 68.38a
15:5	43.93 ± 1.17b	705.13 ± 58.50ab
10:5	47.30 ± 3.58ab	410.50 ± 92.39b
5:5	46.70 ± 1.23ab	617.50 ± 61.69ab

[a] Means followed by the same letter in the same column are not significantly different from one another ((P=0.05) Tukey's Studentized Range Test).
[b] Nonfat Dry Milk.

produced ranged from 410.5 to 951.5. None of the *S. maxima* treatment means were significantly different from the control. The largest numbers of pupae produced were in the 20:5 and 25:5 treatments. The mean number of pupae produced in these treatments were significantly greater than that of the *S. maxima* 10:5 treatment (Table 1).

Treatment means for percent adult emergence, percent male, oviposition and percent eclosion were not significantly different from the control. Percent adult

emergence ranged from 89.6% to 93.4%. Percentage males in the adult stage ranged from 43.1% to 54.2%. Percent eclosion ranged from 81.3% to 94.8% and oviposition ranged from 4.80 mg to 8.24 mg of eggs per female.

This study represents the first attempt to substitute substantial amounts of plant-derived proteins for animal-derived proteins in the larval diet of the screwworm. It demonstrates that a plant-derived alternate protein source can be substituted for most of the nonfat dry milk in the larval diet of the screwworm without detrimental effects on the rearing parameters measured.

Pupal weight and adult size are correlated with larval weight (Gingrich et al. 1971, Peterson and Candido 1987). Adult male screwworm size is positively correlated with field and laboratory quality parameters (Alley and Hightower 1966, Hightower et al. 1972) and is an important criterion in evaluating larval production diets (Gingrich et al. 1971; Gingrich 1972; Harris et al. 1984, 1985; Taylor and Mangan 1987; Taylor 1988). Although the control diet produced pupae with the greatest mean pupal weight, those of the higher concentrations of *S. maxima* were not significantly different. An equally important criterion for evaluating larval screwworm production diets is survival. Values for total number of pupae produced (survival) in the four highest concentrations of *S. maxima* powder (30:5, 25:5, 20:5, 15:5) were greater than the control value although not significantly so.

The higher concentrations of *S. maxima* powder showed the greatest potential for use in the sterile fly production facility due to their positive effect on pupal weight, total number of pupae produced (survival), percent emergence, oviposition and percentage male (sex ratio). Adult emergence, sex ratio, oviposition and percent eclosion vary in importance according to which aspect of the eradication program (i.e., production, eradication, cost, etc.) each is viewed (Gingrich 1972; Harris et al. 1984, 1985; Taylor and Mangan 1987; Taylor 1988). As mentioned, the values for these parameters were not significantly different from the control and they compare favorably to control values reported by Harris et al. (1984, 1985), Taylor and Mangan (1987) and Taylor (1988).

Spirulina spp. have been used in other insect diets in an attempt to lower dietary ingredient costs. Guerra and Garza (1978) reported that larval and pupal development, adult emergence, fecundity and fertility of the moths, *Heliothis virescens* (F.) and *Heliothis zea* (Boddie) reared on diets containing *Spirulina geitleri* J. de Toni were comparable to those reared on casein, soy flour and wheat germ which are the standard protein sources used in the larval diets of these species. The price of the *S. geitleri* used in their study was ca. 80-90% less per kilo than the standard proteins. Similarly, when silkworm, *Bombyx mori* L., larvae were fed *S. platensis* powder substituted for defatted soybean meal they showed comparable weight gains and larval survival to those fed on the standard diet containing defatted soybean meal at all of the concentrations tested except the two lowest (7.5 and 15%) (Hou and Chen 1981).

At present, *Spirulina* spp. produced for human consumption costs $5.00-$20.00/kg while *Spirulina* spp. produced for animal feed could have increased yields and simpler production methods than those currently in use (Vonshak and Richmond 1988). In the future partial or complete substitution of *Spirulina* spp. powder for one or more of the protein sources, such as blood, eggs, nonfat dry milk or calf milk replacer, currently used in the screwworm diet, could represent a cost-savings in dietary ingredients.

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HOW TO COPE WITH AN ALKALOID: LOCOWEED-INSECT HERBIVORE-SYMBIOTIC BACTERIA INTERACTIONS

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ABSTRACT

Three species of insects attacking woolly locoweed, *Astragalus mollissimus var. earlei* (Rydb.) Tidestr., *Delia lupini (Coquillet)*, *Cleonus trivittatus* Say, and *Walshia miscecolorella (Chambers)*, were found to use *Pseudomonas* and *Klebsiella* bacteria to breakdown the toxic alkaloid in locoweed. Aseptic larvae of the three root/stem/crown borers failed to complete development in artificial diets containing either extracts of woolly locoweed alkaloid or swainsonine, a known alkaloid from *Astragalus*.

INTRODUCTION

Locoweeds are a persistent problem in the western United States. *Astragalus mollissimus* var. *earlei* (Rydb.) Tidestr. (woolly loco), a common species in the Trans-Pecos area of west Texas, is a perennial legume with a woody taproot and numerous decumbent stems most abundant in mountain basins on soils of igneous derivation. In low rainfall years (<25 cm), it is often restricted to basin areas and nearby gentle slopes. It occurs over much of the range area on both igneous and limestone soils in high rainfall years (>35 cm). Woolly loco grows in grasslands, regardless of condition, and is commonly associated with the gramma grasses, cane and silver bluestem, and lesser grasses such as *Tridens*, *Buchloe*, and *Muhlenbergia*.

The early history of loco poisoning is reviewed by Crawford (1908), Marsh et al. (1936), and Marshall (1914). The toxic nature of the several species of *Astragalus* is associated with one of the following three factors: (1) the accumulation of selenium in seeds and plant tissues, (2) the presence of alkaloidal substances such as locoine (Fraps and Carlyle 1936) and swainesonine (Davis et al. 1984), and (3) the presence of nitro compounds (James 1983). Although there are 368 species of *Astragalus* in North America (Barnaby 1964), not all exhibit toxic properties.

The similarity of selenium poisoning and locoism has posed problems in determining the "poisonous" factor of various toxic *Astragalus* species. Locoism is a disorder induced by ingestion of locoweeds. Symptoms of locoism vary but animals generally exhibit hallucinations, delirium, defective vision, and other symptoms similar to selenium poisoning (Trelease and Beath 1949). Fraps and Carlyle (1936) isolated the toxic substance locoine from *A. mollissimus* var. *earlei*, but it was not chemically defined. Subsequent research by Faulkner and Smith (1950) and Chervenko and Wender (1950) also failed to chemically

identify the toxic chemical. Swainsonine was first isolated from *Swainsona canescens* F. Muell. in Australia (Colegate et al. 1979). It has also been isolated from *A. lentiginosus* Dougl. (Molyneux and James 1982) and *A. emoryanus* (Rydb.) Cory (Davis et al. 1984).

Locoism is dependent on the amount of locoweed consumed. Mathews (1932) and Kingsbury (1964) reported cattle must ingest 90% of their body weight in two months to exhibit symptoms. Ingestion of 32% body weight in cattle within three months resulted in death. Horses died after consuming 30% body weight in one and a half months. Abortion in livestock is also a common problem with locoism (James 1967, James et al. 1983). Dollahite (1965) estimated losses from locoweed in Texas to be ca. $10 million per year.

Toxic locoweeds are attacked by a number of native insects (Chittenden 1908, Lavigne and Littlefield 1989). Insect species feeding on foliage, seeds, stems, and roots have been considered potential agents for biological control of locoweeds. The principal root-crown feeders are: *Delia* (*Hylemya*) *lupini* (Coquillet) (Diptera: Anthomyiidae), *Cleonus trivittatus* Say (Coleoptera: Curculionidae), and *Walshia miscecollorella* (Chambers)(Lepidoptera: Cosmopterygidae)(Chittenden 1908). This study was undertaken to determine the mechanism allowing the three principal insects attacking *A. mollissimus* to successfully feed and develop on the toxic host tissues.

MATERIALS AND METHODS

Alkaloid extraction. Plant material was collected from natural populations in Brewster and Jeff Davis counties in the Trans-Pecos Area of West Texas. Alkaloidal material was extracted from *A. mollissimus* using the technique described by Harborne (1984). Roots and stems were air dried and pulverized in a food processor. Alkaloids were extracted from dried material with 10% acetic acid in ethanol for 4 hr. The extract was concentrated to 1/4 original volume and precipitated by dropwise addition of concentrated ammonium hydroxide. Alkaloids were collected by centrifugation and washed with 1% ammonium hydroxide. The residue was dissolved in a few drops of ethanol. The presence of alkaloid material was detected by use of three spray reagents: Dragendorff, iodoplatinate, and Marqis (Harborne 1984). Since the alkaloid in woolly loco was not identified as swainsonine, swainsonine was also purchased from Sigma Chemical Co.(St. Louis, MO) to be used as a standard.

Bacterial material. Soil samples were taken from the top 5 cm of soil from stands of woolly locoweed in Brewster and Jeff Davis counties, Texas. Insect-infested locoweeds were collected at the same sites. Soil and plants were washed in distilled water; aliquots of the wash were plated on diagnotic plates (Palleroni 1984). The plants were then dissected in the laboratory. Larval stages of *D. lupini*, *C. trivittatus*, and *W. miscecolorella* were surface sterilized in 70% ethyl alcohol for 30 min. They were then washed through three 5-min. washes in calcium- and magnesium-free phosphate buffered saline and six 5-min washes in physiological saline. Digestive tracts of larvae were aseptically removed and homogenized in 2 ml of physiological saline. After homogenization, serial 10-fold dilutions of each sample were prepared and plated for identification. Bacteria were identified from the soil, plant, and insect sources using standard bacteriological techniques

(Palleroni 1984) and the API 20E identification system (Analytab Products, Plainview, NY).

The ability of the bacteria to metabolize alkaloidal compounds was tested using the auxanographic technique described by Parke and Ornston (1984) and Schmidt (1988). Stock cultures of the *Pseudomonas* and *Klebsiella* species were grown to early stationary phases and then diluted with inorganic salt solution to obtain the initial cell densities used in the experiments. Experiments were conducted in glass-stoppered 125-ml Erlhenmeyer flasks containing 50 ml of the inorganic salt solution and the indicated amount of locoweed alkaloid (or swainsonine). Flasks were incubated at 20^0C without shaking. At regular intervals, numbers of cells were determined using the spread plate technique described by Schmidt (1988). Triplicate 0.1 ml portions of 10-fold dilutions were plated on a medium containing 15 mg of Difco Bacto-agar and 3 mg of Trypticase soy broth with glucose per ml of deionized water. Colony counts were made after 72 hr of incubation at 22^0 C; data represent means of triplicate plate counts from individual flasks. In two sets of experiments, swainsonine or locoweed alkaloid extract at concentrations of 0.0, 0.5, and 1.0 ug/ml was dissolved in the inorganic salt solution.

Insect material. Adults and larval stages of the three principal insects were collected from woolly locoweed populations. Larvae of the three species were reared to adults on the sugarcane rootstalk borer diet (Harley and Wilson 1968). I have used this diet to produce a laboratory culture of these three species that is indistinguishable (behaviorally) from field collected individuals. Eggs of each of the three species were collected from infested plants in the field, from field collected adults on plants in the laboratory, and from laboratory reared cultures. Following surface sterilization with ethanol, none of the eggs were found to harbor bacteria. Laboratory reared larvae were aseptic and field collected larvae were always found contaminated with either *Pseudomonas, Klebsiella*, or both. For each of the three species of borers, individual larvae were placed in 35-ml plastic cups (Bio-Serv, Frenchtown, NJ) containing 15 ml of sugarcane rootstalk borer diet. Larvae were held at room temperature under 18:6 light-dark cycle. Three experimental diets were used: 1) standard diet with 2 ug locoweed alkaloid extract, 2) standard diet with 2 ug swainsonine, and 3) standard diet without alkaloid (control). Bacteria-free, laboratory reared larvae and larvae reared from sterilized field collected eggs and field collected contaminated larvae were tested on the three diets.

RESULTS AND DISCUSSION

Microbial Associations. Bacteria were isolated from field collected specimens of *D. lupini*, *C. trivittatus*, and *W. miscecolorella;* infested plant material; and soil around the plants (Table 1). *Klebsiella oxytoca* and *Pseudomonas aeruginosa* were found on the exoskeleton of insect adults and larvae; in the fore-, mid-, and hindgut areas of the larval stages of all three species of insects, in the feeding channels made by the larvae; on the surface of the infected and uninfected roots and stems; and in the soil around the roots.

Klebsiella and *Pseudomonas* were able to metabolize both the locoweed alkaloid extract and swainsonine as their apparent sole source of carbon and energy (Fig. 1). No growth of either

Pseudomonas or *Klebsiella* was detected in the inorganic salt solutions lacking either the locoweed alkaloid extract or swainsonine (Fig.1).

TABLE 1. Bacteria Isolated from Soil, from the Three Insect Species Attacking Locoweed, and from Locoweed Tissues.

Bacteria	Source(s)
Citrobacter freundii	*Walshia miscecolorella*
Enterobacter agglomerans	*Walshia miscecolorella*
Klebsiella oxytoca	*Walshia miscecolorella*, *Delia lupini*, *Cleonus trivittatus*, Soil, Plant Tissues
Pseudomonas aeruginosa	*Walshia miscecolorella*, *Delia lupini*, *Cleonus trivittatus*, Soil, Plant Tissues

Insect Interactions. None of the three bacterial symbiont-free, insect species completed development in artificial diet containing either swaisonine or the alkaloid extract (Table 2). However, the symbiont-free species completed development in the diets lacking the alkaloids (Table 2). Furthermore, All three species with their symbiotes present completed development in diets with or without the alkaloids (Table 2).

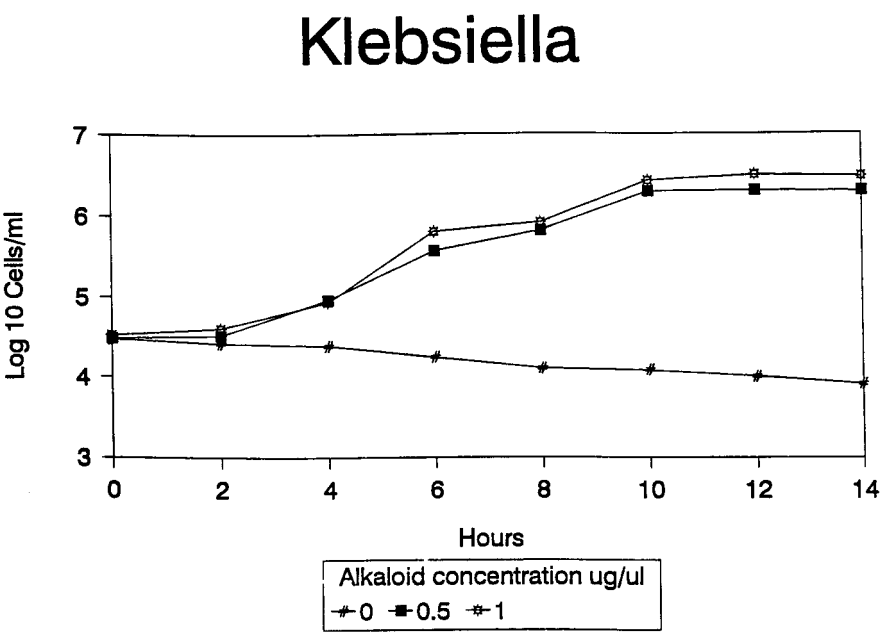
TABLE 2. Growth and Development of Three Species of Root/Stem Borers in Artificial Diet with and without *A. mollissimus* Alkaloid Extract or Swainsonine.

	% Larvae Completing Development[a]		
Species[b]	Control	Extract	Swainsonine
Delia lupini			
Sterilized lab	94	0	0
Sterilized field	96	0	0
Unsterilized field	92	92	94
Cleonus trivittatus			
Sterilized lab	86	0	0
Sterilized field	90	0	0
Unsterilized field	90	92	90
Walshia miscecolorella			
Sterilized lab	86	0	0
Sterilized field	90	0	0
Unsterilized field	94	94	92

[a] N= 50

[b] Sterilized lab = larvae from sterilized lab-reared eggs;
Sterilized field = larvae from field collected eggs surface sterilized in the lab; and
Unsterilized field = field collected larvae.

Given the toxicity of locoweed alkaloids, herbivorous insects utilizing locoweeds must be able deal with the toxic alkaloids. It appears that insects attacking *A. mollissimus* are able to utilize symbiotic bacteria (*Pseudomonas* and *Klebsiella* spp.) to metabolize and detoxify the alkaloids, specifically swainsonine. The ability of both bacteria species to utilize the alkaloid extract and swaisonine as the sole carbon and energy



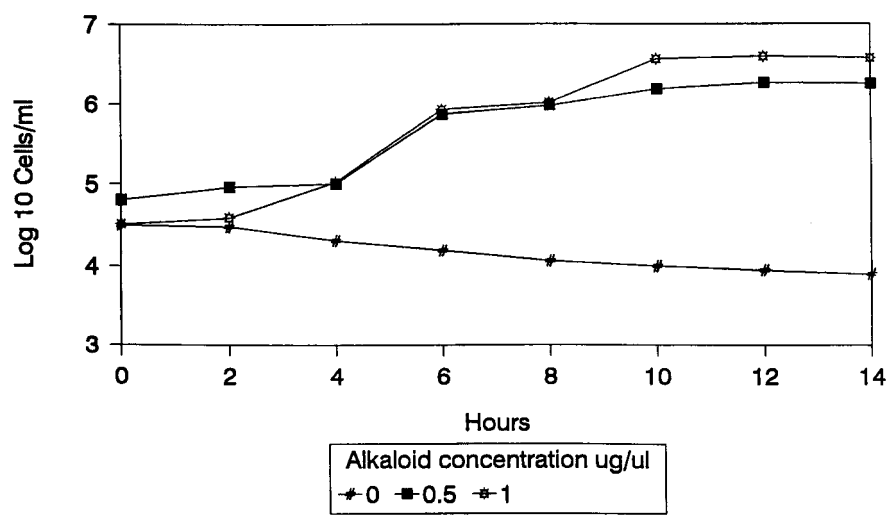


FIG. 1. Growth of *Pseudomonas* and *Klebsiella* on locoweed alkaloid concentration of 0, 0.5, and 1.0 ug/ul at 20°C.

source suggests the presence of an enzyme system capable of hydrolyzing the alkaloids. Other anthrophorid flies, *Tetranops myopaeformis* (von Roder) (Iverson et al. 1984) and *Delia* (*Hylemya*) *antigua* Meigan (Ikeshoji et al. 1980), were reported to utilize symbiotic bacteria in dealing with potentially toxic host plant compounds. Schmidt (1988) reported *Pseudomonas* sp. degrading juglone in the soil beneath black walnut trees.

I am continuing research on this bacteria-alkaloid system to identify the breakdown products of the woolly locoweed alkaloid. It may be possible to protect livestock from the alkaloids in locoweeds either by introducing the enzyme(s) as a dietary supplement (bolus) or by isolating the gene(s) from these symbionts and genetically engineering a candidate ruminant bacteria to produce this enzyme system.

ACKNOWLEDGMENT

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INJECTABLE MOXIDECTIN[1] FOR CATTLE: EFFECTS ON TWO SPECIES OF DUNG-BURYING BEETLES[2]

G. T. Fincher[3] and G. T. Wang[4]

ABSTRACT

Dung from steers injected subcutaneously with the parasiticide moxidectin (0.2 mg/kg body weight) was bioassayed with the dung beetles *Euoniticellus intermedius* (Reiche) and *Onthophagus gazella* (L.). There were no effects on the mean numbers of brood balls produced by either beetle species or on the emergence of adult beetles from brood balls constructed from the dung of injected cattle. The sex ratio of the progeny of both species was not affected by moxidectin.

INTRODUCTION

Moxidectin is a macrocyclic lactone endectocide which is derived from the actinomycete *Streptomyces cyaneogriseus noncyanogenus* (Scholl et al. 1992). The compound is chemically similar to ivermectin and milbemycin and is under development by American Cyanamid Company as an ecto- and endoparasiticide for use in livestock. Moxidectin is reported to be highly effective against common cattle grubs and trichostrongyle nematodes in cattle, and against an ivermectin-resistant strain of an important nematode parasite of sheep (Craig et al. 1992, Pankavich et al. 1992, Scholl et al. 1992). However, there is some concern that systemic insecticides and anthelmintics and/or their metabolites excreted in the dung of cattle might kill nontarget organisms and interfere with beneficial dung-inhabiting insects that degrade cowpats (Wall and Strong 1987). Cattle dung contaminated with these chemicals also might interfere with beneficial dung-inhabiting insects that prey on or compete with immature stages of pest flies developing in cattle dung (Fincher 1991, 1992). The purpose of this study was to determine the effects of dung from cattle injected with moxidectin on the development of two species of dung-burying beetles.

MATERIALS AND METHODS

Two Holstein steers (453.6 and 349.3 kg) were injected subcutaneously with moxidectin at the recommended dose of 0.2 mg/kg body weight. The moxidectin (1% injectable) was provided by the American Cyanamid Company, Princeton, NJ. A similar steer (442.2 kg) was used as a control animal. The cattle were housed

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[1]Mention of a trade name or product does not imply an endorsement of recommendation by USDA.

[2]Coleoptera: Scarabaeidae .

[3]USDA, Agricultural Research Service, Veterinary Entomology Research Unit, Food Animal Protection Research Laboratory, Rt. 5, Box 810, College Station, TX 77845.

[4]American Cyanamid Company, Agricultural Research Division, P.O. Box 400, Princeton, NJ 08540.

indoors in separate concrete-floored pens and fed alfalfa cubes and water. The steers, which had not been previously treated with any anthelmintics or insecticides, were placed on the diet two weeks before the injections. Dung was collected every 2 h on the scheduled days after treatment and placed in separate 40-l plastic buckets until at least a 5,000 g sample was available from each animal. Dung from each steer was collected at 0, 1, 2, 3, 7, 10, 14, 21, 28, 35, and 42 days after injection.

The species of dung beetles bioassayed were *Euoniticellus intermedius* (Reiche) and *Onthophagus gazella* (L.). Two pairs of each species were used to construct brood balls with dung from each steer. Each sample of dung was thoroughly mixed before use. Plastic pails (21.3-cm diameter x 18.5-cm deep) were filled to about 80% capacity with moist sandy loam soil that had been sieved to remove plant roots and rocks. A 400 g pat of dung from each steer was placed on the soil surface in separate pails. Two male-female pairs of either *E. intermedius* or *O. gazella* (7-14 days old) were placed in each pail, and the pails were covered with screen lids. All containers were maintained at 30-34°C, 65-80% RH and a 14:10 (L:D) photoperiod. After 1 wk, all remaining dung on the soil surface of each pail was removed and a plastic cup (5-cm diameter x 5-cm deep) was buried in the soil with the top rim of the cup even with the soil surface. Fresh dung (30-40 g) from the control steer was then placed in the cup of each pail to attract and capture the parent beetles. Water (20-30 ml) was added to the soil surface in each pail every 3-4 days to prevent soil desiccation and to encourage beetle progeny to emerge as soon as possible. Beginning 21 days after the parent beetles were first confined, fresh dung (30-40 g) from the control steer was again placed in the cup of each pail to attract and capture emerging progeny. Dung in the cups was replaced daily and the sex and numbers of captured progeny were recorded. After 5 wk, each pail was emptied and the numbers of complete brood balls constructed by the parent beetles were recorded to determine percentage emergence. Each treatment was replicated six times. Those replicates in which the two pairs of parent beetles constructed less than five brood balls were discarded.

The data were analyzed by a one-way completely randomized analysis of variance (ANOVA) program from the CoStat statistical package and means were separated with Duncan's multiple range test (CoStat Statistical Software 1986).

RESULTS AND DISCUSSION

When compared with the untreated control, there were no significant differences ($P \leq 0.05$) in percentage eclosion or in mean numbers of brood balls constructed from dung of the treated steers by either species of dung beetle (Tables 1, 2). No differences in the sex ratio were detected among *E. intermedius* progeny. Although significant differences ($P \leq 0.05$) in the sex ratio of *O. gazella* progeny were detected at 2 and 21 days posttreatment, the difference most likely was not caused by moxidectin because the ratio of males to females varied greatest in the control beetles on those two dates.

There is great variability in the number of brood balls produced by pairs of dung beetles in laboratory colonies (Blume and Aga 1975, Fincher 1991). Careful attention is given to age and apparent condition of selected parent beetles, but sometimes very few or no brood balls are constructed because of death, injury, or unexplained causes. Years of experience in rearing dung beetles has revealed that this commonly occurs when beetles are reared on dung in the laboratory. For this reason, we discarded all replicates in which the two pairs of parent beetles constructed less than five brood balls because such low production is an indication that there is something wrong with one or more of the parents. Comparisons were made between treatments using the remaining replicates. There were only two replicates rejected from the *E. intermedius* bioassay; one from the control steer and one from treated steer no. 1, both at 35 days after treatment. Six replicates in each of the three treatments were discarded in the *O. gazella* bioassay.

TABLE 1. Mean Numbers of Brood Balls Constructed by Two Pairs of *Euoniticellus intermedius* from Dung of Cattle Injected with Moxidectin, and Percentage Adult Eclosion [a].

	Untreated steer			Injected steer # 1			Injected steer # 2		
Days after treatment	Mean	Range	% Eclosion	Mean	Range	% Eclosion	Mean	Range	% Eclosion
0	21.8	14-27	70.2	24.8	17-32	74.5	25.2	19-30	75.5
1	26.7	22-29	78.1	26.8	19-34	78.9	22.5	19-27	74.8
2	32.5	27-37	84.1	31.0	16-49	75.8	31.3	21-36	85.1
3	34.0	21-41	78.9	40.8	30-54	82.0	34.2	27-42	81.5
7	27.8	24-34	79.0	29.0	24-35	78.2	25.8	23-29	76.1
10	24.2	16-29	83.4	25.0	16-30	85.3	20.2	18-23	89.3
14	29.2	24-31	79.4	28.3	22-36	70.0	24.7	17-39	73.0
21	27.2	20-35	79.1	21.7	16-29	72.3	27.0	20-32	88.2
28	19.2	16-24	74.8	17.2	12-26	81.6	15.8	11-20	83.2
35	30.6	11-40	77.1	20.2	13-27	82.0	20.7	6-28	71.8
42	37.5	34-40	76.9	31.0	18-47	75.3	30.3	22-38	75.8

[a] Values within each row under the mean and % eclosion columns are not significantly different at the $P = 0.05$ level.

TABLE 2. Mean Numbers of Brood Balls Constructed by Two Pairs of *Onthophagus gazella* from Dung of Cattle Injected with Moxidectin, and Percentage Adult Eclosion [a].

	Untreated steer			Injected steer # 1			Injected steer # 2		
Days after treatment	Mean	Range	% Eclosion	Mean	Range	% Eclosion	Mean	Range	% Eclosion
0	40.2	27-56	69.7	40.7	26-61	81.1	42.7	34-52	72.3
1	18.8	11-23	70.2	20.3	5-34	82.0	20.5	16-25	75.6
2	30.0	18-40	80.1	38.0	26-55	80.7	39.5	18-49	84.8
3	55.8	38-62	73.7	37.6	10-62	75.0	53.7	30-69	82.6
7	24.5	5-40	80.3	27.5	14-38	77.0	24.5	15-38	72.8
10	32.5	16-62	85.6	42.0	24-58	79.0	32.8	18-46	81.1
14	20.5	8-33	72.4	22.2	5-38	80.2	23.7	7-33	79.6
21	40.0	26-57	81.3	33.0	20-44	76.3	29.0	16-40	81.0
28	35.0	11-47	81.4	26.7	11-40	75.0	38.2	26-44	85.9
35	33.8	6-54	60.1	26.4	15-40	63.6	18.6	9-29	68.8
42	36.2	17-49	73.7	46.2	20-62	70.0	33.6	19-50	76.8

[a] Values within each row under the mean and % eclosion columns are not significantly different at the $P = 0.05$ level.

There were no apparent effects of moxidectin on the parent beetles since most of the adults were alive when removed from the rearing containers and they lived at least two additional weeks in holding containers before they were discarded.

Results of this study indicate that moxidectin 1% injectable (or its metabolites) had no apparent detrimental effects on two species of dung beetles that fed on dung from cattle treated with the recommended 0.2 mg/kg dosage. For comparative purposes, emergence of adult *E. intermedius* and *O. gazella* from brood balls made with dung from cattle that received the recommended dosage of injectable ivermectin was reduced for 1 and 2 weeks, respectively (Fincher 1992). There were no obvious adverse effects on beetle progeny or changes in the sex ratio when parent beetles constructed brood balls from dung of moxidectin treated cattle. Moxidectin appears to be a compound that is compatible with beneficial, dung-burying beetles when used at the recommended dosage.

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COMPARATIVE LIFE TABLE STATISTICS OF *DIAERETIELLA RAPAE* AND *APHIDIUS MATRICARIAE*[1] ON THE RUSSIAN WHEAT APHID[2]

H. C. Reed[3], D. K. Reed[4], and N. C. Elliott[4]

ABSTRACT

Cohort life tables were developed in the laboratory for two aphidiid parasitoids, *Diaeretiella rapae* McIntosh and *Aphidius matricariae* Haliday, imported into the United States from Asia on Russian wheat aphid hosts. Age-specific survivorship began to decrease at a earlier age for *D. rapae* than for *A. matricariae*. All *D. rapae* died by 27 days, whereas approximately 5% of *A. matricariae* were still alive at that age. Female *D. rapae* began to oviposit at a younger age (13 days) than *A. matricariae* (14 days). Age-specific fecundity for *D. rapae* peaked at approximately 18 eggs per female at 15 days, while age-specific fecundity of *A. matricariae* peaked at approximately 9 eggs per female 3 days later. The overall sex ratio of progeny was more strongly female biased for *D. rapae* (66.9%) than *A. matricariae* (57.2%). The net reproductive rate was 58.6 female offspring per female for *D. rapae* and 37.8 female offspring per female for *A. matricariae*. The mean age at death for *A. matricariae* and *D. rapae* was 23.8 and 19.7 days, respectively. Mean generation time for *D. rapae* was approximately 2.5 days shorter than *A. matricariae*. The intrinsic rate of increase was greater for *D. rapae* (0.263) than *A. matricariae* (0.202).

INTRODUCTION

The Russian wheat aphid, *Diuraphis noxia* (Mordvilko), was first detected in the United States in 1986. It has spread from the initial infestation in west Texas to 16 western states and three western Canadian provinces. Economic losses in the United States due to this insect have exceeded $650 million from 1986 through 1990 (RWA Task Force 1991). Reliance on insecticides for control of the Russian wheat aphid is undesirable because they are expensive and disruptive to the environment (Flickinger et al. 1991). A viable chemical control alternative would be classical biological control by the introduction and establishment of natural enemies.

Several criteria and diverse approaches exist for evaluating and selecting the best agents for controlling a pest (Waage 1990). One method considers life history characteristics as a component of population models for predator-prey interactions and their application to biological control. Demographic statistics that quantify life history attributes such as fecundity, survival, and developmental time under specific conditions are some parameters considered important in this approach. In particular, the intrinsic rate of increase, r_m, combines information on total fecundity, survival, and developmental time from birth to reproductive maturity in a single statistic (Lewontin 1965). The r_m is strongly influenced by the time from birth to first reproduction, and to a lesser extent, by

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[4] USDA, ARS, Plant Science Research Laboratory, 1301 N. Western, Stillwater, OK 74075.

survival and total reproduction (Lewontin 1965). Despite limitations, differences in the values of r_m among species and strains can be compared statistically.

Several species of aphidiid parasitoids have been obtained during foreign explorations in Asia and imported into the United States for Russian wheat aphid biological control. Two species that have occurred commonly in explorations are *Diaeretiella rapae* McIntosh and *Aphidius matricariae* Haliday. Biology and life history studies have been conducted on *D. rapae* and *A. matricariae* using several aphid hosts (Vevai 1942, Shalaby and Rabasse 1979, Hsieh and Allen 1986, Fukui and Takada 1988, Shijko 1989, Hayakawa et al. 1990). However, to our knowledge, no such studies have been conducted using *D. rapae* or *A. matricariae* recently imported into the United States for Russian wheat aphid biological control. Our objective was to compare demographic statistics of Asian *D. rapae* and *A. matricariae* using the Russian wheat aphid as host.

MATERIALS AND METHODS

Parasitoid colonies were established from mummies of *D. rapae* and *A. matricariae* obtained from the USDA, APHIS laboratory located at Mission, TX. The former species was collected in Iraq while the latter was obtained from Syria. Identification numbers assigned to these collections by the quarantine facility at Texas A&M University are T90026 and T89132 for *D. rapae* and *A. matricariae*, respectively.

Wheat plants were grown individually in caged "cone-tainers" (Ray Leach "Cone-tainer Nursery", Canby, OR) in a fritted-clay medium. Cone-tainers were irrigated from below by suspending them over pans containing water and nutrients (Peter's Water Soluble Plant Food) that permeated the growth medium. The Russian wheat aphid infestation on a plant resulted from the reproduction over a 2- or 3-day period by a few adult aphids that were initially placed on the plant. All adults and excess nymphs (>100) were removed with a brush prior to introducing the mated female parasitoid. Preliminary observations indicated that 100 nymphs were a sufficient number to allow maximum oviposition by parasitoids and avoid superparasitism over a 24-h period.

Newly emerged female parasitoids of each species were obtained from the colonies by removing individual mummies, placing them in separate vials, and monitoring emergence daily. Emerging females were placed individually in vials along with a single male and allowed to mate; copulation usually occurred within 5-10 min. After copulation, the female was introduced into the caged plant infested with the aphids. Russian wheat aphids and parasitoids were confined to individual plants with clear vented plastic tubular cages (3.5 by 50 cm). Cone-tainers were placed in a growth chamber (20±1°C) on a 16:8 (L:D) photoperiod. Parasitoids were provided with cotton disks, saturated with a water-honey mixture, placed at the top of the cone-tainer for additional moisture and nutrients.

After 24 h, parasitoids were transferred from the initial cone-tainer to one that contained a wheat plant infested with 100 Russian wheat aphid nymphs by the procedure described above. The procedure of transferring parasitoids to fresh plants was repeated at 24-h intervals until the parasitoid died. Subsequently, all cone-tainers were maintained in the growth chamber until adult parasitoids emerged. All mummified aphids in each cage were counted 10 days after oviposition for *D. rapae* or 11 days after oviposition for *A. matricariae*. The number of mummies in the cage was used as a daily estimate of age-specific fecundity for the individual parasitoid. Mummies were harvested by cutting the plant into sections and placing aphids, plant material, and mummies into a plastic petri dish. Dishes from each day of oviposition were maintained in the environmental chamber, and the number of adults emerging and their sex were recorded daily.

Life table statistics, including intrinsic rate of increase (r_m), were calculated using methods described by Birch (1948). Standard errors for r_m were calculated using a jackknifing algorithm described by Meyer et al. (1986).

RESULTS AND DISCUSSION

The first adult female *D. rapae* eclosed at 13 days; the median age at eclosion was 15 days (Fig. 1). The first *A. matricariae* emerged at 14 days with a median age of 16 days. A greater proportion of *D. rapae* died prior to adult eclosion (14.5%) than that of *A. matricariae* (5.8%). Age-specific fecundity and age-specific survival of *D. rapae* and *A. matricariae* on Russian wheat aphid hosts are illustrated in Fig. 1. All *D. rapae* died by 27 days, whereas approximately 5% of *A. matricariae* were still alive at that age. Female *D. rapae* began to oviposit at a slightly earlier age (13 days) than did *A. matricariae* which laid its first eggs at 14 days. More significantly, age-specific fecundity for *D. rapae* peaked at approximately 18 eggs per female at 15 days, while age-specific fecundity of *A. matricariae* peaked at approximately 9 eggs per female 3 days later (18 days).

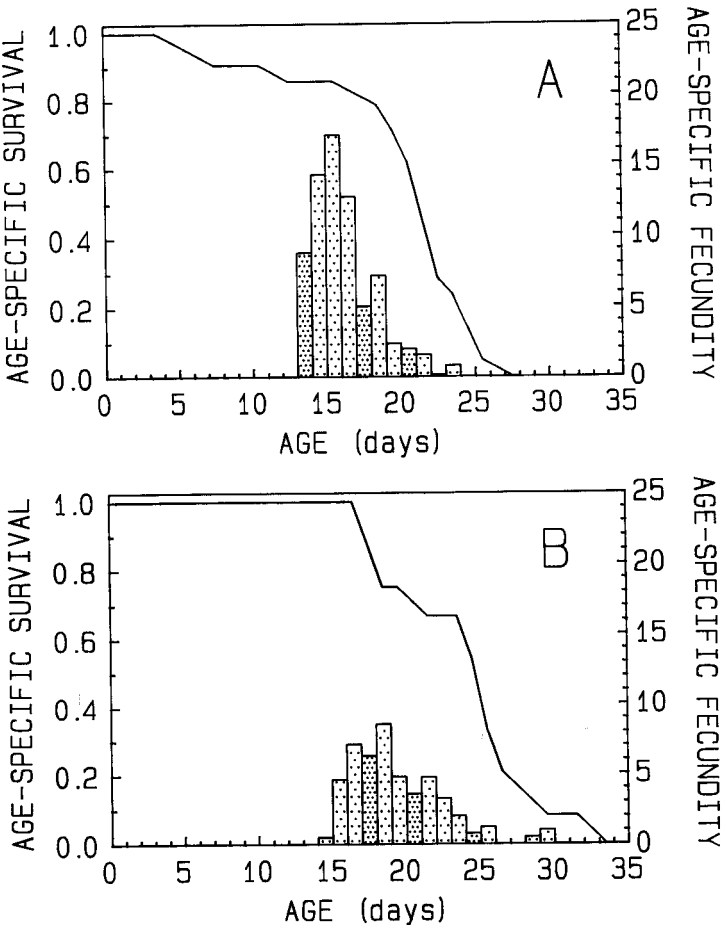

FIG. 1. Age-specific survival and fecundity of Russian wheat aphid parasitoids: A) *Diaeretiella rapae* and B) *Aphidius matricariae*.

The overall sex ratio of progeny was more strongly female biased for *D. rapae* (66.9%; n=1611) than *A. matricariae* (57.2%; n=798). The percent of female progeny among individual females of each species varied from 0 to nearly 80%. Females usually lived 1-2 days after the last day of oviposition.

The net reproductive rate of *D. rapae* (58.6 female offspring per female) was greater than that of *A. matricariae* (37.8) (Table 1). Conversely, the mean age at death for *A. matricariae* (23.8 days) was greater than for *D. rapae*. Mean generation time was approximately 2.5 days shorter for *D. rapae* than for *A. matricariae* (Table 1). Students t-test indicated that the intrinsic rate of increase for *D. rapae* was significantly greater (t=2.83; P<0.01) than that for *A. matricariae* (Table 1).

TABLE 1. Life Table and Related Statistics for *Diaeretiella rapae* and *Aphidius matricariae* on *Diuraphis noxia*[a].

Statistic	*D. rapae*	*A. matricariae*
Number of individuals in cohort	21	12
Mean number of offspring per female	59.4	38.9
Mean age at death	19.7 (1.3)	23.8 (1.3)
Mean generation time	15.5	18.0
Net reproductive rate	58.6	37.8
Intrinsic rate of increase (r_m)	0.263 (0.0145)	0.202 (0.0113)

[a]Numbers in parentheses are standard errors.

To our knowledge, this is the first life table study conducted on *D. rapae* and *A. matricariae* attacking the Russian wheat aphid. However, such studies have been conducted on these parasitoids on other aphid species. Fecundity of *D. rapae* varies widely in laboratory studies. For example, fecundity on *Myzus persicae* at 20°C was much greater than that reported here (235-289 offspring per female) (Hsieh and Allen 1986, Fukui and Takada 1988), while its fecundity on the asparagus aphid, *Brachycorynella asparagi* Mordvilko, at 20°C was lower (17-25 offspring per female) than we observed (Hayakawa et al. 1990). In most of these experiments, like the present study, *D. rapae* females were exposed to at least 100 nymphal hosts. It appears that *D. rapae* has a lower reproductive potential on Russian wheat aphid, although differences among geographic races of the parasitoid or unidentified differences in the experimental conditions might also account for the variation in fecundity among studies. The fecundity of *A. matricariae* was also lower on Russian wheat aphid than that reported for other aphids. Shalaby and Rabasse (1979) documented a mean fecundity of 308 mummies per *A. matricariae* female attacking *Myzus persicae*, while Vevai (1942) recorded only a mean of 86 mummies per female on *Myzus persicae*.

The r_m of *D. rapae* has not been reported previously; however, Shijko (1989) reported a r_m value of 0.155 for *A. matricariae* on greenbug at 20°C. Although estimates of r_m are not always comparable among studies because they are sensitive to differences in experimental conditions (e.g., temperature), it may be enlightening to compare our estimates for *D. rapae* and *A. matricariae* with those reported for other aphidiid parasitoids. Our estimates of r_m, for example, are at the low end of those reported for other aphidiid species which range from 0.15 to 0.48 females per day (Botto et al. 1988, Shijko 1989, Hagvar and Hofsvang 1990, Tripathi and Singh 1990).

Our results indicate that *D. rapae* has a greater rate of population growth on Russian wheat aphid hosts than does *A. matricariae* under the specified experimental conditions. The r_m is strongly influenced by time from birth to first reproduction and is affected by the net reproductive rate to a much lesser extent (Lewontin 1965). The more rapid immature development and correspondingly

shorter generation time are probably responsible for the greater population growth rate potential of *D. rapae* on Russian wheat aphid compared with *A. matricariae*. However, this study examines only two strains of the several races of these species introduced for Russian wheat aphid control. Also, our laboratory experiments do not reflect the multitude of factors encountered by parasitoids in the field, and only results of field releases and field research on the population ecology of the parasitoids can unambiguously demonstrate their utility as biological control agents.

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EFFECTS OF SPOT TREATMENTS OF LOGIC® (FENOXYCARB) ON POLYGYNOUS RED IMPORTED FIRE ANTS: AN INDICATION OF RESOURCE SHARING?

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ABSTRACT

Spot applications of Logic® Fire Ant Bait (fenoxycarb) to the top of mounds of the red imported fire ant, *Solenopsis invicta* Buren, were found to affect developing brood in nearby mounds. Four to five weeks following treatment, mounds up to 7.0 m from the treated site were found to contain reproductive brood only. These findings have implications in the design of fire ant research plots and management approaches using slow-acting fire ant control products.

INTRODUCTION

Food exchange between adjacent nests of the red imported fire ant, *Solenopsis invicta* Buren, was elucidated by Summerlin et al. (1975) using dye-impregnated soybean oil. Bhatkar and Vinson (1987a, 1987b, 1989) demonstrated foraging pattern differences between monogyne and polygyne red imported fire ants by marking foraging ants with non-toxic paint. Results indicated that worker ants moved freely between mounds of the polygynous form but not the monogynous form (Bhatkar and Vinson 1987a).

Logic® Fire Ant Bait contains the 1.0% active ingredient, fenoxycarb, which acts as an insect growth regulator. Ingestion of fenoxycarb by the brood redirects larval development toward winged reproductive castes only (Banks et al. 1978). Queen ant ovaries are also affected (Glancey 1987), and egg production is severely reduced or eliminated. In the absence of worker brood production, colonies treated with Logic slowly decline as worker ants are not replaced. This process can take up to several months. During this period, affected colonies can be easily detected by presence of reproductive brood (large larvae and pupae with wing pads) and the absence of worker brood.

MATERIALS AND METHODS

Test plots were established in native pasture at the edge of an abandoned pecan orchard in Burleson County, Texas. Logic Fire Ant Bait (fenoxycarb) was applied to the tops of individual fire ant mounds and placed randomly between mounds along a transect line at a rate of 20.78 g per spot on 21 September 1990. Treatment spots were

separated by a minimum of 21.33 m. Treatments were replicated six times and marked with plot flags. An additional set of six spots were marked to serve as untreated checks. These plots were randomly selected from an area adjacent to the treatment plots but at least 45.7 m away from any flag. The entire area appeared uniform in terrain, soil type, and vegetation. Adequate moisture was present throughout the test period with temperatures varying greatly, but staying well above 21.1°C. In 1991, a similar trial was conducted in a city park (Raintree subdivision, College Station) in Brazos County, Texas. Logic was applied directly to the tops of six marked fire ant mounds located 29 to 34 meters apart on 18 July.

Four to five weeks following treatment (12 September 1990 and 16 August 1991), all active mounds within a 9.14 m radius were inspected for the presence of worker and reproductive brood. In 1990, all mounds within plots were mapped. In the 1991 Brazos County trial, an additional 30 mounds apart from the treatment area were inspected for the presence of worker and reproductive brood as a control. The total number of mounds with fire ant activity and mounds affected by the Logic treatment, as indicated by the presence of reproductive brood and lack of worker brood, was determined within 0-1.50, 1.50-3.05, 3.05-4.57, 4.57-6.09, 6.09-7.62 and 7.62-9.14 m radii, respectively, from the location of the spot application for each treatment. These values were converted to percent affected mounds and correlated to the mean distance (0.75, 2.25, 3.81, 5.3, 6.85 and 8.38 m) from treatment location.

RESULTS AND DISCUSSION

In the Burleson County trial in 1990, the average mound density was 24.8 mounds per 9.1 m radius plot or 154.3 per hectare, indicating the prevalence of the polygynous form of the the red imported fire ant (Greenberg et al. 1985). Significant negative correlations ($P \leq 0.01$) were found between the percentage of Logic-affected mounds and the mean distance interval (0, 0.8, 2.3, 3.8, 5.3, 6.9 and 8.4 m) from the spot application (Table 1). Linear regression equations [Y, percent affected mounds = (Y intercept) + (slope) X, distance from treatment spot] were:

Central mound treatment:	Y = 83.1 + -11.8X	(r = -0.8990)
Between mound treatment:	Y = 104.4 + -15.0X	(r = -0.8608)

Relationships between treatment location and affected mounds were very similar, regardless of placement of bait on mounds or randomly within an infested area (Fig. 1). A calculated range for the distance from the spot application to a point at which 50% of the mounds were affected by the treatment was 2.8 m and 3.6 m for bait placed on a mound versus randomly placed application, respectively. The maximum distance from treatment spot to affected mound was 7.0 m.

Results from the 1990 trial in Brazos County were similar, but less dramatic. Average mound density was lower, with an average of 12.7 mounds per 9.1 m plot or 103.5 per hectare. A significant negative correlations ($P \leq 0.01$) was found between the percentage of Logic-affected mounds and the distance from the spot application (Table 1), with the equation:

Treated central mound:	Y = 56.0 + -9.5X	(r = -0.9016)

A calculated range for the distance from the spot application to a location at which 50% of the mounds were affected by the treatment was 0.63 m. The maximum distance from treatment spot to affected mound was between 3.1-6.1 m. A random check of 30

mounds outside the treatment area yielded four (13%) with no worker brood. The remainder contained worker brood, but none contained reproductive brood only.

TABLE 1. Number of Red Imported Fire Ant Mounds Affected by Logic® Fire Ant Bait (fenoxycarb) Spot Treatment Four to Five Weeks Following Application (21 September 1991, Burleson County and 16 August 1991, Brazos County, Texas).

	No. of mounds with reproductive brood only/total mounds (percent in parentheses)			
	1990			1991
Radius(m)	Central Mound treatment	Between Mound treatment	Untreated	Treated
1.00-1.50	5/6 (83)	1/1 (100)	0/11 (0)	2/4 (50)
1.50-3.05	10/14 (71)	6/6 (100)	0/17 (0)	3/11 (27)
3.05-4.57	4/26 (15)	3/24 (12.5)	0/41 (0)	6/19 (32)
4.57-6.09	2/45 (4)	0/18 (0.0)	0/36 (0)	0/18 (0)
6.09-7.62	1/38 (3)	1/23 (4.3)	0/34 (0)	0/17 (0)
7.62-9.14	0/44 (0)	0/31 (0.0)	0/31 (0)	0/7 (0)
r	-0.90[a]	-0.87[a]	---	-0.90[b]

[a] Indicates significant correlation between distance from treated mound (0.75, 2.25, 3.81, 5.3, 6.85 and 8.83 m) and percentage of mounds with reproductive brood, only (df = 11, Corr. Coef. = 0.708, P = 0.01).

[b] As above, but for 0.75, 2.25, 3.81 and 5.3 m from treated mounds, only (df = 7, Corr. Coef. = 0.798, P = 0.01).

Clearly, more than a single fire ant mound was affected by spot applications of Logic Fire Ant Bait. In areas infested by the polygyne form, these mounds may represent a single colony comprised of numerous mounds. Results of these trials cannot, however, be used to conclusively demonstrate that food exchange occurred between individuals from adjacent mounds. Conceivably, foraging workers from adjacent mounds or colonies shared a single resource (a spot application of bait) over time, although fire ant baits rapidly decompose in the environment. Regardless, results support the findings of previous studies (Summerlin et al. 1975; Bhatkar and Vinson, 1987a, 1987b, 1989).

Several aspects of these results may have implications to research methodology and fire ant management. The distance between bait treatment location and affected mounds reported here appears to differ with varying densities of fire ant mounds within the test site, being greater where densities are higher. Conceivably, different treatment rates would also cause results to vary. However, when designing studies to compare the effects of individual fire ant mound or broadcast bait treatments, researchers must be aware of the possibility of the exchange and/or sharing of the toxicant-laden bait

between neighboring mounds or colonies. These data suggest that a buffer zone of at least 9.1 m between plots may be necessary to eliminate the possibility of a treatment affecting an adjacent plot.

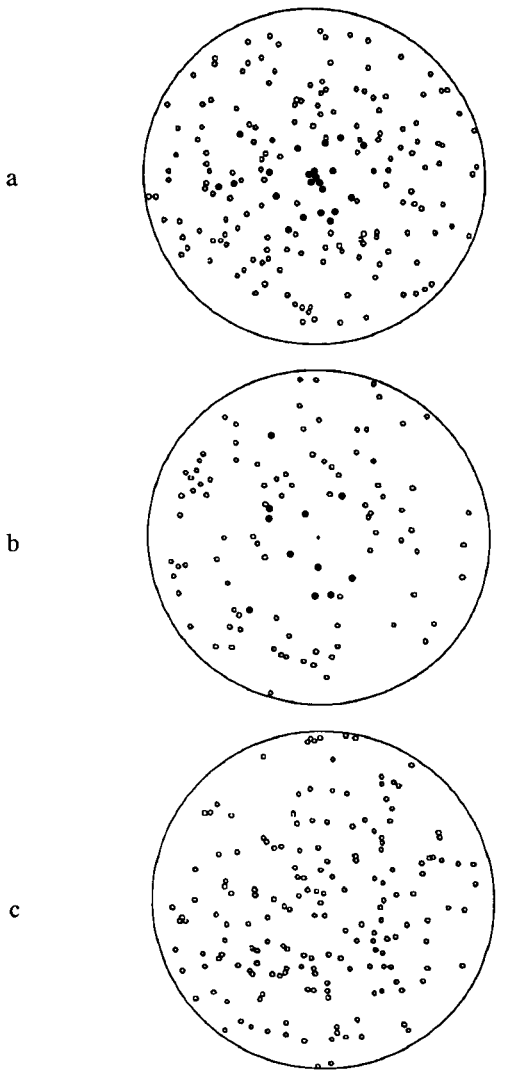

FIG. 1. Composite plot maps from six replicates of three Logic® Fire Ant Bait treatments: a) spot applications directly onto mounds, b) spot applications between mounds (marked with +), and c) untreated. Open circles represent active unaffected red imported fire ant mounds while black dots indicate affected mounds containing reproductive brood, only. Circles are 18.28 m in diameter and top of figure is oriented north (Burleson County, Texas, 1991).

In fire ant management, food exchange and/or resource sharing of a toxicant bait can be beneficial in a number of ways. In small restricted areas, such as home lawns, immigration of colonies can be a problem. Knowledge that a bait application can affect mounds in untreated neighboring areas up to 7.0 m from the edge of the treated area may provide additional justification for the urban use of a slow-acting insect growth regulator such as Logic. Furthermore, documented resource sharing between mounds can be helpful in developing effective application patterns for broadcast treatments and form a basis for the use of bait stations for fire ant management. Finally, resource sharing among ants in polygyne colonies may make them more likely to transmit natural enemy agents, particularly pathogens, from mound to mound and perhaps between colonies.

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PERFORMANCE OF LABORATORY STRAINS OF HELIOTHIS VIRESCENS (F.) (Lepidoptera:Noctuidae) IN FEEDING TESTS AS AFFECTED BY OUTCROSSING TO THE WILD

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ABSTRACT

Two populations of the tobacco budworm, *Heliothis virescens* (F.), colonized in the laboratory for three and 48 generations, respectively, were fed artificial diet treated with gossypol acetic acid (0.0, 0.025, 0.05, and 0.2 % of diet) in 1984. In 1990, three populations, varying in the number of generations in the laboratory since outcrossing to the wild, were fed diet treated with gossypol acetic acid (0.0, 0.025, 0.05, and 0.1% of diet). Three strains were fed on standard artificial diet and cotton leaves in 1991. In 1984, larvae from the population that had been reared in the laboratory the longest were larger and more tolerant of gossypol; larval growth on gossypol treated diets was similar for each strain in 1990. The strain in culture the longest without infusion of wild genes contained individuals that were larger and reached pupation faster than individuals from the other two populations. In 1991, survival and growth of larvae from the strain in the laboratory the longest without infusion of wild genes was greater on artificial diet; however, the survival on cotton leaves was below that of the most recently outcrossed strain.

INTRODUCTION

Gossypol is an important allelochemical in the resistance of cotton plants to the tobacco budworm, *Heliothis virescens* (F.) (Lukefahr and Martin 1966, Stipanovic et al. 1977). Many studies have been conducted using laboratory-reared tobacco budworm larvae to assess the effectiveness of cotton allelochemicals (Chan et al. 1978; Hedin et al. 1981, 1983; Parrott et al. 1983, Shaver and Parrott 1970).

An important consideration when continuously rearing insects in the laboratory involves effects of artificial selection and inbreeding on various aspects of insect biology. Selection and inbreeding could result in a laboratory population considerably different from the wild population. Guthrie and Carter (1972), for example, showed that larvae of the European corn borer, *Ostrinia nubililis* (Hubner), reared continuously on artificial diet lost their ability to survive on a susceptible inbred line of dent corn; however, survival comparable to that of wild borers could be recovered by one backcross to the wild parent. The introduction of wild insects to a laboratory environment generally results in low survival. Boller (1972) suggested that the lack of success in the early stages of laboratory rearing is due to intense

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selection in F_1 and following generations for individuals that are best adapted for laboratory rearing conditions. Adaptation to the laboratory may involve selection for individuals physiologically compatible with an artificial diet or for individuals that accept artificial situations for mating and oviposition.

A common result of this selection is a reduction in genetic variability of laboratory populations. Raulston (1975) showed that a higher percentage of females of a laboratory-adapted strain of *H. virescens* mated, and that they mated more frequently than those of a wild strain. However, with each successive generation in the laboratory, the response of the wild strain came closer to that of the laboratory strain.

Our overall research objective is the development of cotton germ plasms resistant to tobacco budworm. We annually screen several hundred cotton germ plasms using artificial infestation (Jenkins et al. 1982); this requiring mass rearing insects as similar as possible to their wild counterparts. We therefore cross our laboratory females to wild males at the end of each growing season. Offspring from these crosses are placed in diapause until the following growing season, at which time we begin mass rearing for germ plasm screening.

The objective of this study was to compare gossypol tolerance levels of *H. virescens* populations reared in the laboratory for short and long periods of time.

MATERIALS AND METHODS

Strains. In 1984, a test of the growth of tobacco budworm larvae from two laboratory colonies on artificial diet treated with four concentrations of gosspyol was conducted. One population of tobacco budworms had been reared on artificial diet (Bio-Serv 742-A) for three generations at the Entomology Department, Mississippi State University, and will be referred to as MSU. Another population of tobacco budworms had been reared on artificial diet (Bio-Serv F9915) since 1982 at the Southern Field Crop Insect Management Laboratory, USDA-ARS, Stoneville, MS, and will be referred to as SIM. Wild genes were infused annually into SIM. At the time of testing, there had been eight generations since SIM was outcrossed to wild males. Both colonies were started with insects collected near Stoneville.

Strains used in feeding tests in 1990 and 1991 were derived from CS87, a strain received from the Southern Insect Management Laboratory in 1987. This strain had not been outcrossed since 1987. In September 1988, female moths from CS87 were outcrossed to wild males collected in pheromone traps on the Mississippi State Plant Science Farm at Starkville, MS. The F_1's were reared as neonate larvae at 21°C with a 10:14 light:dark photoperiod to induce diapause (Mulrooney et al. 1989). Diapausing pupae were washed in 10% sodium hypochlorite solution and placed in 32 cell plastic trays and sealed (Davis et al. 1990). They were stored at 13°C until March 1989 and then placed at 27°C with a 14:10 light:dark photoperiod until diapause. Progeny of moths emerging from diapause in March 1989 were reared continuously, without further outcrossing, until March 1990, a total of about 12 generations, and are designated CS89. Females from CS89 were mated to wild males from Starkville in September 1987; F_1's were placed into pupal diapause until March 1990 in the same manner as CS89 progeny. Progeny of diapaused pupae emerging in March 1990 were designated CS90 and reared continuously until March 1991. A portion of females from CS90 were mated to wild males collected at Starkville in September 1990. F_1's were placed in diapause until March 1991 at which time diapause was terminated. Progeny were designated CS91.

1984 Feeding Tests. Two strains, MSU and SIM, were used in this feeding test. Gossypol (89%), as gossypol acetic acid, was incorporated into a casein-based artificial diet (Bio-Serv 742-A) at four concentrations: 0.0, 0.025, 0.05, and 0.2% of diet. To ensure uniform dispersion in the diet, gossypol acetic acid at each concentration was dissolved in 50 ml hexane and mixed with the dry ingredients of the diet. Hexane was removed by roto-evaporation. The diet was prepared according to the manufacturer's instructions and poured into clear plastic diet cups (35 ml). One neonate larva was placed into each cup. Cups containing larvae and diet were kept within a growth chamber at 26.7°C, and a L:D 12:12 photoperiod. Larvae were weighed after 4 and 7 days and also after pupation. The design of the experiment was completely randomized with 50 larvae per treatment. Data from each weighing date were analyzed by ANOVA using PROC GLM, and means were compared by the least significant difference (LSD) ($P \leq 0.05$) (SAS Institute 1985).

1990 Feeding Tests. Three strains, CS87, CS89,and CS90, were tested. Gossypol (95%), as gossypol acetic acid, was incorporated into artificial diet (Bio-Serv F9915) at 0, 0.025, 0.05, and 0.1 percent of diet in the same manner as in 1984. Bio-Serv F9915 artificial diet is a soyflour based diet made to the recipe of King and Hartley (1985); whereas, Bio-Serv 742-A is a casein based diet. About 7 ml of diet was poured into each cell (2.22 cm^3) of the insect rearing trays (Dixon Paper Company, Lubbock, TX). Trays were cut into eight-celled sections. One neonate larva was placed into each cell. Trays were sealed with lidding material (Product #27HT12PET1, Oliver Products Company, Grand Rapids, MI) and were kept at 26.7°C under constant light. Larvae were weighed in groups of eight at 4, 7, and 10 days and also after pupation. Individuals nearing the end of the larval stage were checked daily and the time of pupation recorded. These experiments were replicated ten times with eight larvae/replication, and data from each weighing date were analyzed by ANOVA using PROC GLM. Separation of means was by least significant difference (LSD) ($P \leq 0.05$) (SAS Institute 1985).

1991 Feeding Tests. The three strains used in this test were CS87, CS90, and CS91. Larvae were fed either artificial diet (Bio-Serv F9915) or cotton ('DES119') leaf disks punched from recently unfurled leaves on plants grown in the greenhouse. Greenhouse pests such as whiteflies were controlled with nicotine sulfate because of its low residual activity. Diet was prepared and poured into eight-celled insect rearing trays as in 1990. Leaf disks (2.54 cm dia.) were placed in eight-celled trays containing a 0.6-cm layer of 2% agar to prevent dessication of leaf tissue. One neonate larvae was placed into each cell. The trays of larvae were kept in growth chambers at 27°C in complete darkness during the test. At 4 and 7 days, survival and weight of surviving larvae in each 8 celled tray were recorded. After weighing on day 4, larvae fed artificial diet were placed in original rearing cells; whereas, larvae fed leaves were provided with a fresh leaf disk. These experiments were replicated 50 times with eight larvae/replication. Data from each weighing date (larval weight and survival) were analyzed by ANOVA using PROC GLM and the means were compared by the least significant difference (LSD) ($P \leq 0.05$) (SAS Institute 1985).

RESULTS AND DISCUSSION

1984 Feeding Tests. Results of the ANOVA's showed that the strain, gossypol, and strain X gossypol effects were significant ($P<0.05$) for each weighing date . The F values ranged from 3.3 to 255.8. Mean larval weights for each weighing date are recorded in Table 1. The highest level of gossypol (0.2%) significantly reduced the growth of insects from

TABLE 1. Mean Larval and Pupal Weights (mg) of Tobacco Budworms Fed Gossypol-Treated Diet in 1984.

	Day 4		Day 7		Pupation			
% Gossypol	MSU	SIM	MSU	SIM	MSU	N	SIM	N
0	7.3 ±0.4c[a]	8.7 ±0.4b	105.0 ±6.3b	129.5 ±2.9b	266.5 ±6.0c	20	292.0 ±4.9b	41
0.025	4.9 ±0.4d	10.6 ±0.6a	71.3 ±5.1d	133.8 ±3.5a	273.7 ±5.5c	26	310.0 ±5.2a	48
0.05	6.8 ±0.5c	9.6 ±0.5ab	87.7 ±5.8c	131.2 ±3.6a	285.8 ±4.3bc	27	283.8 ±7.4bc	49
0.2	2.7 ±0.2e	2.8 ±0.3e	13.8 ±1.7e	18.7 ±1.8e	231.2 ±8.0d	31	265.1 ±9.2d	41
LSD (P<0.05)	1.2		11.5		20.9			

[a] Strain X gossypol means within a day followed by the same letter are not significantly different (P>0.05, LSD test).

both strains during the larval stage and at pupation. After 4 days, growth of MSU larvae feeding on 0.05% gossypol was not significantly different from that of control larvae. Growth of MSU larvae feeding on all other levels of gossypol was less than that of the control; whereas, the growth of SIM larvae feeding on the 0.025 and 0.05% levels of gossypol during the larval stage equalled or exceeded the growth of control larvae. SIM larvae exhibited an hormetic effect at day 4 when feeding on 0.025% gossypol, at day 7 when feeding on 0.025 and 0.05% gossypol, and at pupation when feeding on 0.025% gossypol. Larvae feeding on these concentrations gained more weight than the control. This effect known as hormesis was proposed by Southam and Ehrlich (1943) to describe "a stimulatory effect of subinhibitory concentrations of any toxic substance on any organism."

At pupation, there were no differences in weight between MSU insects feeding on the 0, 0.025, and 0.05% gossypol treated diets. A comparison of SIM pupal weights showed increased weight gains at the 0.025% level of gossypol but no difference between the control and the 0.05% level.

We expected gossypol tolerance to be an adaptation important for survival in the field; however, the newly introduced strain, MSU, was less tolerant of gossypol than SIM which had been in culture for 48 generations with annual infusion of wild genes. The reason for this response is not clear. Perhaps SIM insects are more adapted to the artificial environment of the laboratory than MSU insects. Larval growth, especially on diets containing lower dosages of gossypol, may have been more dependent on adaptation to artificial diet and rearing conditions than on the ability to tolerate gossypol. The artificial diet into which gossypol was incorporated was the same diet on which the MSU strain had been reared for three generations. Thus the performance of MSU larvae should not have been influenced by the artificial diet used in this feeding test. To our knowledge, the influence of preadaptation to artificial diet on results of feeding studies has not been reported in the literature.

1990 Feeding Tests. The interaction of strain X gossypol was not significant; therefore, main effect means will be discussed. There were significant differences ($P<0.0001$) in the weights of larvae from each strain averaged over gossypol levels (Table 2). F values from the ANOVA's for each weighing date ranged from 11.1 to 18.9. At each weighing date, larvae from CS90, were one generation since infusion of wild genes, were smaller than larvae from CS87 and CS89, which were 12 and 36 generations since infusion of wild genes, respectively. The weight of CS89 larvae was lower than that of CS87 after 10 days on gossypol treated diet. Pupal weights indicated that insects from CS87 were larger than those from CS90 and CS89. Gossypol did not affect the number of days required to reach pupation. However, time to reach pupation was different (main effect $F=39.9$, $P<0.0001$) for each strain. CS87 reached pupation in the shortest time, 15.4 $\pm$ 0.08 days, while CS89 larvae required 15.7 $\pm$ 0.08 days, and CS90, 16.5 $\pm$ 0.11 days. Variation in the time to reach pupation was greatest in CS90 with a standard deviation of 1.7 days. The two strains that had not been recently crossed to the wild, CS89 and CS87, had less variation in time to pupation with standard deviations of 1.23 and 1.34 days, respectively. The uniformity of time to pupation may be an indication of genetic homogeneity brought on by inbreeding that occurs during laboratory colonization.

The major differences between the laboratory strains tested in 1990 are differences in size and the time required to reach pupation. The strain in culture the longest, CS87, contained insects that were larger and reached pupation in a shorter and more uniform time. The uniform

TABLE 2. Mean Larval and Pupal Weights (mg) of Tobacco Budworms from Laboratory Strains Fed Gossypol Treated Diets in 1990.

Strain	Day 4	Day 7	Day 10	Pupation
CS90	4.8 (±0.2)b[a]	51.2 (±3.6)b	249.9 (±13.8)c	294.1 (±5.7)b
CS89	7.7 (±0.5)a	79.3 (±5.7)a	310.8 (±13.1)b	302.3 (±2.4)b
CS87	7.2 (±0.5)a	86.6 (±4.7)a	359.4 (±11.8)a	325.0 (±3.7)a
LSD	1.2	12.1	35.7	11.7

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

TABLE 3. Percent Survival and Larval Weight (mg) of Tobacco Budworms from Different Laboratory Strains Fed Artificial Diet and Cotton Leaves in 1991.

	Artificial Diet		Leaves	
	% Survival[a]	Larval Weight	% Survival	Larval Weight
Strain		Days 0-4		
CS91	85.0 ±3.0b	2.05 ±0.06c	80.0 ±3.5a	0.32 ±0.03a
CS90	92.0 ±2.0a	4.46 ±0.20a	73.0 ±3.3a	0.33 ±0.03a
CS87	93.5 ±2.0a	4.05 ±0.11b	35.0 ±3.8b	0.44 ±0.06a
LSD	6.7	0.38	9.9	0.12
		Days 4-7		
CS91	95.3 ±1.9a	24.42 ±0.98b	69.9 ±4.6a	1.60 ±0.16a
CS90	93.1 ±1.8a	35.41 ±1.19a	71.6 ±4.0a	1.88 ±0.23a
CS87	93.1 ±1.6a	34.52 ±2.83a	77.9 ±5.7a	1.91 ±0.43a
LSD	5.0	5.25	13.6	0.83

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

environment and optimum diet of the laboratory has apparently resulted in the selection of insects that are larger and faster growing.

1991 Feeding Tests. Results obtained in 1984 and 1990 seemed to indicate that larval growth, especially on diets containing lower dosages of gossypol, may have been more dependent on adaptation to artificial diet and rearing conditions than on the ability to tolerate gossypol. In 1991, larvae were fed standard artificial diet and cotton leaves to contrast larval growth on diets indicative of the laboratory and the field.

At 4 days, the survival of CS91 on artificial diet was only 85%; whereas, survival of CS90 and CS87 was 92% and 94%, respectively (Table 3). However, survival of CS91 larvae on cotton leaves was 80% and only 73% and 35% for CS90 and CS89 respectively. These striking contrasts in survival between populations on artificial diet and cotton leaves are strong evidence for adaptations to artificial diet and the laboratory environment that occurred in the non-outcrossed strain, CS87. Larval weight gain after 4 days reflected this adaptation to artificial diet with CS91 gaining only half as much weight as larvae from the other two strains. There were no differences in larval weight gain between strains feeding on leaves.

After 7 days, the only difference between strains was in the amount of weight gained on artificial diet. Larvae from CS91 were significantly smaller that those from CS90 and CS87. Survival between strains during days 4 to 7 was not different.

Results indicate that recently outcrossed larvae should perform better in the screening of cotton germ plasms in the field because of their higher survival on excised cotton leaves. However, survival data collected in the field are needed to substantiate this conclusion. The genetic uniformity of inbred strains that are well adapted to artificial diet and to the laboratory environment are ideal subjects for bioassays and other research requiring little variation between individuals. Whereas an outcrossed strain should provide reliable results in mass releases, plant germplasm screenings, and studies conducted in the field; the culture of two laboratory strains, one outcrossed annually and one maintained without outcrossing, should provide insects that are well suited to most research needs. It is essential for us to continue to cross our colonized insects to wild males in order to keep the genetics of our laboratory population as close as possible to that of the wild insects. Doing so increases the reliability of our germ plasm screening technique which greatly aids in the development of resistant plant varieties.

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INSECT SPECIES INFESTING GRAIN STORED IN RURAL COMMUNITIES IN THE NORTHEAST OF SONORA, MEXICO

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ABSTRACT

The diversity, distribution and abundance of insects in stored grains in six rural townships Northeast of Sonora were determined. Thirty-three insect species (27 Coleoptera, 2 Lepidoptera, 1 Psocoptera, 1 Hemiptera, and 1 Hymenoptera) were detected. Species distribution by stored grains was as follows: 27 in corn, 22 in wheat, 16 in barley and sorghum, and 9 in pinto beans. *Rhyzopertha dominica* and *Tribolium castaneum* were the most abundant species in cereals, while *Acanthoscelides obtectus* was found only in pinto beans.

INTRODUCTION

The state of Sonora is one of the main agricultural areas in Mexico, and it also has the most extensive storage facilities for grains. Some of the grains are stored on site under primitive conditions by farmers. These grains are used for human consumption and for livestock feed. Under these conditions, grain may be exposed to deterioration by abiotic agents such as temperature and moisture as well as biotic agents such as rodents, birds, microorganisms, and insects. Insects cause considerable economical losses during storage and, in many cases, constitute the key factor for successful preservation of stored grain. A key element to any project designed to eliminate insects from stored grain is the specific identification of the infesting species. The objective of this research was to determine the diversity, distribution, and population abundance of insects associated with stored grain in the northeast of Sonora, Mexico.

MATERIALS AND METHODS

This study was conducted in the farm storage areas of the northeastern area of the state of Sonora, Mexico, in the townships of Ures, Baviácora, Aconchi, Huépac, Banamichi, and Arizpe (Fig. 1). Each locality was visited on four occasions (March, May, July, and September, 1989). The first essential step was to obtain a representative sample. Samples of stored grain were obtained following the procedures recommended by "Almacenes Nacionales de Depósito, S.A." (A.N.D.S.A. 1979) and Arias (1981). Samples were collected from the bottom, middle, and near the top using open handle type probes (3, 5, and 10 openings) for bulk grain. Samples from grain stored in sacks were taken using a bag trier from 1/2" to 7 3/8" to reach the center. In the laboratory, samples from all farms of each township were mixed and composite samples of about 1 kg were formed for each grain type, locality, and sampling period. Samples were stored in polyethylene film bags and examined within 15 days of the time of collection. Samples were sieved to separate the insects from grain. Insects were grouped by morphologicla characteristics in petri dishes and identified using the keys of Dobie et al. (1984), Freeman (1980), Gorham (1978), USDA (1979), Weidener and Rack (1984), and Rodríguez (1984). Insect species diversity was reported according to Krebs (1985).

FIG. 1. Map of the State of Sonora, showing the Zone of Study.

RESULTS AND DISCUSSIONS

A total of 33 different species representing 18 families was detected (Table 1). Twenty-seven species belong to the Order Coleoptera, two to the Order Lepidoptera and one each to the Orders Hemiptera, Psocoptera, Diptera, and Hymenoptera. Species represented 7 primary pests, 24 secondary pests, and 2 beneficial species (one predator and one parasite). Five types of stored products (maize, wheat, barley, sorghum, and pinto beans) were sampled. Twenty-seven species were associated with maize, 22 with wheat, 16 with barley and sorghum, and 9 with pinto beans (Table 2).

Rhyzopertha dominica was detected in all localities and grain types, whereas other species were found only in one or two grains. Species such as *Tribolium castaneum* and *Sitophilus zeamais* were found in almost all localities; however, *Plodia interpunctella, Lasioderma serricorne,* and *Trogoderma* spp. were found in only one or two localities. Wheat, barley, and sorghum were infested by *R. dominica* in all localities, as was maize to a lesser degree. *R. dominica* infestation was highest in Ures (about 20 insects/kg), and decreased in the North, possibly due to altitude or temperature (Table 3).

Acanthoscelides obtectus was the most abundant species in pinto beans; the grain stored in Arizpe township presented a heavy infestation of this species. Maize, mostly on the cob, generally was heavily infested by *Sitotroga cerealella. R. dominica* and *S. zeamais* were also detected, although only on maize. *S. cerealella* was present in all localities, but was very scarce in Ures. The most abundant secondary species, which were present in all grain types, were *Tribolium castaneum* and *Cryptolestes ferrugineus.* Flores (1977) and Wong et al. (1987) did not find *Lasioderma serricorne* and *Plodia interpunctella* in Sonora. Moreover, Flores (1987) reported *Sitophilus granarium* as very abundant in northeastern Mexico, but this insect was not found in Sonora either by Wong et al. (1987) or by us.

Table 1. Insect Species Infesting Grain Stored in Rural Communities in the Northeast of Sonora, Mexico.

Genus Species	Common Name	Order	Family	Product[a]	Location [b]
Rhyzopertha dominica (Fabricius)	Lesser grain borer	Coleoptera	Bostrichidae	M, S, W, B	U, BA, AC, BN, A
Sitophilus zeamais Motschulsky	Maize weevil	Coleoptera	Curculionidae	M, S, W, B	U, BA, AC, BN, A
Sitophilus oryzae (Linnaeus)	Rice weevil	Coleoptera	Curculionidae	M, B	BA, AC
Sitotroga cerealella (Olivier)	Angomois grain beetle	Lepidoptera	Gelichidae	M, S, W, B	BA, AC, H, BN, A
Plodia interpunctella (Hubner)	Indian meal moth	Lepidoptera	Pyralidae	M, W	AC
Acanthoscelides obtectus (Say)	Bean weevil	Coleoptera	Bruchidae	PB	BA, H, A
Trogoderma spp.		Coleoptera	Dermestridae	PB	A
Tribolium castaneum (Herbst)	Red flour beetle	Coleoptera	Tenebrionidae	M, S, W, B, PB	U, BA, AC, BN, A
Tribolium confusum Jaquelin duVal	Confused flour beetle	Coleoptera	Tenebrionidae	M, S, B, W	U, BA, AC, BM
Alphitophagus bifasciatus (Say)	Twobanded fungus beetle	Coleoptera	Tenebrionidae	M	BA, AC, BN
Alphitobius diaperinus (Panzer)	Lesser mealworm	Coleoptera	Tenebrionidae	M, S, B, W, PB	U, BA, AC, BN, A
Palorus subdepressus (Wollaston)	Depressed flour beetle	Coleoptera	Tenebrionidae	W, S, B	U, BA, AC
Latheticus oryzae Waterhouse	Long headed four beetle	Coleoptera	Tenebrionidae	M, W, S	U, BA, AC, BN, A
Cryptolestes ferrugineus (Stephens)	Rusty grain beetle	Coleoptera	Tenebrionidae	M, W, S, B, PB	U, BA, AC, BN, A
Cryptolestes pusillus (Schonherr)	Flat grain beetle	Coleoptera	Cucujidae	M, W, S, B	U, BA, AC, BN
Nausibius clavicornes (Kugelan)		Coleoptera	Cucujidae	M	A
Orizaephilus surinamensis (Linnaeus)	Saw-toothed grain beetle	Coleoptera	Silvanidae	M, W, S, B, PB	U, BA, AC, BN, A
Ahasverus advena (Waltl)	Foreign grain beetle	Coleoptera	Silvanidae	M, W	BA, BN, A
Carpophilus spp.	Corn sap beetle	Coleoptera	Nitidulidae	M, W	U, BA, AC, BN, A
Lasioderma serricorne (Fabricius)	Cigarrette beetle	Coleoptera	Anobiidae	M, S	AC, BN
Tiphaea stercorea (Linnaeus)	Hairy fungus beetle	Coleoptera	Mycetophagidae	M, W, PB	A, BA
Anthicus floralis (Linnaeus)		Coleoptera	Anthicidae	M, W	BN, A
Carcinops pumilo (Erichson)		Coleoptera	Histeridae	M, S, W	U, BA, BN, A
Thoractodes heydini Reitter		Coleoptera	Dermestidae	M, W, S, B	BA, AC, BN, A
Attagenus spp.		Coleoptera	Dermestidae	S, B	BN
Antrenus spp.		Coleoptera	Dermestidae	M, S	U, BN, A
Tenebriodes mauritanicus (Linnaeus)	Cadelle	Coleoptera	Trogositidae	W, S	U, BA
Xilocoris flavipes (Reuter)		Hemiptera	Anthocoridae	M, W, B	U, BA, H, AC, A
Liposcelis spp.		Psocoptera	Liposcelidae	M, W, B	U, BA, AC, BN, A
Anisapteromalus calandrae (Howard)		Hymenoptera	Pteromalidae	M, W, S	BA, AC

[a] M = Maize, W = Wheat, S = Sorghum, B = Barley, PB = Pinto Bean

[b] U = Ures, BA = Baviacora, AC = Aconchi, BN = Banamichi, H = Huepac, A = Arizpe

Table 2. Numbers of Insects Infesting Different Grains Stored in Rural Communities in the Northeast of Sonora.

Township	Maize	Pinto Beans	Barley	Wheat	Sorghum
Ures	14	4	12	16	14
Baviácora	21	6	14	20	15
Aconchi	20	4	14	20	15
Huépac	2	1	2	2	1
Banamichi	20	4	13	17	15
Arizpe	19	6	10	15	11

Table 3. Minimum, average, and maximum temperature, pluvial precipitation, clime, and altitude for each locality studied.

Township	Temperature (°C) Min.	Avg.	Max.	Pluvial Precipitation (mm)	Clime[a]	Altitude (m)
Ures	14.7	22.7	30.3	443.5	BSO(h')hw(e')	385
Baviácora	13.5	22.7	30.5	321.5	BW(h')hw(x')(e')	575
Aconchi	13.5	22.7	30.5	321.5	BW(h')hw(x')(e')	610
Huépac	13.5	22.7	30.5	321.5	BW(h')hw(x')(e')	640
Banamichi	13.3	21.4	29.3	424.0	BSOhw(x')(e')	680
Arizpe	11.4	20.1	28.9	421.5	BSOhw(x')(e')	980

[a] According to Koppen System.

Farmers in this region are not trained in the basic principles of grain storage management for insect control, nor do they have access to any technical consultant. They do not use any chemical or biological pest containment treatment. The only pest management procedures practiced are cleaning and sweeping of the storage area and whitewashing before harvest. Grain to be stored is mixed with lime and exposed to the sun to kill the infecting insects (Wong et al. 1991).

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TRICOMAS FOLIARES, CALIDAD DEL ALIMENTO Y EFICIENCIAS DE ALIMENTACION Y CRECIMIENTO DE *LOPHOCERAMICA PYRRHA*[1]

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ABSTRACT

Feeding and growth of larvae of *Lophoceramica pyrrha* (Druce) (Lepidoptera: Noctuidae) fed on diets of two types of leaves of *Wigandia urens* (Ruiz & Pavón) HBK (Hydrophyllaceae) were evaluated. Eight standard parameters of the feeding biology and mean larval weight of the last two larval instars of *L. pyrrha* were compared. Relative biomass consumption was higher for larvae fed smooth leaves than those fed bristly leaves. However, the relative nitrogen accumulation rate for larvae fed smooth leaves was as high as for larvae fed bristly leaves. Although leaf trichomes have been claimed to be an effective defensive mechanism against herbivores, leaf trichomes of *W. urens* did not affect the feeding behavior and growth of *L. pyrrha* larvae.

INTRODUCCION

El papel de los mecanismos de defensa de las plantas contra insectos fitófagos se ha documentado ampliamente; los metabolitos secundarios han influido en la evolución de sistemas digestivos (Rosenthal y Janzen 1979), y las características físicas o mecánicas de las hojas, como los tricomas y la textura, repelen la acción de los insectos fitófagos (Levin 1973, Crawley 1983). Como una respuesta selectiva a esas barreras, la eficiencia de alimentación de los animales puede ser medida por el grado de especialización. Los herbívoros generalistas y especialistas están relacionados con diferentes grupos de plantas (Scriber y Feeny 1979) en un contexto sucesional (Feeny 1976, Rhoades y Cates 1976). La eficiencia de consumo y crecimiento de insectos especialistas y generalistas se ha comparado en diferentes especies de plantas (Waldbauer 1964, Scriber y Feeny 1979). Sin embargo, los estudios que comparan la eficiencia de alimentación en insectos que se alimentan sobre una planta que presenta variación intraespecífica en sus características físicas y químicas son muy escasos. Esto ocurre a pesar de que se sabe que cualquier especie de planta presenta variación en sus propiedades nutricionales y en la concentración de metabolitos secundarios tanto espacial como temporalmente, así como de individuo a individuo y de una parte de la planta a otra del mismo individuo, lo cual puede afectar la conducta alimenticia de los herbívoros (McKey 1979, Denno y McClure 1983).

Para entender los efectos de la variación de una planta sobre las eficiencias de alimentación de los insectos herbívoros, aquí se reporta una comparación de la eficiencia de alimentación de las larvas gregarias de *Lophoceramica pyrrha* (Druce) (Lepidoptera: Noctuidae) cuando son alimentadas con dos tipos de hojas de *Wigandia urens* (Ruiz & Pavón) HBK (Hydrophyllaceae). Las plantas de esta especie presenta dos tipos de tricomas, glandulares y urticantes, y es posible distinguir dos tipos de hojas: híspidas (con ambos tipos de tricomas) y lisas (con tricomas glandulares solamente). Las hojas híspidas (HH) presentan mayor contenido de agua, nitrógeno y fósforo que las hojas lisas (HL) [contenido de agua (% en peso fresco): HH= 72.2 ± 0.6, HL= 61.9 ± 0.9, $t = 5.34$, g.l.= 15,

[1]Lepidoptera: Noctuidae

$P<0.001$; nitrógeno (% en peso seco): HH= 3.25 ± 0.15, HL= 2.63 ± 0.09, t= 9.95, g.l.= 9, $P<0.001$; fósforo (% en peso seco): HH= 0.22 ± 0.01, HL= 0.15 ± 0.01, t= 4.85, g.l.= 9, $P<0.001$; prueba de t para muestras pareadas] (Cano-Santana 1987, Cano-Santana y Oyama 1992). Así, la pregunta principal que trata de contestar este trabajo es: ¿qué tipo de hojas consumen mejor las larvas de *L. pyrrha*: hojas híspidas con tricomas urticantes pero mayor calidad nutricional u hojas lisas sin tricomas urticantes pero relativamente más pobres nutricionalmente?

MATERIALES Y METODOS

Sistema de Estudio. *Wigandia urens* es un arbusto común que coloniza las zonas perturbadas del Valle de México. Los tricomas glandulares de sus hojas son pequeños (0.7-1.0 mm) con un pedicelo de 4-5 células y una cabezuela multicelular que secreta una sustancia pegajosa. Los tricomas urticantes, por su parte, son más largos (3.0-6.0 mm), duros y puntiagudos, y secretan una sustancia tóxica e irritante. Esta planta contiene en sus hojas flavonoides (5,4'-dihidroxi-7-metoxiflavona y 5,4'-dihidroxi-6,7-dimetoxiflavona) y terpenoides (farnesol-quinona y wigandol) (Gómez et al. 1980). Las hojas híspidas son más abundantes en la temporada seca (de noviembre a mayo), en tanto que las hojas lisas lo son en la temporada de lluvias (de junio a octubre) (Cano-Santana 1987), sin embargo en cualquier estación un individuo de *W. urens* puede presentar tanto hojas lisas como híspidas simultáneamente (Cano-Santana 1987).

Lophoceramica pyrrha es un noctuido gregario que se alimenta casi exclusivamente de *W. urens* en el campo. Sus larvas se encontraron una sola vez sobre *Buddleia cordata* HBK (Loganiaceae) y en tres plantas de *Dahlia coccinea* Cav. (Compositae), en observaciones realizadas de 1985 a 1991 (Z. Cano-Santana, obs. pers.) en la Reserva del Pedregal de San Angel, ubicada al SW de la Ciudad de México. Las larvas se alimentan en ambos tipos de hojas en el campo, aunque éstas están presentes solamente de julio a diciembre (Cano-Santana 1987), cuando las hojas híspidas son más escasas. *L. pyrrha* presenta cinco estadios larvarios (Cano-Santana 1987).

Experimentos de Alimentación Larvaria. Las larvas de *L. pyrrha* fueron colectadas en la Reserva del Pedregal de San Angel en el campus de la Universidad Nacional Autónoma de México. Treinta larvas del primer estadio se transfirieron individualmente a cajas de plástico (10 cm de diámetro, 12 cm de altura) y fueron arregladas aleatoriamente en un invernadero. Las larvas fueron divididas en dos grupos de 15 individuos. Un grupo fué alimentado con hojas híspidas y el otro con hojas lisas. Hojas de edad intermedia recién colectadas fueron cambiadas cada dos días evitando la desecación de las hojas. Las hojas híspidas seleccionadas fueron aquellas que presentaron una densidad de tricomas mayor a 15 por cm^2, las hojas lisas eran consideradas como tal cuando carecían totalmente de tricomas urticantes. A cada caja se le colocaba una pieza de algodón humedecido para mantener la humedad relativa en el interior. La temperatura media era de 20° C (día) y 15° C (noche), con un fotoperíodo de 13/11 (día/noche), y aproximadamente 70% de humedad relativa en el interior del invernadero.

Larvas, heces y restos foliares fueron pesados cada 48 h. El alimento no consumido y las heces se secaban en un horno a 45° C durante 48 h. El peso seco del alimento consumido por cada larva por día se calculadó sustrayendo el peso seco de la hoja no consumida del peso seco estimado de la hoja al principio del intervalo de alimentación. Este peso inicial fué estimado de hojas control similares a las utilizadas como alimento de las larvas, pero sin herbívoros (Waldbauer 1968). El crecimiento de las larvas en términos de peso seco fué determinado de la ganancia medida en peso fresco y del contenido de agua larvario, el cual fué estimado para cada estadio en cada experimento secando larvas control adicionales. Debido a que las larvas pierden peso antes de una muda o de la pupación, se calculó el crecimiento larvario en el pico del peso alcanzado en cada estadio.

Nitrógeno. El nitrógeno total de hojas y heces fué medido por duplicado en un Autoanalizador Tecnhicon II (TIS 1977a, b) por la técnica de Kjeldahl (McKenzie y Wallace 1954).

Indices Nutricionales. De nuestros datos calculamos los siguientes parámetros de crecimiento larvario y eficiencia de alimentación (Waldbauer 1968, Slansky y Feeny 1977) de los últimos dos estadios, todos basados sobre pesos secos:

TRCr: Tasa Relativa de Crecimiento = mg de incremento de biomasa por g de masa larvaria media por día.

TRCo: Tasa Relativa de Consumo = mg de alimento ingerido por g de peso larvario medio por día.

DA: Digestibilidad Aproximada o Eficiencia de Asimilación = (mg de alimento ingerido - mg de heces)(100)/mg de alimento ingerido.
ECI: Eficiencia de Conversión de Alimento Ingerido = (mg de biomasa obtenida)(100)/mg de alimento ingerido.
ECD: Eficiencia de Conversión de Alimento Digerido o Eficiencia Neta de Crecimiento = (mg de biomasa obtenida) (100)/(mg de alimento ingerido - mg de heces).
TRCN: Tasa Relativa de Consumo de Nitrógeno = mg de nitrógeno ingerido por g de masa larvaria media por día.
TRAN: Tasa Relativa de Acumulación de Nitrógeno = mg de nitrógeno obtenidos por g de masa larvaria media por día.

La cantidad de nitrógeno obtenido por cada larva experimental fué determinado sustrayendo el nitrógeno total en las heces del nitrógeno ingerido por la larva. La masa larvaria media (BPROM) de cada estadio se calculadó por el método del promedio ponderado (Waldbauer 1964) y fué utilizado para calcular las tasas relativas mencionadas.

La eficiencia para convertir nitrógeno asimilado a nitrógeno corporal de la larva fué considerada de 100%. Sin embargo, una fracción desconocida de nitrógeno asimilado es excretado en la forma de ácido úrico, alantoina, ácido alantoico, y otros compuestos (ver referencias en Scriber y Feeny, 1977).

Análisis Estadístico. Los datos porcentuales fueron transformados como arcsen $\sqrt{x}$% para su tratamiento estadístico (Zar 1984). Para cada parámetro se realizó un Análisis de Varianza (ANOVA) de dos vías para probar el efecto de la dieta (tipo de hoja) y del estadio. Cuando la interacción dieta X estadio fué significativa se aplicó una prueba de Tukey ($P<0.05$), para conocer las diferencias entre celdas (Zar 1984). El efecto de la alimentación con diferente tipo de hojas sobre el contenido de nitrógeno en las heces, el peso de las larvas durante el desarrollo y la duración de cada estadio fué comparada con una prueba de t ($P<0.05$). Todas las medias se presentan junto con sus errores estándar.

RESULTADOS Y DISCUSION

El desarrollo de las larvas en ambos tratamientos se completó entre los 95 y 142 días. El crecimiento en peso de las larvas de *L. pyrrha* no difirió entre tratamientos [peso fresco (g) a los 92 días de desarrollo: hojas lisas= 328.4±34.5, hojas híspidas= 329.1±32.7]. Las larvas mostraron variación en el número de estadios que presentan en su desarrollo, el cual fué de 3 a 7 en larvas alimentadas con ambas dietas. No existió diferencia significativa entre dietas en la duración de cada estadio de desarrollo. La comparación de los parámetros de alimentación y crecimiento larvarios se realizó sobre el penúltimo y último estadios, independientemente del número de ellos que experimentó cada larva.

Las comparaciones de los índices de alimentación y crecimiento mostraron que (Tabla 1): (a) la TRCo fué significativamente mayor con una dieta de hojas lisas, tanto para el penúltimo como para el último estadio (F= 17.88, g.l.= 4,30, $P<0.001$). (b) La TRCN fué mayor con una dieta de hojas híspidas en el penúltimo estadio (q=4.59, g.l.= 4,30, $P<0.05$) pero no para el último estadio (q=1.30, g.l.= 4,30, $P>0.05$). (c) No se presentaron diferencias significativas entre dietas en BPROM, ECI, ECD, AD, TRCr, EUN y TRAN. (d) No hay diferencias significativas entre estadios en la ECI, lo cual se presenta en algunos insectos (Scriber y Slansky 1981). (e) TRCo, TRCr, TRAN, TRCN, DA, y EUN fueron significativamente mayores en el penúltimo estadio que en el último. Esta tendencia en los valores de TRCr, TRCo y DA se ha encontrado, en general, para la mayoría de insectos (Waldbauer 1968, Scriber y Feeny 1979, Scriber y Slansky 1981). (f) La ECD fué significativamente mayor en el último estadio que en el penúltimo, lo cual representa el patrón observado en los insectos (Scriber y Slansky 1981). (g) Hay un efecto significativo de la interacción dieta X estadio en la TRCN (F= 54.73, g.l.= 1,30, $P<0.01$), pero no para ninguno de los demás parámetros de consumo y crecimiento.

La concentración de nitrógeno (% en peso seco) en las heces de larvas alimentadas con hojas híspidas fué significativamente mayor que en las heces de larvas alimentadas con hojas lisas (hojas híspidas= 3.76±0.09, hojas lisas= 2.50±0.09; prueba de t para muestras independientes, t= 9.54, g.l.= 18, $P<0.001$).

TABLA 1. Indices Nutricionales y Masa Larvaria Media para el Penúltimo y Ultimo Estadios de Larvas de *L. pyrrha* alimentadas con Hojas Híspidas y Lisas de *W. urens*.

	Valores de los índices bajo los dos tratamientos[a]			
	Penúltimo estadio		Ultimo estadio	
Indice nutricional[b]	Híspidas (n=7)	Lisas (n=10)	Híspidas (n=7)	Lisas (n=10)
BPROM (mg)	27.76±2.92^{b}	27.63±1.93^{b}	51.64±2.28^{a}	51.82±2.37^{a}
TRCr (mg/g/dfa)	43.80±5.38^{a}	41.93±4.30^{a}	26.21±3.30^{b}	32.22±1.64^{b}
TRCo (mg/g/día)	727.9±20.3^{b}	803.0±26.7^{a}	421.3±20.0^{d}	555.2±19.9^{c}
DA (%)	71.4±2.7^{a}	69.5±3.7^{a}	60.1±3.9^{b}	51.2±3.9^{a}
ECI (%)	5.98±0.63^{a}	5.28±0.57^{a}	6.58±1.17^{a}	5.88±0.39^{a}
ECD (%)	8.52±1.05^{b}	8.27±1.34^{b}	10.71±1.49^{a}	11.62±0.75^{a}
TRCN(mg/g/día)	24.70±1.22^{a}	20.68±0.73^{b}	12.90±0.91^{c}	14.03±0.48^{c}
TRAN (mg/g/día)	16.40±1.47^{a}	14.62±0.78^{a}	6.35±0.49^{b}	7.27±0.32^{b}
EUN (%)	65.64±3.67^{a}	71.17±3.72^{a}	50.26±4.68^{b}	52.04±2.38^{b}

[a]Los datos son medias ± error estándar. n= tamaño de muestra. Los valores seguidos por diferente letra difieren significativamente ($P<0.05$).

[b]BPROM = masa larvaria media; TRCr= tasa relativa de crecimiento; TRCo= tasa relativa de consumo; DA = digestibilidad aproximada; ECI= eficiencia de conversión de alimento ingerido; ECD= eficiencia de conversión de alimento digerido; TRCN= tasa relativa de consumo de nitrógeno; TRAN= tasa relativa de acumulación de nitrógeno; EUN= eficiencia de utilización de nitrógeno.

El papel defensivo de los tricomas contra herbívoros se ha documentado para plantas silvestres (Gilbert 1971, Levin 1973, Johnson 1975) y cultivadas (Broersma et al. 1972, Levin 1973). Algunos insectos han desarrollado diferentes adaptaciones para evitar esas barreras (Rathcke y Poole 1975, Hulley 1988). En este trabajo encontramos que las larvas de *L. pyrrha* tienen la capacidad fisiológica para comer hojas con tricomas urticantes de *W. urens*, aunque con una baja eficiencia de alimentación. El papel defensivo de los tricomas como una barrera física no fué sustentado por este estudio. La pubescencia no siempre representa un mecanismo defensivo de las plantas. Esto está bien ilustrado en estudios de plantas de importancia agrícola (Starks y Merkle 1977, Benedict et al. 1983), aunque no en plantas silvestres.

Las diferencias en el crecimiento de las larvas alimentadas con hojas híspidas y lisas se encontraron en la TRCo y en la TRCN. Esto pudo ser debido a que las hojas híspidas de *W. urens* tienen mayor contenido de nitrógeno, fósforo y agua que las hojas lisas (Cano-Santana 1987, Cano-Santana y Oyama 1992). Es posible pensar que las larvas tratadas con hojas lisas comen más biomasa para compensar la pobreza en contenido de nutrimentos, sobre todo nitrógeno, tal como se ha registrado para *Pieris rapae* (L.) (Lepidoptera: Pieridae) (Slansky y Feeny 1977), que presenta tasas de acumulación de nitrógeno similares cuando se alimentan de hojas con diferente contenido de nitrógeno. El crecimiento de larvas de lepidópteros puede estar limitado por la disponibilidad de nitrógeno y agua en su alimento (Waldbauer 1964, Southwood 1973, Slansky y Feeny 1977), y se ha sugerido que el nitrógeno debe ser concentrado más que otros nutrimentos mayores (Southwood 1973).

En la Tabla 2 se comparan nuestros resultados de crecimiento larvario con otros reportados en la literatura (Scriber y Feeny 1979, Scriber y Slansky 1981, Slansky y Scriber 1982). Según estos datos, DA y EUN están dentro del rango registrado, aunque por encima del límite inferior. Sin embargo, TRAN, ECI, ECD y TRCr están cerca de los valores más bajos reportados. Las tasas de crecimiento relativo y las tasas relativas de acumulación de nitrógeno son ligeramente más bajas que los extremos inferiores registrados. Esos bajos valores en las eficiencias de consumo, en las tasas de crecimiento y en la tasa de acumulación de nitrógeno, sugieren que *W. urens* es un hospedero con hojas de baja calidad que provoca un crecimiento lento en las larvas de *L. pyrrha*. Los valores bajos en la ECI se han relacionado con bajas concentraciones de agua (Feeny 1975), sin embargo, no se encontraron diferencias en este parámetro entre tratamientos a pesar de que las hojas híspidas presentan mayor contenido de agua que las hojas lisas. Los valores de la ECI, que representan una medida de la eficiencia en los procesos digestivos y metabólicos (incluyendo la posibilidad de desintoxicación) (Futuyma y Wasserman 1981), son muy bajos, lo cual sugiere la existencia de muy bajas eficiencias en los procesos metabólicos de *L. pyrrha* al alimentarse de hojas de *W. urens*.

TABLA 2. Comparación de los Indices Nutricionales de *L. pyrrha* al alimentarse de *W. urens*, con los registrados en la Literatura para Lepidópteros.

	Rango de datos		
Indice nutricional[a]	*L. pyrrha*	Registrado	Referencia[b]
TRCr (mg/g/dfa)	26-42	30-1500	1,2
TRCo (mg/g/día)	421-803	270-10700	1
DA (%)	51-71	12-97	1
ECI (%)	5-7	1-78	1
ECD (%)	8-12	2-93	1
TRAN (mg/g/día)	6.4-16.4	7.2-81.7	3
EUN (%)	50.3-71.2	28.2-93.5	3

[a]Igual que en Tabla 1.
[b]1= Slansky y Scriber (1982), 2= Scriber y Slansky (1981), 3= Scriber y Feeny (1979).

Estos resultados deben interpretarse en el entendido de que son el producto de un experimento realizado con larvas criadas aisladamente, ya que la conducta de alimentación de *L. pyrrha* en el campo es gregaria. En el caso de *L. pyrrha*, es probable que haya un efecto negativo de la cría en aislamiento. El desarrollo larvario de *L. pyrrha* criada de manera aislada se cumplió en 95-142 días, mientras que en otros ensayos realizados con grupos de 25 larvas muestran un desarrollo de 48-99 días alimentándose de hojas de *W. urens* (hojas híspidas= 72.1 ± 2.3 días, hojas lisas= 88.8 ± 3.4 días; Cano-Santana 1987, Cano-Santana y Oyama, datos no publicados). Probablemente esta condición de aislamiento pudo haber estado involucrada en las variaciones observadas en el número de estadios de desarrollo en las larvas. De cualquier manera, las larvas de *L. pyrrha* criadas de manera agregada presenta altos índices de mortalidad y bajas tasas de crecimiento al alimentarse de hojas de *W. urens*, comparados con los que presenta al alimentarse de las hojas de *Buddleia cordata*, su hospedero "alternativo" (Cano-Santana 1987, Cano-Santana y Oyama, datos no publicados). Lo anterior reafirma que las bajas eficiencias ecológicas observadas en *L. pyrrha* en este experimento se deben, en gran parte, a la baja calidad nutricional de las hojas de *W. urens* para este noctuido.

En conclusión, los tricomas urticantes de las hojas de *W. urens* no afectan negativamente la biología alimenticia de las larvas de *L. pyrrha*. Por el contrario, la mayor calidad nutricional de las hojas que presentan tricomas urticantes, le permiten a las larvas de esta especie alcanzar los mismos niveles de acumulación de nitrógeno consumiendo menor cantidad de follaje, que si se alimentara de hojas lisas.

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UNA NUEVA ESPECIE DEL GENERO *GEOMYLICHUS* [1] FAIN 1970, DE ISLA CERRALVO; BAJA CALIFORNIA SUR, MEXICO.

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ABSTRACT

A new species of listrophorid mite of the genus *Geomylichus* associated with *Perognathus arenarius siccus* (Rodentia: Heteromidae) in Cerralvo, Baja California Sur, Mexico, is described male and female and illustrations are given.

RESUMEN

Se describe una especie nueva de listrofórido del género *Geomylichus*, asociada a *Perognathus arenarius siccus* (Rodentia: Heteromidae) roedor endémico de la Isla Cerralvo, Baja California Sur, México. Se dan figuras del macho y la hembra.

INTRODUCCION

Los ácaros del género *Geomylichus*, se incluyen dentro de la superfamilia Listrophoroidea, representada por cinco familias, la cual agrupa ectoparásitos altamente específicos. La especie que ahora se describe, pertenece a la familia Listrophoridae, que se caracteriza por presentar estructuras quitinizadas en la región propodosomal que los ácaros utilizan para fijarse al pelo de los hospederos, así como algunas modificaciones en patas anteriores para esta misma función (Fain 1984, Hoffmann y Servín 1990). Esta familia incluye 20 géneros y alrededor de 120 especies registradas en las regiones: Holártica, Neártica y Oriental. En las regiones Australiana y Etiopica están ausentes con excepción de la especie *Leporacarus gibbus*, la cual fue introducida por el hombre en Australia junto con su hospedero el conejo doméstico (Fain 1984).

Los listrofóridos se han encontrado asociados con diversos mamíferos de los órdenes Insectivora, Lagomorpha, Rodentia y Carnívora, como simbiontes permanentes de sus hospedantes durante todo su ciclo de vida (Hoffmann y Servín 1990).

El género Geomylichus se encuentra bien representado en Norte América e incluye en total 20 especies, todas de roedores de las familias Geomyidae, Heteromyidae y Cricetidae, excepto la especie *G. sylvilagus* la cual está presente en conejos de la familia Leporidae (Fain 1981). Para México las especies registradas son: *G. dipodomius* (Radford 1953), *G. floridanus* (Radford 1949), G. *mexicanus* Fain 1976, *G. postscutatus* Fain 1976, *G. sylvilagus* Fain 1976 y *G. comitanensis* Hoffmann y Servín 1990. Esta última especie se ha colectado en la región continental de Baja California Sur frente a la Isla Cerralvo, sobre *Perognathus arenarius sublucidus* Nelson y Goldman, ratón considerado como endémico de esta región.

En el presente trabajo se presenta una nueva especie de *Geomylichus*, colectado en ratones endémicos de la Isla Cerralvo B.C.S., como parte de un proyecto que se realiza actualmente en el Centro de Investigaciones Biológicas de B.C.S.

1/ Acarida:Listrophoridae

MATERIALES Y METODOS

La Isla Cerralvo se encuentra en el Golfo de California al oeste de la ciudad de La Paz, B.C.S. a los 24° 09' y 24° 22' N; y a los 109° 47' y 109° 56' W. Esta Isla tiene una superficie total de 155 km^2 con una longitud mayor de 24 km del NNW al SSE. El sitio donde se realizó la colecta presenta una vegetación de tipo matorral costero, con dominancia de las siguientes especies: *Paullinia sonorensis*, *Cardiospermum tortuosa*, *Jatropha cinerea*, *Stenocereus gummosus* y *Ferocactus diguetii*.

Para la captura de los ratones se utilizaron trampas Sherman, las cuales permiten colectar vivos a estos mamíferos, evitando de esta manera que los ectoparásitos abandonen a sus hospedantes. Los ratones fueron trasladados vivos al laboratorio, en donde recién sacrificados se examinaron con ayuda de un microscopio estereoscópico, para colectar los ácaros ectoparásitos los cuales se colocaron en sustancias aclarantes y posteriormente fueron preparados en laminillas fijas en líquido de Hoyer, con sus datos respectivos. Los ejemplares fueron identificados, medidos e ilustrados con ayuda de un microscopio de contraste diferencial de interferencia. Las medidas obtenidas de los ejemplares están dadas en micrones.

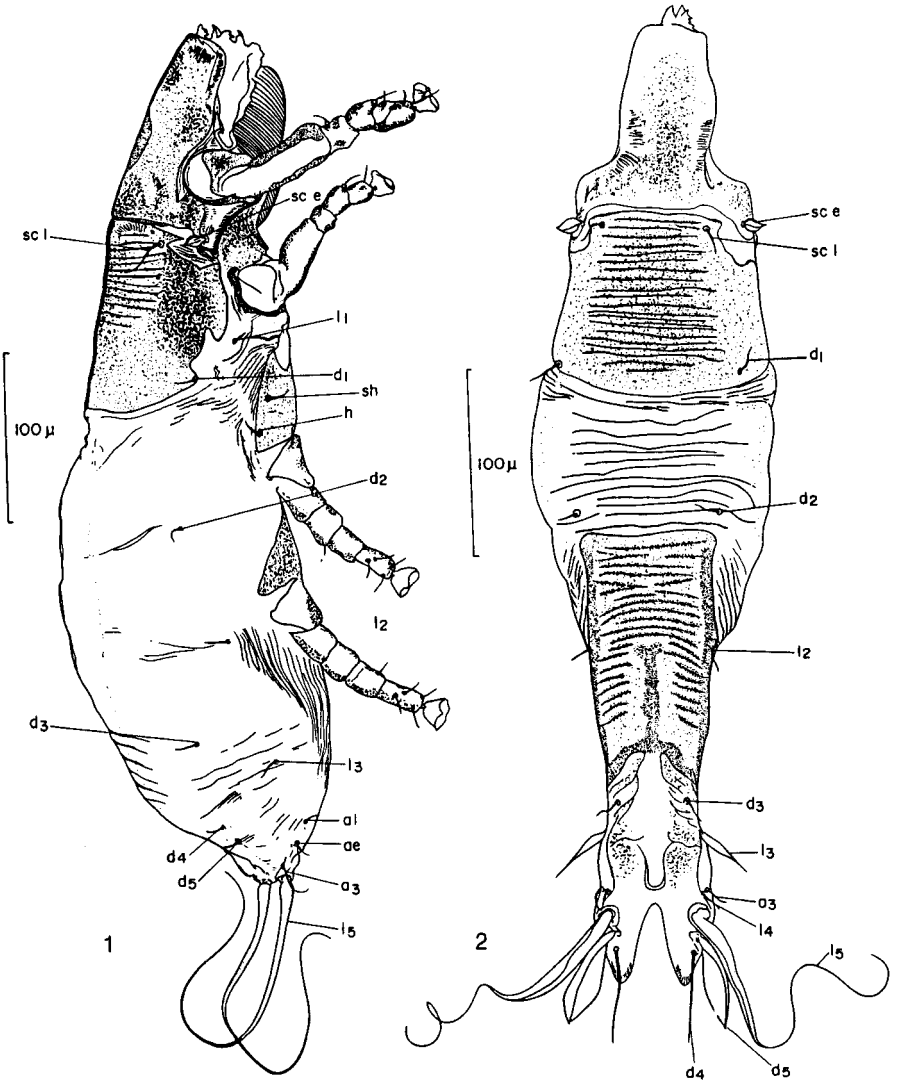
Geomylichus bassolsi sp. nov.
(Figs. 1-6)

Hembra adulta (Fig. 1): El holotipo presenta cuerpo alargado de forma ovalada, mide 502 de largo por 151 de ancho; 10 paratipos hembras miden de 473 a 524 de largo por 128 a 152 de ancho. Las placas prescapular y postescapular difieren muy poco en tamaño, midiendo a lo largo en la parte media del cuerpo 120 y 118 respectivamente, mientras que en los paratipos la longitud de la prescapular es de 113 a 131 y la postescapular de 113 a 125. Los bordes laterales de esta última placa, además de ser irregulares, presentan una amplia proyección lobular a la altura de las patas II, y alrededor de 13 estrías concentradas en la región media anterior. En ambas placas también se aprecian zonas punteadas principalmente en la región lateral de las mismas. Histerosoma sin placa histerosomal, con estrías transversales poco definidas, restringidas a la región posterodorsal. Ventralmente, en la región posterior se presentan finas estrías longitudinales, a partir de las coxas IV y entre las sedas l_2 y l_3. Las sedas del cuerpo son pequeñas y delgadas con excepción de la escapular externa, que en este caso mide 13 de largo por 7 de ancho (Fig. 5), y la l_5 que igual que en la mayoría de las especies se encuentra insertada en un cono terminal, cuya longitud es de 234. Las membranas coxales I y II miden de largo 92 y 39; y de ancho 34 y 18 respectivamente. Las patas IV tienen una longitud de 157 en el holotipo y de136 a 165 en los paratipos. Finalmente, en la parte posterior del cuerpo se observa la bolsa copuladora cuya forma se representa en la figura 4.

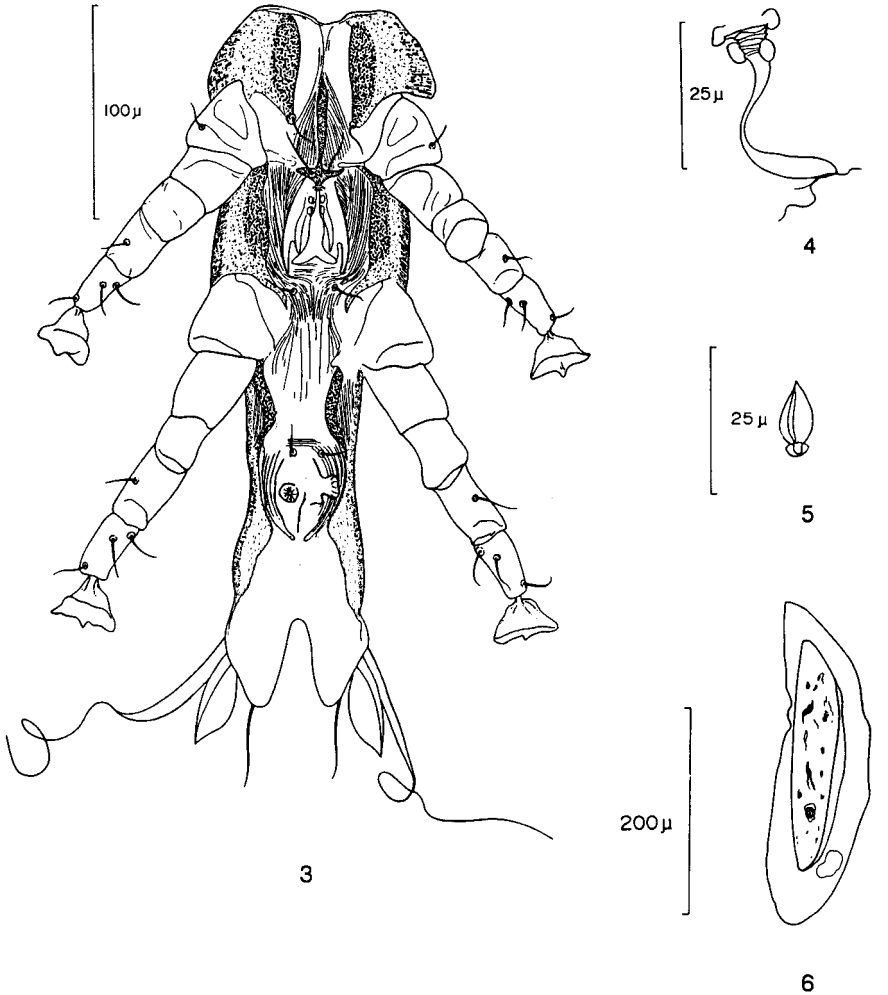
Macho (Figs. 2-3). Diez paratipos machos, son de menor tamaño que las hembras, de largo miden de 490 a 524 y de ancho 117 a 142. Las placas prescapular y postescapular al igual que en la hembra son aproximadamente del mismo tamaño; la primera mide de 97 a 109 y la segunda de 96 a 108. Ambas placas presentan zonas punteadas, pero la postescapular presenta además alrededor de 20 estrías ubicadas en la región media de ésta (Fig. 2). Posterior a esta placa se observa una zona descubierta con estrías transversales, las cuales no son más de 20. Esta área se continúa con la placa histerosomal ubicada después de las sedas d_2, la cual también presenta estrías transversales, principalmente en la región media anterior, combinadas con puntos los cuales se distribuyen de manera uniforme en toda la placa. Los bordes laterales de esta placa, son fuertemente esclerosados y termina en dos lóbulos característicos del género. Las sedas dorsales son como sigue: la escapular externa es de forma similar a la de la hembra, mide de 10 a 18 de largo por 6 a 8 de ancho (Fig. 2); la l_5 de los machos se distingue de la de las hembras, porque presenta en su parte basal una membrana que tiene aproximadamente la mitad del tamaño de esta seda. La d_5 es una amplia membrana de forma triangular, que mide de 55 a 57 de largo por 15 a 17 de ancho. Las membranas de las coxas I miden de 87 a 94 de largo por 22 a 30 de ancho, las membranas coxales II miden de 37 a 48 de largo por 11 a 21 de ancho. Ventralmente se observa un par de sedas en el esclerito delante del pene, otro par detrás de la región genital y otro par de pequeñas sedas, las a_1 delante de los acetábulos anales (Fig. 3).

Huevo (Fig. 6). Al igual que en otras especies, el huevo es alargado y alcanza a medir 307, lo cual casi corresponde al tamaño del histerosoma de la hembra. Estos huevos fueron colectados en la misma región del ratón, en donde se colectaron los ácaros adultos.

Afinidades. Siguiendo la clave de Fain y Whitaker (1987), *G. bassolsi* presenta afinidad con *G. brevispinosus*, coincidiendo con los bordes irregulares de la placa postescapular, con la posición de las estrías y con el tamaño de las sedas l_5, pero se diferencia en la forma y tamaño de la seda escapular externa, presentan una longitud similar las placas prescapular y postescapular y las membranas coxales II son en este caso de tamaño mayor. Por otro lado los hospedantes son diferentes, situación que debe tomarse en cuenta, dada la alta especificidad de estos ácaros.



FIGS. 1-2 *Geomylichus bassolsi, sp. nov.* 1 Hembra adulta, *habitus lateral.* 2 Macho adulto, habitus dorsal.



FIGS. 3-6. *Geomylichus bassolsi, sp. nov.*. 3 Región postero-ventral del macho. 4 Espermateca de la hembra. 5 Seda escapular externa de la hembra. 6. Huevo

Datos del material tipo: Holotipo hembra y todos los paratipos se colectaron sobre *Perognathus arenarius siccus* Osgood (Mammalia: Rodentia: Heteromyidae) en Isla Cerralvo, Baja California Sur, México, el 19 de octubre de 1991, R. Servín y R. Aguilar *leg.* El tipo y 10 paratipos están depositados en la Colección Acarológica del Centro de Investigaciones Biológicas de B.C.S. Cinco paratipos están en la Colección Acarológica de la Escuela Nacional de Ciencias Biológicas del I.P.N. y seis paratipos están incluídos en la Colección Acarológica del Instituto de Biología de la U.N.A.M.

Etimología: Se dedica esta especie a la Dra. Isabel Bassols, como un reconocimiento a su labor dentro de la Acarología en México.

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SCIENTIFIC NOTE

STATUS OF THE PUNCTUREVINE SEED WEEVIL[1] IN THE TEXAS SOUTHERN HIGH PLAINS

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Puncturevine, *Tribulus terrestris* L., is cosmopolitan in distribution and probably was introduced into the United States with livestock imported from the Mediterranean region (Johnson 1932). This weed pest, commonly referred to as goathead, has long been recognized in the southwestern US as a serious competitor in row crops.

The puncturevine seed weevil, *Microlarinus lareynii* (Jac. du Val), which infests seed burs of the puncturevine plant, was first introduced into the US in 1961 when weevils collected near Catania, Sicily, were released in Arizona, California, Colorado, Nevada, Utah and Washington (Maddox 1976).

Daniels and Wiese (1967) released 250 puncturevine seed weevils at sites near Amarillo and Big Spring, TX, in 1961. These authors reported that infestations did not become established at the Amarillo release site but weevils survived and multiplied at the Big Spring location. During the fall of 1963, weevil-infested puncturevine plants taken near Big Spring were distributed at several locations near Amarillo and Wellington, TX. In 1964, puncturevine seed weevil infestations were readily detected near Wellington, but infestations again failed to establish at Amarillo.

During 1964, infested puncturevine plants were introduced into numerous uninfested areas by both farmers and researchers, and by the fall of 1966, puncturevine seed weevil infestations had spread throughout the Texas Rolling Plains and the southern Texas High Plains (Daniels and Wiese 1967). Maddox (1976) reasoned that severe natural selection had allowed the development of a cold-hardy strain of weevils after surveys conducted during 1973 showed that infestations had spread throughout the Texas Panhandle and north to the Nebraska border.

Maddox (1976) listed several arthropod predators and parasitoids known to attack *M. lareynii*. In west Texas, C. E. Rogers, USDA, ARS (personal communication), reared the parasitoids, *Zatropis* sp., *Bracon mellitor* Say, and *Trimeromicrus maculatus* Gahan from larvae and pupae of the puncturevine seed weevil. Puterka et al. (1986) reported that in the Texas Rolling Plains, parasitism of the puncturevine seed weevil by *B. mellitor* occurred from early June through October. Natural enemies did not, however, prevent this species from becoming firmly established in west Texas. By the mid-1970's, the puncturevine seed weevil had apparently reached an ecological balance with its host, with both populations existing at a low level. From a practical standpoint, puncturevine was no longer an economic weed pest in west Texas.

However, during the mid-1980's puncturevine infestations again began to increase rapidly in west Texas. Producer calls received at the Texas Agricultural Experiment Station at Lubbock indicated that puncturevine had returned as an economic pest by 1987 (J. R. Abernathy, personal communication). We speculate that the resurgence of the puncturevine population resulted from a severe reduction of the *M. lareynii* population by the extremely cold winter of 1983-84, one of the coldest winters on record for west Texas. During December 1983, there were 24 days in which the minimum temperature at Lubbock, TX, was 0° C or lower and 10 days in which the maximum temperature did not exceed 0° C. During January 1984, there were 28 days in which the minimum temperature was 0° C or lower and 4 days in which the maximum temperature did not exceed 0°C (NOAA 1983,1984). Studies of

[1]Coleoptera: Curculionidae

overwinter boll weevil, *Anthonomus grandis* Boh., survival in Stonewall Co., TX, during this period showed a survival rate of only 0.06% (D. R. Rummel, unpublished data). It is likely that the severe winter weather during December and January of 1983-84 had the same devastating effect on the overwintering puncturevine seed weevil population.

Limited surveys conducted in a nine county area during July 1991 indicated that while infestations were quite variable, puncturevine seed weevils were still relatively abundant (Table 1). Because of producer concern over the increase in the puncturevine population, Plains Cotton Growers Inc., in cooperation with the Texas Agricultural Experiment Station, developed a program to release *M. lareynii* in the High Plains area. The weevils were obtained from a California insectary and resold to producers at cost.

TABLE 1. Results of 1991 and 1992 Puncturevine Seed Weevil Surveys.

	1991			1992		
County	Date	No. burs	Percent Infested Burs	Date	No. burs	Percent infested burs
Bailey[a]	--	--	--	01 July	200	47.5
Borden	--	--	--	02 July	150	57.3
Briscoe[a]	--	--	--	26 June	200	27.0
Castro	--	--	--	26 June	200	11.5
Cochran[a]	--	--	--	01 July	200	45.0
Crosby[ab]	24 July	151	42.4	23 June	88	36.4
Dawson[ab]	--	--	--	02 July	200	55.0
Dickens[ab]	--	--	--	23 June	137	40.9
Floyd[ab]	24 July	131	9.9	29 June	200	2.0
Gaines[a]	--	--	--	06 July	200	29.0
Garza[b]	17 July	311	30.0	29 June	200	35.0
Hale[ab]	24 July	204	25.5	26 June	200	30.5
Hockley[ab]	23 July	200	50.0	01 July	185	21.6
Kent	--	--	--	29 June	125	16.0
Lamb[ab]	23 July	200	27.0	01 July	200	60.0
Lubbock[ab]	18 July	192	34.4	10 July	200	28.0
Lynn[ab]	17 July	193	3.6	02 July	200	51.0
Motley	--	--	--	23 June	77	64.9
Parmer[a]	--	--	--	06 July	200	51.5
Scurry[b]	--	--	--	26 June	200	63.5
Swisher[a]	--	--	--	26 June	200	16.0
Terry[b]	23 July	205	44.9	06 July	200	26.0
Yoakum	--	--	--	07 July	175	32.0

[a]1991 weevil releases. Total no. released = 28,000.

[b]1992 weevil releases. Total no. released = 22,000.

In August 1991, 28,000 weevils were released by producers into puncturevine infested areas. Because of the rapid spread of *M. lareynii* after the 1961 releases, we reasoned that releasing new weevils in the area might speed the recovery of the existing population.

During the period 23 June to 7 July 1992, a comprehensive survey for puncturevine seed weevil infestation was conducted in 23 High and Rolling Plains counties. Puncturevines were collected at random from three locations in each county, returned to the laboratory and seed burs dissected and examined for evidence of weevil infestation. The number of seed burs examined depended upon the number of seed available on the collected vines. Due to the

cool wet spring of 1992, the growth of puncturevines was somewhat delayed and the number of seed burs was limited in some areas. In most instances, 200 seed burs from each county were examined (Table 1).

Puncturevine seed weevil infestations were detected at all locations sampled, but varied considerably from a low of 2.0% infested seed burs in Floyd Co. to a high of 64.9% in Motley Co. (Table 1). Infestation data collected in 1992 offered no evidence that puncturevine seed weevil releases enhanced infestation levels. However, present data indirectly indicates population enhancement. For example, surveys in Floyd Co. showed a puncturevine seed weevil infestation level of 9.9 and 2.0% in 1991 and 1992, respectively. Only 200 weevils were released in Floyd Co. in August of 1991, and the release obviously had no effect on the 1992 infestation level. However, the percentage infested puncturevine rate in Lynn Co. increased from 3.6% in 1991 to 51.0% in 1992 after 16,400 puncturevine weevils were released by farmers in August of 1991. Infestation data from 1991 and 1992 are not directly comparable because sample sites were not always the same and the 1991 survey was rather limited. However, the survey data from Lynn Co. indicates that the release of large numbers of puncturevine seed weevils may have influenced the puncturevine infestation rate in that county.

The present study shows that *M. lareynii* is distributed throughout the 23 county study area. While the infestation rate varied among counties, the species appeared to be relatively abundant throughout the area. We conclude that the *M. lareynii* population is in a state of resurgence following a period of severe depression caused by long periods of low temperatures during the winter of 1983-84.

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