EFFECT OF TRAP DESIGN AND COLOR IN EVALUATING ACTIVITY OF *TABANUS ABACTOR* PHILIP[1] IN TEXAS ROLLING PLAINS HABITATS

T. R. Moore, J. E. Slosser, J. Cocke, Jr.[2], and W. H. Newton[3]
Texas Agricultural Experiment Station
P.O. Box 1658, Vernon, Texas 76385

ABSTRACT

The ability to monitor *Tabanus abactor* Philip was enhanced in a study conducted in the Texas Rolling Plains in 1994. Redberry juniper, *Juniperus pinchoti* Sudw., honey mesquite, *Prosopis glandulosa* Torr., and a mixture of the two were determined to be preferred habitats after examining four different habitat types based on dominant vegetation in the area. Brown boards on the ground and white buckets in the air, both coated with Tanglefoot® stickum, were used to capture the flies. These two types of traps can be successfully used to determine fly emergence, fly concentrations, and numbers of flights during a season. Color was determined to be a visual attractant in two separate tests using sticky traps, and the location of the traps on the ground or in the air was critical when selecting color. Brown and red colors were significantly more effective at ground level than in the air, while cyan and white colors were significantly more effective in the air than at ground level. Color within trap type can be used to trap flies more efficiently. Female flies were trapped more efficiently on bucket traps in the air, and male flies were trapped more efficiently on board traps at ground level.

INTRODUCTION

Tabanus abactor Philip, a horse fly commonly referred to in the Texas Rolling Plains as the "cedar fly", is a damaging pest of livestock. A study in 1978 and 1979 in the Texas Rolling Plains indicated 98% of all species captured, using modified Manitoba fly traps, were *T. abactor* (Davis and Sanders 1981). Animals attacked by flies become irritated and experience blood loss, and diseases may be transmitted from an infected animal bitten previously. In the Texas coastal region, tabanids were responsible for significant economic loss in cattle due to decreased weight gain, blood loss and death of animals (McGregor and Schomberg 1952). In Tennessee Goodwin et al. (1985) reported that loss in weight gain and lower milk production can be attributed to reduced grazing and nervous activity as a result of the vicious biting habits of feeding female tabanids. Yearling heifers exposed to tabanids were found to be 16.9% less feed efficient, resulting in a

---

[1]Diptera: Tabanidae
[2]Texas Agricultural Extension Service, P.O. Box 292, Stephenville, Texas 77806.
[3]College of Agriculture, Tarleton State University, Stephenville, Texas 77806.

potential weight gain loss value per year of $10.00/head compared to those not exposed (Perich et al. 1986).

Seasonal occurrence of *T. abactor* activity ranges from early June to late September. Large concentrations occur in June and July, and a smaller peak population occurs in mid-August (Wright et al. 1984).

Montandon et al. (1993) found that larvae were associated with juniper tree litter, but habitat association of adults has not been reported. Thompson (1969) indicated that male flies were not commonly captured in traps. *Tabanus abactor* response to color has not been studied, but Kingston et al. (1986) collected large numbers of flies that were apparently attracted to a light blue vehicle. In 1993 white and brown colors were found to attract flies, but it was not known if these were the most attractive colors. Therefore, the objectives of this study were to evaluate (1) flight activity in different vegetation habitats, (2) fly response to trap type and color, and (3) sex ratio of captured flies.

MATERIALS AND METHODS

During 1994, experiments were conducted on the Y Experimental Ranch in Foard and Cottle counties in the Texas Rolling Plains.

Trap Type. Based on preliminary trials in 1993, a white bucket and a brown board, with stickum®, were found to attract and capture adult *T. abactor*. These traps were more productive than variations of the Manitoba trap (Davis 1981). Therefore, two types of sticky traps were developed to capture flies. The bucket trap was constructed by attaching a 6" long x 1 3/4" diameter plastic pipe vertically to the inside wall of a 4-gallon white plastic food container (food containers obtained from Golden Corral, Vernon and Stephenville, TX). This container was cylindrical with an enclosed bottom and an open top. The bucket trap was inverted over a metal T-post in the ground. The pipe inside the bucket was positioned in a manner that the bottom portion of the trap rested on the T-post. Ground traps were constructed from 1/2 " plyboard cut into 18.25" squares. Surface areas of the bucket and board traps were identical. The board was painted with a flat white exterior latex as an undercoat, followed by flat brown exterior latex. The white bucket trap was not painted.

Polyisobutlene (pib), "Tangle Foot" stickum®, was utilized to ensnare flies on the traps. A 1/16"-layer of stickum was applied with a putty knife to the painted area of the trap. Trapped flies were held in place by the stickum until they could be counted and recorded. The flies were counted as they were removed with forceps twice weekly. Several species of Diptera were captured, but *T. abactor* were easily recognized by the three parallel rows of triangles along the length of the dorsum of the abdomen and by a faint spot at the furcation (branching) of wing vein R4+5 (Philip 1931). Traps were cleaned with citrus oil, and stickum was reapplied approximately every two weeks.

Habitat Study. The dominate tree species in the study area were honey mesquite, *Prosopis glandulosa* Torr., and redberry juniper, *Juniperus pinchoti* Sudw. The four habitats studied to determine the flight activity of *T. abactor* included juniper, mesquite, a mixed juniper-mesquite and cleared sites. Each habitat type was replicated three times for a total of 12 site locations. In the juniper sites, tree size ranged from saplings to mature trees up to 15 feet tall. In the mesquite sites, trees were predominately immature basal sprouted regrowth over 10' tall. One cleared site had a sparse amount of juniper regrowth two to three feet tall. The habitat study was conducted by placing one white plastic bucket trap three feet above the ground and one brown board trap on the ground at each of the 12 locations. The two traps were stationed within 5 feet of each other at each location. The study began with the first captured *T. abactor* on 2 June and concluded 17 October 1994. The traps were operated for 137 days and were inspected twice weekly.

Color Study. The color study, involving two tests, was conducted using three moderately dense juniper sites (about 56 trees per acre) two miles apart. At each site, nine colors of the suspended bucket and nine corresponding colors of the ground board trap were evaluated for *T. abactor* preference; colors consisted of red, yellow, green, blue, white, brown, black, cyan, and magenta. Cyan (blue-green) was derived by mixing one oz. blue with 5/16 oz. green and blending into one pint Sherwin Williams interior flat latex base. Magenta (red-purple) was derived by mixing 1/16 oz. white with one pint Sherwin Williams latex rose base. The remaining colors were Sherwin Williams generic (Color All Spray Enamel). Bucket traps were color-sprayed over the original white plastic; board traps were sprayed with flat white paint prior to the color overspray. Test 1 was operated for 26 days beginning 9 June and ending 5 July when *T. abactor* activity ceased. Identical colors of the bucket and board traps were placed side-by-side, and each set of paired traps was separated by five feet. Traps were arranged in a straight line in each replication. The test was replicated three times, with a replication in each of three different juniper locations.

Test 2 began after increased fly activity was detected in the study area on 28 July, and the traps were operated for 43 days and terminated 9 September. In this study board and bucket traps of the same color were placed adjacent to one another and separated from paired traps of different colors by 45 feet to eliminate interference between trap colors. The same colors were used as in Test 1, but the traps were randomly arranged in a circular pattern. In both Test 1 and Test 2, the traps were inspected twice weekly and trap colors were rotated to the next position at each trap inspection to minimize position effects within each replication.

In early August, light reflectance measurements were taken with the use of a Personal Spectrometer II (Analytical Spectral Devices, Boulder, Co.). Measurements of the reflectance of nine colors of the bucket traps with the stickum applied were taken from the four cardinal compass directions around the trap. These locations represented four different sun angles for the measurements. The mean light reflectance of these four locations was used to calculate average reflectance values.

Sex Ratio Study. The sex ratio study was conducted using the *T. abactor* captured during the habitat and color studies at the juniper sites. From 6 to 30 June 1994, all *T. abactor* from the habitat study were retained and transported to the laboratory for sex determination. The flies were kept separate according to trap type. Flies captured in the color study from 23 June to 30 June were retained and brought to the laboratory for sex determination. The flies from the color study were kept separate according to color and trap type. The flies were identified as females if their eyes were separated medially and had two green horizontal bands traversing each eye, or males if they had eyes contiguous with only one horizontal band (Philip 1931).

Experimental Design and Analyses. A randomized complete block design with a split-plot treatment arrangement and three replications was used for the habitat study. The four habitats represented the whole plots, and the two trap types represented subplots within each habitat. Trap color tests were designed as randomized complete block experiments with a split-plot treatment arrangement. The two trap types represented the whole plots, and the nine trap colors represented the subplots within each trap type. Treatments were replicated three times.

In the sex ratio study conducted 6-30 June, fly response to trap type was analyzed as a split-plot experiment, with trap type (brown board and white bucket) being whole plots and sex as the subplots. Although the habitat test was replicated three times, the flies from each trap type in the three juniper sites were pooled on each sampling date to increase sample size. Flies were removed from the traps eight times during the 6-30 June interval, and the eight sampling dates were used as the source of variation for replication

in the analysis. In the second sex ratio study conducted 23-30 June, fly response to trap type and color was analyzed as a split split-plot experiment, where trap colors represented the whole plots. Trap types were treated as subplots, because board and bucket traps of the same color were adjacent to each other in this test. Fly sex was treated as sub-subplots. The three sampling dates of 23, 27 and 30 June were treated as replications in the analysis.

Analyses of variance and mean separation tests were performed using the FACTOR and RANGE programs, respectively, of MSTAT-C (MSTAT Development Team 1988).

RESULTS AND DISCUSSION

Habitat Study. Board and bucket traps captured significantly lower numbers of *T. abactor* in cleared habitats compared to juniper, mesquite, and juniper/mesquite mix habitat types (Table 1). There were no significant differences among fly numbers in the juniper, mesquite, and juniper/mesquite mix sites; however, *T. abactor* larvae were discovered only under juniper tree leaf litter which apparently provides a suitable habitat for developing larvae (Montandon et al. 1993). These results indicate that *T. abactor* adults prefer tree habitats compared to clearings and suggest that flies are indiscriminate in their preference of juniper or mesquite after they emerge. Our results are supported by those of Kingston et al. (1986) who reported significantly greater numbers of resting flies in habitats with dense cover compared with numbers in habitats lacking cover.

Color Study. In two tests, there were no differences in numbers of flies caught between trap types (Table 2). However, within trap types, color significantly affected numbers captured. In Test 1, red boards trapped significantly more flies than any other color except brown boards. Cyan buckets trapped significantly more flies than any other color except white buckets. In Test 2, the red, brown and black boards trapped significantly higher number of flies. Difference in captures on buckets in Test 2 were not as pronounced as in Test 1, but highest numbers were again trapped on the cyan buckets. In both tests, the yellow boards and buckets were, in general, the least attractive.

Reflectance spectra from each color of the bucket traps are shown in Figs. 1a and 1b. Reflected light intensity was highest from the white and yellow traps and lowest from the black traps. High reflected light intensity does not account for numbers of flies captured because yellow buckets captured low numbers of flies in these tests. Apparently the flies were responding to trap color, not reflected light intensity. Light in the 489-495 μm range, which is common to both the white and cyan buckets, seems to be the most attractive range of wavelength for female flies. The polyisobutlene stickum may have altered reflectance patterns of the traps, but Brach and Trimble (1985) reported the greatest influence of the adhesive was when it was applied to fluorescent colored traps which we did not have.

Sex Ratio Study. In the 6-30 June study, sex by trap type interaction was significant (LSD=40.8, df=14, P=0.10); significantly more males than females were captured on brown boards, while significantly more females than males were captured on white buckets (Table 3). In the color study combined over trap type, more females than males were captured on cyan and white traps, but sex ratios were not different on other colors (Table 4). More flies were captured on cyan and white colored buckets as compared with catches on cyan and white colored boards (Table 5). These results indicate that cyan and white buckets are highly attractive to female flies. Red and brown boards captured more flies than red and brown buckets (Table 5), but there were no significant differences between male and female captures on these colors (Table 4). However, the highest numbers of males were caught on the red and brown colors. Thompson (1969)

TABLE 1. Average Number of *Tabanus abactor* Captured on Two Types of Sticky Traps in Four Different Habitats. Y-Ranch, Foard and Cottle Counties, Texas, 1994.

Trap	Habitat Type				
Type	Juniper	Mesquite	Clearing	Juniper/Mesquite	Mean[a]
Board	17.6	22.1	6.3	19.1	16.2 A
Bucket	9.2	12.1	1.7	8.6	7.9 B
Mean[b]	13.4 a	17.1 a	4.0 b	17.8 a	

[a] Values in a column followed by different uppercase letters (A-B) are significantly different ($P<0.01$, F-test).
[b] Values in a row followed by a different lowercase letter (a-b) are significantly different ($P\leq0.10$, LSD).

TABLE 2. Average Number of *Tabanus abactor* Captured in Two Tests with Nine Colors with Two Types of Sticky Traps. Y-Ranch, Foard and Cottle Counties, Texas, 1994.

Trap	Test 1[a,b]		Test 2[a,b]	
Color	Board	Bucket	Board	Bucket
Red	14.4 A	3.6 BC	47.9 AB	15.7 AB
Magenta	8.6 BC	3.1 BC	30.9 BC	14.2 AB
Cyan	1.5 D	11.0 A	1.3 D	26.0 A
Brown	11.2 AB	3.4 BC	54.2 A	13.4 AB
Black	8.4 BC	2.1 C	49.2 A	10.7 AB
Green	4.3 CD	2.2 C	30.7 C	14.4 AB
Yellow	0.2 D	0.6 C	4.2 D	0.2 B
Blue	2.6 D	4.3 BC	13.3 D	14.5 AB
White	1.1 D	8.2 AB	0.4 D	20.5 A
Mean[c]	5.8 a	4.3 a	25.8 a	14.4 a

[a] Values for response to colors are compared within trap type within test, and means with a different uppercase letter are significantly different ($P<0.01$, LSD).
[b] Test 1 conducted with paired traps adjacent to each other and Test 2 conducted with paired traps separated from each other.
[c] Main effect for trap type is compared within Test 1 or Test 2; values with a common lower case letter are not significantly different ($P>0.10$, F-test).

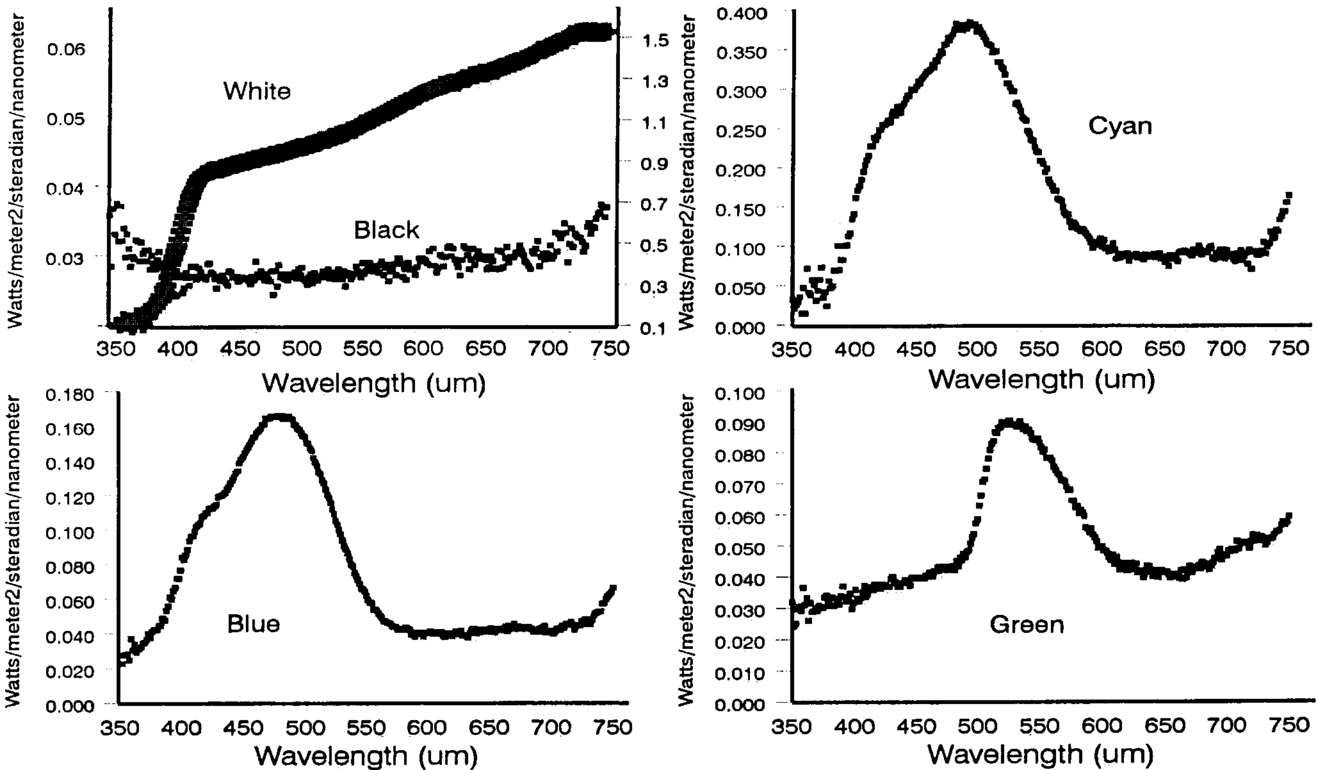


FIG. 1a. Reflectance spectra for colored bucket traps for capturing *Tabanus abactor*. Y-Ranch, 1994.

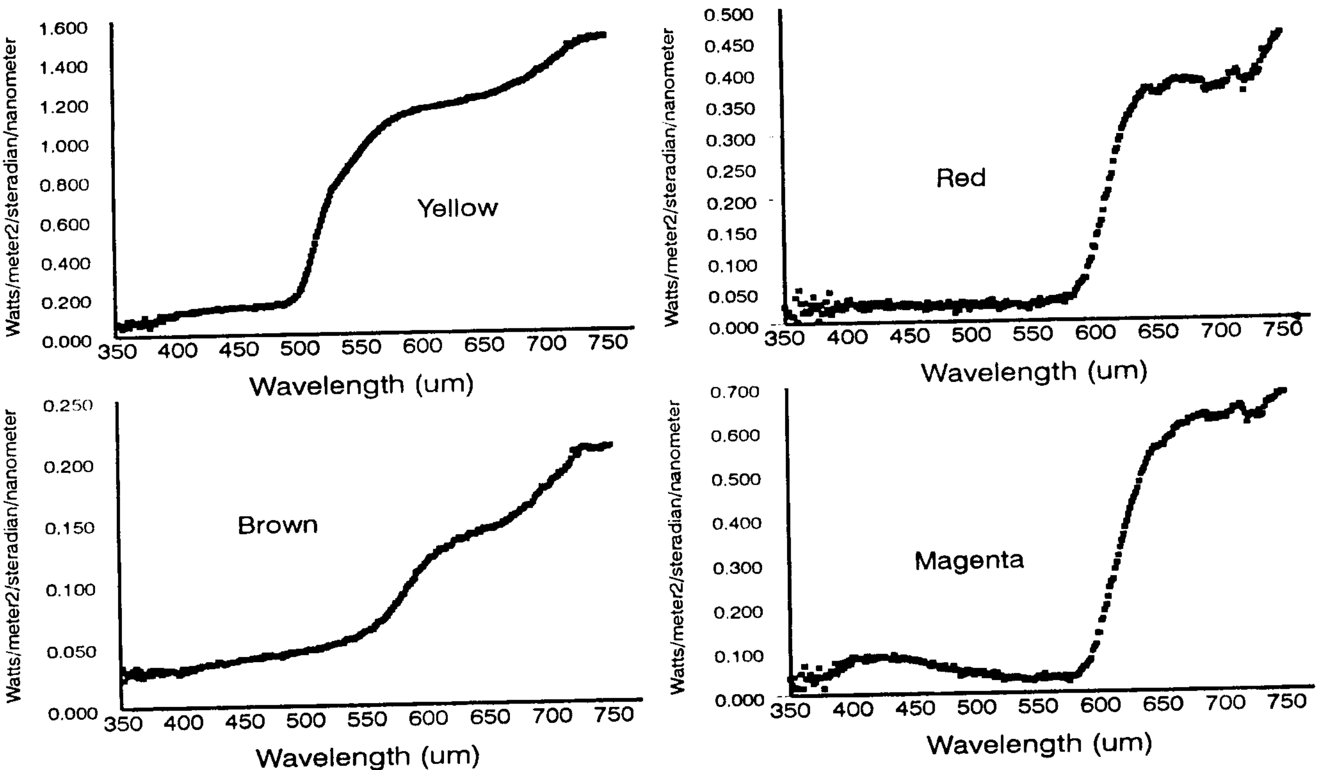


FIG. 1b. Reflectance spectra for colored bucket traps for capturing *Tabanus abactor*. *Y-Ranch, 1994.*

TABLE 3. Average Number of Male or Female *Tabanus abactor* Captured on Two Types of Sticky Traps. Y-Ranch, Foard and Cottle Counties, Texas, 1994.

Trap Type	Sex of Fly		
	Male	Female	Mean[a/]
Brown Board[b/]	56.5 a	14.9 b	35.7 A
White Bucket[b/]	0.5 b	101.4 a	50.9 A
Mean[c/]	28.5 a	58.1 b	

[a/] Values for trap type main effect with a common uppercase letter are not significantly different (P>0.10, F-test).
[b/] Interaction values for sex within trap type with a different lowercase letter are significantly different (P≤0.10, LSD).
[c/] Values for sex main effect with a different lowercase letter are significantly different (P<0.10, F-test).

TABLE 4. Average Number of Male or Female *Tabanus abactor* Captured on Nine Colors of Sticky Traps. Y-Ranch, Foard and Cottle Counties, Texas, 1994.

Trap Color	Sex[a/]		
	Male	Female	Mean[b/]
Red	10.5 a	11.0 a	10.8 A
Magenta	7.5 a	12.5 a	10.0 AB
Cyan	0.3 b	19.8 a	10.1 AB
Brown	13.5 a	7.8 a	10.7 A
Black	4.3 a	5.7 a	5.0 BC
Green	4.3 a	6.3 a	5.3 ABC
Yellow	0.3 a	1.2 a	0.8 C
Blue	1.8 a	9.7 a	5.8 ABC
White	0.2 b	16.7 a	8.4 AB
Mean	4.8 b	10.1 a	

[a/] Male and female are compared within color, and interaction values with a different lowercase letter are significantly different (P<0.05, LSD); main effect values are significantly different (P<0.01, F-test).
[b/] Trap colors are compared with uppercase letters, and values with a different letter are significantly different (P<0.05, LSD).

TABLE 5. Average Number of Male and Female *Tabanus abactor* Captured with Nine Colors on Two Types of Sticky Traps. Y-Ranch, Foard and Cottle Counties, Texas, 1994.

Trap	Trap Type[a]		
Color	Board	Bucket	Mean[b]
Red	18.5 a	3.0 b	10.8 A
Magenta	16.5 a	3.5 a	10.0 AB
Cyan	1.2 b	19.0 a	10.1 AB
Brown	19.7 a	1.7 b	10.7 A
Black	8.3 a	1.7 a	5.0 BC
Green	7.7 a	3.0 a	5.3 ABC
Yellow	0.3 a	1.2 a	0.8 C
Blue	4.7 a	6.8 a	5.8 ABC
White	0.2 b	16.7 a	8.4 AB
Mean	8.6 a	6.3 a	

[a] Trap types are compared within colors, and interaction values with a different lowercase letter are significantly different ($P<0.01$, LSD); main effect values for trap type are not different ($P>0.10$, F-test).
[b] Trap colors are compared with uppercase letters, and values with a different letter are significantly different ($P<0.05$,LSD).

reported that the ratio of male to female captures is frequently a characteristic of trap type, but male captures were uncommon in three trap types described as a modified Manitoba, a modified animal trap, and an aerial sweep net. These three types of traps are generally thought to capture females seeking blood meals. As males do not bite, they would not likely be captured in these collecting devices. Our results, however, demonstrate that male flies are frequently captured, and red or brown boards were the most attractive colors for males (Table 4). These results suggest that female flies were attracted to an above ground trap with a cyan or white color, while males were attracted to a ground-level trap colored red or brown.

Seasonal activity. Results of trap catches indicated that seasonal flight activity began 2 June and ended 17 October. There were three distinct fly emergence flights, and each lasted approximately one month (Fig. 2). These peak fly concentrations were comparable to peaks which occurred during June, July and mid-August as reported by Wright et al. (1984). However, our results indicate the third flight peaked in mid-September based on board and bucket traps. Sanders (personal communication) found that fly activity increased 4-5 days after a substantial rainfall, especially following a relatively dry period. These results indicate the second flight, which peaked 1 August, was the largest in number and shortest in duration. Seasonal meteorological variances from year to year may influence time and duration of flight activity.

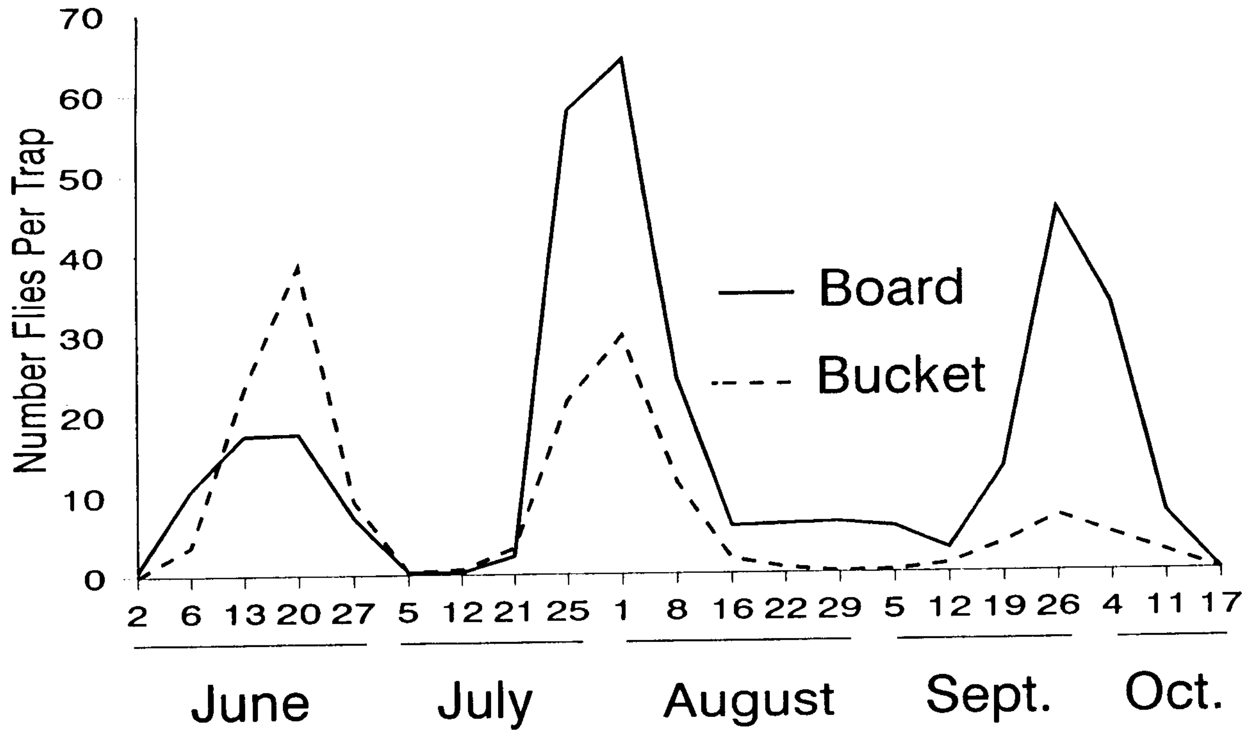


FIG. 2. *Tabanus abactor* activity in juniper sites, Y-Ranch, 1994.

ACKNOWLEDGMENT

We express our appreciation for the effort and contribution made by Mikel J. Moran and the assistance and encouragement of Bobby Idol and Roxanne Montandon.

LITERATURE CITED

Brach, E. J. and R. M. Trimble. 1985. Effect of adhesive on the spectral reflectance of insect traps. Can. Entomol. 117: 1565-1568.

Davis, S. G. and D. P. Sanders. 1981. Seasonal and geographical distribution of *Tabanus abactor* Philip in the Texas Rolling Plains. Southwest. Entomol. 6: 81-86.

Goodwin, J. T., B. A. Mullens, and R. R. Gerhart. 1985. The Tabanidae of Tennessee. Univ. of Tenn. Agric. Exp. Stn. Bull. 642.

Kingston, S. R., J. K. Wangberg, and D. P. Sanders. 1986. Flight behavior and nocturnal resting sites of *Tabanus abactor* Philip (Diptera: Tabanidae) in the Texas Rolling Plains. J. Kan. Entomol. Soc. 59: 337-342.

McGregor, W. S. and O. C. Schomberg. 1952. A partial annotated list of species of the Tabanidae. J. Econ. Entomol. 45:746

Montandon, R., J. E. Slosser, and D. Lucia. 1993. Habitat of larval *Tabanus abactor* Philip in the Texas Rolling Plains. Southwest. Entomol. 18: 61-62.

MSTAT Development Team. 1985. MSTAT: a microcomputer program for the design, management and analysis of agronomic research experiments. Version 4.0. Michigan State University, East Lansing.

Perich, M. J., R. E. Wright, and K. S. Lusby. 1986. Impact of horse flies (Diptera: Tabanidae) on beef cattle. J. Econ. Entomol. 79: 128-131.

Philip, C. B. 1931. New Tabanidae (horseflies) with notes on certain species of the longus group of Tabanus. Ohio J. Sci. 36: 149-156.

Thompson, P. H. 1969. Collecting methods for Tabanidae (Diptera). Ann. Entomol. Soc. Amer. 62: 50-57.

Wright, R. E., R. K. Whittle, M. J. Perich, and A. L. Hollander. 1984. Seasonal occurrence of horse flies (Diptera: Tabanidae) in North Central Oklahoma. J. Kan. Entomol. Soc. 57: 209-215.

QUARANTINE EVALUATION OF EXOTIC PARASITOIDS OF THE SWEETPOTATO WHITEFLY, *BEMISIA TABACI* (GENNADIUS)[1]

John Goolsby[2], Jesusa Crisostomo Legaspi[3], Benjamin C. Legaspi, Jr.[4]

ABSTRACT

Nineteen strains of parasitoids belonging to the genera *Eretmocerus* and *Encarsia* were evaluated as potential biological control agents of the sweetpotato whitefly, *Bemisia tabaci* on melons. Of these strains, three are undescribed species of *Eretmocerus*, two collected from Texas and one from Taiwan. Percentage parasitism and numbers of hosts attacked were measured on melon plants in the greenhouse. Percentage parasitism was found to decline with host numbers and was described by an exponential decay function. Highly significant differences were found in both numbers of hosts attacked and in percentages of parasitism among the different strains, but no strong evidence was found to indicate that *Eretmocerus* species are better parasitoids than *Encarsia* species, or vice versa. The evaluations indicate that a species of *Encarsia* nr. *pergandiella* from Brazil (identification number M94055) appeared to be the most promising parasitoid of *B. tabaci* in a melon crop.

INTRODUCTION

The sweetpotato whitefly, *Bemisia tabaci* (Gennadius) (Biotype "B") also known as *Bemisia argentifolii* Bellows & Perring (Homoptera: Aleyrodidae), is an extremely destructive pest of field, ornamental and greenhouse crops throughout the world. *B. tabaci* inhabits a wide geographical range (Cock 1986, 1993), possesses a high fecundity and a short life-cycle (Baumgartner and Yano 1990), attacks a broad range of host plants (Cock 1986, Byrne et al. 1990), and serves as a vector for plant viruses (Brown et al. 1992). The whitefly also produces honeydew that promotes growth of sooty mold which contributes to crop loss. Moreover, populations of *B. tabaci* have displayed resurgence and resistance to conventional insecticides (Dittrich et al. 1990).

In the Lower Rio Grande Valley of Texas, *B. tabaci* infested over 100,000 acres of cotton in 1991, causing an estimated crop loss of about $80 million (Faust 1992). Coupled with losses in fruit and vegetable production, total crop loss in the Lower Rio Grande Valley in 1991 was estimated to exceed $100 million. In the same year, *B. tabaci* in Texas caused $250 million in economic losses and more than 6000 lost jobs (Perring et al. 1993, D. Riley pers. comm.). Additionally, damage in 1991 was estimated at $37 million in Arizona, $120 million and 3400 jobs lost in California, and over $140 million in Florida.

This study used melon as a host plant to evaluate 17 exotic and two native strains of parasitoids as possible biological control agents of *B. tabaci*. Melons were selected as a host because they are the first crop in the spring to harbor a large population of whiteflies. These early populations migrate to other crops and overwhelm attempts at control (J. C. Legaspi, unpubl. data). In addition, native parasitoids have not been found to be effective control

---

1 Homoptera: Aleyrodidae

2 Mission Biological Control Center, USDA-APHIS-PPQ, PO Box 2140, Moore Air Base, Mission TX 78573

3 Texas Agricultural Experiment Station, 2415 East Highway 83, Weslaco, TX 78596

4 Joint affiliation: Biological Control of Pests Research Unit, USDA-ARS and Texas Agricultural Experiment Station, 2413 East Highway 83, Weslaco, TX 78596

agents of *B. tabaci* on melons (M. Ciomperlik, Mission Biological Control Center, unpubl. data). Parasitoids may have been hampered by the hirsute leaves of melons. Because of the prohibitive costs of maintaining numerous parasitoid strains, this evaluation is necessary in order to prioritize cultures for rearing and to identify the most promising parasitoids for later field evaluations in melons.

MATERIALS AND METHODS

The whiteflies used as hosts in this experiment were taken from a colony established from material collected from cotton in Weslaco, TX, in 1994. This whitefly colony was maintained in environmental growth chambers which held fluctuating daily temperature from 24 to 29°C, a 50-70% RH, and a 14:10 L:D photoperiod. The colonies was maintained using hibiscus (*Hibiscus rosa-sinensis*) as a host plant.

The parasitoids evaluated in this study represented the greatest geographical and genetic diversity available in culture at the Mission Biological Control Center (MBCC). These whitefly parasitoids were collected from all parts of the world as shown in Table 1. The 19 strains/species of parasitoids of *B. tabaci* are tabulated according to site and country of collection, collectors, host plant on which material was collected, date of the collection and identification of the parasitoid. Pending futher genetic and morphological assessment, the strains/species are classified according to a unique "M" number assigned by MBCC. The parasitoids are species of *Encarsia* and *Eretmocerus*. All parasitoid cultures were reared using the whitefly colonies on hibiscus, and were maintained in similar environmental growth chambers held at the same conditions.

Three of the strains represent new species of *Eretmocerus* (Table 1). Strain M94002 was collected by M. Rose and S. Stauffer [Dept. of Entomology, Texas A&M University (TAMU)] on tomato, and strain M94003 was collected by J. Rodriguez in Mission, Texas, on cabbage. Strain M93058 was collected by C. Moomaw (TAMU) in Taiwan on tomatoes. All three species are currently being described by M. Rose. The *Encarsia* collected in Parbhani, India, (M92018) will be described as a new species by J. Heraty (UC Riverside).

The host plant chosen for the evaluations was melon (*Cucumis melo* var. "Perlita"). The melons were planted in pots (15cm diameter) which were held in a quarantine greenhouse maintained at 27°C and 50-60% RH and 12:12 L:D photoperiod. After four weeks, the melons were infested with about 10,000 adult whiteflies. After exposure for three days, individual leaves were covered with homemade sleeves to prevent further whitefly oviposition. The sleeves consisted of two sheets of organza mesh (≈ 8x8 cm, ≈ 60 mesh size) glued together on all sides, except for one. The remaining unglued side was secured using Velcro® strips. The whitefly immature stages were allowed to develop for 14 days, i.e. to second and third instars, before they were exposed to parasitoids.

Two adult female parasitoids of a given strain were collected from mixed age (1 to 7-days old) cultures, and this constituted a replicate. Each replicate was placed in a sleeve for 48 h, thus allowing parasitism. After the exposure period, the parasitoids were removed from the sleeves. Each of the 19 strains was replicated ten times. The developing parasitoids were held in their respective sleeve for 14-16 days to develop to the pupal stage. After this interval, numbers of parasitized and unparasitized whiteflies were recorded. Parasitism was determined by the presence of an immature parasitoid or characteristic exit hole from the host exuvium. The entire experiment was performed twice.

All statistical analyses were performed using the Systat® Statistical Package, version 5.2 (Wilkinson et al. 1992). Numbers of hosts attacked and percentage parasitism were analyzed by One-Way ANOVA using strain/species as the treatment. Replicates were excluded when the sum of parasitized and unparasitized whiteflies totalled less than ten. In order to test whether *Eretmocerus* spp. or *Encarsia* spp. exhibited greater numbers of hosts attacked or percentage parasitism, the data were divided according to genus and analyzed for effect of species using ANOVA. Data from both trials were also pooled to test for a possible effect of trial number on numbers attacked and percentage parasitism using a Two-Way ANOVA. When ANOVAs were significant, means were separated using Tukey's test corrected for experiment wise error and all error limits were set at $p = 0.05$. The data from both trials were pooled and nonlinear regressions were performed using total numbers of hosts available as predictors for both numbers of hosts attacked and percentage parasitism.

TABLE 1.Parasitoids of Sweetpotato Whitefly, *Bemisia tabaci,* Evaluated as Biological Control Agents

Strain No.	Collection Site	Collector(s)	host plant	Collection date	Identification
M92014	Murcia, Spain	A. Kirk K. Chen R. Sobhian	cotton	1992	*Eretmocerus* nr. *mundus*
M92018	Parbhani, India	R. Nguyen		1992	*Encarsia* sp. nov.
M92019	Padappai, India	A. Kirk L. Lacey	eggplant	1992	*Eretmocerus* nr. *mundus*
M93003	Murcia, Spain	A. Kirk L. Lacey	lantana	1992	*Encarsia transvena*
M93005	Thirumala, India	A. Kirk L. Lacey		1992	*Eretmocerus* nr. *mundus*
M93058	Tainan, Taiwan	C. Moomaw	tomato, poinsettia	12-4-93	*Eretmocerus* sp. nov.
M93064	Mazotos, Cyprus	A. Kirk L. Lacey	lantana	10-4-93	*Encarsia lutea*
M94002	College Station, TX	M. Rose S. Stauffer	tomato	1994	*Eretmocerus* sp. nov.
M94003	Mission, TX	J. Rodriguez	cabbage	1994	*Eretmocerus* sp. nov.
M94014	Benguet, Philippines	J. Legaspi, R. Carruthers T. Poprawski	white potatoes	3-8-94	*Encarsia transvena*
M94016	Shan-Hua, Taiwan	J. Legaspi, R. Carruthers T. Poprawski	poinsettia	3-14-94	*Encarsia transvena*
M94017	Shan-Hua, Taiwan	J. Legaspi, R. Carruthers T. Poprawski	soybean, tomato	3-14-94	*Encarsia transvena*
M94019	Shan-Hua, Taiwan	J. Legaspi R. Carruthers T. Poprawski	soybean, tomato	3-14-94	*Encarsia transvena*
M94023	Sai Noi Klong Ha Roi, Thailand	A. Kirk L. Lacey	eggplant, melons	3-9-94	*Eretmocerus* sp.
M94036	Chiang Mai, Thailand	A. Kirk L. Lacey	*Chromolaena odorata*	3-25-94	*Eretmocerus* sp.
M94047	Kuala Lumpur, Malaysia	A. Kirk L. Lacey	*Mussaenda* sp.	3-28-94	*Encarsia transvena*
M94055	Sete Lagoas, Brazil	M. Rose	soybean	2-20-94	*Encarsia* nr. *pergandiella*
M94056	Sete Lagoas, Brazil	M. Rose	soybean	2-25-94	*Encarsia* nr. *hispida*
M94120	Golan Heights, Israel	A. Kirk L. Lacey	melons	10-94	*Eretmocerus* nr. *mundus*

RESULTS AND DISCUSSION

Statistical analyses will be presented in the following sequence: for each trial, we present the effects of species/strain on numbers attacked and percentage parasitism, followed by the comparison between *Eretmocerus* and *Encarsia* species. Finally, we present the comparisons between trials and the nonlinear regressions using the pooled data.

Trial 1. The results of the first trial are summarized in Table 2 which shows mean numbers of hosts attacked (± SE), in descending order, by each strain of parasitoid and mean percentage parasitism (± SE, arc sin transformation). The column for "N" indicates the number of replicates used from the initial ten following the exclusion of replicates with low total whitefly counts.

The numbers of whiteflies attacked were significantly different among the strains of parasitoids ($F = 4.38$; $df = 18, 153$; $r^2 = 0.34$; $P < 0.01$). The highest number of hosts attacked was recorded for strain M94055, a species of *En.* nr. *pergandiella* collected from Brazil. Percentage parasitism was also significantly different among strains of parasitoids ($F = 3.91$; $df = 18, 153$; $r^2 = 0.32$; $P < 0.01$). Using this measure of attack, the best performing strains were M92018 (*Encarsia* from India) and M94019 (*Encarsia* from Taiwan).

Numbers of hosts attacked and percentage parasitism were grouped according to genus to test whether higher attack rates could be found for either *Eretmocerus* spp. or *Encarsia.* spp. The numbers of hosts attacked were not significantly affected by genus ($F = 3.83$; $df = 1, 170$; $r^2 = 0.022$; $P > 0.05$). Percentage parasitism likewise was not found to be affected by parasitoid genus ($F = 2.55$; $df = 1, 170$; $r^2 = 0.015$; $P > 0.05$).

Trial 2. Results of the second trial are shown in Table 3. In the second trial, strain M94017 was eliminated completely from the analyses because of insufficient data caused by low whitefly oviposition. Numbers attacked were again significantly affected by strain of parasitoid ($F = 9.39$; $df = 17, 161$; $r^2 = 0.49$; $P < 0.01$). The highest numbers of hosts attacked were found for strain M92014 (*Er. mundus* from Spain) followed by M94055 (*Encarsia* from Brazil), the strain which exhibited the highest numbers attacked in the first trial. As in the first trial, percentage parasitism was again significantly affected by parasitoid strain ($F = 4.28$; $df = 17, 161$; $r^2 = 0.31$; $P < 0.01$). The clear best performer was again the *Encarsia* from Brazil (M94055), which was significantly different from all other strains.

In the test for differences between the two genera, the number of hosts attacked was found to be significantly affected by genus ($F = 14.1$; $df = 1, 177$; $r^2 = 0.074$; $P < 0.05$). The *Eretmocerus* strains attacked a mean of 22.3 hosts (SE 2.2, n = 90) compared to a mean of 12.4 (SE 1.4, n = 89) for the *Encarsia* strains. However, percentage parasitism was again not found to be affected by parasitoid genus ($F = 1.3$; $df = 1, 177$; $r^2 = 0.007$; $P > 0.05$).

Comparison between trials. In the Two-Way ANOVA for parasitoid strain and trial number, the strain eliminated from Trial 2 due to low numbers of usable replicates (M94017) was also eliminated from Trial 1, as required in the statistical analysis. The test on strain and trial number showed that numbers of whiteflies attacked was again significantly affected by the strain of parasitoid ($F = 8.1$; $df = 17, 305$; $r^2 = 0.42$; $P < 0.01$) and trial number ($F = 5.2$; $df = 1, 305$; $r^2 = 0.42$; $P < 0.05$). Fewer hosts were attacked in the first trial (AVE 13.4, SE 1.5, n = 162) compared to the second (AVE 17.4, SE 1.4, n = 179) ($F = 4.0$, $df = 1, 339$; $r^2 = 0.012$, $P < 0.05$). The difference in numbers attacked may be due to the greater numbers of whiteflies present in the second trial. The mean number of whiteflies per trial was calculated by pooling total numbers of parasitized and unparasitized whiteflies across the different strains. In the first trial, mean number of whiteflies was 178.0 (SE 11.9, n = 172), which increased significantly in the second trial to 348.6 (SE 17.9, n = 179) ($t = 7.9$, $df = 308$, $P < 0.01$).

In the analysis of percentage parasitism, parasitoid strain again proved to be a highly significant factor ($F = 3.6$; $df = 17, 305$; $r^2 = 0.3$; $P < 0.01$). The effect of trial number on percentage parasitism was insignificant ($F = 1.5$; $df = 1, 305$; $r^2 = 0.31$; $P > 0.05$). Percentage parasitism was 15.4 (SE 1.3, n = 162) in the first trial and 14.0 (SE 0.9, n = 179) in the second. The general conclusion from the joint analysis between the two trials is that the effect of the parasitoid strain on both numbers of hosts attacked and percentage parasitism is highly significant. No difference in percentage parasitism was found between the two trials.

TABLE 2. First Trial: Evaluation of Parasitoids against *B. tabaci* in Greenhouse melons

Strain number	Number attacked	Tukey's HSD	Percentage Parasitism	Tukey's HSD	N
M94055 *Encarsia* (Brazil)	41.7 ± 11.1	a	19.1 ± 3.0	a,b,c	10
M94019 *Encarsia* (Taiwan)	32.2 ± 4.3	a,b	35.1 ± 7.6	a	6
M94056 *Encarsia* (Brazil)	26.4 ± 5.6	a,b,c	27.9 ± 5.3	a,b	9
M92014 *Eretmocerus* (Spain)	24.4 ± 10.7	a,b,c	23.8 ± 7.3	a,b,c	8
M93064 *Encarsia* (Cyprus)	16.6 ± 6.1	b,c	22.6 ± 5.5	a,b,c	10
M94023 *Eretmocerus* (Thailand)	14.1 ± 4.0	b,c	17.1 ± 4.2	a,b,c	8
M94036 *Eretmocerus* (Thailand)	13.9 ± 6.0	b,c	13.2 ± 3.3	a,b,c	9
M92018 *Encarsia* (India)	13.7 ± 2.7	b,c	30.7 ± 6.1	a	10
M94120 *Eretmocerus* (Israel)	11.9 ± 5.3	b,c	12.5 ± 4.0	a,b,c	10
M94014 *Encarsia* (Philippines)	10.8 ± 2.9	b,c	16.0 ± 3.8	a,b,c	8
M93005 *Eretmocerus* (India)	9.2 ± 4.7	b,c	11.3 ± 3.9	a,b,c	9
M93003 *Encarsia* (Spain)	7.7 ± 4.7	b,c	5.5 ± 2.9	b,c	10
M92019 *Eretmocerus* (India)	6.3 ± 2.7	b,c	13.6 ± 5.3	a,b,c	10
M94002 *Eretmocerus* (Texas)	4.4 ± 2.8	b,c	11.5 ± 6.2	a,b,c	10
M94016 *Encarsia* (Taiwan)	4.3 ± 1.9	b,c	8.0 ± 4.0	b,c	9
M94003 *Eretmocerus* (Texas)	4.0 ± 3.7	b,c	6.3 ± 5.1	b,c	10
M94047 *Encarsia* (Malaysia)	3.4 ± 2.3	c	4.6 ± 3.1	c	10
M94017 *Encarsia* (Taiwan)	1.6 ± 1.5	c	2.3 ± 1.7	c	10
M93058 *Eretmocerus* (Taiwan)	0.5 ± 0.3	c	2.6 ± 1.8	c	6

TABLE 3. Second Trial: Evaluation of parasitoids against *B. tabaci* in greenhouse melons

Strain number	Number attacked	Tukey's HSD	Percentage Parasitism	Tukey's HSD	N
M92014 *Eretmocerus* (Spain)	55.0 ± 9.2	a	16.9 ± 2.0	b	10
M94055 *Encarsia* (Brazil)	37.6 ± 4.0	a,b	37.5 ± 6.7	a	10
M92019 *Eretmocerus* (India)	29.1 ± 4.4	b,c	16.4 ± 2.1	b	10
M94120 *Eretmocerus* (Israel)	26.8 ± 7.1	b,c,d	16.2 ± 4.0	b	10
M93058 *Eretmocerus* (Taiwan)	25.9 ± 6.6	b,c,d	15.3 ± 3.0	b	10
M94036 *Eretmocerus* (Thailand)	20.7 ± 2.9	b,c,d,e	14.4 ± 1.3	b	10
M94016 *Encarsia* (Taiwan)	13.8 ± 4.2	c,d,e	13.7 ± 2.5	b	10
M94002 *Eretmocerus* (Texas)	13.6 ± 3.4	c,d,e	16.5 ± 2.5	b	10
M93005 *Eretmocerus* (India)	12.9 ± 4.1	c,d,e	16.4 ± 6.0	b	10
M93003 *Encarsia* (Spain)	12.5 ± 3.0	c,d,e	11.9 ± 2.4	b	10
M94047 *Encarsia* (Malaysia)	10.9 ± 3.3	c,d,e	10.0 ± 2.7	b	10
M94056 *Encarsia* (Brazil)	10.0 ± 2.8	c,d,e	12.3 ± 3.9	b	10
M93064 *Encarsia* (Cyprus)	9.3 ± 3.0	c,d,e	8.4 ± 3.0	b	9
M92018 *Encarsia* (India)	8.6 ± 1.9	c,d,e	8.5 ± 1.4	b	10
M94003 *Eretmocerus* (Texas)	8.4 ± 2.9	c,d,e	9.4 ± 2.3	b	10
M94023 *Eretmocerus* (Thailand)	8.2 ± 3.1	c,d,e	13.4 ± 4.4	b	10
M94014 *Encarsia* (Philippines)	5.8 ± 1.4	d,e	9.0 ± 1.7	b	10
M94019 *Encarsia* (Taiwan)	3.0 ± 0.9	e	5.0 ± 1.3	b	10

Nonlinear regressions. Using the pooled data from both trials, a nonlinear regression was performed on the total number of hosts as a predictor for numbers attacked. The form of equation chosen was a Type II functional response (Hassell 1978):

$$N_a = N_t \left[1 - \exp\left\{ - \frac{a'TP_t}{1 + a'T_h N_t} \right\} \right]$$

where N_a is the number of hosts attacked, N_t is the number of hosts available, a' is the searching efficiency, P_t is number of parasitoids searching, T is the searching time and T_h is the handling time required to attack the host. N_t was estimated by using the total of parasitized and unparasitized hosts; P_t was set to two parasitoids and T was set to two days. A significant fit was found ($F = 46.3$; $df = 2, 170$; $r^2 = 0.35$; $P < 0.01$) which yielded an estimate of 0.0793 for a' and 0.19 for T_h. The numbers of hosts attacked and the disc equation estimate are shown in Fig. 1A.

A simple exponential decay function was used to estimate total number of hosts as a predictor for percentage parasitism (arc sin transformed). The equation used was:

$$Y = c \exp(\alpha N_t)$$

where N_t again is total hosts available estimated by using the sum of parasitized and unparasitized hosts, and c and a are constants. The regression yielded estimates of 23.35 for c and -0.0033 for a ($F = 84.7$; $df = 2, 170$; $r^2 = 0.5$; $P < 0.01$). Arc sin percentage parasitism and the exponential decay equation are shown together in Fig. 1B.

Although the experimental protocol was identical for both trials, it appears that the number of hosts available was higher in the second. Host density may have a significant effect on attack rates as measured using both number of hosts attacked and percentage parasitism. Higher host densities may result in higher numbers of hosts attacked but lower percentages of parasitism. Because host densities cannot be held constant across different trials, the interpretation of results across trials must be made with caution.

In summary, the method of evaluating the performance of the parasitoids produced reasonably consistent results as similar results were obtained in both trials. The data indicate highly significant differences in both numbers of hosts attacked and in percentages of parasitism among the different strains, but no strong evidence was found to indicate that *Eretmocerus* species are better parasitoids of *B. tabaci* than *Encarsia* species, or vice versa. Obviously, a limited set of experiments such as those described are insufficient to select a single "best" strain from among those tested and laboratory evaluations such as those described may be poor indicators of success in the field. Any general conclusions must take into consideration the experimental conditions used because other strains may perform better using different host plants or temperature conditions, for example. Performance may also be affected by interspecific competition, especially against the local parasitoid complex. Furthermore, we were unable to measure host feeding which is clearly an important factor in determining the efficacy of a parasitoid of *B. tabaci.* With these limitations, the evaluations indicate that the *Encarsia* nr. *pergandiella* from Brazil (M94055) appears to be the most promising parasitoid of *B. tabaci* in melons. This strain and others which performed well in these evaluations merit further consideration using different crops and experimental conditions and may prove effective in mass release field trials.

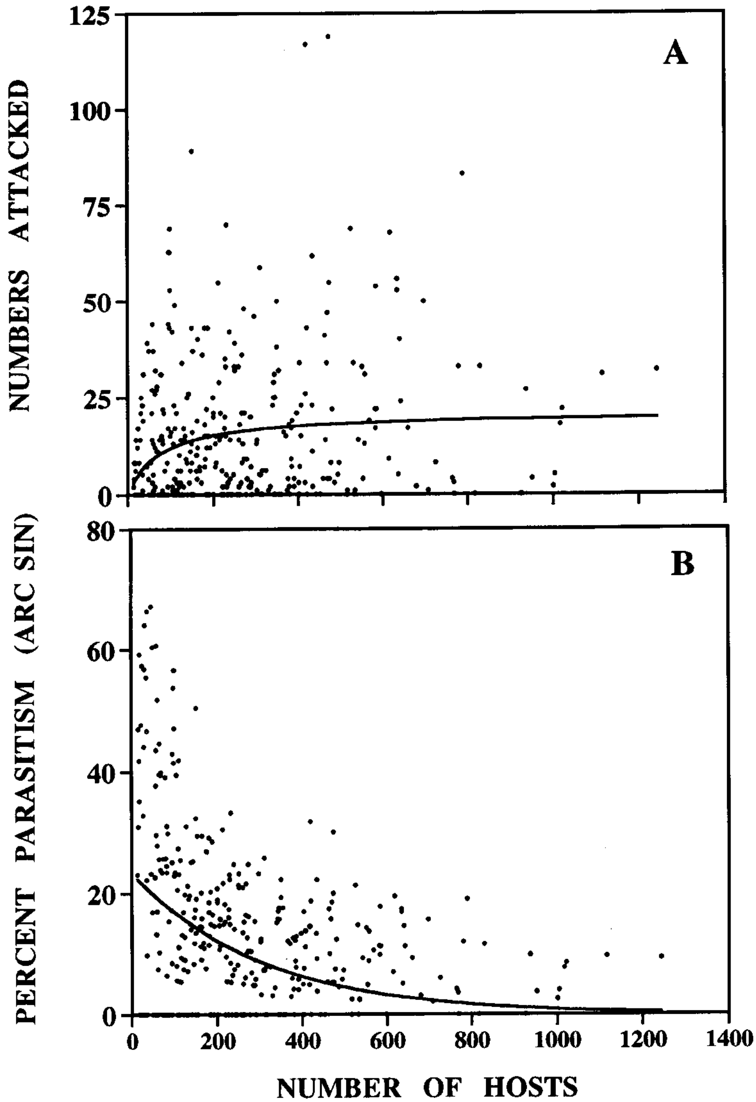


FIG 1. Nonlinear regressions of host numbers as predictors of number of hosts attacked and percentage parasitism (arc sin transformation). A) Number of hosts attacked is described by a Type II functional response equation where a' = 0.0793, Th = 0.19, T = 2, Pt = 2 ($F = 46.3$; df = 2, 170; $r^2 = 0.35$; $P < 0.01$). B) Percentage parasitism was described using an exponential decay function: $Y = 23.35 \exp(-0.0033 N_t)$ ($F = 84.7$; df = 2, 170; $r^2 = 0.5$; $P < 0.01$).

ACKNOWLEDGMENT

We thank R. Carruthers, J. Gould, C. Pickett, G. Simmons, L. Wendel, R. N. Huettel and an anonymous reviewer for useful comments on this manuscript. Technical assistance was provided by J. Hadman, J. Rivera, R. Sheetz, P. Silva and M. Torres. Approved for publication by the Texas Agricultural Experiment Station.

LITERATURE CITED

Baumgartner, J., and E. Yano. 1990. Whitefly population dynamics and modelling. pp. 123-146. *In* D. Gerling [ed.] Whiteflies; Their Bionomics, Pest Status and Management. Intercept Ltd. Andover, Hants., UK.

Brown, J. K., H. S. Costa, and F. Laemmlen. 1992. First report of whitefly-associated squash silverleaf disorder of *Cucurbita* in Arizona and of white streaking disorder of *Brassica* species in Arizona and California. Plant Dis. 76:426.

Byrne, D. N., T. S. Bellows and M. P. Parrella. 1990. Whiteflies in agricultural systems, pp. 227-262. *In* D. Gerling [ed.] Whiteflies; Their Bionomics, Pest Status and Management. Intercept Ltd., Andover, Hants., UK.

Cock, M. J. W. 1986. *Bemisia tabaci* - A Literature Survey on the Cotton Whitefly with an Annotated Bibliography - FAO, CAB IIBC. 121 pp.

Cock, M. J. W. 1993. *Bemisia tabaci* - An Update on the Cotton Whitefly with an Annotated Bibliography - IIBC, CSC, FAO, IICA. 78 pp.

Dittrich, V., S. Uk, and G. H. Ernst. 1990. Chemical control and insecticide resistance of whiteflies, pp. 263-286. *In* D. Gerling [ed.] Whiteflies; Their Bionomics, Pest Status and Management. Intercept Ltd., Andover, Hants., UK.

Faust, Robert M. 1992. Conference Report and 5-Year National Research and Action Plan for Development of Management and Control Methodology for the Sweetpotato Whitefly. Houston, Texas, February 18-21, 1992. USDA-ARS, ARS-107, 165 pp.

Hassell, M. P. 1978. The Dynamics of Arthropod Predator-Prey Systems. Monographs in Population Biology No. 13. Princeton Univ. Press. 237 pp.

Perring, T. M., A. D. Cooper, R. J. Rodriguez, C. A. Farrar, and T. S. Bellows, Jr. 1993. Identification of a whitefly species by genomic and behavioral studies. Science. 259: 74-77.

Wilkinson, L., M. Hill, and E. Vang. 1992. SYSTAT: Statistics, Version 5.2 Edition. Evanston, IL. Systat, Inc. 724 pp.

ACTIVITY PATTERNS OF ADULT MEXICAN RICE BORERS (LEPIDOPTERA: PYRALIDAE) IN SUGARCANE

D. W. Spurgeon,[1] T. N. Shaver, J. R. Raulston,[2] and P. D. Lingren

Crop Insect Pests Management Research Unit, USDA-ARS, College Station, TX 77845

ABSTRACT

Nocturnal collections of *Eoreuma loftini* (Dyar) adults in sugarcane were used to evaluate the effects of synthetic pheromone applications on nightly activity patterns. The pheromone treatment differentially influenced captures of the sexes; usually, more females were collected from treated than untreated plots while the opposite trend was observed for males. More flying than sitting moths were captured in both pheromone treated and untreated plots. Peaks of female activity occurred early in the night (2200-2400 h CDT) while males were most active late in the night (0400-0600 h). Pheromone treatment did not influence nightly collection patterns of flying or sitting females or sitting males. Although a change in the collection pattern of flying males in response to the pheromone was demonstrated, differences were small relative to the overall nightly pattern of collection. These results indicate that optimal sampling times for *E. loftini* adults differ according to moth sex. This information will be useful in developing and evaluating control strategies involving mating disruptants, attractants, or adulticides.

INTRODUCTION

The Mexican rice borer, *Eoreuma loftini* (Dyar), was first detected in the Lower Rio Grande Valley in 1980 (Johnson 1984) and has become the key pest of sugarcane, *Saccharum* spp., in the region (Johnson 1984, Meagher et al. 1994). *E. loftini* larval populations and associated damage can be reduced by multiple, prophylactic insecticide treatments (Johnson 1985, Meagher et al. 1994), but such treatments are economically unsatisfactory because of the high costs of multiple applications and reductions in sugarcane yield and quality that occur despite intensive control measures (Ring et al. 1991). The limitations presented by conventional insecticidal control strategies have promoted efforts to investigate alternative methods, such as mating disruption using synthetic pheromones (Shaver and Brown 1993); however, proper design and evaluation of these strategies requires a thorough understanding of *E. loftini* adult behavior and biology.

Most information regarding *E. loftini* adult behavior has been obtained from laboratory studies. Nightly patterns of calling and mating by females, and responses of males to ovipositor extracts were investigated by Brown et al. (1988). Spurgeon et al.

---

[1]Present address: USDA, ARS, Crop Insects Research Unit, 2413 E Highway 83, Weslaco, TX 78596.

[2]USDA, ARS, Crop Insects Research Unit, 2413 E Highway 83, Weslaco, TX 78596.

(1995a) further examined effects of moth age on these patterns. Nightly adult emergence patterns, oviposition patterns, and the potential for multiple mating have also been defined in the laboratory (Spurgeon et al. 1995b, 1995c, Shaver et al. 1994). Field observations of *E. loftini* adult behavior patterns have been limited to observations of activity patterns of females and male responses to traps (Shaver et al. 1994) and evaluations of pheromone trap designs (Shaver et al. 1991). Our objectives were to define the nightly activity patterns of *E. loftini* adults, and investigate the effects of synthetic pheromone on these patterns in Lower Rio Grande Valley sugarcane.

MATERIALS AND METHODS

Pheromone-treated and untreated areas of 4 ha each were established at west and east ends, respectively, of a 15-ha field of sugarcane (cultivar '321'). These areas were separated by >200 m of untreated sugarcane, and oriented so the treated area was normally downwind from the untreated area. Each area was divided into four sections, each section 21 rows wide (32 m) by about 320 m long. Sections were separated by an alley row in which stalks were cut and subsequently maintained at a height of 0.5-1.0 m. Sampling was conducted in the alleys which were each divided into four 79-m-long plots, giving a total of 12 plots in each area. Plots were considered replicates.

Pheromone components were obtained from Bedoukin Research, Danbury, CT, and impregnated into rubber chips (each about 34 mg and with about 45 mm^2 of surface area) by soaking the chips for 2 h in a hexane solution of pheromone [90 mg pheromone, 8.0:1.0:1.3 (Z)-13-octadecenyl acetate:(Z)-11-hexadecenyl acetate:(Z)-13-octadecenal, per ml hexane]. Excess solution was decanted and the chips were air dried. Finished chips contained 13.5% pheromone (W/W). Pheromone-impregnated chips were aerially applied to the treated area at a rate of 99 g AI (approximately 21,500 chips) in 3,740 ml 'Biotac' polybutene-based adhesive (Scentry, Buckeye, AZ) per ha on 12 May and again on 8 July 1992 using Scentry 'Nomate' application equipment.

Adult *E. loftini* populations were sampled one to three nights per week from 11 May (a single pre-treatment sample) to 12 August, using headlamps and sweepnets. Each plot was sampled for 10 min of each hourly sampling period, with the first period beginning shortly after dusk (2100 h CDT) and the last period beginning about an hour before dawn (0500 h). Samplers were randomly assigned to treatments and alleys each hour. Ordinary headlamps were used during 26 nights. Samplers scanned the alleys and adjacent plants for moths and attempted to collect each moth observed. Because of the presence of other species of small Lepidoptera in the plots, moths that escaped could not be positively identified and were not recorded. Collected moths were placed in 29.5-ml plastic cups each closed with a paper lid and labeled with the time of collection, treatment, plot, collector, and moth activity at the time of detection. Moth activity was designated as flying or sitting because other activities (calling, mating, feeding, or ovipositing) were difficult to monitor accurately or were observed very rarely. On five additional nights (29 July and 3, 5, 10, and 12 August), red cellophane filters were used to cover the headlamp lenses to minimize moth response to the lights, and only sitting moths were collected. Captured moths were subsequently sexed in the laboratory.

Because both days of collection and nightly sampling periods represented repeated observations of the experimental units (plots), data were analyzed by repeated measures analysis of variance using the "REPEATED" statement in the SAS procedure GLM (SAS Institute 1988). Data collected on 11 May (pre-treatment), and while using red-filtered headlamps, were analyzed separately. In each analysis a sphericity test was applied to each repeated effect and their interactions to determine if the data satisfied the Huynh-Feldt condition (SAS Institute 1988). When the Huynh-Feldt condition was accepted ($P>0.05$),

the repeated effects (day and period) and associated interactions were assessed using the usual univariate F tests. When the Huynh-Feldt condition was rejected at $0.05 > P > 0.0001$, or when multivariate analyses could not be performed because of insufficient degrees of freedom, probability levels adjusted by the Greenhouse-Geisser epsilon (G-G adjusted P) were used for hypothesis testing. When the Huynh-Feldt condition was rejected at $P \leq 0.0001$, or a sphericity test could not be conducted because of a determinant <0 or insufficient error degrees of freedom, multivariate tests (Wilk's Lambda) were used to test the significance of repeated factors and their associated interactions. Because significant interactions were often difficult to interpret, additional analyses were conducted on subsets of the data (by sex, and by sex and activity) to facilitate their interpretation.

RESULTS AND DISCUSSION

Pre-treatment Collections. Seventy moths were collected on the night before the pheromone treatment was applied (11 May). Collections contained more males (64) than females (6) (F=45.68; df=1, 88; P<0.01), more flying (63) than sitting moths (7) (F=42.59; df=1, 88; P<0.01), and more moths in the plots designated as untreated (44) than in the plots to be treated (26) (F=4.40; df=1, 88; P=0.039). A significant sex by activity interaction (F=42.59, df=1, 88; P<0.01) was observed because most of the males collected (94%) were flying compared with only half of the females. A marked periodicity in captures was observed (Wilk's Lambda=0.457; F=12.05; df=8, 81; P<0.01). Significant interactions between periodicity and sex, activity, and sex and activity were also observed.

Although all females were captured within the first 4 h of sampling, collections were too small to reliably demonstrate periodicity in the captures (Wilk's Lambda=0.898; F=1.17; df=4, 41; P=0.338). Further, no significant ($P \leq 0.05$) interactions between period and any other factors were observed. Periodicity in male captures was observed (Wilk's Lambda=0.293; F=19.28; df=5, 40; P<0.01) with most (97%) being caught in the last three sampling periods (0300 - 0600 h) (Fig. 1). The period by activity interaction indicated differences between activities (Wilk's Lambda=0.349; F=14.95; df=5, 40; P<0.01); periodicity of capture was signficant for flying males (Wilk's Lambda=0.167; F=23.67; df=4, 19; P<0.01), but not for sitting males (Wilk's Lambda=0.857; F=1.11; df=3, 20; P=0.368).

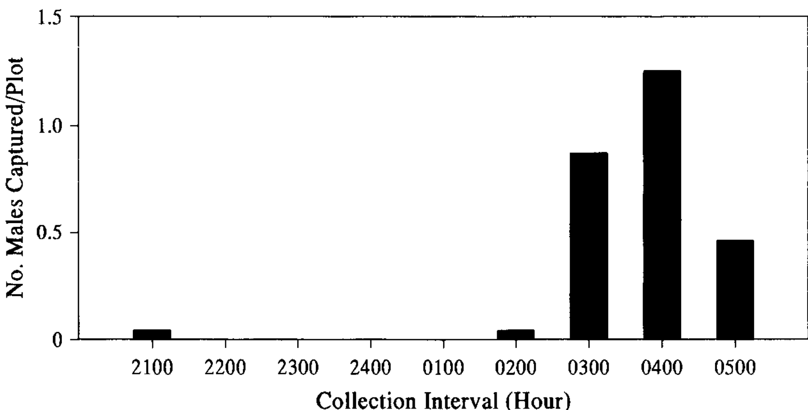


FIG. 1. Periodicity of pre-treatment captures of *E. loftini* males in sugarcane.

Collections with Standard Headlamps. During 25 nights of collecting, 8,315 moths were captured. Nightly captures ranged from 35 moths on 12 May to 563 moths on 13 July, but generally increased between mid-May and mid-June (Wilk's Lambda=0.013; F=213.59; df=24, 65; P<0.01). More males (6,858) were captured than females (1,457) (F=876.81; df=1, 88; P<0.01) and more flying moths (7,201) were captured than sitting moths (1,114) (F=1,113.69; df=1, 88; P<0.01), but overall captures were not signficantly different between untreated (4,270) and treated plots (4,045) (F=1.52; df=1, 88; P=0.221). A significant periodicity of capture was observed (Wilk's Lambda=0.025; F=396.20; df=8, 81; P<0.01), but most interaction terms were also highly significant (P<0.01), indicating that patterns of capture and other main effects were not the same for all combinations of sex, activity, and treatment.

Analyses of subsets of the data indicated that more females were captured in the pheromone treated (826) than the untreated plots (631) (F=23.43; df=1, 44; P<0.01), and more flying females (961) were captured than sitting females (496) (F=133.24; df=1, 44; P<0.01). Although the activity by treatment interaction was significant (F=5.10; df=1, 44; P=0.029) the difference between treatments in the proportion of captured females that were flying was small (pheromone treated, 67%; untreated, 65%). Periodicity in female captures was observed (Wilk's Lambda=0.063; F=68.26; df=8, 37; P<0.01), and the period by treatment (Wilk's Lambda=0.810; F=1.09; df=8, 37; P=0.393) and period by activity by treatment (Wilk's Lambda=0.785; F=1.27; df=8, 37; P=0.291) interactions indicated that patterns of hourly captures of females were not affected by the pheromone treatment. However, a significant period by activity interaction (Wilk's Lambda=0.555; F=3.71; df=8, 37; P<0.01) indicated differences in periodicity of captures between activities. Despite evidence of differences in patterns of capture of flying and sitting females, highest capture rates generally occurred during the second and third collecting periods (2200-2400 h), and lowest captures during the last two periods (after 0400 h), regardless of female activity (Fig. 2). The day by period interaction was also significant for both flying (F=3.24; df=192, 4,224; G-G adjusted P<0.01) and sitting (F=2.13; df=192, 4,224; G-G adjusted P<0.01) females, indicating variation in the pattern of capture among days (Fig. 2).

Analyses of male collections indicated that slightly more males were captured in the untreated (3,639) than pheromone treated plots (3,219) (F=5.57; df=1, 44; P=0.022), and more flying males (6,240) were captured than sitting males (618) (F=998.75; df=1, 44; P<0.01). A significant activity by treatment interaction (F=5.79; df=1, 44; P=0.020) indicated that the effects of the pheromone treatment on male captures varied with activity type. Slightly more flying males were collected in the untreated (3,332) than in the pheromone treated plots (2,908) (F=6.11; df=1, 22; P=0.022), but captures of sitting males were similar between treatments (pheromone treated, 311; untreated, 307; F=0.01; df=1, 22; P=0.933).

A strong pattern was observed in the male captures (Wilk's Lambda=0.009; F=527.96; df=8, 37; P<0.01), but significant period by activity (Wilk's Lambda=0.009; F=485.10; df=8, 37; P<0.01) and period by treatment (Wilk's Lambda=0.415; F=6.53; df=8, 37; P<0.01) interactions indicated that the patterns of capture varied among combinations of activity and treatment. Captures of flying males tended to be low for the first six collecting periods (2100-0300 h), and then increased each hour until sampling was terminated (Wilk's Lambda=0.003; F=558.54; df=8, 15; P<0.01). The period by treatment interaction (Wilk's Lambda=0.183; F=8.39; df=8, 15; P<0.01) indicated that treatment affected the periodicity of capture of flying males, but those effects were very subtle. Over 70% of all flying males were collected during the last two collection periods (0400-0600 h) regardless of treatment. Only 13.9 and 17.5% of males were collected

during the first through sixth collection periods (2100-0300 h) in the untreated plots and pheromone treated plots, respectively (Fig. 3). Differences among days in the patterns of capture were indicated by the day by period interaction (F=13.28; df=192, 4,224; G-G adjusted P<0.01), but these differences were small relative to the general trend in periodicity of capture (Fig. 3).

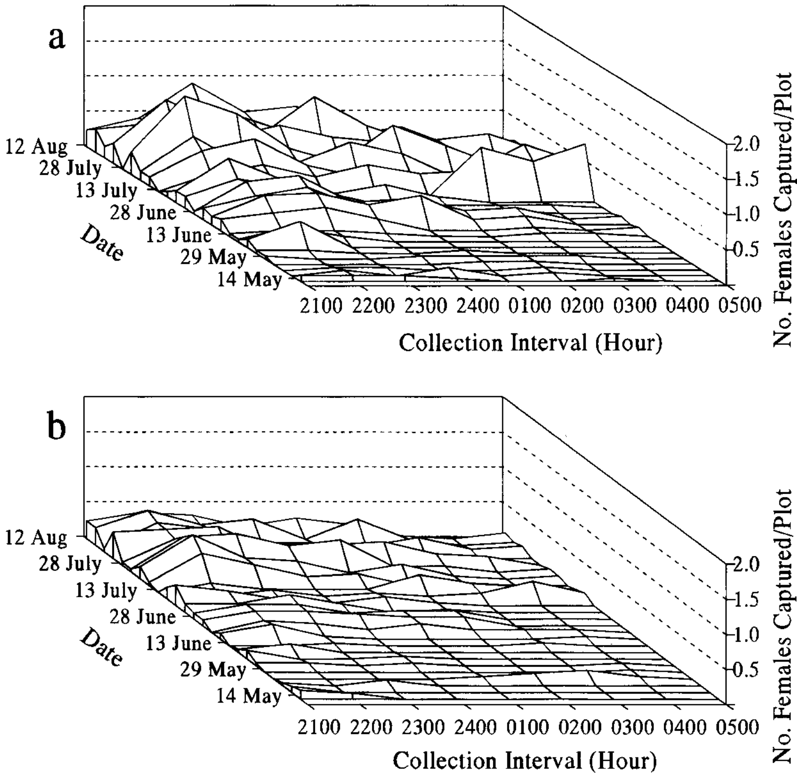


FIG. 2. Periodicity of (a) flying and (b) sitting *E. loftini* females captured in sugarcane using standard headlamps.

Periodicity of capture of sitting males was also significant (F=4.36; df=8, 176; G-G adjusted P<0.01) and not affected by treatment (period by treatment; F=0.41; df=8, 176; G-G adjusted P=0.818) or day of collection (day by period interaction; F=1.50; df=192, 4224; G-G adjusted P=0.102). Peak captures of sitting males tended to occur during the sixth to eighth collection periods (0200-0500 h). While statistically significant, patterns of capture of sitting males were not well defined (Fig. 4) relative to other demonstrated patterns of capture (Fig. 2, Fig. 3).

Collections with Red-filtered Headlamps. During five nights of sampling, 757 moths were captured (122-193 moths per night). More males (491) were collected than females (266) (F=43.63; df=1, 44; P<0.01), and more moths were collected from pheromone treated (433) than untreated plots (324) (F=10.24; df=1, 44; P<0.01). The sex by treatment interaction (F=3.42; df=1, 44; P=0.071) indicated that effects of treatment were similar for both sexes.

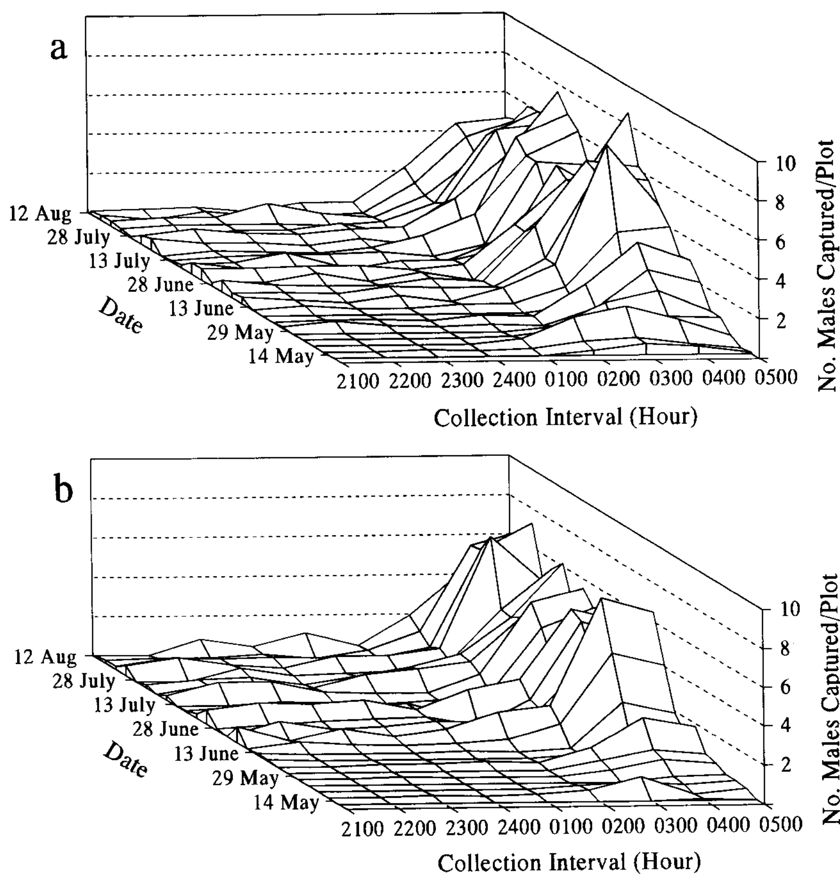


FIG. 3. Periodicity of flying *E. loftini* males captured in (a) untreated and (b) pheromone treated sugarcane using standard headlamps.

Periodicity in captures was indicated in the initial analysis (Wilk's Lambda=0.227; F=15.71; df=8, 37; P<0.01), but the period by sex interaction (Wilk's Lambda=0.180; F=21.11; df=8, 37; P<0.01) indicated the pattern of capture differed between sexes. The period by treatment interaction (Wilk's Lambda=0.931; F=0.342; df=8, 37; P=0.943) failed to demonstrate any effect of pheromone treatment on patterns of capture. Hourly captures of females combined over nights ranged from 21-36 moths and significant periodicity of captures was not observed (F=0.99; df=8, 176; P=0.444). Periodicity of male captures was detected (F=20.08; df=8, 176; G-G adjusted P<0.01) and was unaffected by the pheromone treatment (period by treatment interaction; F=0.74; df=8, 176; G-G adjusted P=0.588). Captures of males were greatest during the seventh and eighth sampling periods (0300-0500 h); 61% were caught in the last three periods (0300-0600 h). Periodicity of male captures also varied somewhat among days (day by period interaction; F=2.08; df=32, 704; P=0.023) (Fig. 5).

Several factors influenced the interpretation of our data, including the absence of true statistical replication in our experimental design. It is often impractical to replicate broadcast treatments of pheromone or other attractants because of the special nature of these

treatments; thus, field studies of effects of synthetic pheromone on mating disruption or behavior commonly involve pseudoreplicated experimental designs (e.g., Curtis et al. 1985, Flint et al. 1985, Kehat et al. 1986, McLaughlin et al. 1994, Moffitt and Westigard 1984, Shaver and Brown 1993, Suckling and Shaw 1992, Taschenberg and Roelofs 1977, Zvirgydins et al. 1984). Pheromones are typically volatile and depend on bulk air movement for their dispersal and the experimental subjects (insects) are frequently mobile. Evaluation of these treatments requires careful spatial arrangement of treated and untreated areas, and study arenas large enough to minimize movement between these areas or to ensure temporary restriction of the experimental subjects to respective treatment areas. The expenses and difficulties associated with simultaneous treatment and sampling of spatially distant, multiple study sites, rather than the use of subsamples (plots) as replicates, are often prohibitive.

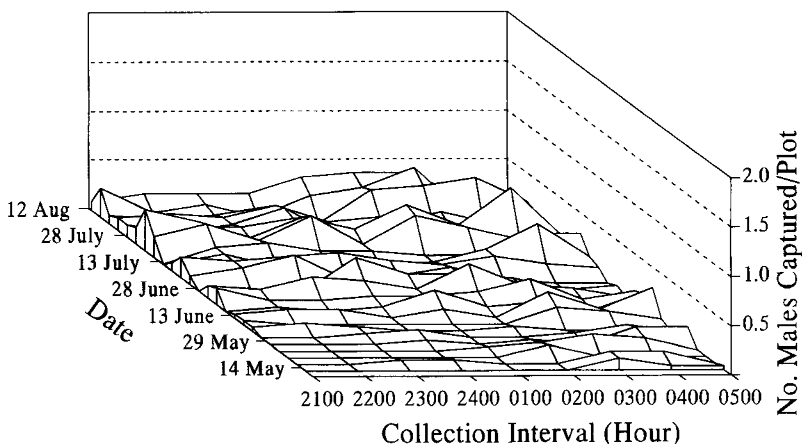


FIG. 4. Periodicity of sitting *E. loftini* males captured in sugarcane using standard headlamps.

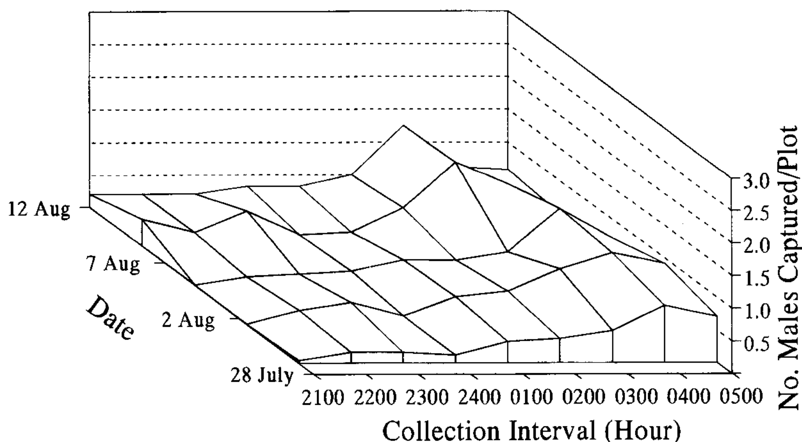


FIG. 5. Periodicity of *E. loftini* males captured in sugarcane using red-filtered headlamps.

Interpretation of the data was also affected by potential limitations in our sampling scheme. We sampled from fixed sites (alleys) to overcome difficulties associated with observing and collecting mobile insects from a crop characterized by extremely dense stands of tall plants. Consequently, collections of sitting insects were limited to individuals undetected during a previous sampling period or captured after moving to alleys between sampling periods. Moths captured while sitting were therefore likely to have flown recently.

A further constraint to interpretation of the data arose from the preponderance of flying males during the later sampling periods. The availability of large numbers of flying males, some of which appeared to be attracted to the headlamps, probably diminished samplers' abilities to search for sitting insects. Sample dates involving the use of apparently less attractive red-filtered headlamps were included to assess the impact of this factor.

Pre-treatment samples were of limited usefulness because of the low magnitude of *E. loftini* populations, except that patterns of male capture and relative magnitudes of male versus female and flying versus sitting moths were similar to those observed after pheromone application. The low magnitudes of pre-treatment populations and the lack of true replication precluded an accurate assessment of the effects of pheromone treatment on the relative sizes of *E. loftini* populations.

The apparent effects of the pheromone treatment on collections were opposite for the sexes, indicating that the pheromone differentially influenced moth behavior, and consequently our ability to collect male and female moths. More females were generally collected from treated than untreated plots regardless of sampling method. This increase was probably a consequence of a slight behavioral change in response to the pheromone; females have been observed to call for mates from more exposed sites in pheromone-treated than in untreated plots (D.W.S., unpublished data). Effects of the pheromone treatment on male captures were more difficult to interpret. While captures of flying males tended to be greater in untreated than treated plots, captures of sitting males were not different between treatments regardless of collection method. Although our data demonstrate subtle behavioral effects of pheromone treatments on flying males, they are not sufficient to determine whether observed differences resulted from differences in population levels or differences in respective proportions of time spent flying and sitting.

The lack of replication posed fewer problems in interpretation of activity patterns. Collections consistently indicated more flying than sitting moths. Sampling from fixed alleys may have slightly biased collections in favor of flying moths, but more importantly, *E. loftini* are very cryptic when resting, and flying moths are much more apparent to samplers. The greater apparency of flying moths and the availability of large numbers of flying males in the later sampling periods may have biased collections in favor of flying moths. Therefore, activity patterns indicated from collections of flying moths are probably more reliable than those from collections of sitting moths. This conclusion is supported by the observation that activity patterns for sitting females differed between light sources; periodicity in captures was observed with standard headlamps, but not with red-filtered headlamps. Also, periodicity of sitting males, regardless of light source, was poorly defined relative to that of flying males or females.

Patterns of collection for flying moths indicated that females were most active during early sampling periods while males were most active during late periods. Our observations of female activity patterns are consistent with those reported by Shaver et al. (1994). Peak captures of males corresponded to time periods when males respond to ovipositor extracts (Brown et al. 1988), pheromone traps (Shaver et al. 1994), and calling females (Spurgeon et al. 1995a). Therefore, mate seeking was probably responsible for activity peaks of flying males.

Periodicity of female activity was not affected by the pheromone treatment. While

the pheromone resulted in a detectable change in male activity pattern, actual differences between treatments were small and of little practical consequence. Variations in activity patterns of both males and females among days may have been caused by differences in conditions such as temperature or wind speed. In addition, Spurgeon et al. (1995a) found that moth age affected temporal patterns of female calling and associated male response. Therefore, changes in the age distributions of field populations could have resulted in subtle changes in observed activity patterns. Regardless of their causes, day to day variations in activity patterns were relatively small during our study.

Our data describe in detail the activity patterns of *E. loftini* adults. These results and our observations concerning techniques and potential sources of error in monitoring *E. loftini* populations should be useful in maximizing efficiency of studies requiring collection of adults, and in developing and evaluating control strategies involving mating disruptants, attractants, or adulticides.

ACKNOWLEDGMENT

We express our appreciation to T. W. Popham, Biometrician (USDA-ARS, Stillwater, OK) for his assistance in analyzing the data. This research was funded in part by Consep Membranes, Inc., Bend, OR, and Rio Grande Valley Sugar Growers, Inc., Santa Rosa, TX. This article reports the results of research only. Mention of a proprietary product does not constitute an endorsment for its use by USDA.

LITERATURE CITED

Brown, H. E., L. T. Wood, T. N. Shaver, and J. Worley. 1988. Behavioral responses of male *Eoreuma loftini* (Lepidoptera: Pyralidae) to ovipositor extracts. J. Econ. Entomol. 81: 184-188.

Curtis, C. E., P. J. Landolt, and J. D. Clark. 1985. Disruption of navel orangeworm (Lepidoptera: Pyralidae) mating in large-scale plots with synthetic sex pheromone. J. Econ. Entomol. 78: 1425-1430.

Flint, H. M., J. R. Merkle, and A. Yamamoto.1985. Pink bollworm (Lepidoptera: Gelechiidae): field testing a new polyethylene tube dispenser for gossyplure. J. Econ. Entomol. 78: 1431-1436.

Johnson, K.J.R. 1984. Identification of *Eoreuma loftini* (Dyar) (Lepidoptera: Pyralidae) in Texas, 1980: forerunner for other sugarcane boring pest immigrants from Mexico? Bull. Entomol. Soc. Am. 30: 47-52.

Johnson, K.J.R. 1985. Seasonal occurrence and insecticidal suppression of *Eoreuma loftini* (Lepidoptera: Pyralidae) in sugarcane. J. Econ. Entomol. 78: 960-966.

Kehat, M., E. Dunkelblum, S. Gothilf, N. Bar Shavit, D. Gordon, and M. Harel. 1986. Mating disruption of the Egyptian cotton leafworm, *Spodoptera littoralis* (Lepidoptera: Noctuidae), in cotton with a polymeric aerosol formulation containing (*Z,E*)-9,11-tetradecadienyl acetate. J. Econ. Entomol. 79: 1641-1644.

McLaughlin, J. R., E. R. Mitchell, and P. Kirsch. 1994. Mating disruption of diamondback moth (Lepidoptera: Plutellidae) in cabbage: reduction of mating and suppression of larval populations. J. Econ. Entomol. 87: 1198-1204.

Meagher, R. L., Jr., J. W. Smith, Jr., and K.J.R. Johnson. 1994. Insecticidal management of *Eoreuma loftini* (Lepidoptera: Pyralidae) on Texas sugarcane: a critical review. J. Econ. Entomol. 87: 1332-1344.

Moffitt, H. R. and P. H. Westigard. 1984. Suppression of the codling moth (Lepidoptera: Tortricidae) population on pears in southern Oregon through mating disruption with sex pheromone. J. Econ. Entomol. 77: 1513-1519.

Ring, D. R., H. W. Browning, K.J.R. Johnson, J. W. Smith, Jr., and C. E. Gates. 1991. Age-specific susceptibility of sugarcane internodes to attack by the Mexican rice borer (Lepidoptera: Pyralidae). J. Econ. Entomol. 84: 1001-1009.

SAS Institute. 1988. SAS user's guide: statistics, version 6.03 ed. SAS Institute, Cary, NC.

Shaver, T. N., H. E. Brown, J. W. Bard, T. C. Holler, and D. E. Hendricks. 1991. Field evaluations of pheromone-baited traps for monitoring Mexican rice borer (Lepidoptera: Pyralidae). J. Econ. Entomol. 84: 1216-1219.

Shaver, T. N., and H. E. Brown. 1993. Evaluation of pheromone to disrupt mating of *Eoreuma loftini* (Lepidoptera: Pyralidae) in sugarcane. J. Econ. Entomol. 86: 377-381.

Shaver, T. N., H. E. Brown, and J. R. Raulston. 1994. Mating potential of feral and laboratory-reared *E. loftini* (Lepidoptera: Pyralidae). J. Econ. Entomol. 87: 1213-1216.

Spurgeon, D. W., P. D. Lingren, J. R. Raulston, and T. N. Shaver. 1995a. Age-specific mating activities of Mexican rice borers (Lepidoptera: Pyralidae). Environ. Entomol. 24: 105-109.

Spurgeon, D. W., P. D. Lingren, J. R. Raulston, and T. N. Shaver. 1995b. Pupal development and adult emergence patterns of the Mexican rice borer (Lepidoptera: Pyralidae). Environ. Entomol. 24: 76-79.

Spurgeon, D. W., P. D. Lingren, T. N. Shaver, and J. R. Raulston. 1995c. Realized and potential fecundity of the Mexican rice borer (Lepidoptera: Pyralidae) as a function of pupal weight. Environ. Entomol. 24: 94-98.

Suckling, D. M., and P. W. Shaw. 1992. Conditions that favor mating disruption of *Epiphyas postvittana* (Lepidoptera: Tortricidae). Environ. Entomol. 21: 949-956.

Taschenberg, E. F., and W. L. Roelofs. 1977. Mating disruption of the grape berry moth, *Paralobesia viteana*, with pheromone released from hollow fibers. Environ. Entomol. 6: 761-763.

Zvirgydins, A., P. D. Lingren, T. J. Henneberry, C. E. Nowell, and J. M. Gillespie. 1984. Mating disruption of a wild population of tobacco budworm (Lepidoptera: Noctuidae) with virelure. J. Econ. Entomol. 77: 1464-1469.

TRI-TROPHIC INTERACTIONS AND PREDATION RATES IN *CHRYSOPERLA* SPP.[1] ATTACKING THE SILVERLEAF WHITEFLY[2,3]

Jesusa Crisostomo Legaspi[4], Donald A. Nordlund and Benjamin C. Legaspi, Jr.

Biological Control of Pests Research Unit, USDA, ARS
Subtropical Agricultural Research Laboratory, 2413 East Highway 83
Weslaco, TX 78596

ABSTRACT

We investigated tri-trophic interactions among the host plant, the silverleaf whitefly (SLWF), *Bemisia argentifolii* Bellows and Perring, and the predatory lacewings *Chrysoperla rufilabris* (Burmeister) and *C. carnea* (Stephens). *B. argentifolii* females avoided ovipositing on leaves on which *C. rufilabris* larvae were previously located. This tendency appeared to increase with increasing exposure time of the predators to the leaves. We measured the effects of host plant on body weight, developmental duration and survival of the lacewing. SLWF reared on cantaloupes and cucumber appeared to be better quality prey than those reared on poinsettia or lima bean. Lacewings that fed on SLWF reared on cucumbers and cantaloupes developed more rapidly, showed increased survival, and weighed more as newly-emerged adults, compared to those reared on poinsettia and lima bean. Lacewings feeding on SLWF reared on poinsettia and lima bean did not survive to the pupal stage. We concluded that SLWF reared on poinsettia or lima bean may have been nutritionally inadequate for *C. rufilabris* development, or that SLWF may have accumulated plant compounds which were detrimental to the development of the lacewings. There was little difference in predation rates between larvae of *C. carnea* and *C. rufilabris*, although *C. carnea* may consume significantly more whiteflies during certain intervals. Both species consumed from 25 to 75 SLWF daily.

INTRODUCTION

The silverleaf whitefly (SLWF), *Bemisia argentifolii* Bellows and Perring (Homoptera: Aleyrodidae) [sweetpotato whitefly, *Bemisia tabaci* (Gennadius) Biotype "B"] caused crop losses estimated at over $500 million in 1991 (Perring et al. 1993). Crop losses due to this pest in the Imperial Valley of California alone from 1991 to 1994 were estimated at over $300 million (Birdsall et al. 1995). SLWF causes crop loss by direct feeding on phloem, vectoring viral plant pathogens, and by the production of honeydew exudate which is a medium for the growth of sooty mold fungi. SLWF also has a relatively high reproductive potential and a wide host range. Chemical control of SLWF is often insufficient because of insecticide resistance (Dittrich et al. 1990) and because the pest is often situated on the undersides of leaves where insecticides are difficult to apply.

Of the known predators, *Chrysoperla (= Chrysopa) rufilabris* (Burmeister) and *C. carnea* (Stephens) (Chrysopidae) are available commercially. Elkarmi et al. (1987) compared

[1] Neuroptera: Chrysopidae

[2] Homoptera: Aleyrodidae

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

[4] Current address: Texas Agricultural Experiment Station, 2415 East Hwy 83, Weslaco, TX 78596

life histories of the two predators to assess the possibility of mass rearing and also described much of the biology and life history of *C. rufilabris*. Breene et al. (1992) released first and second-instar *C. rufilabris* larvae against SLWF on *Hibiscus rosa-sinensis* L. in the greenhouse and found that releases of 25 or 50 larvae per plant at two-week intervals maintained the plants in marketable condition. Legaspi et al. (1994) studied prey preference and the effect of diet on development of *C. rufilabris* larvae provided SLWF, and a variety of diets, including lepidopteran eggs, aphids and an artificial diet. Lacewing larvae consumed an average of 532 SLWF (mostly eggs) daily, but showed increased survival and development when fed *Sitotroga cerealella* (Olivier) (Gelechiidae) or *Helicoverpa zea* (Boddie) (Noctuidae) eggs.

This is a report of our investigations of the possibility that *C. rufilabris* larvae release materials that reduce oviposition by SLWF. We also investigated the effect of host plant on development, survival and body weight of *C. rufilabris,* and we compared predation rates of *C. rufilabris* and *C. carnea.*

MATERIALS AND METHODS

C. rufilabris larvae were obtained from the USDA-ARS Biological Control of Pests Research Unit rearing facility at Weslaco, TX, and from the Rincon-Vitova Insectary (Oak View, California). Larvae were maintained following the methods described by Nordlund and Morrison (1992). *S. cerealella* eggs used for feeding *C. rufilabris* were also obtained from Rincon-Vitova. The experiments were conducted in an environmental growth chamber at 27°C, 50-60% RH and 14:10 L:D photoperiod, except where noted.

Oviposition deterrents of C. rufilabris. To prevent SLWF infestation, lima beans (*Phaseolus limensis* L.) (cv. 'Jackson Wonder') were enclosed in organdy nets in the greenhouse. Third-instar *C. rufilabris* larvae were isolated into 4-cm diameter petri dishes secured with rubber bands and lined with damp filter paper. Prior to the start of the experiment, larvae were fed *S. cerealella* eggs and an artificial diet, using the methods of Hassan and Hagen (1978) (see Legaspi et al. 1994). A single lima bean leaf was removed from the plant and placed in a plastic petri dish (15-cm diameter) lined with damp filter paper. Leaves were kept moist by surrounding the stem with damp cotton. To confine the predators, Tree Tanglefoot® (The Tanglefoot Company, Grand Rapids, MI) was applied on the leaf perimeter. Ten *C. rufilabris* larvae were placed on each leaf. To prevent cannibalism, *S. cerealella* eggs were placed on each leaf, after which the dishes were secured using rubber bands. The treatments consisted of placing the predators on the bean leaves for 2, 3 or 4-d durations. Each treatment had a corresponding control consisting of bean leaves with *S. cerealella* eggs and Tanglefoot and held for the same duration as the corresponding treatment. All treatments and controls were replicated ten times.

After each exposure treatment, the predators and the petri dish covers were removed. SLWF were collected from tomato (*Lycopersicon esculentum*) and cantaloupe (*Cucumis melo cantalupensis*) cv. 'Perlita', plants in a greenhouse using a modified hand vacuum. The treatment and control leaves were then placed randomly in a cage (≈ 75 x 45 x 45 cm) and exposed to SLWF. Length of exposure to the whiteflies was equal to the length of exposure to the predators, e.g. leaves exposed to the predators for 2 d were also exposed to the whiteflies for 2 d. Number of eggs laid on each leaf were then recorded.

Effect of host plant on development, survival and body weight of C. rufilabris. First to second-instar *C. rufilabris* were isolated individually in plastic petri dishes (4 cm diameter) lined with damp filter paper and secured with a rubber band. *B. argentifolii* were provided as prey by excising the plant tissue containing the immatures and placing this in the petri dishes with the predators. SLWF were reared from poinsettia (*Euphorbia pulcherrima*) cv. 'V-14 Glory' (Ecke Farms, Encinitas, CA), cucumber (*Cucumis sativus*), cantaloupe, and lima bean grown in the greenhouse. Each treatment was replicated ten times. Developmental time was recorded as days required for green lacewing larvae to molt from one instar to the next. Body weight of the larvae was recorded every 3-5 d using a Mettler® (Mettler Instrument Corp., Princeton, NJ) analytical balance AT200 (precision ± 0.01 mg) until the pupal stage was reached. Also, the body weight of the newly-emerging adult was recorded. Survival was calculated as the proportion of larvae alive at specific times.

Comparison of predation and body weights between C. rufilabris and C. carnea. This experiment was conducted in the laboratory at ambient temperatures (mean = 24.4°C, range = 23.3 - 26.7°C). Second to fourth-instar SLWF were used as prey. Second-instar *C. rufilabris* and *C. carnea* were separated individually in plastic petri dishes (4-cm diam) lined with damp cellulose support pads and secured with a rubber band. The predator larvae were provided SLWF prey ad libitum (about 50-100 prey per d) on leaf discs throughout their life. Each treatment had ten replicates. After each 24-hr feeding period, the number of prey attacked were recorded. Additional measurements included longevity (number of days the predator was alive), survival (number of predators alive at specific intervals), and developmental time from one larval stage to the next. The predator larvae were weighed twice a week.

Statistical analysis was performed using the Systat© package (version 5.2) (Wilkinson et al. 1992). All tests were judged at $P = 0.05$, and means were separated using Tukey's HSD test. The effect of host plant on body weights was analyzed using a General Linear Model analysis (see Wilkinson et al. 1992). A regression model was defined for body weight as a function of time and type of host plant, where host plant was specified as categorical data.

RESULTS AND DISCUSSION

Oviposition deterrents of C. rufilabris. The effect of exposure of leaves to *C. rufilabris* on SLWF oviposition is shown in Fig. 1. Exposure time of 2 d did not produce significant differences between treatment and control. Leaves treated with the predator were found to have a mean of 72.7 (SE 24.0) eggs compared to the control which had 40.4 (SE 11.9) ($t = 1.2$, $N = 10$, $P > 0.05$). However, exposure times >2d produced significant reductions in the mean numbers of eggs laid by SLWF females. Leaves exposed for 3 d were found to contain 42.9 (SE 16.2) eggs in the treatment, compared to 154.3 (SE 41.8) eggs in the control ($t = 2.48$, $N = 10$, $P < 0.05$).

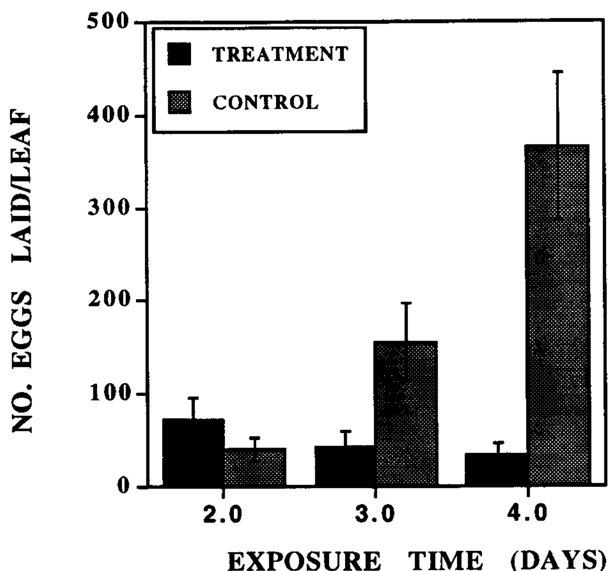


FIG. 1. Oviposition of *B. argentifolii* on control lima bean leaves and leaves on which *C. rufilabris* larvae were confined for 2, 3, or 4 d.

The tendency of SLWF to avoid ovipositing in the treatment was even more pronounced in the 4-d treatment; mean number of eggs on treated leaves was 34.9 (SE 12.5) compared to 365.9 (SE 79.2) on control leaves ($t = 4.13$, $N = 10$, $P < 0.01$). These results indicate that *B. argentifolii* females tended to avoid ovipositing in leaves on which *C. rufilabris* larvae were previously located. Moreover, this tendency appears to increase with increasing exposure time of both predators and whiteflies to the leaves. These results are in agreement with those found by Butler and Henneberry (1988) and may indicate the presence of an oviposition deterrent (kairomone) produced by the *C. rufilabris* larvae.

Effects of host plant on development, survival and body weight of C. rufilabris. The effect of host plant on the mean body weight (mg ± SE) of *C. rufilabris* is shown in Fig. 2A; means excluded larvae that had either died or pupated. Fig. 2B indicates the size of each sample.

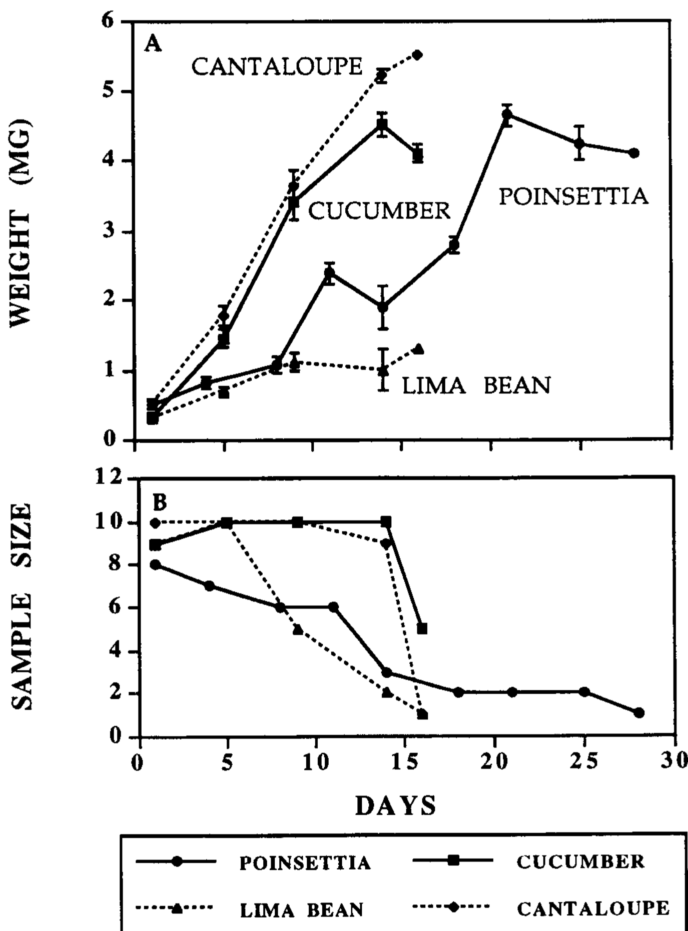


FIG. 2. Effect of host plant on body weights of *C. rufilabris*. Predator larvae feeding on whiteflies on cucumbers and cantaloupes had significantly higher mean body weights (± SE) than those with poinsettias and lima beans as the host plant (Fig. 2A). Fig. 2B indicates the numbers of lacewings represented by the corresponding means.

Body weights of *C. rufilabris* on cantaloupe and cucumber were similar. Predators reared on lima beans were smallest among the different host plants. Predators reared on poinsettias were smaller than on cantaloupe or cucumber, but survived longer and pupated later than those on the other host plants. The General Linear Model analysis supported these conclusions. Both time ($F = 428.9$, df = 1, 143, $P < 0.01$) and host plant type ($F = 44.3$, df = 3, 143, $P < 0.01$) were highly significant factors affecting predator body weight. Mean body weight was highest on cantaloupe and cucumber, and lowest on poinsettias and lima beans (Tukey's test, $P < 0.05$) (Fig. 3).

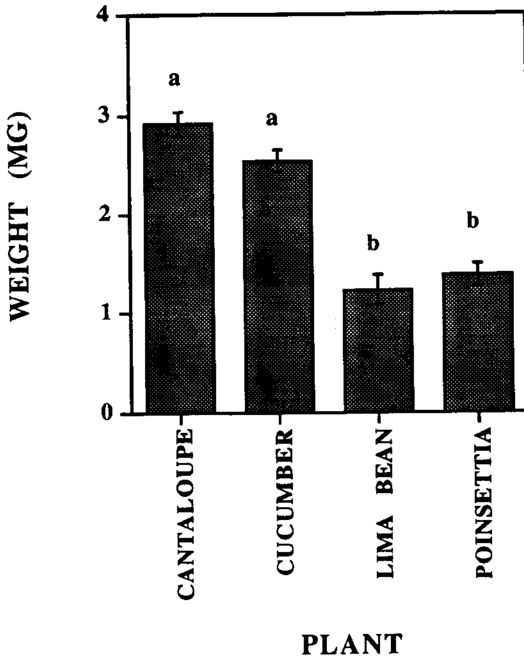


FIG. 3. Effect of host plant on mean body weight (± SE) of *C. rufilabris*. Mean body weights are calculated over total time on the host plant prior to death or pupation. The effect of host plant on body weight is highly significant ($F = 44.3$, $P < 0.01$). Means with the same letters are not significantly different (Tukey's HSD, $P = 0.05$).

Development of first and second instars of *C. rufilabris* differed according to the host plants upon which their prey SLWF were reared (Fig. 4). Only *C. rufilabris* larvae provided SLWF from cucumbers and cantaloupes reached the adult stage, with an adult weight of 2.23 mg (SE 0.63, $N = 3$) for cucumber and 3.1 mg ($N = 1$) for cantaloupe. *C. rufilabris* provided larvae from poinsettia and lima bean lived only to the third instar and died before reaching the pupal stage. Survival of *C. rufilabris* provided SLWF from the different host plants is shown in Fig. 5. The survival curve for larvae that were provided SLWF reared on poinsettia and lima beans shifted to the left, indicating a much lower survival compared with larvae that were provided SLWF from cantaloupe and cucumber plants.

Comparison of predation between C. rufilabris and C. carnea. Larval body weights of both species increased from about 0.25 mg per larvae to a maximum of about 2.0 mg (Fig. 6). Body weights did not differ between the two species ($F = 0.056$, $P = 0.81$). Numbers of whiteflies consumed by the two species of lacewings are shown in Fig. 7.

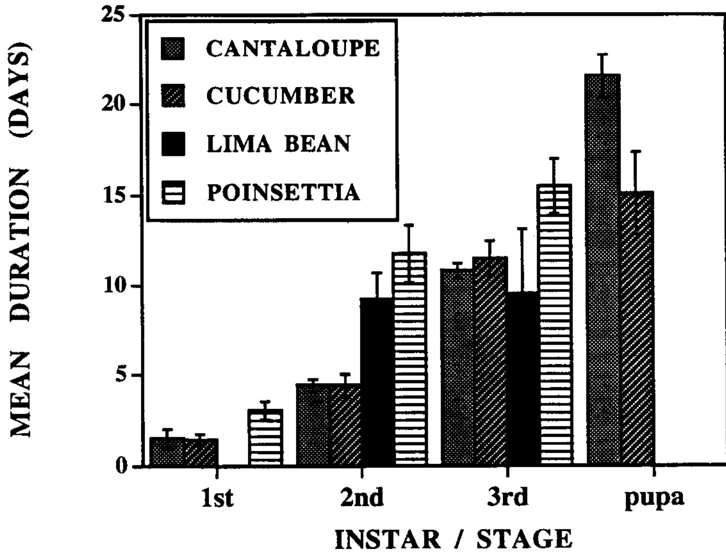


FIG. 4. Developmental times of *C. rufilabris* as affected by host plant (±SE). The lima bean treatment was started using 2nd instar predataors. *C. rufilabris* in lima bean and poinsettia treatments did not survive to pupation.

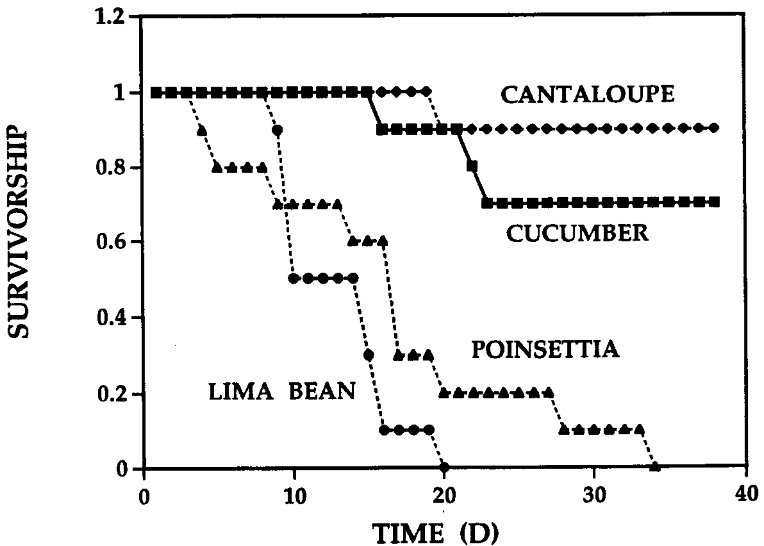


FIG. 5. Survival of *C. rufilabris* as affected by host plant. The predator displayed higher survival on cantaloupe and cucumber than on poinsettia and lima beans.

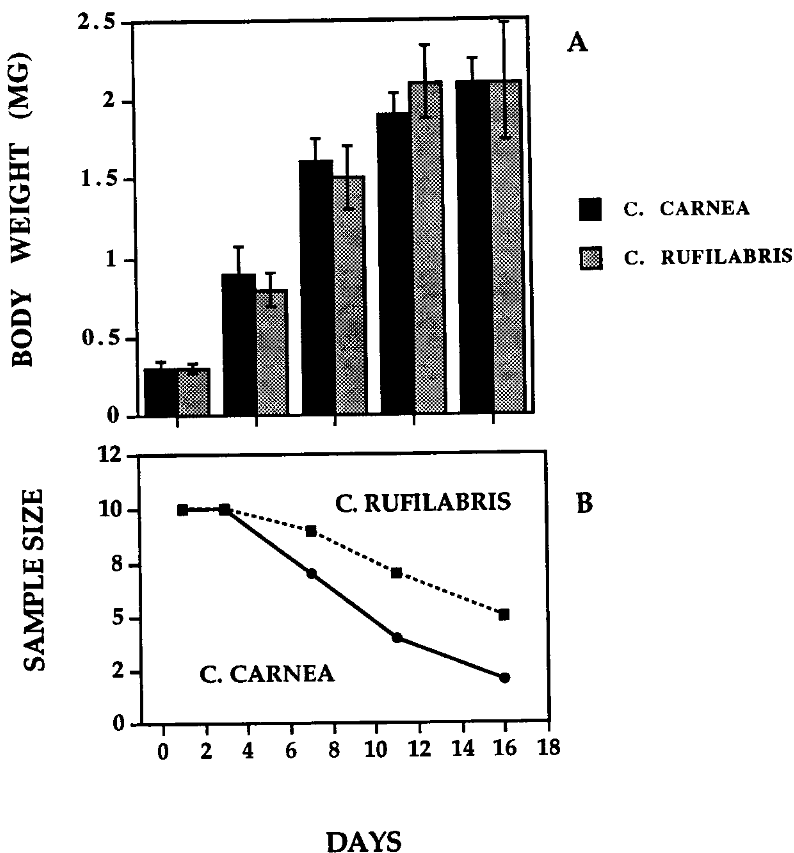


FIG. 6. Comparison of body weights of *C. rufilabris* and *C. carnea*. Body weights (± SE) of *C. rufilabris* and *C. carnea* were not significantly different using poinsettia as the host plant ($F = 0.056$, $P = 0.81$) (Fig. 6A). Fig. 6B indicates the numbers of lacewings represented by the corresponding means.

Statistical analysis of the numbers of whiteflies consumed over the entire experiment indicates a significantly higher number of whiteflies consumed by *C. carnea* than by *C. rufilabris* ($F = 4.7$, $P < 0.05$). The significant difference in predation between the two species was due largely to the increased predation rate by *C. carnea* during the 5-d period from days 12 to 16. Analysis of the data from days 1 to 11 only produced no significant differences in numbers of whiteflies consumed between the two species ($F = 0.1$, $P = 0.75$). However, the differences in predation rates are highly significant for the subset of data collected from days 12 to 16 ($F = 6.7$, $P < 0.01$). Based on these results, the most prudent conclusion is that there is little difference between predation rates between larvae of *C. carnea* and *C. rufilabris*, although *C. carnea* may consume significantly more whiteflies during certain intervals. More tests are necessary to demonstrate conclusively if differences exist in predation rates between species. Both predators consumed an average of 25 - 75 whiteflies daily (Fig. 7).

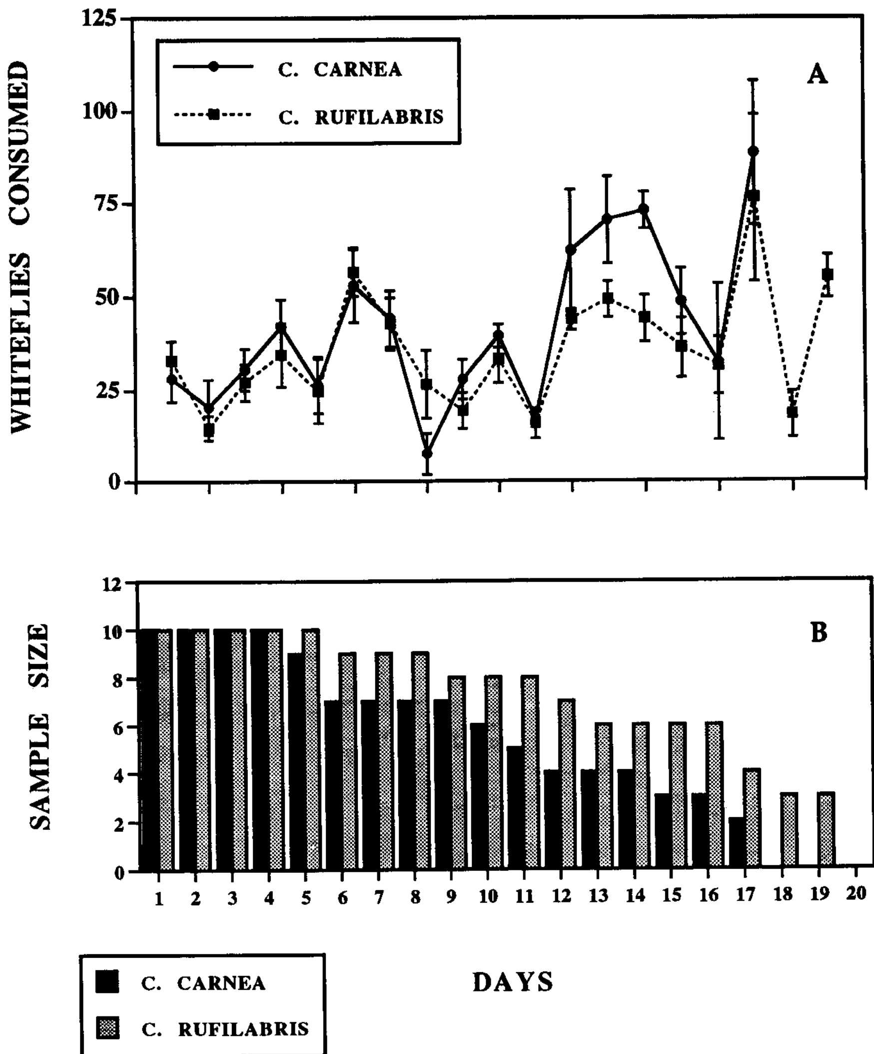


FIG. 7. Comparison of predation between *C. carnea* and *C. rufilabris* on poinsettia. Daily numbers of whiteflies consumed are shown (± SE) as a function of time (Fig. 7A). A significantly higher number of whiteflies was consumed by *C. carnea* than by *C. rufilabris* ($F = 4.7$, $P < 0.05$). Fig. 7B indicates the numbers of lacewings represented by the corresponding means.

Results of these experiments demonstrate the effect of tri-trophic interactions between the host plant, phytophagous pest and entomophagous insect. The presence of the predator on a host plant was shown to deter oviposition by the whitefly after the predators had been removed from the plant. The adaptive significance of this behavior may relate to improving the survival of the whitefly offspring by avoiding sites infested with predators. The precise chemical cues which cause this behavior and possible applications in biological control programs will require further study.

The host plant can affect body weight and survival of the predator, presumably through the sequestration of plant compounds into the phytophagous prey, or by influencing the nutritional quality of the prey. In these experiments, SLWF feeding on cantaloupes and cucumber appeared to be better quality prey than those feeding on poinsettia or lima bean. *B. argentifolii* and the plants that they were reared on apparently affect *C. rufilabris'* development, survival, and body weight. Lacewings that preyed on SLWF that were reared on cucurbits such as cucumbers and cantaloupes developed more rapidly, showed increased survival, and weighed more as newly-emerged adults, compared to those from poinsettia and lima bean. Lacewings feeding on the latter two plants did not reach the pupal stage. This phenomenon supports the findings of Legaspi et al. (1994) who speculated that the SLWF reared on poinsettia or lima bean were nutritionally inadequate (see also Hydorn and Whitcomb 1979) for *C. rufilabris* development, or *B. argentifolii* reared on these plant hosts may have an accumulative toxic effect on *C. rufilabris*. However, because the predators were in contact with the lima bean and poinsettia foliage, reduced survival could also be attributed to direct effects of the plant rather than nutritional or allelochemical effects via the host. Further nutritional studies on the quality of *B. argentifolii* and *C. rufilabris* as well as a biochemical analysis of the plants will increase our understanding of the tri-trophic interaction between predator, prey and plants.

ACKNOWLEDGMENT

We are grateful to H. J. McAuslane, K. A. Hoelmer, R. L. Meagher, Jr. and two anonymous reviewers for helpful comments on this paper. We thank P. Silva and C. Bennett for collection and entry of data. J. Rivera reared the plants in the greenhouse and J. Correa, R. Buenrostro and I. Torres reared the green lacewings. Paul Ecke Poinsettias, Encinita, CA provided the poinsettia seedlings. This paper is approved for publication by the Texas Agricultural Experiment Station.

LITERATURE CITED

Birdsall, S. L., D. Ritter, and P. L. Cason. 1995. Economic impact of the silverleaf whitefly in Imperial Valley, California. p. 162. *In* T. J. Henneberry, N. C. Toscano, R. M. Faust, and J. R. Coppedge (eds.) Silverleaf Whitefly: 1995 Supplement to the 5-year National Research and Action Plan. USDA-ARS 1995-2.

Breene, R. G., R. L. Meagher Jr., D. A. Nordlund, and Y. T. Wang. 1992. Biological control of *Bemisia tabaci* (Homoptera: Aleyrodidae) in a greenhouse using *Chrysoperla rufilabris* (Neuroptera: Chrysopidae). Biol. Control. 2: 9-14.

Butler, G. D., Jr. and T. J. Henneberry. 1988. Laboratory studies of *Chrysoperla carnea* predation on *Bemisia tabaci.* Southwest. Entomol. 13: 165-170.

Dittrich, V., S. Uk, and G. H. Ernst. 1990. Chemical control and insecticide resistance of whiteflies. pp. 263-285 *In* D. Gerling [ed.] Whiteflies: Their Bionomics, Pest Status and Management. Intercept Ltd.

Elkarmi, L. A., M. K. Harris, and R. K. Morrison. 1987. Laboratory rearing of *Chrysoperla rufilabris* (Burmeister), a predator of insects of pecans. Southwest. Entomol. 12: 73-78.

Hassan, S. A. and K. S. Hagen. 1978. A new artificial diet for rearing *Chrysopa carnea* larvae (Neuroptera, Chrysopidae). Z. Angew. Entomol. 86: 315-320.

Hydorn, S. B., and W. H. Whitcomb. 1979. Effects of larval diet on *Chrysopa rufilabris.* Fla. Entomol. 62: 293-298.

Legaspi, J. C., R. I. Carruthers, and D. A. Nordlund. 1994. Life history of *Chrysoperla rufilabris* (Neuroptera: Chrysopidae) provided sweetpotato whitefly, *Bemisia tabaci* (Homoptera: Aleyrodidae) and other food. Biol. Control. 4: 178-184.

Nordlund, D. A., and R. K. Morrison. 1992. Mass rearing of *Chrysoperla* spp. pp. 427-439 *In* T. E. Anderson and N. C. Leppla [eds.] Advances in Insect Rearing for Research and Pest Management. Westview Press, Boulder, CO.

Perring, T. M., A. D. Cooper, R. J. Rodriguez, C. A. Farrar and T. S. Bellows, Jr. 1993. Identification of a whitefly species by genomic and behavioral studies. Science. 259: 74-77.

Wilkinson, L., M. Hill, and E. Vang. 1992. SYSTAT: Statistics, Version 5.2 Edition. Evanston, IL. Systat, Inc. 724 pp.

AN ANALYSIS OF EXCRETION IN THE STABLE FLY, *STOMOXYS CALCITRANS* (L.)*

Andrew C. Chen and Paul G. Schleider

Food Animal Protection Research Laboratory and Southern Crops Research Laboratory
Agricultural Research Service
U.S. Department of Agriculture, 2881 F&B Road, College Station, TX 77845

ABSTRACT

Excretion immediately after a blood meal was studied in the stable fly, *Stomoxys calcitrans* (L.) by a new, non-invasive technique. The process of excreta formation was recorded on video tape and later analyzed as digitized images. The formation of the excretory droplets takes approximately 0.25 sec. Droplet size, estimated by assuming a spherical shape, ranged from 100 to 400 nl. Size of excretory droplets gradually decreased with time while the time interval between droplets increased. The average rate of excretion was ca. 21 nl/min during the 2-h observation period. This was in close agreement with the loss of body weight during the same period.

INTRODUCTION

Excretion is an important physiological function in insects and many aspects of it have been studied extensively (See Bradley 1985 for review). Bloodsucking insects face a special challenge. As intermittent feeders, they usually take in a meal that exceeds their own body weight (Lester and Lloyd 1928, Wigglesworth 1931, DeLoach and Spates 1979, Stobbart 1977). Individuals become sluggish after such a meal, and their speed of flight can be reduced by as much as 75% (Glasgow 1961). These insects, as a rule, have developed amazingly efficient systems to remove excess fluid following a blood meal (Lester and Lloyd 1928, Wigglesworth 1931, Gee 1975, Williams et al. 1983).

Excretion in insects is controlled by diuretic hormones (Petzel et al. 1985, Schooley et al. 1987, Coast 1988). Studies on insect diuretic hormones generally use in vitro bioassay systems to detect and verify the diuretic activity. However, recently Spring and Kim (1995) have questioned the validity of such assays and suggested that in vivo assays might be better suited for such studies. Early studies on insect excretion had employed in vivo systems by using weight loss as a means of measuring rate of excretion (Lester and Lloyd 1928, Wigglesworth 1931, Lanley and Pimley 1973, Gee 1975). Here, we introduce a new method for studying insect excretion by analyzing digitized video images of diuretic insects and report results on stable fly excretion using this method.

---

*Mention of a commercial or proprietary product in this paper does not constitute an endorsement of this product by the USDA, nor does it imply the recommendation of the product by the USDA to the exclusion of similar products.

MATERIALS AND METHODS

The stable fly, *Stomoxys calcitrans* (L.), was maintained under controlled conditions of 27°C, 50% RH and a 12D:12L photoperiod. Larvae were reared on a semi-defined diet (Bridges et al. 1984). Adults emerging within a 2-h time period 1 h after lights-on were collected for the experiments and kept in a stainless steel cage with wire screening (12 in W × 12 in H × 16 in D). No food or water was offered during the first 24 h of adult eclosion because the stable fly does not readily feed during this period (Spates 1983).

At the end of 24 h, flies were anesthetized by chilling at 4°C and females were selected and transferred to 8-oz polystyrene cups with wire screening in the lids. When flies recovered from anesthesia upon returning to room temperature, they were offered bovine blood soaked in a dental roll through the wire screening. Immediately after they ceased feeding, they were anesthetized again at 4°C and transferred to observation chambers.

The setup for recording excretory activity is shown in Fig. 1. Observation chambers were constructed from 12 mm × 75 mm polystyrene tubes. The lower 40 mm of the tubes was removed with a saw. The remaining part was erected and kept in a vertical position by fitting over a short piece of acrylic rod (3/8 in diameter) which is glued to a base. The chamber can revolve around this stump as necessary. The base is mounted on a platform that travels on an optic bench by a helical rack and pinion mechanism (G61,284 and G61,286, Edmund Scientific, Barrington, NJ). After the introduction of an individual fly, the chamber was plugged with another short piece of 3/8 in acrylic rod glued to a short piece of acrylic which prevented it from falling into the chamber. A space of 15 mm was left between the two pieces of acrylic rods. Two chambers were mounted side by side so that the activity of two flies could be recorded simultaneously. Video taping started immediately after the flies recovered from anesthesia. The flies were under constant surveillance during the 2-h taping session. When the fly moved to a position where the posterior end was away from the camera, the chamber was rotated so that the excreted droplet was always clearly visible from the camera. The study was carried out at 22°C.

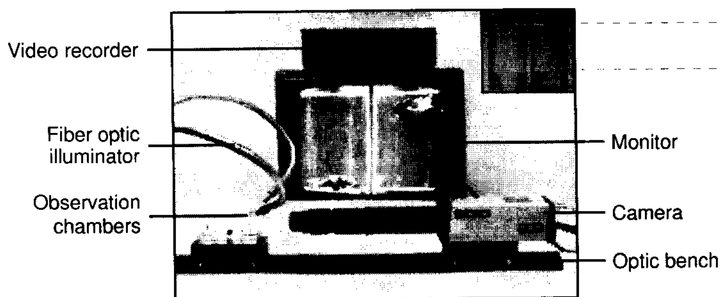


FIG. 1. The setup for recording the in vivo excretion of the stable fly. The inset shows a close-up view of the observation chambers. The dash lines demarcate the space between the ceiling and the floor of the chambers.

Video recording of excretion was carried out with a high resolution 8-mm recorder (EV-C100, Sony, Tokyo) equipped with a high resolution (525 lines) charge-coupled device (CCD) camera (Ultrichip Mod.JE-7442, Javelin, Torrance, CA). The camera was outfitted with a 6.5× zoom lens through a 0.67× adapter. Recording was monitored on a high resolution monitor (BWM 12, Javelin). The camera was mounted on the same optic bench on which the observation chambers were mounted. At 1× zoom, the entire observation

chamber was in the field of view and therefore, no adjustment was required to follow the movement of the fly in the chamber. Because of the short distance between the front and back walls of the chamber, constant focusing was not necessary once the initial adjustment was made. Illumination of the flies was provided by two optic fibers ca. 7 cm from the chambers, ca. 2.5 cm in front of the plane of the chambers and at an angle of ca. 15° above the horizontal line. This illumination was optimum for the elucidation of the excretory droplet. Excretion was recorded for 2 h for each fly at a rate of 30 frames per sec against a dark background. A stationary image of a stage micrometer (2 mm divided into 0.01 mm) was also recorded at the same magnification and distance for calibration of the droplets.

After excretion was recorded, the tape was played back and each time a droplet of excreta was extruded, the image was frozen, digitized and captured on a microcomputer. Because it took ca. 0.25 sec (7-8 frames) for the droplet to develop to its maximal size, the last frame immediately prior to dropping was used. An IBM compatible microcomputer (80486-33 Mhz) equipped with a digitizing card (Smart Video Recorder; Intel, Hillsboro, OR) was used to capture frozen images. For measuring the size of excretory droplets, a program written in Visual BASIC was used where the image of the droplet hanging from the tip of the abdomen and that of the micrometer were projected on the computer monitor in two separate windows simultaneously. The diameter of droplets was then calculated against the micrometer standard. The diameter was used in a spreadsheet (Excel; Microsoft, Redmond, WA) to calculate the droplet size. The weight of the flies immediately before placing in the observation chamber and after the 2-h recording period was also recorded.

RESULTS AND DISCUSSION

Fig. 2 shows an example of a female stable fly with a fully developed excretory droplet hanging from the end of her ovipositor. The droplet appears to be spherical and its outline is clearly discernible for easy measurement. Initially, studies were performed in larger chambers (4-cm cube). Preliminary observations showed that excretion began 3 to 7 min after cessation of feeding. Anesthesia by chilling at 4°C did not appear to have affected

FIG. 2. An example of a frozen video frame of a female stable fly excreting.

the lag time between cessation of feeding and beginning of excretion. Because it was difficult to track the fly in such a large space, smaller chambers were adopted. During the first 2-h after the blood meal that excretion was studied, the droplet ranged between 0.6 and 0.9 mm in diameter. Excreta begin as a clear fluid and continued to appear clear until almost the end of the 2 h study period by which time the excreta began to become brown. Fig. 3 shows the excretion in three stable flies recorded with the video method. The size of excretory droplets gradually decreased with time while the time interval between droplets increased. Individual variation in both the excretory rates and interval between excretory droplets was observed.

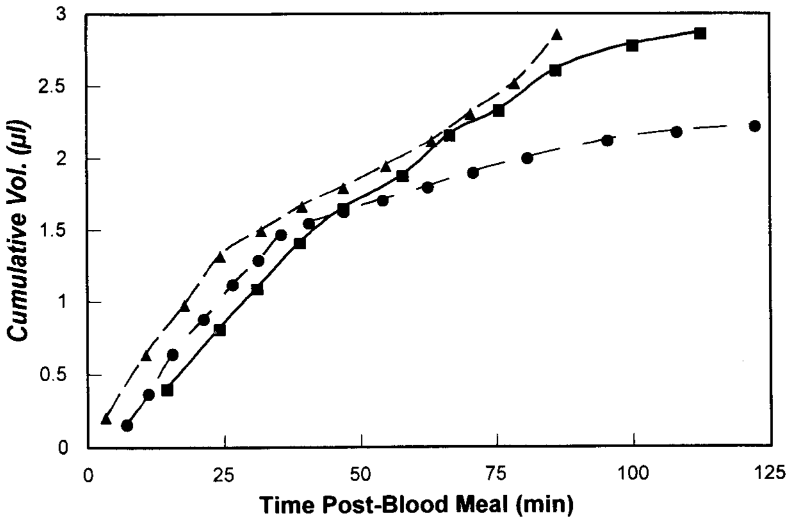


FIG. 3. Excretion in three individual stable flies. Cumulative volume of excretion in each fly is plotted against time after a blood meal.

Excretion by intact insects has been studied in some bloodsucking insects, most notably in tsetse flies. Lester and Lloyd (1928) first reported rapid excretion that amounted to 43% of the blood meal in 1 h in *Glossina morsitans*. Langley and Pimley (1973) found that although the rate of excretion varied depending on the source of blood meal, *G. morsitans* eliminates 40-60% of the fluid in the meal in the first hour after a blood meal. A similar rate (38% in 1 h) was reported in a different species of tsetse fly, *G. austeni* (Gee 1975). In *Rhodnius prolixus* (Wigglesworth 1931), the rate of excretion shows a large variation among individuals ranging from ca. 16 to 34% in 1 h (calculated from the data given). The rate of excretion in the stable fly is much less than tsetse flies and reduviid bugs, amounting to less than 3 µl in 2 h (<30%) (Fig 3). However, the individual variation found in the *S. calcitrans* is similar to that in *R. prolixus* as well as another reduviid bug, *Triatoma infestans* (Schnitker et al. 1988).

In all of the reported studies involving tsetse flies and reduviid bugs, the rate of excretion was measured by weight loss at fixed times after a blood meal. This is a useful method, but it does not provide detailed information, such as how frequently the insect excretes or the size of each excretory droplet. With the recording method that we report here, we were able to determine the rate of excretion over a fixed period of time and determine that, over time, the interval between formation of excretory droplets increased. Further, we were able to demonstrate that size of droplets decreased over time. In their study on *T. infestans*, Schnitker *et al.* (1988) analyzed the excreta size by measuring droplets after collection under liquid paraffin. No experimental details were given as to how the bugs were held over liquid paraffin. We had initially attempted to collect the excreta in liquid paraffin. However, the stable fly invariably fell from the walls or the stopper (ceiling) of the container and perished in liquid paraffin. Lester and Lloyd (1928) removed the wings from the tsetse fly for easier weighing. It is not clear how much disturbance was caused by such trauma and how much weight loss was due to evaporation through the cut.

In order to assure precision and accuracy of measurement, a close-up lens was used in this study to maximize image size. Greater image size could have been achieved with the system employed, but the 1× zoom was selected for optimal results. At this magnification, accurate measurement was accomplished while a reasonable space was available for the fly to move without leaving the field of view of the camera. Reproducibility as determined by measuring an excretory droplet several times was within 3.8%.

To limit the range of movement of test flies such that they were easier to follow with the close-up camera lens, flies were initially tethered by gluing the wings to the end of a wooden applicator stick with wax (Dethier and Chadwick 1947). However, flies refused to feed in such an arrangement whether the blood was offered to them by touching the tarsi or directly to the proboscis. Since tethering created such an unnatural environment for the fly, this idea was abandoned. Further, flies would not imbibe when a blood-soaked dental roll was offered directly in the observation chamber. However, they flew to the blood pad and commenced feeding to engorgement immediately either in the rearing cage or in the 8-oz plastic cup. The reason for the reluctance for the stable fly to feed in the first two situations is not understood. Space limitation may play a role, but other environmental factors cannot be ruled out. For example, when the blood was offered in the dental roll in the observation chamber, the microclimate in the chamber was changed as well (e.g., the humidity becomes more saturated). This high humidity may affect their feeding behavior. The mechanism(s) of inhibition of feeding can only be revealed by future studies.

In mosquitoes, excretion begins very quickly after the commencement of blood feeding; it begins while the mosquito is still feeding (Williams et al. 1983). Such is not the case in the stable fly. In fact, it took a minimum of 3 min after cessation of feeding before the first excretory droplet is developed. This made it possible to record the excretion of the very first droplet even though it was not possible to use the same chamber for both feeding and observation of excretion.

It took less than 2 min for the stable fly to engorge. The average volume of the blood meal in this study was less than 10 µl (assuming a density of 1.05 for the bovine blood, Seunaga 1965). This is considerably smaller than that reported previously for individuals from the same lineage (DeLoach and Spates 1979). The flies used in the current study were notably smaller than those used in the past (6 mg vs. 9 mg, personal observation). It is obvious that smaller insects would ingest less blood.

Although both sexes of the stable fly are blood feeders, only females were used in this study because as the excretory droplets formed, the ovipositor was extruded. This allowed the excreta to become spherical momentarily before it was discharged from the body, making it possible to measure the size more accurately.

The only disadvantage with the video recording method for analyzing in vivo excretion is that, in order for the excreta to be visible for measurement, the observation chambers have to be rotated to keep the fly in proper orientation. However, if undisturbed, the stable fly is relatively motionless after a blood meal. Therefore, the chambers do not have to be rotated frequently. Although this method was designed to analyze in vivo excretion in the stable fly, it can easily be adapted for other types of behavior such as feeding in other species of insects as well.

LITERATURE CITED

Bradley, T. J. 1985. The excretory system: structure and physiology, pp. 421-465. *In* G. A. Kerkut and L. I. Gilbert [ed] "Comprehensive Insect Physiology, Biochemistry and Pharmacology", Vol. 4, , Vol. 4, pp. 421-465. Pergamon Press, Oxford.

Bridges, A. C., J. W. Summerlin, and G. E. Spates. 1984. A new and more economical base medium for rearing larvae of the stable fly, horn fly and housefly. Southwest. Entomol. 9: 388-390.

Coast, G. M. 1988. Fluid secretion by single isolated Malpighian tubules of the house cricket, *Acheta domesticus*, and their response to diuretic hormone. Physiol. Entomol. 13: 381-391.

DeLoach, J. R., and G. Spates. 1979. Rate of digestion of ^{51}Cr-hemoglobin by *Stomoxys calcitrans* (Diptera: Muscidae). J. Med. Entomol. 16: 493-496.

Dethier, V. B., and L. E. Chadwick. 1947. Rejection thresholds of the blowfly for a series of aliphatic alcohols. J. Gen. Physiol. 30: 247-253.

Gee, J. D. 1975. Diuresis in the tsetse fly *Glossina austeni*. J. Exp. Biol. 63: 381-390.

Glasgow, J. P. 1961. The feeding habit of *Glossina swynnertoni*. J. Anim. Ecol. 30: 77-85.

Langley, P. A., and R. W. Pimley. 1973. Influence of diet composition on feeding and water excretion by the tsetse fly, *Glossina morsitans*. J Insect Physiol. 19: 1097-1109.

Lester, H. M. O., and L. Lloyd. 1928. Notes on the process of digestion in tsetse flies. Bull. Ent. Res. 19: 39-60.

Petzel, D. H., H. H. Hagedorn, and K. W. Beyenbach. 1985. Preliminary isolation of mosquito natriuretic factor. Am. J. Physiol. 249: R379-R386.

Schnitker, A., G. A. Schaub, and H. P. Maddrell. 1988. The influnce of *Blastocrithidia triatomae* (Trypanosomiatidae) on the reduviid bug *Triatoma infestans*: *in vivo* and *in vitro* diuresis and production of diuretic hormone. Parasitol. 96: 9-17.

Schooley, D. A. C. A. Miller, and J. P. Proux. 1987. Isolation of two arginine vasopressin-like factors from ganglia of *Locusta migratoria*. Arch. Insect Biochem. Physiol. 5: 157-166.

Seunaga, O. 1965. A rearing method of stable fly and quantity of blood taken up by a fly. Endem. Dis. Bull. Nagasaki. 1: 296-301. [In Japanese.] Rev. Appl. Entomol. (1968) 56: 196. Abstrct 709.

Spates, G. E. 1983. Midgut proteolytic and hemolytic activities, and feeding habits of young adult stable flies *Stomoxys calcitrans* (L.). Southwest. Entomol. 8: 178-185.

Spring, J. H., and I. Kim. 1995. Differential effects of neuropeptides on the distal and mid-tubules of the house cricket. Arch. Insect Biochem. Physiol. 29: 11-23.

Stobbart, R. H. 1977. The control of diuresis following a bloodmeal in females of the yellow fever mosquito *Aedes aegypti* (L.). J. Exp. Biol. 69: 53-85.

Wigglesworth, V. B. 1931. The physiology of excretion in a bloodsucking insect, *Rhodnius prolixus* (Hemiptera, Reduviidae). J. Exp. Biol. 8: 411-427.

Williams, J. C., Jr., H. H. Hagedorn, and K. W. Beyenbach. 1983. Dynamic changes in flow rate and composition of urine during the post-bloodmeal diuresis in *Aedes aegypti* (L.). J. Comp. Physiol. B 153: 257-265.

EFFECTS ON CONTROL OF ONION THRIPS[1] FROM CO-APPLICATION OF ONION PESTICIDES

Saleh A. Al-Dosari[2], Whitney S. Cranshaw[3], and Frank C. Schweissing[4]

ABSTRACT

A series of trials were conducted during 1994 and 1995 to determine effects of co-application of pyrethroid insecticides (cypermethrin, lambda-cyhalothrin) with fungicides and a bactericide used for pathogen management for onion thrips control. In two out of four trials, co-application of insecticides with the chlorothalonil fungicide formulation Bravo 720 reduced control of onion thrips. Control was also reduced in the single trial when the bactericide Kocide DF was applied with lambda-cyhalothrin. Other fungicide formulations did not show reduction of onion thrips control in co-application with an insecticide, and the fungicides did not have direct effects on onion thrips populations. Sequential application of lambda-cyhalothrin (Warrior 1E) with chlorothalonil (Bravo 720) did not reduce thrips control while co-application did.

INTRODUCTION

Onion thrips, *Thrips tabaci* Lindeman, is the major insect pest of onion in Colorado and is currently managed by the use of pyrethroid insecticides. In addition, the crop is regularly attacked by a number of pathogenic fungi, including downy mildew (*Peronospora destructor* Berk), purple blotch (*Alternaria porri* Ellis), botrytis blight and neck rot (caused by *Botrytis allii* Munn, *B. squamosa* Walker and *B. byssoidea* Walker) (Schwartz et al. 1995). Various fungicides are critical to effective management of these pathogens and are widely used.

For economic reasons it is often advantageous to use a mixture of an insecticide and fungicides during pesticide applications. However, other than identification of those combinations that are incompatible in tank mixes, interactions of chemicals applied to control fungi and insects are rarely studied. Pilling and Jepson (1993) tested a group of fungicides (flutriafol, penconazole, imazalil, triadimenol, myclobutanil, triadimefon, propiconazole) and found all of them to increase the toxicity of lambda-cyhalothrin to honey bees when the mixture was applied topically. Mixing lambda-cyhalothrin with fungicides also significantly reduces the dosage amount between a sublethal repellent effect and a lethal effect, which is the primary factor in preventing pyrethroid-induced mortality in honeybees in the field (Rieth and Levin 1987).

---

[1] *Thrips tabaci* Lindeman (Thysanoptera: Thripidae).

[2] Department of Plant Protection, College of Agriculture, King Saud University, P.O. Box 2460, Riyadh, Saudi Arabia, 11451.

[3] Department of Entomology, Colorado State University, Ft. Collins, CO 80523.

[4] Arkansas Valley Research Center, Rocky Ford, CO 81067.

Formulations of co-applied pesticides also may adversely affect efficacy. Target sites for pesticide applications on onions differ somewhat, with foliar protection being important in fungicide applications on onion, while coverage of the interior of the neck is optimal for onion thrips control. Formulations may be designed to optimize this coverage; Bravo 720, (chlorothalonil), for example, is being currently marketed as a formulation that strongly resists wash-off after application to foliage (Weather-Stik^{R}).

An understanding of such pesticide interactions is important in developing Integrated Pest Management (IPM) systems for a crop. Therefore, studies were conducted to determine the effects of fungicides and bactericides used for managing onion pathogens on onion thrips and thrips management in Colorado onion production.

METHODS AND MATERIALS

The original study was conducted during 1994 at the Arkansas Valley Research Center, Rocky Ford, Colorado. Onions (c.v. 'Colorado 6') were seeded 21 March to double row beds at 40-in centers. Individual plots consisted of 50 feet of bed, and the experimental design was a randomized complete block with four replications. Treatments consisted of sprays of Ammo 2.5E (cypermethrin), Ammo 2.5E + Crop Oil Concentrate (soybean oil), or Ammo 2.5E + Bravo 720 (chlorothalonil). Applications were made over the top of the plants 1 July using a CO_2 compressed air sprayer delivering 23.5 gal/acre at 45 psi. Temperature at the time of application was 92^{0}F. Plot evaluations were made 8 July by counting all thrips on ten consecutive plants.

Trials were again established at the Arkansas Valley Research Center during 1995 with plots of similar design as in the 1994 study. Cultivar was again 'Colorado 6', but individual plot length was decreased to 20 ft of bed. Treatments consisted of a variety of commercially used fungicides (Bravo 500, Bravo 720, Maneb 75DF, Ridomil MZ58, Bravo 50/Ridomil 80W) and a bactericide (Kocide DF) with and without the insecticide Warrior 1E (lambda-cyhalothrin). Treatment rates of Warrior 1E were 0.01 lbs AI/acre on the first application (5 July) which is below recommended use rates; application rates on the second application date (13 July) were 0.02 lbs AI/acre, within the recommended rate. Fungicides and bactericides were applied at the same rates on both dates. All treatments were applied in a water volume of 20 gal/acre, in a flat-fan pattern over the top of the plants at 40 psi, using a CO_2 compressed air sprayer. Plot evaluations were made 13 July and 25 July by counting all thrips on 10 consecutive plants per plot.

A second trial, also at the Arkansas Valley Research Center, focused on the interaction of Warrior 1E in co-application with the chlorothalonil fungicide Bravo 720, with and without various spray adjuvants (Crop Oil Concentrate, Kinetic). Plot procedures and experimental design were similar to the above trial except the cultivar was 'Tango' and the number of replications was expanded to five. Treatments were applied 13 July using a CO_2 compressed air sprayer delivering 25 gal/acre at 45 psi. Plot evaluations were made 25 July by counting all thrips on ten consecutive plants per plot.

A final trial was conducted on transplant onions (c.v. 'Vega') at the Horticulture Field Research Center, Ft. Collins, Colorado. Plantings, established 28 April, were double beds at 32 in between row spacing. Individual plots consisted of a 20-ft length of a single bed, and experimental design was a randomized complete block with the number of replications increased to six. Treatments consisted of applications of Warrior 1E alone or in combination with Bravo 720. In addition, trials were conducted to determine effects of co-application versus sequential application on thrips control. For the latter treatments, the first spray (either the insecticide or fungicide) was made approximately 90 min prior to the second spray (either fungicide or insecticide), which was sufficient to allow drying of sprays between the two treatments. Applications were made 26 July and 8 August using a CO_2

compressed air sprayer delivering 20 gal/acre, in a single pass over the top of each row of plants. Plot evaluations were made 1, 8, and 14 August by counting all thrips on ten consecutive plants/plot.

Treatment differences in control of onion thrips were analyzed by Analysis of Variance (ANOVA) using the Student Newman Keuls (SNK) test to determine separation of significant treatment values (SAS 1985). In trials using insecticides, the analysis did not include the untreated control. Thrips populations on the control were used as a measure of percentage control of the treatments.

RESULTS AND DISCUSSION

In the original 1994 study, there were significant differences in thrips control among plots treated with cypermethrin (Ammo 2.5E) (Table 1). Co-application with the fungicide Bravo 720 reduced control from 72.1 to 48.9 %, compared to use of the insecticide alone. The addition of Crop Oil Concentrate did not improve thrips control.

TABLE 1: Evaluation of Crop Oil Concentrate and a Co-applied Fungicide (Bravo 720) on Control of Onion Thrips with Cypermethrin (Ammo 2.5E). Arkansas Valley Research Center. Rocky Ford, Colorado, 1994.

Treatment	Mean no. thrips/plant[1]	Mean no. thrips/plant[2] (control)
Ammo 2.5E 0.1 lb AI/acre	18.0 a	18.0 a (72.1%)
Ammo 2.5E 0.1 lb AI/acre + Crop Oil Concentrate 1 gal	14.4 a	14.4 a (77.7%)
Ammo 2.5E 0.1 lb AI/A + Bravo 720 3 pt	33.0 a	33.0 b (48.9%)
Untreated Control	64.5 b	

[1] Mean of 40 plants (four replications). Evaluation made 8 July. Numbers followed by the same letter within a column that are not significantly different (P = 0.05) by SNK test.
[2] Analyzed without the untreated control. Percent control based on the untreated control.

In the first 1995 study at the Arkansas Valley Research Center, treatment differences (P = 0.05) existed among plots receiving application of Warrior 1E (lambda-cyhalothrin) (Table 2). Following the first application involving the low rate of lambda-cyhalothrin, there were significantly higher numbers of thrips on plots co-applied with the bactericide Kocide DF compared to plots co-applied with either the fungicides Maneb 75DF or Ridomil MZ58. These differences were maintained following the second application as well. Thrips populations on plots treated with Warrior 1E alone tended to be lower but were never significantly different from those co-applied with the various chlorothalonil (Bravo) formulations. None of the fungicides affected thrips populations on plots that were not treated with insecticides, indicating that they do not have direct effects on the insects.

In the subsequent trial, co-application of chlorothalonil (Bravo 720) with lambda-cyhalothrin (Warrior 1E) did not result in effects on thrips control (Table 3). The addition of spray adjuvants similarly did not affect control.

In the final study, significant treatment differences were observed among pesticide treatments when analyzed alone (Table 4), but not when combined with the untreated control. On the first evaluation date, co-application of Bravo 720 and Warrior 1E reduced thrips control from 94.6 to 66.0% compared to application of Warrior 1E alone. Co-application

TABLE 2: Onion Thrips Populations Resulting from Application of Fungicides, a Bactericide (Kocide DF) and the Insecticide Lambda-cyhalothrin (Warrior 1E), Alone and in Co-application. Arkansas Valley Research Center, Rocky Ford, Colorado, 1995.

Treatment and Rate/Acre	Mean no. thrips/plant[1] (%control) 13 July	25 July
With Insecticide		
Warrior 1E 0.01/0.02 lbs AI[2]	38.8 ab (43.6%)	13.9 bc (72.6%)
Bravo 500 4.5 pts + Warrior[2]	51.5 ab (24.7%)	12.8 ab (74.8%)
Bravo 720 2 pts + Warrior[2]	51.9 ab (24.7%)	16.9 ab (66.7%)
Maneb 75DF 3 lbs + Warrior[2]	35.0 b (49.2%)	8.8 c (82.7%)
Bravo 50/Ridomil 81W 2 lbs + Warrior[2]	41.6 ab (39.6%)	17.7 bc (65.2%)
Ridomil MZ58 2 lbs + Warrior[2]	33.7 b (51.1%)	9.4 c (81.5%)
Kocide DF 2 lbs + Warrior[2]	52.2 a (24.2%)	21.2 a (58.3%)
Without Insecticide		
Bravo 500 4.5 pts	66.0 a	37.9 a
Maneb 75DF 3 lbs	60.2 a	41.9 a
Bravo 50/Ridomil 81W 2 lbs	69.5 a	36.4 a
Ridomil MZ58 2 lbs	76.5 a	41.2 a
Untreated Control	68.9 a	50.8 a

[1] Mean of 40 plants (four replications). Number within a column that are followed by the same letter are not significantly different ($P < 0.05$) by SNK test.
[2] Warrior 1E (lambda-cyhalothrin) was applied at a rate of 0.01 lb AI/acre on the first application and 0.02 lb AI/acre on the second application.

TABLE 3: Evaluation of Effects on Onion Thrips Control of Co-application of a Chlorothalonil Fungicide (Bravo 720) and Lambda-cyhalothrin (Warrior 1E), With or Without Spray Adjuvants. Arkansas Valley Research Center, Rocky Ford, Colorado, 1995.

Treatment and Rate/Acre	Mean no. thrips/plant[1] (%control)
Warrior 1E 0.02 lbs AI	7.0 b (90.5%)
Warrior 1E + Bravo 720 3 pts	11.5 b (84.5%)
Warrior + Bravo 720 2 pts	11.5 b (84.5%)
Warrior 1E + Bravo 720 2 pts + Crop Oil Concentrate 1 gal	11.6 b (84.3%)
Warrior 1E + Bravo 720 2 pts + Kinetic 1.25 v%/v	11.2 b (84.9%)
Untreated Control	74.1 a

[1] Mean of four replications/treatment (40 plants). Numbers within a column followed by the same letter are not significantly different ($P < 0.05$) by SNK test.

TABLE 4: Effect on Onion Thrips Density of Lambda-cyhalothrin (Warrior 1E) Application in Combination (+) or in Sequence (fb) with a Chlorothalonil Fungicide (Bravo 720). Horticulture Research Farm. Fort Collins, Colorado, 1995.

Treatment and Rate/A	Mean no. thrips/plant[1] (%control) 1 August	8 August
Warrior 1E 0.02 lbs AI	4.5 b (94.6%)	20.9 a (66.7%)
Warrior 1E 0.02 lbs AI fb Bravo 720 3 pts	9.2 b (88.4%)	40.6 a (35.2%)
Bravo 720 3 pts fb Warrior 1E 0.02 lbs AI	5.8 b (92.7%)	28.6 a (54.4%)
Bravo 720 3 pts + Warrior 1E 0.02 lbs AI	27.1 a (66.0%)	44.9 a (28.4%)
Untreated Control	79.7	62.7

[1] Mean of six replications/treatment (60 plants). Numbers within a column followed by the same letter are not significantly different ($P < 0.05$) by SNK test.

also resulted in reduced control compared to sequential application of the same pesticides, with time allowed between applications to allow drying. These significant treatment differences were not present on the latter two sample dates.

Mixtures of insecticides and fungicides, or other pesticide mixtures, are often applied in the field. However, it is important that the components of these mixtures are compatible in order to avoid plant injury, loss of pesticidal activity, and resulting economic loss. Incompatibility may be either physical or chemical. Physical incompatibility results from conflicting physical properties of the solutes of a system and can be measured visually when flocculation, coagulation, creaming, sedimentation or coalescence occurs (Tarwater 1984). Coalescence results from the disruption of the properties of the liquid boundaries of the droplets, as droplets lose their individual integrity and nonuniform application results (Long 1992). The coalescence may be one possible reason for the reduction in the effectiveness of the lambda-cyhalothrin formulation Warrior 1E when co-applied with the fungicide Bravo 720 and the bactericide Kocide DF in this study. However, no physical incompatibility was observed during any of the co-applications in this trial.

Reduced efficacy of pyrethroid insecticides from co-application with fertilizers also has been reported by Long (1992). He noted significant reduction in tobacco budworm mortality as a result of co-applying a lambda-cyhalothrin formulation (Karate 1E) with a urea solution, compared to the water control. He indicated that the distribution of this insecticide in the fertilizer solution was adversely affected. When mixed into water, its distribution was relatively equally distributed throughout the solution; however, when mixed into a fertilizer solution, the majority (70%) of the insecticide was found in the top layer of the fertilizer solution within five min.

An alternative explanation for the reduced efficacy may be binding and immobilization by the formulation of the co-applied fungicide. The pyrethroids used in this trial have very low water solubility: 0.005 mg/l for lambda-cyhalothrin (Metcalf and Metcalf 1993) and 0.009 mg/l for cypermethrin (Sayler et al. 1994). The also bind readily to organic materials, such as would be found in stickers used in formulated pesticides. [The Koc value of cypermethrin is 160,000 gr/ml (Sayler et al. 1994).] Advertising literature for Bravo 720 strongly features its wash-off resistance, which allows it to remain on foliage and act as an effective protectant fungicide on foliage. However, onion thrips populations are concentrated in the neck of the plant, feeding on new growth. Binding to the fungicide

formulation which is then retained on upper foliage might immobilize the pyrethroids and prevent them from moving in sufficient concentration to control thrips in the neck of the onion plant.

Regardless of the mode of action, this study strongly suggests that co-application of insecticides and pesticides used for plant disease control on onions may affect insect control. Further research is needed to determine the exact means by which such effects on control can occur, but their potential effects on onion thrips management have been demonstrated in these trials.

ACKNOWLEDGMENT

We would like to thank Casey Sclar, Aaron Spriggs and Jason Bishop for assisting with field work and analysis. This study was funded, in part, by Colorado Agricultural Experiment Station Project 319.

LITERATURE CITED

Long, D.W. 1992. Co-application of insecticides and foliar urea fertilizer: Effects on chemical deposition, biological efficacy, chemical stability and physical and compatibility. M.S. Thesis. Louisiana State University. 112 pp.

Metcalf, L.M., and R.A. Metcalf. 1993. Destructive and Useful Insects: Their Habits and Control. McGraw Hill. New York. 981 pp.

Pilling, E.D., and P.C. Jepson. 1993. Synergism between EBI fungicides and a pyrethroid insecticide in the honeybee (*Apis mellifera*). Pestic. Sci. 39: 293-297.

Rieth, J.P., and M.D. Levin. 1987. The repellent effect of two pyrethroid insecticides on the honeybee. Physiol. Entomol. 13: 213-18.

SAS Institute. 1985. SAS User's Guide: Statistics. SAS Institute, Cary, N. C.

Sayler, L.A., J.W. Allen, D.A. Rutz, and M.A. Kamrin. 1994. EXTOXNET (Extension Toxicology Network).

Schwartz, H.F, P. Westra, and W.S. Cranshaw. 1995. Colorado Onion IPM. Colorado State Coop. Exten. Bull. 547A. 22 pp.

Tarwater, O.P. 1984. Compatibility and Tank-mix Testing of Pesticides. American Chemical Society Symposium Series 254. American Chem. Soc. Washington D.C.

PUPATION AND ADULT LONGEVITY OF *PHYLLOPHAGA CRINITA*, *ANOMALA FLAVIPENNIS* AND *A. FORAMINOSA* (COLEOPTERA: SCARABAEIDAE)

Luis A. Rodríguez-del-Bosque

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

ABSTRACT

Duration of pupal stage and adult longevity in the laboratory were estimated for full grown field-collected larvae of *Phyllophaga crinita* (Burmeister), *Anomala flavipennis* Burmeister, and *A. foraminosa* Bates in northern Tamaulipas, Mexico. Duration of pupation averaged 14.6, 15.8, and 21.5 d for *A. foraminosa*, *A. flavipennis*, and *P. crinita*, respectively. Adult longevity averaged 13.3 and 15.3 d for *A. foraminosa* and *A. flavipennis*, respectively. Longevity of *P. crinita* averaged 25.4 and 38.1 d for males and females, respectively. One female *P. crinita* lived 87 d, showing the ability of this species to survive in the pupal cell for long periods of time, waiting for the occurrence of rainfall to trigger adult emergence. Bivoltinism of *A. flavipennis* and *A. foraminosa* was corroborated in northern Tamaulipas, with adults having both spring and fall emergence.

RESUMEN

Se estimó la duración del período pupal y la longevidad de adultos en el laboratorio a partir de larvas de *Phyllophaga crinita* (Burmeister), *Anomala flavipennis* Burmeister y *A. foraminosa* Bates, colectadas en campos infestados del norte de Tamaulipas, México. El estado pupal promedió 14.6, 15.8 y 21.5 d para *A. foraminosa*, *A. flavipennis* y *P. crinita*, respectivamente. La longevidad de adultos promedió 13.3 y 15.3 d para *A. foraminosa* y *A. flavipennis*, respectivamente. Por su parte, la longevidad de *P. crinita* promedió 25.4 y 38.1 d para machos y hembras, respectivamente. Una hembra de *P. crinita* vivió durante 87 d, demostrando la capacidad de esta especie de sobrevivir en la celda pupal por largos períodos de tiempo, esperando la ocurrencia de lluvias, lo cual estimula la emergencia de los adultos. Se corroboró el bivoltinismo de *A. flavipennis* y *A. foraminosa* en el norte de Tamaulipas, con la emergencia de adultos tanto en primavera como en otoño.

INTRODUCTION

The most common species of white grubs causing crop losses in field corn and grain sorghum in northern Tamaulipas, Mexico, are *Phyllophaga crinita* (Burmeister), *Anomala flavipennis* Burmeister, and *Anomala foraminosa* Bates (Rodriguez-del-Bosque et al. 1995). The biology and habits of *P. crinita* have been studied under laboratory, greenhouse, and field conditions in northeastern Mexico and Texas. This species has a one-year life cycle in northern Tamaulipas and southern Texas, although populations in northern Texas may have a two-year life cycle (Reinhard 1940, Teetes et al. 1976, Huffman and Harding 1980, Rodriguez-del-Bosque 1988). Spring emergence of *P. crinita* adults is triggered by rainfall;

reproductive flight activity peaks 3-4 d after the occurrence of precipitations (Gaylor and Frankie 1979), suggesting that teneral adults wait passively in soil pupal cells until rainfall occurs.

The two distinctive flight periods (spring and fall) of *A. flavipennis* and *A. foraminosa* suggests a bivoltine life cycle for these species in northern Tamaulipas (Rodriguez-del-Bosque et al. 1995). *Anomala flavipennis* has been reported to be univoltine in Kansas (Hayes and McColloch 1924). The objectives of this investigation were to determine the pupal survival and adult longevity of *P. crinita*, and to corroborate bivoltinism in the two species of *Anomala* in northeastern Mexico.

MATERIALS AND METHODS

To study pupation and adult emergence for the spring generation, 180, 127, and 188 full grown larvae of *P. crinita*, *A. flavipennis*, and *A. foraminosa*, respectively, were collected from infested fields at the Campo Experimental Rio Bravo (INIFAP), near Rio Bravo, Tamaulipas, during February-March of 1994. Additional larvae of *Anomala* (156 *A. flavipennis* and 143 *A. foraminosa*) were collected during July 1994, at the Campo Experimental for the study of pupation and adult emergence of the fall generation. In both cases, larvae were placed individually in 30-ml plastic cups with soil and closed with plastic lids, maintained in the laboratory at 25 $\pm$ 1°C, and observed three times a week for survival and pupation. Soils in the cups were moistened once a week to avoid desiccation. At the time of pupation, individuals were placed in new cups containing a moistened cotton ball separated by a piece of wax paper. Pupae were observed daily for adult emergence. After adults emerged, cotton balls were removed from the cups, and adults were maintained unfed (simulating the "waiting in the soil" condition), and observed daily for longevity. Sex was determined only for *P. crinita* adults.

Percentage survival, pupal duration, and adult longevity were estimated for all species in the spring generation. Pupation and adult emergence of the species of *Anomala* during the fall generation were recorded, but neither pupal duration nor adult longevity was estimated. The purpose for observing the second generation was to corroborate the bivoltinism of species of *Anomala* in northern Tamaulipas. Survival was compared among species by using χ^2 test ($P < 0.05$), and pupal duration and adult longevity by t tests ($P < 0.05$) (SAS Institute 1988).

RESULTS AND DISCUSSION

Survival of field-collected larvae to pupal stage and adulthood was moderate for species of *Anomala*, and poor for *P. crinita* (Table 1). It was not possible to determine whether these differences were due to rearing conditions or could be attributed to actual differences in vital statistics among the species. Duration of the pupal stage averaged 14.6 and 15.8 d for *A. foraminosa* and *A. flavipennis*, respectively. The pupal stage of *P. crinita* averaged 21.0 and 21.9 for males and females, respectively (Table 2). Duration of the pupal stage of *A. flavipennis* and *P. crinita* in this study were similar to those recorded for the same species elsewhere by Hayes and McColloch (1924) and Reinhard (1940), respectively. Although pupation habits and the average length of the pupal stage in the genus *Phyllophaga* may be different between species, those differences are not as variable as in the genus *Anomala*. For example, *Anomala nigropicta* Casey and *A. binotata* Gyllenhal pupate in the fall, overwinter as adults, and emerge early the next spring; in the same locality, *A. innuba* (F.) overwinters as a full grown larva, pupates in the spring, and adults emerge in June (Ritcher 1958). High variability has been reported even within the same species;

Table 1. Survival of Field-Collected Larvae of *Anomala foraminosa, A. flavipennis*, and *Phyllophaga crinita* to Pupal Stage and Adulthood.

Species	No. larvae collected	% Survival to pupal stage[a]	% Survival to adulthood[a]
A. foraminosa	188	75.7 a	52.1 a
A. flavipennis	127	78.8 a	56.5 a
P. crinita	180	53.8 b	30.8 b

[a] Percentages within a column followed by the same letter are not significantly different ($P <$ 0.05; χ^2 test).

Table 2. Pupal Duration and Adult Longevity of *Anomala foraminosa, A. flavipennis*, and *Phyllophaga crinita* at 25 $\pm$ 1°C.

		Pupal duration (d)			Adult longevity (d)		
Species (sex)	n	Mean[a] $\pm$ SEM	Min	Max	Mean[a] $\pm$ SEM	Min	Max
A. foraminosa (m+f)	98	14.6 $\pm$ 0.3 a	9	20	13.3 $\pm$ 0.5 a	4	24
A. flavipennis (m+f)	72	15.8 $\pm$ 0.3 b	10	20	15.3 $\pm$ 1.0 b	4	38
P. crinita (m)	14	21.0 $\pm$ 0.6 c	18	25	25.4 $\pm$ 3.0 c	13	46
P. crinita (f)	20	21.9 $\pm$ 0.5 c	19	26	38.1 $\pm$ 5.8 c	5	87

[a] Means within a column followed by the same letter are not significantly different ($P < 0.05$; *t* test).

pupation of *Anomala transvaalensis* Arrow ranged from 42 to 271 d (Donaldson 1981). This high variability in duration of the pupal stage was not the case for the two species of *Anomala* and *P. crinita* in this study (Table 2).

Adult longevity differed among species, ranging from an average of 13.3 d for *A. foraminosa* to 38.1 d for *P. crinita* females (Table 2). Maximum adult longevity was observed for a *P. crinita* female, which lived almost three months (Table 2). This suggests that teneral adults of *P. crinita* are able to wait in the pupal cells for long periods of time before rainfall triggers emergence. Evidently *P. crinita* has evolved to synchronize "readiness for emergence" (i.e., completion of pupation) with the rainy season. Flight activity of *P. crinita* occurs mostly from late April to early June (Rodriguez-del-Bosque 1995), a period with high probability of rains in northeastern Mexico. The ability of *P. crinita* adults to survive in the soil for long periods of time allows the species to cope with drought periods.

The apparently shorter longevity observed for species of *Anomala* may not be an ecological disadvantage because adult emergence of *A. flavipennis* and *A. foraminosa* are not as dependent on rainfall (unpublished data). All surviving full grown larvae of *A. flavipennis* and *A. foraminosa* collected during either February-March or July pupated, and adults emerged during the respective spring or fall generations. This demonstrated the

existence of two distinct annual generations for both of these species in northeastern Mexico. None of the larvae collected during the first generation emerged during the second generation, and vice versa, diminishing the possibility for the existence of populations with "short" and "long" life cycles, as suggested for *Anomala cupripes* Hope in Taiwan (Chu et al. 1986).

In conclusion, this study demonstrated the ability of *P. crinita* adults to survive for long periods of time before emergence, a trait adaptive for coping with drought. In addition, bivoltinism of *A. flavipennis* and *A. foraminosa* in northeastern Mexico was evidenced, in contrast with the univoltinism of *A. flavipennis* in Kansas (Hayes and McColloch 1924), a situation probably related to the slower accumulation of heat units in northern latitudes that preclude the possibility of having two cycles a year.

ACKNOWLEDGMENT

The assistance in field collections and laboratory observations by Rosalio Navarro and Julian Fuentes, and the helpful comments to an early version of this manuscript by Robert L. Crocker and David G. Riley (Texas A&M University), are greatly appreciated. Approved by the Centro de Investigación Regional del Noreste as INIFAP-CIRNE-A014.

LITERATURE CITED

Chu, Y. I., J. L. Juang, and C. J. Shih. 1986. Studies on the development of larval stage of *Anomala cupripes* Hope. Pl. Prot. Bull. (Taiwan) 28: 335-341.

Donaldson, J.M.I. 1981. Laboratory rearing technique and life cycle of *Anomala transvaalensis* (Coleoptera: Scarabaeidae). J. Ent. Soc. Sth. Afr. 44: 283-288.

Gaylor, M. J., and G. W. Frankie. 1979. The relationship of rainfall to adult flight activity, and of soil moisture to oviposition behavior and egg and first instar survival in *Phyllophaga crinita*. Environ. Entomol. 8: 591-594.

Hayes, Wm. P., and J. W. McColloch. 1924. The biology of *Anomala kansana* (Scarabaeidae, Coleop.). J. Econ. Entomol. 17: 589-594.

Huffman, F. R., and J. A. Harding. 1980. Biology of *Phyllophaga crinita* (Burmeister) in the Lower Rio Grande Valley sugarcane. Southwest. Entomol. 5: 59-64.

Reinhard, H. J. 1940. The life history of *Phyllophaga lanceolata* (Say) and *Phyllophaga crinita* Burmeister. J. Econ. Entomol. 33: 572-578.

Ritcher, P. O. 1958. Biology of the Scarabaeidae. Ann. Rev. Entomol. 3: 311-334.

Rodriguez-del-Bosque, L. A. 1988. *Phyllophaga crinita* Burmeister (Coleoptera: Melolonthidae): Historia de una plaga del suelo (1855-1988). Memorias de la III Mesa Redonda sobre Plagas del Suelo. Sociedad Mexicana de Entomología. Morelia, Michoacán, México. pp. 53-79.

Rodriguez-del-Bosque, L. A., R. L. Crocker, and E. J. Riley. 1995. Diversity and abundance of *Phyllophaga* and *Anomala* species in agroecosystems of northern Tamaulipas, Mexico. Southwest. Entomol. 20: 55-59.

SAS Institute. 1988. SAS/STAT user's guide, release 6.03 edition. SAS Institute, Cary, N.C.

Teetes, G. L., L. J. Wade, R. C. McInyre, and C. A. Schaefer. 1976. Distribution and seasonal biology of *Phyllophaga crinita* in the Texas High Plains. J. Econ. Entomol. 69: 59-63.

SUSCEPTIBILITY STATUS OF BOLL WEEVILS[1] FROM LOUISIANA TO ELEVEN INSECTICIDES

S.H. Martin, J.B. Graves, B.R. Leonard, E. Burris, S. Micinski, J.D. Powell, and J. Roberson[2]

Louisiana State University Agricultural Center
Baton Rouge, LA 70803

ABSTRACT

Topical bioassays of 11 insecticides were conducted with the R. T. Gast Insect Rearing laboratory colony (GAST-LAB) and 22 field collections of boll weevils, *Anthonomus grandis grandis* Boheman, from 11 parishes in Louisiana. The order of toxicity of the insecticides tested against the GAST-LAB colony from most to least toxic was cyfluthrin > zeta-cypermethrin > fipronil [(±)-5-amino-1-(2, 6-dichloro-α,α,α-trifluoro-*p*-tolyl)-4-tri-fluoromethylsulfinylpyrazole-3-carbonitrile] > deltamethrin > azinphosmethyl > cypermethrin > methyl parathion > Pirate® [4-bromo-2-(4-chlorophenyl)-1-(ethoxymethyl)-5-(trifluoromethyl)-pyrrole-3-carbonitrile] > oxamyl > endosulfan > malathion. There was no conclusive evidence of resistance to any of the 11 insecticides bioassayed against field-collected boll weevils, although LD_{50}'s for cypermethrin, cyfluthrin, malathion, and oxamyl were highly variable. The LD_{50} values for deltamethrin, endosulfan, fipronil, oxamyl, Pirate®, and zeta-cypermethrin will serve as baseline data for future studies.

INTRODUCTION

The boll weevil, *Anthonomus grandis grandis* Boheman, was the most destructive pest of cotton in the United States in 1993, causing estimated losses in cotton production of 314,226 bales (Williams 1994). Management strategies have been developed to minimize the use of insecticides for control of this pest. However, chemical control remains the most effective strategy for managing boll weevils (Lincoln and Graves 1978, Graves and Wolfenbarger 1995).

Within a decade after organochlorine insecticides were introduced for control of the boll weevil, field populations from Louisiana were found to be resistant to these insecticides (Roussel and Clower 1957). In contrast, organophosphorus insecticides have been used successfully to control boll weevils without development of resistance since 1955 (Graves and Roussel 1962; Hopkins et al. 1975, 1984; Graves and Wolfenbarger 1995). Although boll weevil control

---

[1]*Anthonomus grandis grandis* Boheman (COLEOPTERA: CURCULIONIDAE)

[2]USDA-ARS, R. T. Gast Insect Rearing Laboratory

failures with organophosphorus insecticides have not been reported in the United States, laboratory studies have demonstrated a significant decrease in toxicity of azinphosmethyl to field collections from the Rio Grande Valley in Texas (Wolfenbarger et al. 1986). More recently, Kanga et al. (1995) reported substantial tolerance to organophosphorus, carbamate, and pyrethroid insecticides in boll weevils from the Brazos Valley in Texas.

Pyrethroid insecticides have been used since 1978 to control bollworm, *Helicoverpa zea* (Boddie), and tobacco budworm, *Heliothis virescens* (F.), in cotton and also to suppress boll weevil populations (Hopkins et al. 1984, Leonard et al. 1989, Graves and Wolfenbarger 1995). Several studies have established LD_{50} values for boll weevils using pyrethroids (Rathinam 1979, Pavloff 1982, Hopkins et al. 1984, Leonard et al. 1989).

Recently, concern has been expressed by some cotton producers and agricultural consultants that pyrethroids are not providing satisfactory efficacy against some populations of boll weevil. This study was undertaken to evaluate several populations of boll weevils collected throughout Louisiana for their susceptibility to selected pyrethroids and other classes of commercial and experimental insecticides. The data from this study are compared to previously reported reference values (Hopkins et al. 1975, Rathinam 1979, Pavloff 1982, Hopkins et al. 1984, Wolfenbarger et al. 1986, Leonard et al. 1989) to determine if any changes in boll weevil susceptibility to these insecticides have occurred. This study also established reference toxicological values for oxamyl, a carbamate insecticide registered for boll weevil control, and several other insecticides that may be used for control of boll weevil or other cotton pests in the near future. Additionally, spring and fall field collections of boll weevil were compared to determine if seasonal variation in responses to insecticides is occurring.

MATERIALS AND METHODS

The R. T. Gast Insect Rearing Laboratory (Mississippi State, MS) colony of boll weevils (GAST-LAB), which has been in continuous laboratory culture without exposure to insecticides or supplementation with field strains for more than 15 years, was used as a reference colony. GAST-LAB weevils were collected as adults from larval rearing trays and fed diet pellets (Roberson and Wright 1984).

Field collections of adult boll weevils from 11 Louisiana parishes (counties) were made by using Scentry® pheromone traps baited with grandlure (Scentry Division, Ecogen Inc., Langhorne, PA). The parishes chosen for the study ranged from those with the lowest (Bienville and De Soto) to the highest (Franklin and Tensas) cotton acreage and from the most northern (Bossier and Caddo) to the most southern (Pointe Coupee and Rapides) cotton production areas. Generally, boll weevils were trapped from several cotton fields in each parish and pooled. The adult weevils were removed from the traps, placed in 3.7-liter cardboard cartons, and supplied diet pellets (Roberson and Wright 1984). The insects then were transported to the Cotton Insect Pest Management Laboratory, Department of Entomology, Louisiana State University

Agricultural Center in Baton Rouge, LA. Collections were made from May-June of 1991, 1992, and 1993 and from September-November of 1992 and 1993.

Technical grade azinphosmethyl and cyfluthrin (Bayer Corporation, Kansas City, MO); cypermethrin, endosulfan, and zeta-cypermethrin (FMC Corporation, Middleport, NY); malathion and Pirate® [4-bromo-2-(4-chlorophenyl)-1-(ethoxymethyl)-5-(trifluoromethyl)-pyrrole-3-carbonitrile] (American Cyanamid Company, Princeton, NJ); methyl parathion (Chem Sources, West Chester, PA); oxamyl (Dupont E.I. de Nemours and Company, Wilmington, DL), and deltamethrin and fipronil [(±)-5-amino-1-(2,6-dichloro-α,α,α-trifluoro-p-tolyl)-4-trifluoromethylsulfinylpyrazole-3-carbonitrile] (Rhone-Poulenc Ag. Co., Research Triangle Park, NC) were obtained from the manufacturers for the topical bioassays.

Adult boll weevils weighing 10-18 mg were placed into 300-ml Nyman® (Nyman Mfg. Co., E. Providence, RI) cups (10 weevils/cup) and supplied with fresh diet pellets. All insecticides were applied according to the standard Entomological Society of America method of determining insecticide resistance in boll weevil (Anonymous 1968). Serial dilutions were prepared from technical grade insecticides in acetone solutions. Insects were treated on the dorsal surface of the thorax with 1-μl aliquots of acetone alone (control) or technical grade insecticide dissolved in acetone.

Dose-mortality lines for all insecticides were determined from four or five doses per insecticide with ten insects treated per dose (two-three replicates). After treatment, the weevils were held at 27±3°C at 55-65% RH and mortality was determined after 48 h. The criterion for mortality was inability of weevils to upright themselves within 30-60 seconds after being prodded.

All data were corrected for control mortality using Abbott's (1925) formula. Control mortality was generally less than 10%. Results were analyzed using a microcomputer-based probit analysis (POLO-PC, LeOra Software, 1119 Shattuck Ave., Berkeley, CA). Chi-square values for all dose-mortality lines were not significant, which indicated that all of the data sets fit the probit model. LD_{50}'s were considered significantly different if 95% confidence limits (CL) did not overlap. Toxicity ratios (TR) were calculated by dividing LD_{50}'s of field collections by LD_{50}'s of the GAST-LAB reference colony (Robertson et al. 1995).

RESULTS

The relative toxicity of 11 insecticides to the GAST-LAB reference colony ranged from a 48 h LD_{50} of 0.003 for cyfluthrin to 0.586 μg/weevil for malathion, a 195X difference (Tables 1-11). Cyfluthrin was the most toxic compound followed by zeta-cypermethrin, fipronil, deltamethrin, azinphosmethyl, cypermethrin, methyl parathion, Pirate®, oxamyl, endosulfan, and malathion, respectively. Slopes of dose-mortality lines ranged from 1.6 (fipronil) to 5.1 (methyl parathion) with most values ranging from 2-4.

LD_{50}'s of field-collected boll weevils to azinphosmethyl ranged from 0.011 (1991 spring Bossier) to 0.107 μg/weevil (1992 fall Bossier), a 9.7X difference (Table 1). Five

TABLE 1. Toxicity of Azinphosmethyl to Boll Weevils from Selected Locations. LD_{50} Values are Expressed in μg of Insecticide/Weevil.

Colony or Collection	Number tested	48-Hour LD_{50} (95% CL)	Slope±SE	TR[a]
GAST-LAB	460	0.029 (0.023-0.037)	3.1±0.3	
		Spring 1991		
Franklin	145	0.020 (0.014-0.025)	3.7±0.8	0.7
Rapides	181	0.026 (0.016-0.037)	2.7±0.6	0.9
Bossier	210	0.011 (0.007-0.015)	2.7±0.7	0.4
Tensas	254	0.017 (0.011-0.023)	2.6±0.4	0.6
		Spring 1992		
Bienville	200	0.031 (0.018-0.043)	3.4±0.5	1.0
Caddo	200	0.072 (0.049-0.141)	2.8±0.6	2.5
Concordia	138	0.027 (0.022-0.033)	3.8±0.6	0.9
Franklin	105	0.029 (0.023-0.034)	7.0±1.6	1.0
Rapides	177	0.073 (0.056-0.110)	2.0±0.4	2.5
Tensas	120	0.027 (0.019-0.033)	4.7±1.0	0.9
		Fall 1992		
Bossier	200	0.107 (0.088-0.168)	3.7±1.0	3.6
Caddo	150	0.078 (0.062-0.111)	5.0±0.8	2.6
Franklin	291	0.028 (0.022-0.032)	5.3±1.0	0.9
		Spring 1993		
Bossier	390	0.041 (0.026-0.061)	2.6±0.6	1.4
Caddo	150	0.059 (0.045-0.070)	5.3±1.4	2.0
Concordia	130	0.017 (0.005-0.035)	2.3±0.4	0.6
Franklin	181	0.028 (0.022-0.037)	2.4±0.3	1.0
Grant	130	0.024 (0.018-0.032)	2.9±0.5	0.8
Natchitoches	130	0.020 (0.015-0.026)	4.1±0.9	0.7
Rapides	180	0.029 (0.015-0.045)	1.5±0.4	1.0
Tensas	181	0.025 (0.015-0.040)	2.6±0.3	0.8

[a]TR (toxicity ratio) is LD_{50} of field collection/LD_{50} of GAST-LAB colony.

collections of weevils had LD_{50}'s that were significantly higher than the GAST-LAB colony (0.029 μg/weevil). The Bossier 1991 spring collection had a LD_{50} that was significantly lower than the GAST-LAB colony, while the other 15 collections had LD_{50}'s that were not significantly different from that for the GAST-LAB colony. TRs ranged from 0.4-3.6X. The LD_{50} of the Bossier 1992 fall field collection was significantly higher than the LD_{50}'s of the Bossier 1991 and 1993 spring field collections. However, no significant differences were observed among LD_{50}'s of spring and fall collections of boll weevils from Caddo Parish or among the LD_{50}'s of spring and fall collections of boll weevils from Franklin Parish. Furthermore, boll weevils collected in the spring of 1991 from Bossier Parish were significantly more susceptible to azinphosmethyl than the collection made in this parish in the spring of 1993. Similarly, weevils collected from Rapides Parish in the spring of 1991 and 1993 were less susceptible than weevils collected from the same parish in 1992.

LD_{50}'s of field-collected boll weevils to malathion ranged from 0.076 (1991 Tensas) to 3.786 (1992 Bossier) μg/weevil, a 50X difference (Table 2).

TABLE 2. Toxicity of Malathion to Boll Weevils from Selected Locations. LD_{50} Values are Expressed in µg of Insecticide/Weevil.

Colony or Collection	Number tested	48-Hour LD_{50} (95% CL)	Slope±SE	TR[a]
GAST-LAB	414	0.586 (0.454-0.739)	3.3±0.4	
		Spring 1991		
Tensas	180	0.076 (0.052-0.111)	2.4±0.4	0.1
		Spring 1992		
Bienville	320	0.244 (0.206-0.275)	5.9±1.4	0.4
Tensas	115	0.294 (0.208-0.394)	2.4±0.4	0.5
		Fall 1992		
Bossier	340	3.786 (2.281-10.42)	1.5±0.3	6.5
Caddo	150	3.299 (1.381-173.8)	1.0±0.3	5.6
Franklin	440	0.176 (0.102-0.257)	2.1±0.3	0.3
		Spring 1993		
Bossier	390	0.593 (0.289-1.422)	1.9±0.5	0.9
Caddo	200	0.780 (0.623-1.053)	3.7±1.0	1.3
Concordia	240	0.132 (0.067-0.238)	3.0±0.4	0.2
De Soto	180	0.178 (0.100-0.255)	2.5±0.5	0.3
Franklin	298	0.128 (0.104-0.160)	3.4±0.5	0.2
Grant	120	0.151 (0.073-0.253)	1.8±0.4	0.3
Natchitoches	211	0.138 (0.080-0.199)	2.5±0.5	0.2
Rapides	150	0.416 (0.275-0.807)	2.0±0.5	0.7
Tensas	120	0.141 (0.108-0.174)	4.2±0.8	0.2

[a]TR (toxicity ratio) is LD_{50} of field collection/LD_{50} of GAST-LAB colony.

TABLE 3. Toxicity of Methyl Parathion to Boll Weevils from Selected Locations. LD_{50} Values are Expressed in µg of Insecticide/Weevil.

Colony or Collection	Number tested	48-Hour LD_{50} (95% CL)	Slope±SE	TR[a]
GAST-LAB	750	0.087 (0.073-0.104)	5.1±0.7	
		Spring 1992		
Tensas	167	0.031 (0.019-0.02)	4.0±0.6	0.4
		Fall 1992		
Bossier	310	0.063 (0.051-0.074	6.4±1.0	0.7
Caddo	150	0.069 (0.056-0.10)	3.5±1.0	0.8
Franklin	302	0.056 (0.048-0.063)	4.5±0.7	0.7
		Spring 1993		
Bossier	390	0.034 (0.024-0.042)	3.5±0.9	0.4
Caddo	130	0.034 (0.020-0.043)	3.9±1.0	0.4
Concordia	180	0.031 (0.026-0.036)	7.0±1.5	0.4
Franklin	180	0.065 (0.052-0.068)	2.4±0.4	0.8
Grant	190	0.028 (0.016-0.036)	4.2±1.1	0.3
Natchitoches	270	0.039 (0.030-0.046)	4.6±0.9	0.5
Rapides	210	0.047 (0.029-0.062)	5.4±0.8	0.5
Tensas	180	0.035 (0.020-0.053)	3.7±0.5	0.4
		Fall 1993		
Tensas	111	0.050 (0.037-0.060)	5.3±1.2	0.6

[a]TR (toxicity ratio) is LD_{50} of field collection/LD_{50} of GAST-LAB colony.

Three field collections had significantly higher LD_{50}'s than the GAST-LAB colony (0.586 μg/weevil), three collections had LD_{50}'s not significantly different from the GAST-LAB colony, while ten collections had LD_{50}'s that were significantly lower. TRs ranged from 0.1-6.5X. Boll weevils collected from Tensas Parish in the spring of 1992 were significantly less susceptible to malathion than boll weevils collected in the spring of 1991 or spring of 1993. Also weevils collected from Bossier and Caddo parishes during the fall of 1992 were significantly less susceptible to malathion than those collected from these same parishes in the spring of 1993.

LD_{50}'s of the field collections of boll weevil for methyl parathion ranged from 0.028 (1993 Grant) to 0.069 (1992 Caddo) μg/weevil, a 2.5X difference (Table 3).

Eleven of the 13 LD_{50} values for methyl parathion were significantly lower than the LD_{50} for the GAST-LAB colony (0.087 μg/weevil), and none was higher. TRs ranged from 0.3-0.8X. The LD_{50}'s of the 1992 Bossier and Caddo fall collections were significantly higher than LD_{50}'s of the field collections made in these parishes during the spring of 1993. However, no significant difference was observed between the LD_{50} of the 1992 fall Franklin Parish collection and that of the 1993 spring field collection. All boll weevil collections from Tensas Parish had similar LD_{50}'s regardless of collection time.

LD_{50} values for oxamyl for the field-collected boll weevils ranged from 0.024 (1993 Natchitoches) to 0.397 (1992 Bossier) μg/weevil, a 16.5X difference (Table 4). Nine of the 17 field collections had LD_{50}'s significantly lower than the GAST-LAB colony (0.205 μg/weevil), but none was significantly higher. TRs ranged from 0.1-1.9X. Significantly higher LD_{50}'s were observed for field collections of boll weevils during the fall of 1992 from Bossier Parish compared to the spring of 1993. However, no significant difference was observed between the Caddo field collection made in the fall of 1992 and the spring collection in 1993. The LD_{50} response for the 1992 fall Franklin Parish collection was significantly higher than that of the 1993 spring Franklin collection but not significantly different compared to the 1992 spring Franklin collection.

Seven field collections of boll weevils had LD_{50}'s for endosulfan ranging from 0.032 (Franklin) to 0.08 (Concordia) μg/weevil, a 2.5X difference (Table 5). All LD_{50}'s for the seven field collections were significantly lower than the LD_{50} (0.435 μg/weevil) for the GAST-LAB colony. TRs ranged from 0.1-0.2X.

LD_{50}'s of the field-collected weevils for cypermethrin ranged from 0.015 (1992 fall Tensas) to 0.764 (1993 Caddo) μg/weevil, a 51X difference (Table 6). Fifteen of the 22 field collections of boll weevils had LD_{50}'s significantly higher than the GAST-LAB colony (0.029 μg/weevil) and none had significantly lower LD_{50} values. TRs ranged from 0.5-26.4X. None of the fall field collections of boll weevil exhibited significantly higher LD_{50}s compared with spring field collections from the same locations. Significantly higher LD_{50}'s were observed for spring field collections from Caddo (1993), Franklin (1991 and 1993), and Tensas (1991, 1992, and 1993) compared with fall field collections from the same locations.

TABLE 4. Toxicity of Oxamyl to Boll Weevils from Selected Locations. LD_{50} values are Expressed in μg of Insecticide/Weevil.

Colony or Collection	Number tested	48-Hour LD_{50} (95% CL)	Slope±SE	TR[a]
GAST-LAB	460	0.205 (0.130-0.346)	2.5±0.3	
		Spring 1991		
Tensas	180	0.036 (0.023-0.051)	2.3±0.4	0.2
		Spring 1992		
Bienville	290	0.110 (0.089-0.135)	4.10.8	0.5
Concordia	131	0.108 (0.087-0.153)	3.5±0.8	0.5
Franklin	113	0.074 (0.056-0.094)	3.4±0.7	0.4
Rapides	95	0.101 (0.080-0.145)	3.9±1.0	0.5
Tensas	150	0.093 (0.059-0.176)	3.2±0.5	0.5
		Fall 1992		
Bossier	340	0.397 (0.261-0.595)	2.5±0.3	1.9
Caddo	165	0.202 (0.121-0.458)	2.5±0.4	1.0
Franklin	421	0.110 (0.076-0.150)	4.3±0.9	0.5
		Spring 1993		
Bossier	240	0.048 (0.033-0.063)	2.4±0.5	0.2
Caddo	252	0.071 (0.049-0.163)	2.0±0.5	0.4
Concordia	240	0.050 (0.035-0.078)	1.8±0.4	0.2
Franklin	120	0.030 (0.024-0.035)	6.6±1.5	0.2
Grant	290	0.057 (0.036-0.098)	2.1±0.4	0.3
Natchitoches	420	0.024 (0.016-0.032)	1.8±0.3	0.1
Rapides	180	0.058 (0.042-0.081)	2.5±0.5	0.3
Tensas	180	0.040 (0.032-0.050)	2.8±0.4	0.2

[a]TR (toxicity ratio) is the LD_{50} of field collection/LD_{50} of GAST-LAB colony.

TABLE 5. Toxicity of Endosulfan to Boll Weevils from Selected Locations. LD_{50} Values are Expressed in μg of Insecticide/Weevil.

Colony or Collection	Number tested	48-Hour LD_{50} (95% CL)	Slope±SE	TR[a]
GAST-LAB	170	0.435 (0.348-0.524)	3.9±0.8	
		Spring 1993		
Bossier	181	0.049 (0.034-0.066)	2.1±0.4	0.1
Concordia	130	0.080 (0.056-0.104)	2.8±0.6	0.2
Franklin	250	0.032 (0.016-0.053)	2.3±0.4	0.1
Grant	240	0.072 (0.056-0.089)	3.8±0.8	0.2
Natchitoches	209	0.050 (0.031-0.072)	2.3±0.5	0.1
Rapides	120	0.048 (0.020-0.097)	2.9±0.5	0.1
Tensas	240	0.039 (0.028-0.052)	2.1±0.3	0.1

[a]TR (toxicity ratio) is LD_{50} of field collection/LD_{50} of GAST-LAB colony.

Eight field collections of boll weevils had LD_{50}'s for cyfluthrin that ranged from 0.005 (1993 Grant) to 0.092 (1992 Bossier) μg/weevil, a 18X difference (Table 7). Four of the eight weevil collections had LD_{50} values significantly higher than the GAST-LAB colony (0.003 μg/weevil), and none had LD_{50} values that were significantly lower. TRs ranged from 1.4-

TABLE 6. Toxicity of Cypermethrin to Boll Weevils from Selected Locations. LD_{50} Values are Expressed in μg of Insecticide/Weevil.

Colony or Collection	Number tested	48-Hour LD_{50} (95% CL)	Slope±SE	TR[a]
GAST-LAB	540	0.029 (0.020-0.039)	2.1±0.2	
		Spring 1991		
Franklin	189	0.091 (0.057-0.121)	2.9±0.7	3.1
Rapides	150	0.056 (0.035-0.080)	2.6±0.7	1.9
Tensas	209	0.067 (0.027-0.125)	2.1±0.4	2.3
		Spring 1992		
Bienville	190	0.060 (0.044-0.080)	2.0±0.3	2.0
Caddo	170	0.427 (0.219-2.496)	1.3±0.3	14.7
Concordia	120	0.107 (0.059-0.198)	1.7±0.6	3.7
Franklin	59	0.031 (0.017-0.048)	2.3±0.6	1.1
Rapides	118	0.081 (0.059-0.117)	2.2±0.4	2.8
Tensas	180	0.058 (0.032-0.082)	2.1±0.5	2.0
		Fall 1992		
Bossier	340	0.368 (0.264-0.539)	1.3±0.2	12.8
Caddo	170	0.116 (0.060-0.280)	1.4±0.3	4.0
Franklin	421	0.018 (0.011-0.024)	3.4±0.3	0.6
Tensas	121	0.015 (0.007-0.024)	1.3±0.3	0.5
		Spring 1993		
Bossier	389	0.417 (0.192-1.834)	0.6±0.1	14.5
Caddo	300	0.764 (0.381-2.721)	1.1±0.2	26.4
Concordia	240	0.078 (0.053-0.156)	1.9±0.5	2.7
Franklin	150	0.065 (0.053-0.082)	3.5±0.6	2.2
Grant	180	0.094 (0.063-0.197)	1.6±0.3	3.2
Natchitoches	419	0.092 (0.040-0.191)	1.3±0.2	3.2
Pointe Coupee	113	0.073 (0.052-0.173)	2.7±1.0	2.5
Rapides	260	0.038 (0.026-0.059)	1.5±0.3	1.3
Tensas	180	0.055 (0.041-0.084)	1.8±0.3	1.9

[a]TR (toxicity ratio) is LD_{50} of field collection/LD_{50} of GAST-LAB colony.

TABLE 7. Toxicity of Cyfluthrin to Boll Weevils from Selected Locations. LD_{50} Values are expressed in μg of Insecticide/Weevil.

Colony or Collection	Number tested	48-Hour LD_{50} (95% CL)	Slope±SE	TR[a]
GAST-LAB	200	0.003 (0.002-0.006)	2.4±0.3	
		Fall 1992		
Bossier	120	0.092 (0.050-0.147)	1.4±0.3	26.9
Franklin	371	0.010 (0.003-0.017)	1.4±0.2	2.8
		Spring 1993		
Bossier	382	0.038 (0.024-0.116)	1.5±0.4	11.3
Concordia	190	0.008 (0.005-0.012)	1.8±0.4	2.4
Franklin	220	0.009 (0.005-0.018)	1.1±0.3	2.8
Grant	150	0.005 (0.003-0.007)	1.8±0.4	1.4
Natchitoches	241	0.027 (0.017-0.070)	1.6±0.4	7.9
Tensas	210	0.012 (0.009-0.016)	2.6±0.6	3.6

[a]TR (toxicity ratio) is LD_{50} of field collection/LD_{50} of GAST-LAB colony.

TABLE 8. Toxicity of Deltamethrin to Boll Weevils from Selected Locations. LD_{50} Values are Expressed in µg of Insecticide/Weevil.

Colony or Collection	Number tested	48-Hour LD_{50} (95% CL)	Slope±SE	TR[a]
GAST-LAB	150	0.017 (0.012-0.021)	3.6±0.7	
		Spring 1993		
Bossier	332	0.032 (0.023-0.046)	2.8±0.6	1.9
Concordia	280	0.022 (0.014-0.029)	1.8±0.3	1.3
Franklin	250	0.010 (0.005-0.016)	1.2±0.3	0.6
Natchitoches	209	0.060 (0.033-0.232)	1.2±0.3	3.6
Tensas	190	0.021 (0.012-0.044)	1.3±0.3	1.2

[a]TR (toxicity ratio) is LD_{50} of field collection/LD_{50} of GAST-LAB colony.

TABLE 9. Toxicity of Zeta-cypermethrin to Boll Weevils from Selected Locations. LD_{50} Values are Expressed in µg of Insecticide/Weevil.

Colony or Collection	Number tested	48-Hour LD_{50} (95% CL)	Slope±SE	TR[a]
GAST-LAB	120	0.012 (0.009-0.015)	4.1±0.8	
		Fall 1992		
Franklin	160	0.011 (0.006-0.019)	1.3±0.3	1.0
		Spring 1993		
Bossier	562	0.040 (0.023-0.099)	2.1±0.3	3.3
Concordia	330	0.018 (0.013-0.024)	1.7±0.3	1.5
Franklin	250	0.016 (0.011-0.021)	1.8±0.3	1.3
Natchitoches	270	0.010 (0.007-0.014)	2.3±0.4	0.9
Tensas	330	0.019 (0.014-0.025)	2.0±0.3	1.6

[a]TR (toxicity ratio is LD_{50} of field collection/LD_{50} of GAST-LAB colony.

TABLE 10. Toxicity of Fipronil to Boll Weevils from Selected Locations. LD_{50} Values are Expressed in µg of Insecticide/Weevil.

Colony or Collection	Number tested	48-Hour LD_{50} (95% CL)	Slope±SE	TR[a]
GAST-LAB	210	0.013 (0.009-0.019)	1.6±0.3	
		Spring 1993		
Bossier	300	0.009 (0.007-0.014)	2.4±0.6	0.7
Caddo	144	0.012 (0.009-0.019)	2.4±0.6	0.9
Concordia	241	0.006 (0.004-0.007)	2.9±0.6	0.5
Franklin	250	0.008 (0.006-0.009)	3.7±0.7	0.6
Grant	270	0.004 (0.001-0.005)	2.6±0.8	0.3
Natchitoches	269	0.006 (0.005-0.007)	3.8±1.0	0.5
Rapides	381	0.010 (0.009-0.013)	4.7±1.3	0.8
Tensas	470	0.009 (0.006-0.012)	3.1±0.4	0.7

[a]TR (toxicity ratio) is LD_{50} of field collection/LD_{50} of GAST-LAB colony.

26.9X. No significant differences between LD_{50} values were observed for fall (1992) field collections from Bossier or Franklin Parish compared with spring field collections (1993) from the same locations.

LD_{50} values for deltamethrin for five field collections of boll weevils ranged from 0.010 (Franklin) to 0.060 (Natchitoches) µg/weevil, a 6X difference (Table 8). Two LD_{50} values for field collections were significantly higher than the LD_{50} (0.017 µg/weevil) for the GAST-LAB colony, but none of the LD_{50} values for the field collections was significantly lower than the GAST-LAB colony. TRs ranged from 0.6-3.6X.

Six field collections had LD_{50} values for zeta-cypermethrin that ranged from 0.010 (Natchitoches) to 0.040 (Bossier) µg/weevil, a 4X difference (Table 9). Only one (Bossier) of the six field collections had an LD_{50} value that was significantly higher than the LD_{50} (0.012 µg/weevil) of the GAST-LAB colony. TRs ranged from 0.9-3.3X. No significant differences in LD_{50} values were observed between the 1992 fall field collection from Franklin Parish and the 1993 spring collection.

LD_{50} values for to fipronil ranged from 0.004 (Grant) to 0.012 (Caddo) µg/weevil, a 3X difference (Table 10). Three of the eight field collections of weevils had LD_{50}'s that were significantly lower than that of the GAST-LAB colony (0.013 µg/weevil), and none had significantly higher LD_{50} values. TRs of field collections ranged from 0.3-0.9X.

Seven field collections had LD_{50} values for Pirate® ranging from 0.013 (Franklin) to 0.050 (Tensas), a 3.8X difference (Table 11). Six of the seven LD_{50} values for the field collections of boll weevils were significantly lower than that of the GAST-LAB colony (0.151 µg/weevil), and none had significantly higher LD_{50} values. TRs ranged from 0.3-0.9X.

TABLE 11. Toxicity of Pirate® to Boll Weevils from Selected Locations. LD_{50} Values are Expressed in µg of Insecticide/Weevil.

Colony or Collection	Number tested	48-Hour LD_{50} (95% CL)	Slope±SE	TR[a]
GAST-LAB	220	0.151 (0.116-0.219)	2.9±0.6	
		Spring 1993		
Bossier	422	0.022 (0.014-0.033)	1.4±0.2	0.4
Concordia	190	0.043 (0.004-0.128)	1.1±0.2	0.9
Franklin	190	0.013 (0.008-0.017)	3.0±0.7	0.3
Grant	300	0.020 (0.009-0.035)	1.2±0.2	0.4
Natchitoches	210	0.028 (0.012-0.050)	1.2±0.2	0.6
Rapides	101	0.029 (0.011-0.049)	1.9±0.5	0.6
Tensas	240	0.050 (0.016-0.106)	1.3±0.2	0.3

[a]TR (toxicity ratio) is LD_{50} of field collection/LD_{50} of GAST-LAB colony.

DISCUSSION

The order of toxicity of the 11 insecticides bioassayed by topical application against the GAST-LAB colony of boll weevils from the most toxic to least toxic was cyfluthrin > zeta-cypermethrin > fipronil > deltamethrin > azinphosmethyl> cypermethrin > methyl parathion > Pirate® > oxamyl > endosulfan > malathion. The pyrethroids as a class were generally the most toxic insecticides to boll weevils. However, the order of toxicity of insecticides in laboratory bioassays is not necessarily indicative of their performance in the field. Field performance is determined not only by inherent toxicity but also by dosage rate as well as the chemical and physical characteristics for each insecticide as it is formulated.

GAST-LAB Reference Colony. This colony of boll weevils has been reared for over 15 years at the R.T. Gast Rearing Laboratory because its coloration is darker than normal [designated ebony by Bartlett (1967)]. This darker coloration has facilitated research on sterilization and population dynamics of boll weevils (Wright and Villavaso 1983). However, the use of this colony as a "susceptible" reference strain in estimating TRs or RRs for insecticide resistance studies appears questionable based on the data reported herein. In the case of endosulfan, fipronil, methyl parathion, oxamyl, and Pirate®, most of the LD_{50} values for field collections of boll weevils were significantly lower than the LD_{50} for the GAST-LAB colony. Consequently, more emphasis was placed on comparison of the range of LD_{50} values of field collections of boll weevils obtained for each insecticide to previously published LD_{50} values than on TRs in trying to determine the relative susceptibility of field collections of boll weevils to insecticides.

Organophosphorus Insecticides. The 48-h LD_{50} values for the GAST-LAB colony and all of the field colonies for azinphosmethyl were similar to the 48-h values of 0.01-0.12 (Rathinam 1979), 0.04-0.08 (Hopkins et al. 1984), 0.02-0.18 (Pavloff 1982), 0.0092-0.13 (Wolfenbarger et al. 1986), and 0.04 (Leonard et al. 1989) reported earlier. Variation found in the responses of field collections of boll weevils (0.011 to 0.107 μg/weevil, a 10X difference) in this study is similar to the 12X difference noted by Rathinam (1979), the 9X difference observed by Pavloff (1982), and the 14X difference reported by Wolfenbarger et al. (1986). Four of the five field collections that had the highest LD_{50}'s were made in either Bossier or Caddo Parish, which are located in northwest Louisiana.

In a previous study, Rathinam (1979) compared the toxicity of malathion to boll weevils from nine states. The 48-h LD_{50} values reported in that study ranged from 0.22 μg/weevil exhibited by weevils from Scott County, MS, to 1.15 μg/weevil for weevils from Weslaco, TX. The GAST-LAB colony and all but two of the field collections in this study exhibited 48-h LD_{50}'s within the range reported by Rathinam (1979) and the value (1.24 μg/weevil) reported by Hopkins et al. (1975) for a laboratory colony. The Bossier (1992) and the Caddo (1992) field collections exhibited significantly higher LD_{50}'s compared with the GAST-LAB colony and all of the other field collections in the present study including

collections from the same locations made during 1993. The LD_{50} values of the 1992 collections from Bossier and Caddo Parishes were 6X greater than that of the GAST-LAB colony and 4X greater than the LD_{50} values determined for collections from the same parishes in 1993.

The 48-h LD_{50} values for the GAST-LAB colony (0.087 µg/weevil) and all of the field collections (range was 0.028-0.069 µg/weevil) for methyl parathion were similar to the values 0.03-0.08, 0.04-0.13, 0.05-0.06, and 0.05 presented by Rathinam (1979), Pavloff (1982), Hopkins et al. (1984), and Leonard et al. (1989), respectively. Additionally, there has been little if any change in methyl parathion toxicity during the last 30 years based on LD_{50} data (0.03-0.06 µg/weevil) from the early 1960's (Graves and Roussel 1962).

The organophosphorus insecticides, particularly azinphosmethyl and methyl parathion, have been the insecticides of choice for boll weevil control for nearly four decades. Malathion has been used almost exclusively in the boll weevil eradication programs that have proven to be successful in the southeastern and southwestern parts of the Cotton Belt. All previous published data (Brazzel and Shipp 1962; Graves and Roussel 1962; Fye et al. 1970; Hopkins et al. 1975, 1984; Rathinam 1979; Pavloff 1982; Wolfenbarger et al. 1986; Leonard et al. 1989; Martin et al. 1993) and the results presented herein do not provide clear and convincing evidence of resistance to the organophosphorus insecticides in boll weevils from the United States. However, Laboucheix and Gonzales (1987) and Swezey and Salamanica (1987) reported extremely high LD_{50} values (up to 1.73 µg/weevil) for methyl parathion against weevils from Nicaragua. Thus at least for methyl parathion, published LD_{50} values for boll weevils from Central America are much higher than those reported from the United States.

Carbamate Insecticides. Data on the susceptibility of the boll weevil to oxamyl (Table 5) reported herein [data from 1991 and 1992 previously reported in Martin et al. (1993)] serve as baseline reference data. The LD_{50} of the GAST-LAB colony to oxamyl was 0.211 µg/weevil and was significantly higher than that for 13 of the 18 field collections tested. Caddo (1992 spring) and Bossier (1992 fall) were the only field collections to exhibit higher LD_{50}'s than the GAST-LAB colony although they were not significantly higher. No evidence of resistance in the boll weevil to oxamyl was observed.

Cyclodiene Insecticides. After less than a decade of widespread use against boll weevils and other insect pests of cotton, resistance was documented in Louisiana (Roussel and Clower 1957) to cyclodiene insecticides such as endrin, dieldrin, and heptachlor. No baseline toxicity data were found for endosulfan in previous studies, and it is not known whether boll weevils ever exhibited resistance to this particular cyclodiene. However, no evidence of resistance to endosulfan was found in any of the seven field collections tested during 1993. In fact, all of the LD_{50} values for the field collections were significantly less than that for the GAST-LAB colony. Endosulfan has not been used extensively for boll weevil control in Louisiana, but its use as an aphicide on cotton has increased recently.

Pyrethroid Insecticides. The 51X range in LD_{50} values for

the 22 field collections tested with cypermethrin in 1991, 1992, and 1993 plus the fact that 15 of the 22 colonies had LD_{50}'s that were significantly higher than that of the GAST-LAB colony suggest that cypermethrin resistance may be developing in boll weevils particularly in Bossier and Caddo parishes of Louisiana. However, Rathinam (1979) reported 43X and 261X ranges in 48-h LD_{50}'s of boll weevils from nine states to permethrin and fenvalerate, respectively. These tests were done prior to the widespread use of pyrethroids for bollworm/tobacco budworm control in cotton.

Seventeen of the 22 field collections tested with cypermethrin in this study had LD_{50} values that were not significantly different from the LD_{50} reported by Leonard et al. (1989) for an Alexandria, LA (Rapides Parish) field collection (0.13 μg/weevil; 95% CL 0.054-0.253). Similarly, 18 of the 22 field collections had LD_{50}'s that were either less than or equal to the LD_{50} of a laboratory colony (0.14 μg/weevil; 95% CL 0.121-0.157) reported by Hopkins et al. (1984) prior to the widespread use of pyrethroids. The LD_{50} reported by Leonard et al. (1989) for a St. Joseph (Tensas Parish) collection was 0.43 μg/weevil (95% CL 0.336-0.593) and was significantly higher than the LD_{50}'s of the laboratory colonies tested by Hopkins et al. (1984). This LD_{50} response of the St. Joseph collection also was significantly higher than all but four of the field collections tested in this study. The four field collections that had LD_{50} values similar to that of the St. Joseph (Tensas Parish) collection reported by Leonard et al. (1989) were collected in Bossier and Caddo parishes. Furthermore, slopes of dose-mortality lines for these four collections were rather flat (0.6-1.3). However, LD_{50}'s of boll weevils collected in Tensas Parish in 1991, 1992, and 1993 were significantly lower than that reported by Leonard et al. (1989). In context of historical data reported by Hopkins et al. (1984) and Leonard et al. (1989), there was no conclusive evidence of resistance to cypermethrin in the field collections of boll weevil made during 1991, 1992, and 1993 from 11 Louisiana parishes.

LD_{50}'s for cyfluthrin (eight field collections), deltamethrin (five collections), and zeta-cypermethrin (six collections) were less variable than that observed for cypermethrin. The range of the LD_{50}'s for the field collections to cyfluthrin, deltamethrin, and zeta-cypermethrin was 18X, 6X, and 4X, respectively. However, boll weevils from Bossier Parish again had the highest LD_{50}'s of all the field collections. These LD_{50}'s were all significantly higher than that of the GAST-LAB colony. Deltamethrin is not registered for use on cotton in the United States and zeta-cypermethrin has only been used on cotton since 1992. Cyfluthrin has been used for several years but not as long as cypermethrin. Because no previous LD_{50} data were found for boll weevils to cyfluthrin, deltamethrin, and zeta-cypermethrin using topical bioassays, the data reported herein serve as a baseline for further studies.

Experimental Insecticides. No indications of cross-resistance to fipronil, a phenyl pyrazole, and Pirate®, a pyrrole, were observed. In fact, none of the field colonies tested with fipronil or Pirate® had LD_{50}'s significantly higher than that of the GAST-LAB colony. These data will serve as baseline responses for further studies.

Seasonal Variation. Seasonal variation in the effectiveness of insecticides against boll weevils was first documented by Rainwater and Gaines (1951). They found that cyclodienes were only about 50% as effective when applied to cotton fields in October as in July. Reiser et al. (1953) explained this phenomenon as being due to the liposoluble cyclodienes sequestered in lipids, which were highest in boll weevils late in the year. In a study to evaluate variation in seasonal susceptibility to the pyrethroid permethrin, Pavloff (1982) found that topical LD_{50}'s generally were higher in August than in May or July. However, comparisons in this study between responses to azinphosmethyl, cyfluthrin, cypermethrin, malathion, methyl parathion, oxamyl, and zeta-cypermethrin of spring and fall field collections from the same location do not indicate decreased susceptibility during the late season to these insecticides. In fact, there was generally as much variation in LD_{50}'s of boll weevils from the same location from spring to spring as there was from spring to fall.

Implications. Based on the laboratory bioassays reported herein, there was no evidence of resistance to carbamate, cyclodiene, organophosphorus, pyrrole, and phenyl pyrazole insecticides detected in boll weevils collected from 11 Louisiana parishes during 1991-1993. Although the LD_{50}'s of field collections of boll weevil to pyrethroids, particularly cypermethrin, varied greatly, there was no conclusive evidence of resistance [see Robertson et al. (1995) for detailed discussion regarding natural variation in bioassay results]. Additionally, replicated field tests evaluating insecticide efficacy indicates that pyrethroids still are efficacious against boll weevils in Louisiana (Leonard et al. 1993, Martin et al. 1994).

Kanga and Plapp (1992) have reported data (using the adult vial residual film test) on the responses of boll weevils from southeast Arkansas that suggest that cyfluthrin resistance may be developing in that location. Additionally, Kanga et al. (1995) reported tolerance to carbamate, organophosphorus, and pyrethroid insecticides in boll weevils collected in 1988 from the Brazos Valley, TX. However, tolerance to organophosphates and pyrethroids in Brazos Valley weevils was not found in 1989 and 1990. Further monitoring in 1991 and 1992 by Kanga et al. (1995) in Texas, Oklahoma, Arkansas, Mississippi, and Mexico indicated tolerance to organophosphates and pyrethroids in several boll weevil populations. The highest LD_{50} values for the majority of the insecticides tested in this study were from Bossier and Caddo parishes in northwest Louisiana. Continued monitoring of boll weevils from northwest Louisiana is needed to determine if resistance to the pyrethroids is developing in this area.

ACKNOWLEDGMENT

Appreciation is expressed to Carol Pinell-Alison, Dr. Grady Coburn, and Robert Goodson for their assistance in collecting boll weevils. The assistance of Scott Brush, Larry Daigle, Mike Fowler, Lauren Kim, James Pope, and Roy Von Holden in setup of the bioassays is greatly appreciated. Critical reviews of this manuscript by Y.A.I. Abdel-Aal and J.A. Ottea were very helpful. This manuscript is approved for

publication by the Director of the Louisiana Agricultural Experiment Station as manuscript 95-17-9242.

REFERENCES CITED

Abbott, W. S. 1925. A method of computing the effectiveness of an insecticide. J. Econ. Entomol. 18: 265-267.

Anonymous. 1968. First conference on test methods for resistance in insects of agricultural importance. Bull. Entomol. Soc. Am. 14: 31-37.

Bartlett, A. C. 1967. Genetic markers in boll weevil. J. Heredity 58: 159-163.

Brazzel, J. R., and O. E. Shipp. 1962. The status of boll weevil resistance to chlorinated hydrocarbon insecticides in Texas. J. Econ. Entomol. 55: 941-944.

Fye, R. E., C. L. Cole, and D. L. Bull. 1970. Populations of boll weevil in selected fields in Presidio County, Texas and Ojinaga, Chihuahua, Mexico in late 1968 subsequent to reproductive-diapause control programs in 1965-1967. J. Econ. Entomol. 63: 1084-1086.

Graves, J. B., and J. S. Roussel. 1962. Status of boll weevil resistance to insecticides in Louisiana during 1961. J. Econ. Entomol. 55: 938-940.

Graves, J. B., and D. A. Wolfenbarger. 1995. Chemical control. *In* Proc. Boll Weevil Centennial Symposium. USDA-ARS. In press.

Hopkins, A. R., H. M. Taft, and W. James. 1975. Reference LD_{50} values for some insecticides against the boll weevil. J. Econ. Entomol. 68: 189-192.

Hopkins, A. R., R. F. Moore, and W. James. 1984. Contact and residual toxicities of pyrethroids and organophosphorus compounds to the boll weevil (Coleoptera: Curculionidae). J. Georgia Entomol. Soc. 19: 27-34.

Kanga, L. B., and F. W. Plapp, Jr. 1992. Development of a glass vial technique for monitoring resistance to organophosphate and carbamate insecticides in the tobacco budworm and the boll weevil, pp. 731-734. *In* Proc. Beltwide Cotton Conf., National Cotton Council, Memphis, TN.

Kanga, L. H. B., F. W. Plapp, Jr., M. L. Wall, M. A. Karner, R. L. Huffman, T. W. Fuchs, G. W. Elzen, and J. L. Martinez-Carrillo. 1995. Monitoring tolerance to insecticides in boll weevil populations (Coleoptera: Curculionidae) from Texas, Arkansas, Oklahoma, Mississippi, and Mexico. J. Econ. Entomol. 88: 198-204.

Laboucheix, J., and D. F. Gonzales. 1987. Estimate of the effectiveness of methyl parathion against *Anthonomus grandis grandis* Boheman in Nicaraguan cotton fields. Cot. Fib. Trop. Vol. XLII: 51-53.

Leonard, B. R., E. Burris, J. B. Graves, and A. M. Pavloff. 1989. Toxicity of pyrethroids tested against boll weevil (Coleoptera: Curculionidae) adults in laboratory and field trials. J. Agric. Entomol. 6: 265-274.

Leonard, B.R., J.B. Graves, and D.W. Long. 1993. Control of boll weevil in cotton with selected insecticides, 1991. Insecticide and Acaricide Tests 18: 242-243.

Lincoln, C., and J. B. Graves. 1978. Insecticides: resistance and new insecticides, formulations and applications technology, pp. 74-84. *In* The boll weevil: management strategies. Southern Cooperative Series Bull. 229. Fayetteville, AR.

Martin S. H., J. B. Graves, B. R. Leonard, E. Burris, J. D. Powell, and J. L. Roberson. 1993. Toxicity of selected insecticides to boll weevils from Louisiana, pp. 903-907. *In* Proc. Beltwide Cotton Conf., National Cotton Council, Memphis, TN.

Martin, S.H., J.B. Graves, C.A. White, B.R. Leonard, and P.A. Clay. 1994. Efficacy of selected pyrethroid insecticides against boll weevil and tarnished plant bug in cotton, 1993. Arthropod Management Tests 19: 233.

Pavloff, A. M. 1982. Some factors affecting the toxicity of pyrethroid and organophosphorus insecticides to the boll weevil. *Anthonomus grandis grandis* Boheman. M. S. Thesis, Louisiana State University, Baton Rouge. 91 pp.

Rainwater, C. F., and J. C. Gaines. 1951. Seasonal decline in the effectiveness of certain insecticides against the boll weevil. J. Econ. Entomol. 44: 974-976.

Rathinam, T. 1979. Responses of boll weevils from nine southern states to selected insecticides. M. S. Thesis, Louisiana State University, Baton Rouge. 72 pp.

Reiser, R., D. S. Chadbourne, K. A. Kviken, C. F. Rainwater, and E. E. Ivy. 1953. Variations in lipid content of the boll weevil and seasonal variation in its resistance to insecticides J. Econ. Entomol. 46: 337-340.

Roberson, J., and J. E. Wright. 1984. Production of boll weevils, *Anthonomus grandis grandis* Boheman. pp. 188-192. *In* E. G. King and N. Leppla [eds.] Advances and challenges of insect rearing. USDA Tech. Bull.

Robertson, J. L., H. K. Preisler, S. S. Ng, L. A. Hickle, and W. D. Gelernter. 1995. Natural variation: a complicating factor in bioassays with chemical and microbial pesticides. J. Econ. Entomol. 88: 1-10.

Roussel, J. S., and D. F. Clower. 1957. Resistance to the chlorinated hydrocarbon insecticides in the boll weevil. J. Econ. Entomol. 50: 462-468.

Swezey, S. L., and M. L. Salamanica. 1987. Susceptibility of the boll weevil (Coleoptera: Curculionidae) to methyl parathion in Nicaragua. J. Econ. Entomol. 80: 358-361.

Williams, M. R. 1994. Cotton insect losses in 1993, pp. 743-763. *In* Proc. Beltwide Cotton Conf., National Cotton Council, Memphis, TN.

Wolfenbarger, D. A., A. M. Pavloff, R. Thiagrasan, J. B. Graves, and D. F. Clower. 1986. Variation in response of the boll weevil to methyl parathion and azinphosmethyl in the lower Rio Grande Valley, Texas and Mexico. Southwest. Entomol. 11: 95-99.

Wright, J.E., and E.J. Villavaso. 1983. Boll weevil sterility, pp. 153-177. *In* Cotton Insect Management with Special Reference to the Boll Weevil, USDA-ARS Handbook No. 589.

ABUNDANCE AND DISTRIBUTION OF INSECT IN STORED WHEAT GRAIN IN SONORA, MEXICO

Wong-Corral, F., M.O. Cortez-Rocha[1], and J. Borboa-Flores.

Departamento de Investigación Posgrado Alimentos. Universidad de Sonora. Apartado postal #1658. Hermosillo, Sonora. México.

ABSTRACT

Twenty-three species of insects were recovered from commercial storage facilities in Sonora, Mexico. The most common were lesser grain borer, *Rhyzopertha dominica* Fab., and the red flour beetle, *Tribolium castaneum*. Abundance was greatest in wheat stored on the ground and lowest in semi-underground and country metal silos. Diversity was very high due in part to the warm and dry climate; the highest incidence of pests were in the Southern Region.

INTRODUCTION

The state of Sonora is the largest wheat producing area in Mexico, with about 48% of national production. Consequently, a large number of storage facilities are distributed throughtout this area; however, few studies of insects species infesting stored grain in this state have been carried out. Wong et al. (1993) reported the presence of insect species in different stored products in rural communities of Sonora, but no information is available concerning the species present in commercial storage facilities. The objective of our study was to ascertain the insect species present in commercial wheat storage facilities in Sonora and to determine their distribution during three climatic seasons.

MATERIAL AND METHODS

The study areas selected were the Northern Region (San Luis Rio Colorado, Sonoita, Altar, and Caborca), Central Region (Hermosillo and Guaymas), and Southern Region (Vicam, Ciudad Obregon, Navojoa, and Huatabampo) of the state of Sonora (Fig. 1). The type of storage structure varied extensively with the region and volume of production; therefore, we selected those which were common to all regions, used for bulk storage, and had a capacity of thousands of tons, as follows: exposed on the ground, country concrete elevators, country metal elevators, semi-underground storage (70% of capacity is underground), flat metal storage, and flat concrete storage. All of the above facilities receive grain directly from producers.

[1]Department of Grain Science and Industry. Kansas State University. Manhattan, Kansas. 66502

FIG. 1. Map showing the localities of Sonora, Mexico, where storage facilities were sampled.

Representative samples from all (40) storage facilities were obtained according to the techniques described by the International Seed Testing Association (1966). Samples (15-kg) were collected (March, June, September 1990) from the bottom, middle and surface using open handle probes with 10, 5 and 3 openings, respectively. Each sample was deposited in a plastic bag, sealed, labeled, and transported to the laboratory for analysis. Samples were homogenized in a Boerner divisor to obtain a 1-kg aliquote. Insects present in the 1-kg subsample were removed using a 5/64" aluminum sieve. Identification was based on keys by Dobie et al. (1984), Weidner and Rack (1984), Rodríguez (1985), Gorham (1978), and Freeman (1980). Numbers of insects in each sample were recorded and reported as abundance per 1-kg of sample according to Krebs (1985).

RESULTS

A total of 23 insect species [primary pests (4), secondary pests (17), and beneficials (2: one predator and one parasite)] were found infesting stored wheat (Table 1). The two species of beneficial insects were considered insignificant due to the fact they were recovered in only a few sites and only in the South and Central regions. Most of the detected species belonged to the orders Coleoptera and Lepidoptera, Psocoptera, and Hemiptera; Hymenoptera was represented by just one individual. A total of 16 families were represented (Table 1). Statistical analysis indicated a significant difference between sampling period and localities. We noted that the insects were most widely distributed in Sonora.

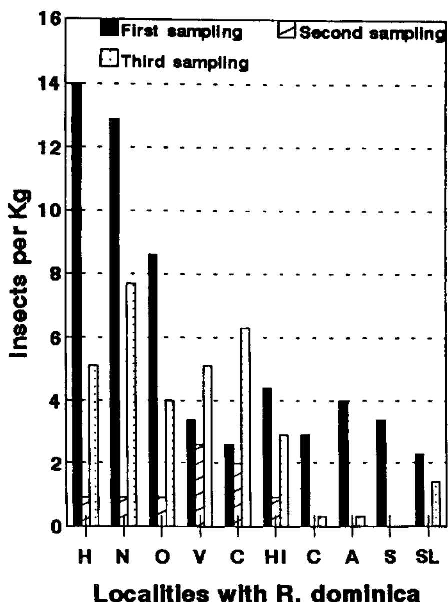
Table 1. Stored Products Insects Found in Samples of Wheat Collected in Localities of Sonora, Mexico.

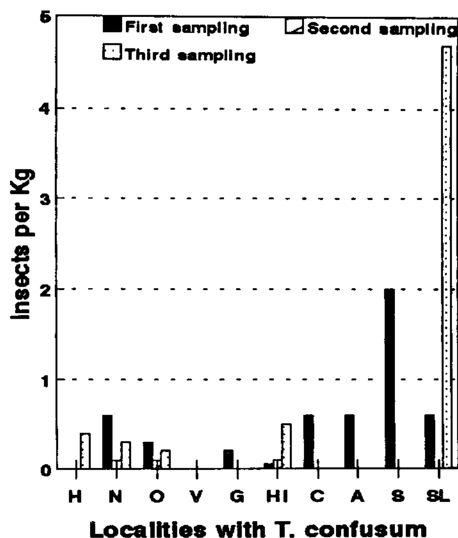
Scientific name	Family	Localities
Primary pests		
Rhyzopertha dominica (F.)	Bostrichidae	H, N, O, V, G, HI, C, A, S, SL
Sitophilus zeamais (L.)	Curculionidae	H, N, O, V, G, C, SL
Secondary pests		
Oryzaephilus surinamensis (L.)	Silvanidae	H, N, O, V, G, HI, C
Oryzaephilus mercator (Fauvel)	Silvanidae	O, C, SL
Cryptolestes ferrugineus (Stephens)	Cucujidae	H, N, O, V, G, HI, C, A, S, SL
Cryptolestes pusillus (Schonherr)	Cucujidae	H, N, O, V, G, HI, C
Tribolium castaneum (Herbst)	Tenebrionidae	H, N, O, V, G, HI, C, A, S, SL
Tribolium confusum Jacquelin duVal	Tenebrionidae	H, N, O, V, G, HI, C, SL
Latheticus oryzae Waterhouse	Tenebrionidae	H, N, O, V, G, HI, C, A, SL
Palorus subdepresus (Wollaston)	Tenebrionidae	N, HI, SL
Alphitobius diaperinus (Panzer)	Tenebionidae	H, N, O, G, HI, C
Lasioderma serricorne (F.)	Anobiidae	O, HI
Trogoderma parabile (F.)	Dermestidae	HI
Trogoderma spp.	Dermestidae	H, N, HI
Thorictodes heydini Reiter	Dermestidae	O, HI
Ephestia spp.	Pyralidae	H, G
Liposcelis spp.	Liposcelidae	H, N, O, V, G, HI, C
Tiphaea stercorea (L.)	Mycetophagidae	N, O, G, HI
Carpophilus hemipterus (L.)	Nitulidae	N, O, V, G, HI
Carcinopus pumilo	Histeridae	H, N, O, V
Anthicus floralis	Anthicidae	H, N, HI
Beneficial species		
Anisapteromalus calandre (Howard)	Pteromalidae	H, N, O, HI, C
Xylocoris flavipes (Reuter)	Anthocoridae	H, N, O, V, HI, SL

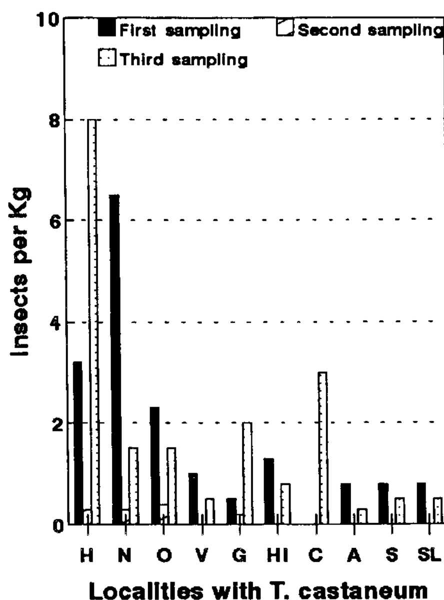
H= Huatabampo, N= Navojoa, O= Ciudad Obregon, V= Vicam, G=Guaymas, HI= Hermosillo, C= Caborca, A= Altar, S= Sonoita, SL= San Luis Rio Colorado.

The highest insect populations were detected in the Southern Region, and the lowest were found in samples from the Northern Region. Abundance tended to diminish from south to north (Figs. 2, 3). It appeared that climatic differences between the regions might be significant. The Southern Region is characterized by a subtropical and humid climate while a pronounced dry climate prevails in the Northern region and temperature fluctuations are extremes (≈ 120°F in summer and ≈32°F in winter). The climate of the Central Region is somewhat an average of the two extreme regions.

The highest number of species was found in wheat stored on the ground (18) followed by concrete flat storage (16); the lowest was found in the semi-underground storage (11). The most abundant internal feeding insect species was the lesser grain borer, *Rhyzopertha dominica*. It was detected in large numbers in concrete silos, but it was less abundant in semi-underground facilities (Fig. 4). The rest of the storage systems showed intermediate values. ANOVA indicated that abundance was statistically different ($P \leq 0.05$) between cities and storage systems. Among the external infestors, the red flour beetle, *Tribolium castaneum*, was most abundant in flat concrete storage, flat metal storage, and country concrete silos, respectively (Fig. 4). There was a significant difference







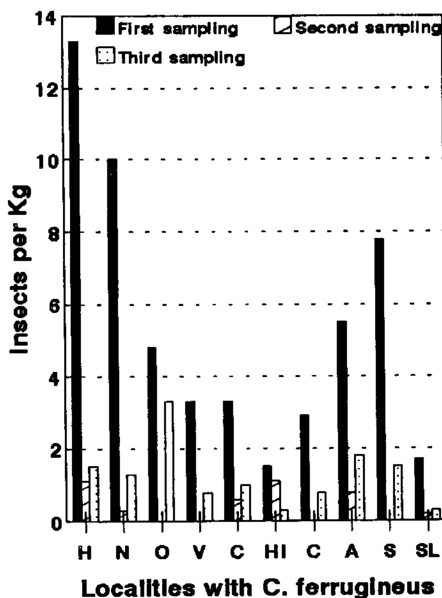
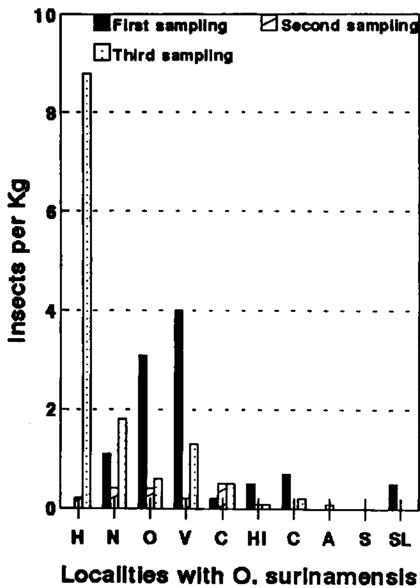
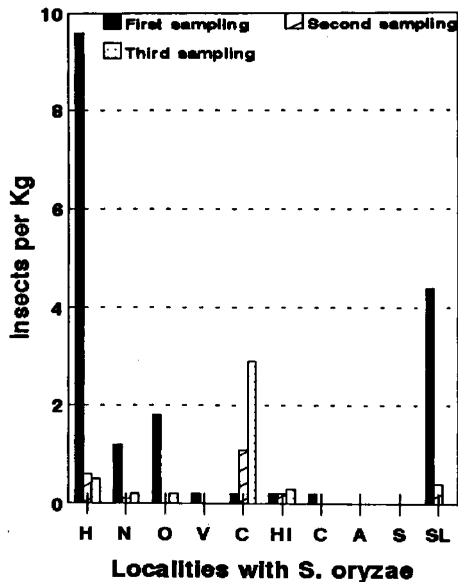
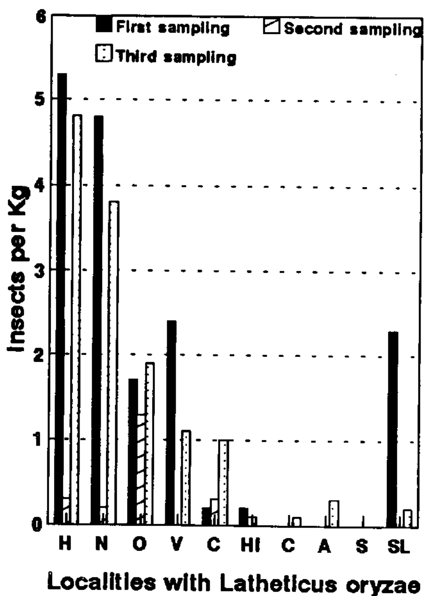


FIG. 2. Insect distribution in stored wheat in localities of Sonora, México. H= Huatabampo, N= Navojoa, O= Ciudad Obregón, V= Vicam, G= Guaymas, HI= Hermosillo, C= Caborca, A= Altar, S= Sonoita, SL= San Luis Rio Colorado.







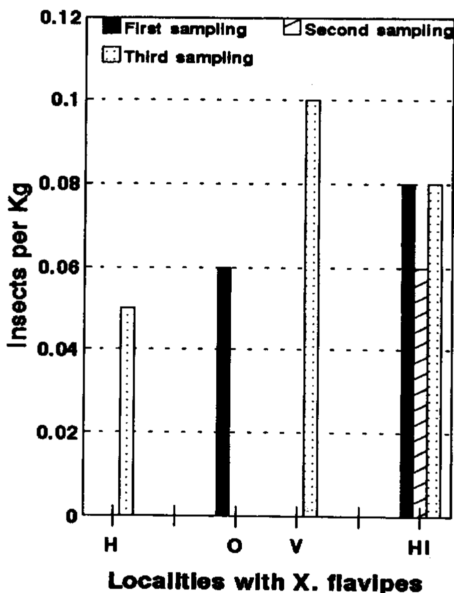
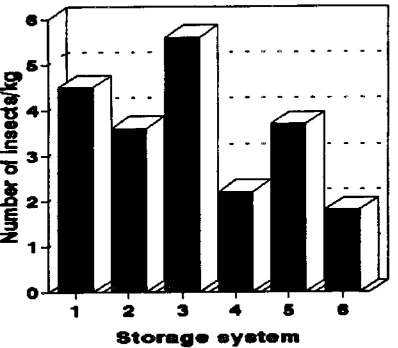
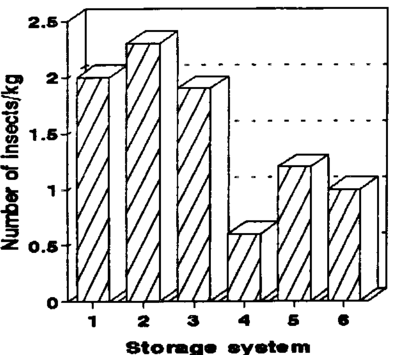
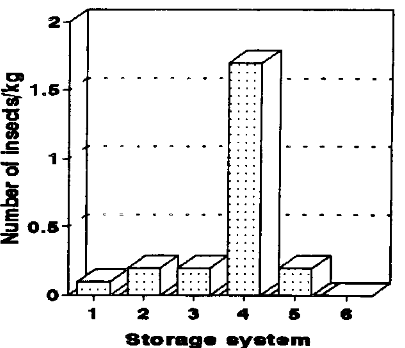
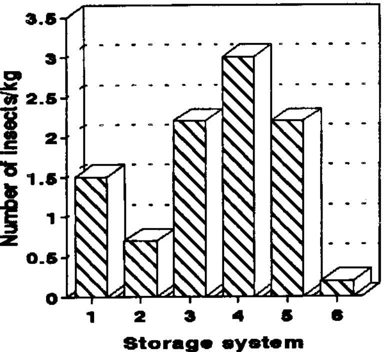


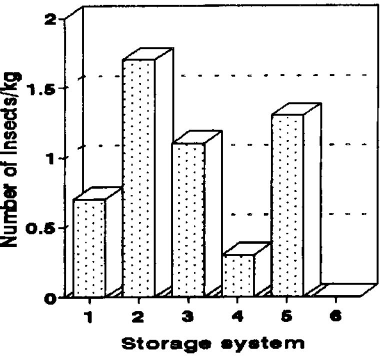
FIG. 3. Insect distribution in stored wheat in localities of Sonora, México. H= Huatabampo, N= Navojoa, O= Ciudad Obregón, V= Vicam, G= Guaymas, HI= Hermosillo, C= Caborca, A= Altar, S= Sonoita, SL= San Luis Rio Colorado.











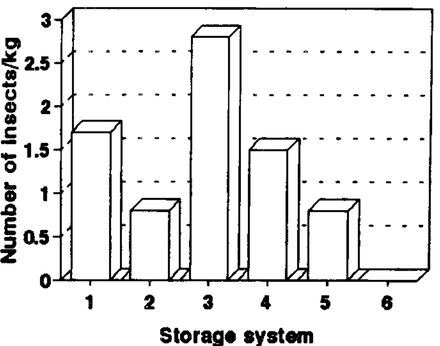


FIG. 4. Abundance of Insects in Stored Wheat Kernels in Commercial Facilities of Sonora, México. Storage Systems (1= Flat Metal Elevator, 2= Flat Concrete Elevator, 3= Country Concrete Elevator, 4= Country Metal Elevator, 5= On the Ground, and 6= Semi-Under Ground.

($P \leq 0.05$) among cities and sampling season. The confused grain beetle, *Tribolium confusum*, was abundant only in country metal silos; it was not detected in semi-underground storage and was scarce in other storage systems (Fig. 4). The confused grain beetle was present in high numbers during the third sampling season (September) in San Luis Rio Colorado and during January in Sonoita (both cities are located in the Northern Region). There were significant differences ($p \leq 0.05$) between localities and sampling periods. The rusty grain beetle, *Cryptolestes ferrugineus*, was present in semi-underground storage and concrete flat storage in very low numbers, and at high density in others (Fig. 4). This species showed the same pattern as the lesser grain borer. The sawtoothed grain beetle, *Oryzaephilus surinamensis*, was abundant in flat concrete storage, on ground storage, and also in country concrete elevators (Fig. 4). However, it was scarcely present in country metal storage and was absent in semi-underground storage; this same trend was apparent for all study periods, all cities and all seasons. The longheaded flour beetle, *Latheticus oryzae*, was not detected in semi-underground storage in any sampling season; it was scarcely present in flat concrete storage and in ground storage, whereas it was very abundant in the rest of storage systems (Fig. 4). The benefical species found were the predator *Xylocoris flavipes* (Fig. 4) and the parasite *Anisapteromalus calandrae*. They were present, however, in only very low levels and in very few localities.

According to the information presented here, we concluded that many insect species are present in the different wheat storage systems in Sonora, Mexico, and, that they are widely distributed. A knowledge of the diverse insect species infesting stored wheat will enable better management and pest control methods to be applied in order to maintain wheat quality.

LITERATURE CITED

I.S.T.A. 1966. International rules for seed testing. International Seed Testing Assoc. Wageningen, Netherland. pp. 12-25

Dobie, P., C.P. Haines, R.G. Hodges, and P.F. Prevett. 1978. Insects and arachnids of tropical stored products. Development and Research Institute, London. pp. 24-112

Krebs, J.Ch. 1985. Estudio de la distribución y abundancia. Segunda Edición. Ed. HARLA, S.A. México, D.F. pp. 147-148

Freeman, P. 1980. Common insects pests of stored food products. Butler Tanner Ltd, London. pp. 5-36.

Gorham, R.J. 1978. Training manual for analytical Entomology in the food industry. FDA Technical Bull. No. 2, Washington, D.C. pp. 25-50

Rodríguez, R.R. 1985. Guía de insectos dañinos de productos almacenados. INIA-Zona Sur, México, D.F. pp. 5-73

Weidner, H., and G. Rack. 1984. Tables de determination des principauz ravageurs des denrees dntreposees dans les pays chauds. Deutsche Gesseeschaft (GTZ). GMBH. Eschborn.

Wong-Corral, F.J., M.O. Cortez-Rocha, J. Borboa-Flores, and F. Bustamante-Andrade. 1993. Insect species found in grain stored in rural communities of Sonora, México. Southwest. Entomol. 17:327-331

VOL. 21 NO. 1 SOUTHWESTERN ENTOMOLOGIST MAR. 1996
SCIENTIFIC NOTE

SUSCEPTIBILIDAD DEL ARADOR DE LOS CITRICOS, *PHYLLOCOPTRUTA OLEIVORA*[1] Y EL ACARO DEPREDADOR, *EUSEIUS MESEMBRINUS*[1], AL ACARICIDA DICOFOL

A. E. Flores[2], S. Flores[2], M. H. Badii[2], E. Aranda[3] y E. Hernández[2]

El arador de los cítricos, *Phyllocoptruta oleivora* Ashmead, es considerado plaga importante en el noreste de México, particularmente en Nuevo León y Tamaulipas ya que provoca una considerable reducción de la calidad de los frutos. Hasta ahora el único método efectivo de control de la plaga se ha basado exclusivamente en el empleo de acaricidas químicos convencionales tales como el ethion, oxamil y dicofol ó también productos inorgánicos como el azufre.

El acaricida dicofol ha sido ampliamente utilizado tanto en Nuevo León como en Tamaulipas para el control de la araña texana, *Eutetranychus banksi* (McGregor). Recientemente Flores et al. (1994) encontraron que esta plaga ha desarrollado cierto grado de tolerancia a este producto. Debido a los antecedentes de desarrollo de tolerancia al dicofol en la araña texana de los cítricos y a la carencia de información de base acerca del efecto de este producto sobre *P. oleivora* y el ácaro depredador *E. mesembrinus* (Flores et al. 1996), se realizaron bioensayos con el objetivo principal de cuantificar la susceptibilidad de poblaciones de campo de ambas especies. Adicionalmente, se comparó la respuesta tóxica de *E. mesembrinus* en bioensayos de película residual en hoja arena (técnica de hoja-arena descrita por Childers et al. 1991) y en microcámara (Flores et al. 1996).

Con el fin de establecer la relación concentración-mortalidad de la respuesta de ambas especies al dicofol, se realizaron varias colectas de especímenes durante el período enero de 1993 a diciembre de 1994 en una huerta comercial de naranjos ubicada en Buenavista, Allende, N.L., México. Las poblaciones de ácaros se mantuvieron separadamente sobre hojas de naranjo en unidades de cría utilizando la técnica de hoja-arena y éstas en una cámara ambiental Biotronette® Mark III a temperatura de 24 ± 2 °C, 60-70% H.R. y 12:12 L:O.

La técnica de bioensayo utilizada en las pruebas de toxicidad para ambas especies fue la de película residual en hoja (Busvine 1971). Diferentes diluciones en agua fueron preparadas a partir de un líquido emulsificable comercial del acaricida dicofol (Kelthane® 18%) y utilizadas para tratar por inmersión hojas de naranjo donde se expusieron los ácaros. La mortalidad se cuantificó 24, 48 y 72 horas después de la exposición y los resultados (tabla 1) se procesaron mediante el análisis probit de máxima verosimilitud (Finney 1962).

La utilización del dicofol para el control del arador de los cítricos a la concentración de campo recomendada (1.5-2 ml/lt de Kelthane® al 18% de i.a.), en teoría reduciría grandemente las poblaciones de ácaros sin discriminar entre especies plaga y no plaga. Los resultados de Flores et al. (1994) indican que las poblaciones susceptibles de *E. banksi* son intermedias en su respuesta al acaricida,

---

[1]ACARI: ERIOPHYIDAE, PHYTOSEIIDAE
[2]Universidad Autónoma de Nuevo León, Facultad de Ciencias Biológicas. Ap. Postal 391, 66450, San Nicolás de los Garza, N. L. México.
[3]Programa de Graduados en Agricultura, Instituto Tecnológico y de Estudios Superiores de Monterrey (ITESM), Campus Monterrey, Sucursal de Correos "J" C.P. 64849 Monterrey, N.L., México.

en comparación con las especies aquí estudiadas. Sin embargo, las poblaciones tolerantes de la araña texana son menos afectadas por el acaricida en comparación con *P. oleivora* y *E. mesembrinus*.

TABLA 1.- Concentraciones Letales 50 y 90 para Adultos de *P. oleivora* y *E. mesembrinus* después de la Exposición al Dicofol en Bioensayos de Microcámara y Hoja-arena. Monterrey, N.L, México.

Tiempo (h)	CL_{50}	I.C.*	CL_{90}	I.C.	Ajuste (p)	b
			P. oleivora en hoja-arena			
24	13.47	11.82-15.44	252.86	195.44-338.18	$9x10^{-6}$	1.00
48	3.42	3.09- 3.78	30.92	26.24- 37.07	$9x10^{-6}$	1.33
72	1.70	1.53- 1.89	16.79	14.22- 20.21	$9x10^{-6}$	1.28
			E. mesembrinus en hoja-arena			
24	102.19	82.78-130.47	972.49	602.83-2211.23	0.0012	1.31
48	28.24	24.12- 32.57	106.70	88.13- 135.97	$1x10^{-6}$	2.22
72	19.10	16.27- 21.97	59.54	50.40- 73.37	$1x10^{-6}$	2.59
			E. mesembrinus en microcámara			
24	115.53	81.52-180.50	1702.97	780.65-6563.76	0.3411	1.10
48	13.59	9.94- 18.52	120.71	77.69- 219.76	0.0079	1.35
72	3.82	2.81- 5.05	22.28	15.41- 37.35	$7x10^{-6}$	1.67

*I. C. Intervalo de confianza, p= probabilidad de ajuste, b= pendiente de la línea

El rango de susceptibilidad al dicofol mostrado por las tres especies de ácaros de los cítricos mencionadas en el párrafo precedente podría ser importante en un programa de manejo de plagas en este cultivo. Esto sería, seleccionando cuidadosamente dosis que redujeran selectivamente las poblaciones del arador, se permitiría al depredador subsistir alimentándose de los ácaros araña que sobrevivieran a la aplicación. Esta estrategia encuentra soporte en el hecho de que diversos autores coinciden al señalar que la principal causa de la disminución de poblaciones de depredadores es la falta de alimento después de una aplicación de acaricida (Croft y Morse 1979).

LITERATURA CITADA

Busvine, J. R. 1971. A Critical Review of the Technique for Testing Insecticides. Commonwealth Internat. Bureau Inst. Entomol. London. 288 pp.

Childers, C. C., M. M. Abou-Setta and M. S. Nawar. 1991. Biology of *Eutetranychus banksi*: Life tables on "marsh" grapefruit leaves at different temperatures (Acari:Tetranychidae). Internat. J. Acarol. 17: 29-35.

Croft, B. A. and J. G. Morse. 1979. Research advances on pesticide resistance in natural enemies. Entomophaga. 24: 3-11.

Finney, D. J. 1962. Probit Analysis. 2nd ed. Harvard University Press, Cambridge. 318 pp.

Flores, A. E., M. H. Badii, E. Aranda H. and S. Flores B. 1994. Tolerance to dicofol in a field population of *Eutetranychus banksi* (McGregor). Southwest. Entomol. 19: 161-165.

Flores, A. E., S. Flores, M. H. Badii y E. Hernández. 1996. Efecto del dicofol en la oviposición y parámetros reproductivos de *Euseius mesembrinus* (Dean) (Acari: Phytoseiidae). Agrociencia (en prensa), México.

SOUTHWESTERN ENTOMOLOGICAL SOCIETY

PERSPECTIVE

****
Southwestern Entomologist provides this section for contirubtions of general interest with an emphasis on papers of a theoretical or historical nature.
****

CURRENT STATUS OF BACKCROSS STERILITY IN *HELIOTHIS VIRESCENS* (F.)

D. D. Hardee and M. L. Laster

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

ABSTRACT

Because of insecticide resistance, high cost of controls, and the need to reduce pesticide use, attempts have been made to manage tobacco budworm (TBW), *Heliothis virescens* (F.), with non-insecticidal techniques. One such approach has been the use of sterile male interspecific hybrids produced from crosses between TBW males and *Physalis* fruitworm, *Heliothis subflexa* (Guenee) females. In this review, we summarize 20 years of research to develop this technique into a usable system to manage TBW and outline needs for future development.

INTRODUCTION

During 1994, cotton farmers lost almost $1 billion to cotton insects when the amount of lost yield and actual expenses incurred for their control were combined (Hardee and Herzog 1995). The two most important insects inflicting damage were the tobacco budworm (TBW), *Heliothis virescens* (F.), and cotton bollworm (CBW) (corn earworm or tomato fruitworm, *Helicoverpa zea* (Boddie)) (Williams 1995). Over ten years ago, control costs for these two insects in Mississippi were estimated at $113.77/ha (Simpson 1983). In the United States, damage to all crops (cotton, corn, soybean, tobacco, tomato, lettuce and other economic and ornamental plants) attacked by TBW and CBW has been estimated to exceed $1 billion annually (Agricultural Research Service 1976). Because of their similar behaviors, (i.e., CBW and TBW often occur at the same time on the same crop, especially cotton, and inflict similar damage), it is difficult to separate losses caused by either insect. TBW is of particular concern because of its tendency to develop resistance to insecticides (Elzen et al. 1992). In addition to resistance, increasing concern over the role of pesticides in environmental pollution and public health and the desire by growers to manage economic pests effectively within individual fields, and more recently on an area-wide basis, have strongly motivated an intensive search for alternate strategies to insecticides for management of these pests.

DISCUSSION

Knipling (1960) proposed that strains of an insect with inferior or lethal genetic characteristics might be developed, released, and adopted as a means of autocidal control. Subsequent reviews have explored theoretical appraisals of potential usage in area-wide management of CBW and TBW by manipulation of host plants (Knipling and Stadelbacher 1983) and genetic sterility (Agricultural Research Service 1985; Klassen et al. 1970;

Knipling 1966, 1970; Knipling and Klassen 1976; LaChance 1974, 1985; LaChance and Knipling 1962; LaChance et al. 1967; Laster et al. 1988a; Lingren et al. 1977; North 1975; Proshold and Bartell 1970). In addition, Carpenter, Gross, Mannion, and co-workers (Carpenter 1991, 1992; Carpenter and Gross 1989, 1993; Carpenter et al. 1987a, 1987b, 1987c, 1989; Mannion et al. 1994) reported results of studies of inherited sterility in suppression of the CBW. In this report, we summarize accomplishments in the specific area of autocidal control of TBW with backcross (BC) hybrid sterility.

Prior to Hardwick's (1965) memoir on the CBW complex, CBW and TBW were both classified as members of the genus *Heliothis*. Hardwick separated the two species by placing CBW in the genus *Helicoverpa* which is currently in use. Another species of Heliothine, *Heliothis subflexa* (Guene$\acute{e}$), sometimes called the *Physalis* fruitworm (PFW) (Laster 1972, Ignoffo et al. 1983), occurs exclusively on *Physalis* sp., primarily cutleaf ground cherry, *Physalis angulata* L. (Laster 1972) or *P. heterophylla* Nees (Sitchawat and Thurston 1980) in Mississippi; it is not a pest of any economically important crop. PFW was originally described by Guene$\acute{e}$ in 1852 and was relegated to the status of variety or geographical race of TBW (Laster 1972). It was restored to specific rank by McElvare (1941) who provided sufficient characterization to separate *virescens* from *subflexa*. Forbes (1954), Kimball (1965), Furr et al. (1974), and Todd (1978a, 1978b) provided additional characters to support McElvare's action. Brazzel et al. (1953), however, disagreed with part of McElvare's description and suggested using host plant to separate TBW and PFW since the latter (1) occurs only on *Physalis* spp. and (2) is not observed attacking cotton, tobacco, and other crops. Mitchell and Heath (1987) successfully discovered an ovipositional stimulant for PFW in *Physalis*, and Mitchell et al. (1988) were successful in developing a laboratory rearing procedure for PFW.

Realizing the close similarity between TBW and PFW and having been inspired by theories projected by Knipling (1960, 1966, 1970), Laster (1972) was the first to demonstrate sterility in male interspecific hybrids between PFW females and TBW males. Proshold and LaChance (1974) verified the production of sterile male hybrids and further showed that the reciprocal cross also produced hybrid males with some sterility. However, by selecting and mating females from this cross, full fertility was rapidly restored. Both studies showed that F_1 females from PWF females x TBW males were fertile, and when they were backcrossed to TBW males, they continued to produce sterile progeny (Laster 1972, Proshold and LaChance 1974, Laster et al. 1976, Karpenko and Proshold 1977), a characteristic that has held consistent for 229 generations as of 23 February 1995. When hybrid females were backcrossed to PFW males, however, fertility of male offspring was restored by the third generation (Karpenko and Proshold 1977). BC progeny were able to utilize host plants that were acceptable to TBW (Laster et al. 1982). Laster et al. (1988b) obtained the same hybridization results with TBW and PFW from Argentina as Laster (1972) did in his original work with U.S. insects. The powerful potential of using this sterility character as a method of control stimulated considerable research along two lines: (1) to determine the behavior and competitive ability of the BC insect in comparison with pure TBW, and (2) to collect basic biological and genetic data to establish the basis for the sterility (Proshold et al. 1982b).

Several facts emerged from this research. The major cause of sterility in the hybrid and backcross males was their inability to fill the female spermatheca with eupyrene sperm (LaChance 1984; LaChance and Karpenko 1981, 1983; Goodpasture et al. 1980a, b). This is due to a variety of causes. F_1 hybrid males mate and the eupyrene sperm are very often included in the spermatophore, but these sperm usually do not leave the bursa copulatrix of the female and are not found in the spermathecae (Proshold and LaChance 1974, Proshold et al. 1975). F_1 males produce a large proportion of grossly abnormal sperm that have a single head and two tails (Richard et al. 1975); double-tailed sperm were also reported by

Goodpasture et al. (1980a). Proshold and LaChance (1974) reported reduced chromosome pairing in the first meiotic division of F_1 hybrid males; thus, in the F_1 hybrids and early backcross generations, meiotic abnormalities and the production of two-tailed sperm characterize the sterility of the males. However, chromosome pairing at the first meiotic division is almost normal by the sixth backcross (LaChance 1984), and the production of two-tailed sperm virtually disappears by the 4th backcross (Goodpasture et al. 1980a). The basis of male sterility in the later backcross generations was unknown until Goodpasture et al.(1980b) showed that many of the eupyrene sperm bundles in the testis of adult males in later backcross generations had other abnormalities, such as swollen mitochondrial derivatives and degenerating bundles. LaChance (1984) maintained that absence of eupyrene sperm bundles in the duplex or abnormalities in the sperm tails most likely accounts for the sterility of backcross males. LaChance and Olstad (1988a, 1988b) describe the ultrastructural changes in reproductive organs that take place in normal prepupae, pupae and adult TBW males in a step-by-step comparison with the process in sterile BC males (LaChance 1984).

At least two separate types of sterility are associated with *Heliothis* hybrids (Proshold et al. 1982b). One is associated with chromosome desynapsis and subsequent spermiogenic abnormalities. This sterility is lost when females are crossed with an appropriate male so that the species genome is placed into the same species cytoplasm. More importantly from a control standpoint, there is persistent BC male sterility when the TBW genome is present in PFW cytoplasm. This cytoplasmic sterility is maternally inherited but does not appear to be associated with maternally transmitted cytoplasmic microorganisms, such as bacteria or rickettsia (LaChance and Karpenko 1981), but probably with mitochondrial DNA (Miller and Huettel 1986, Miller et al. 1986) in *Heliothis*.

In contrast to hybrid males, hybrid females differ greatly in appearance, biology, and reproduction, depending upon the type of interspecific cross. When PFW is the female in an interspecific cross, about 40% of the hybrid females enter an intense diapause from which few emerge (Laster 1972, Proshold and LaChance 1974). Most females that do not diapause contain few or no mature eggs and do not mate with males of other species. Those that do mate lay only about one-half and one-fourth the number of eggs oviposited by PFW and TBW females, respectively. If mated, percentage egg hatch is as great as expected of females of other species (Proshold and LaChance 1974). At least three generations are required before mating frequency is as prevalent in BC females as that in pure TBW (Laster et al. 1977).

In contrast, hybrid females from reciprocal crosses (TBW female x PFW male) do not enter diapause. Further, they mate readily with males of either species and deposit about the same number of eggs as PFW females if mated. However, eggs from these females do not hatch as frequently as eggs from either TBW or PFW females (Proshold and LaChance 1974). In artificial rearing studies, Brewer and King (1979) showed that CBW consumed more of a soybean-flour-wheat germ diet and gained more weight than TBW and BC insects.

When hybrid and BC females are crossed with TBW males, about half of the remaining PFW chromosomal DNA is replaced by TBW each generation. Thus, chromosomal DNA of BC_3 insects should be nearly 94% TBW. Persistence of BC male sterility allows the use of insects that have been backcrossed to the point of being genetically similar to TBW. Thus, most research comparing behavior and competitive ability of BC insects with that of TBW has been done with BC_3 or later generation insects (Proshold et al. 1982b).

Pheromone traps baited with BC females (one/trap) capture as many native TBW males as do those baited with TBW females (Laster et al. 1978a). Females previously mated to BC males seem to attract more males than virgin females, which indicates that BC males do not satisfy the mating urge of females and they will readily remate. The fact that more

males are trapped in the first instance is best explained by selection. Not all virgin females placed in traps have a propensity to mate. These females probably would be precluded in traps baited with females mated to BC males. Pair et al. (1977a) found that females mated with BC males remated about twice as frequently as those mated with TBW males. Further, Pair et al. found synchrony in mating time between BC and TBW females; however, PFW females mated earlier in the scotophase. For the first mating, BC and TBW females were found in copula with both types of males in about equal proportions, but rematings occurred more frequently with BC males than TBW males for both types of females (Pair et al. 1977a).

Female TBW mated with BC males readily remate. Pair et al. (1977b) found that if the next mating was with a TBW male, the female was fully fertile. In contrast, if females mated with TBW males and then with BC males, the females were infertile or only partially fertile. The authors suggested this was caused by apyrene sperm of the second mating replacing eupyrene sperm of the first mating.

Raulston et al. (1979) released BC insects in a cotton field and observed interaction between released and native TBW. BC females mated readily with native males, but native females were rarely observed in copula with BC males which were competing with native males for BC females but not for native females. In general, the BC insects' behavior was similar to that of the laboratory colony from which they were derived. Carpenter et al. (1979) observed similar results with BC females. Thus, the behavior of BC insects under field conditions seemed to be dependent on the genetic background from which the BC was developed. Raulston et al. (1979) suggested that this could allow the engineering of behavior in released insects.

In a limited cage study in which TBW and BC insects were released in ratios of 1:1 or 1:5, Laster et al. (1978b) collected first and second generation eggs and larvae. Hatchability of first generation eggs was lower in cages with both types of insects than in cages with only TBW. When adult males reared from eggs or larvae from various cages with BC and TBW insects were crossed with TBW females, 59-100% failed to reproduce, indicating infusion of male sterility. Infusion of male sterility was also observed following a one-time release in a semi-isolated plot of cotton and sesame (Martin et al. 1981b). Emergence from diapause of BC insects appeared to be in synchrony with that of TBW (Laster et al. 1987a, Martin et al. 1981a, Stadelbacher and Martin 1981). Scheck and Gould (1995) studied in detail the oviposition preferences of *H. virescens, H. subflexa*, and the backcross. Tillman and Laster (1995) found no differences in parasitism rates of *Microplitis croceipes* (Cresson) on *H. virescens* and the *H. virescens/H. subflexa* backcross.

BC females have been observed actively flying, feeding, and mating earlier than native females; however, no difference in time of oviposition or preference for oviposition sites was detected (Proshold et al. 1983c). Activity periods of BC and native males appeared to coincide and mating was random between native and BC insects when the latter were placed in the field as pupae and allowed to emerge. When BC adults were released, greater than expected intramating frequencies were observed, apparently as a result of inadequate dispersal of insects. Mating interaction between native and BC insects was verified by an increase in sterility of native females with increased numbers of released insects. BC females with eupyrene sperm in their spermathecae were commonly collected (Proshold et al. 1983c). Of released males captured, fewer than 15% were trapped farther away than 1.6 km; males placed in release cages as pupae dispersed farther than those released as adults; eggs oviposited by released females were collected as far as 8 km from the release site, although most were collected near release sites. Ratios of eggs oviposited by released or native females were similar on pigeon pea, *Cajanas cajan* (L.), and on *Bastardia viscosa*

(Koth.), the two most important hosts for TBW on the island of St. Croix (Proshold et al. 1983b) where most release studies were made; therefore, the need for frequent release sites in future suppression programs with BC-TBW has been demonstrated.

A black body mutant that was dominant to wild type in TBW, inherited as a simple Mendalian trait, and lethal when homozygous has been transferred to PFW, apparently by chromosome displacement; the genetic expression was similar in both species but the mutant PFW did not appear to be the same as in TBW (Proshold et al. 1983a). A mutant PFW line obtained by crossing mutant females with mutant males could not be maintained beyond one generation because of low fertility and survival (Proshold et al. 1983a).

Simultaneously, with on-going research, models have been developed to demonstrate the potential for suppression of TBW by release of sterile backcross insects (Laster et al. 1976; Parvin et al. 1976; Levins et al. 1981; Levins and Parker 1983; Makela and Huettel 1979, 1981; Roush and Schneider 1985). Other research showed that insects carrying the sterile male trait were infused into natural populations, did not differ in mating synchrony or frequency of mating, entered diapause, survived the winter, and emerged the following spring in synchrony with TBW populations (Pair et al. 1977a, Martin et al. 1981a). A study conducted in Puerto Rico in 1976 (Martin et al. 1984) showed that BC insects mated with wild insects, suggesting the potential for infusion of sterility into the native population.

Due to the importance of Laster's (1972) discovery, the economic damage of this insect, and the amount of supporting research, USDA-ARS in cooperation with the Mississippi Agriculture and Forestry Experiment Station and the Federal Experiment Station in St. Croix, U.S. Virgin Islands, initiated a pilot test in 1977 to infuse a measurable degree of the sterile male trait into the feral TBW population on St. Croix and subsequently evaluate population suppression (Proshold 1983, King et al. 1985). Although it was known from previous studies (Haile et al. 1975) that St. Croix was not isolated enough to prevent migration and emigration, much was known about the island and its insect fauna (Snow et al. 1969, 1974; North et al. 1975), and it represented the most isolation known at that time. Backcross larvae would feed on the same plants as TBW, and a slight rise in the population in wild host plants early in the season could be expected. Reproduction, however, would be limited by the mating of normal males, and the total population should be declining when agricultural crop plants are large enough for second generation attack. Thus, the population in a release program should continue to decline through successive generations; whereas, a natural, uncontrolled population normally would continue to increase (Martin et al. 1981a). This research culminated after four years (1977-1980) in an all-island release from August through December of 1980 (Proshold and Smith 1982). Greater than 90% emergence of BC insects was obtained, beginning in early evening hours; females emerged prior to males, and numbers of males trapped after release declined rapidly, suggesting a survival of 3-5 days and an intermating period of no more than two nights (Proshold et al. 1986). During this period, the backcross frequency increased linearly at 4-5% per week, and resulted in a backcross frequency of about 94% during early 1981; TBW egg and larval populations showed a 75% reduction during the spring of 1981 compared to previous years (Proshold and Smith 1982). Thus, through rearing (Hartley et al. 1982, Smith et al. 1980) and release of backcross insects, sterility was infused into feral males in frequencies >90% (Martin et al. 1981b, Proshold et al. 1982b, Proshold 1983). Thereafter, the backcross phenotype became extinct within 5 years of the last release, apparently because of genetic drift and selection; as the frequency of backcross declined, TBW populations returned to pre-release levels (Proshold and Smith 1990).

The relationship of pheromones produced by CEW, TBW, PFW, and backcross insects to the effectiveness of the hybrid in management has been studied in some detail. Identification of pheromone components has been reported for the CBW (Klun et al. 1980a), TBW (Klun et al. 1980b, Roelofs et al. 1974, Teal and Tumlinson 1989, Tumlinson et al.

1975), PFW (Heath et al. 1990, 1991; Klun et al. 1982; Teal et al. 1981), TBW and PFW backcross hybrids (Teal and Oostendorp 1993, 1995a, 1995b), and general Heliothine chemistry (Klun et al. 1979; Teal and Tumlinson 1987, 1990; Tingle et al. 1978). Sheck and Gould (1995) recently reported oviposition preferences of TBW, PFW, and BC. Key to the use of the backcross hybrid in management of the TBW was the determination that released backcross females were competitive with TBW females in attracting TBW males from the natural population (Carpenter et al. 1979, Laster et al. 1978a, Laster and Stadelbacher 1991, Raulston et al. 1979), and that TBW females mated to backcross males and then placed in traps attracted more males than females mated with normal males (Laster et al. 1978a, Raulston et al. 1979). Raina and Stadelbacher (1990) also showed that TBW females mated with normal and sterile backcross males reacted the same (i.e., sex pheromone production was terminated and titer of the pheromone reached a very low level within 2 h following mating). The complete courtship and mating behavior of the PFW and hybrid are described by Cibrian-Tovar and Mitchell (1991). Firko and King (1991) developed a lineage of TBW-backcross strain that was resistant to a pyrethroid insecticide by repeatedly backcrossing TBW-backcross females to resistant tobacco budworm males and selecting for tolerance. Ignoffo et al. (1983, 1985a, 1985b) found that PFW was 1000-fold less susceptible to baculoviruses than TBW, but that BC was equal to TBW, suggesting that this character was controlled by a single gene.

Backcross sterility has been demonstrated to be maternally inherited (Laster 1972, Proshold and LaChance 1974, Miller et al. 1986) and was therefore transmitted in common to succeeding generations; however, the mechanism of BC sterility is still not completely understood. Models have been proposed invoking microorganisms (Degrugillier 1989, Degrugillier and Newman 1993), mitochondria (Miller and Huettel 1986, Miller et al. 1986), and Y-autosome chromosome incompatability (Roehrdanz 1990, Roehrdanz and LaChance 1981) as causative agents for BC sterility. Methods have been described to monitor dispersal of and to separate sterile BC males from normal males (Gassner and Proshold 1978; Gassner et al. 1979; Goodpasture et al. 1980a, 1980b; LaChance 1987; Roehrdanz 1994).

From the time of the last release of BC hybrids in 1980 on St. Croix (Proshold 1983) until 1992, essentially no attempts have been made to evaluate this program in a true agricultural environment in the United States, primarily due to a shortage of research funds. As supporting research, a population density study for TBW in 1981-1983 was conducted to determine the overwintered density of TBW. Results (Laster and Smith 1982, Smith et al. 1985) showed that 138, 5, and 10 feral moths emerged per ha in 1981, 1982, and 1983, respectively. These data provided estimates on which to base rearing programs for anticipated future releases. Finally, from 1991-1994, pilot test funds were allocated to study the value of released sterile hybrids in reducing TBW populations in the Mississippi Delta (Laster 1995; Laster and Roberson 1990; Laster and Hardee 1992; Laster et al. 1993a, 1993b, 1994a, 1995a). Results (Laster et al. 1994a, 1995a) obtained with traps (Hartstack and Witz 1981, Hartstack et al. 1979) showed that in a 3:1 released:wild ratio in 1992 produced a 29.9% sterility carryover in 1993, and a 2.6:1.0 released:wild ratio in 1993 produced a 12.1% sterility carryover, with 12.1% sterility also in the 1992 release area in 1994. These results showed that higher ratios of released:wild must be attained to achieve the best results; however, even with low released numbers, results were very favorable, considering the survival (Laster et al. 1978a) and migration potential (Raulston et al. 1982, Hendricks et al. 1973) of these insects. These results corroborated the need for large experimental areas emphasized by Schneider (1989) and Schneider et al. (1989).

The future of sterile hybrid releases in management of TBW is totally dependent on (1) decreased costs of rearing, (2) decreased cost of separating the sexes for making the backcross, (3) improved released techniques, and (4) the ultimate development of a sterile

backcross hybrid for CBW. Over the past 10 years, improvements in rearing of CBW and TBW have reduced cost of mass production of these insects by about 30%, but considerable reductions are still needed. Two major challenges to rearing cost reductions are (1) loss of qualified mass rearing personnel (death, retirement, down-sizing, etc.), and (2) an apparent loss of interest in research personnel in continuance of rearing research. Attempts have been made to separate sexes of TBW and BC hybrids via mechanical means, such as greater light transmission through abdomens of males versus females, but procedures have never exceeded 85% accuracy (Hardee, unpublished) which is unacceptable. If interest in a BC release program is re-kindled in the future, perhaps light transmission and other computerized procedures can be re-visited. Placement of release boxes over a large area, as was done in the recent pilot program (Laster et al. 1996a), would be cost prohibitive, and thus improved release techniques should be investigated. CBW and TBW are usually grouped together as an insect complex when discussing costs associated with these insects (Hardee and Herzog 1995), so solving the TBW problem with sterile BC releases does not solve the CBW problem. Laster (1979), Laster and Hardee (1995), Laster and Sheng (1995), and Laster et al. (1985, 1987b, 1994b, 1996b) all reported unsuccessful attempts to produce a sterile hybrid in CBW by mating CBW with *Helicoverpa armigera* from Australia, Russia, and China and *H. assulta* from Pakistan and Thailand. Additional studies are planned with species from China and the south Pacific which are closer to CBW in size and morphology. The use of Bt transgenic cottons (beginning in 1996) is being suggested as a possible means of solving problems associated with both CBW and TBW (Perlak et al. 1990). In addition, Hardee and Bell (1996) report that the use of insect pathogens in a large area-wide program may delay the development of CBW/TBW problems by one entire generation, which would mean major strides in solving the CBW/TBW problems. If either or both of these methodologies fails to resolve problems with these two insects, the sterile BC technique still remains a possible procedure in the future. However, advancements in all four of the above areas will be prerequisites for further testing and use of BC approach to managing TBW.

LITERATURE CITED

Agricultural Research Service. 1976. Report of ARS National *Heliothis* Planning Conference. New Orleans, LA, 19-21 January 1976.

Agricultural Research Service. 1985. An assessment of genetic methods for the control of *Heliothis virescens* and *Heliothis zea*. *In Heliothis* research planning: genetic control - a workshop. Stoneville, MS, 12-14 June 1984.

Brazzel, J. R., L. D. Newsom, J. S. Roussel, C. Lincoln, F. J. Williams, and G. Barnes. 1953. Bollworm and tobacco budworm as cotton pests in Louisiana and Arkansas. La. Agric. Exp. Stn. Tech. Bull. 482: 1-47.

Brewer, F. D., and E. G. King. 1979. Consumption and utilization of soyflour-wheat germ diets by *Heliothis* spp. Ann. Entomol. Soc. Am. 72: 415-417.

Carpenter, J. E. 1991. Effect of radiation dose on the incidence of visible chromosome aberrations in *Helicoverpa zea* (Lepidoptera: Noctuidae). Environ. Entomol. 20: 1457-1459.

Carpenter, J. E. 1992. Sperm precedence in *Helicoverpa zea* (Lepidoptera: Noctuidae): Response to a substerilizing dose of radiation. J. Econ. Entomol. 85: 779-782.

Carpenter, J. E., and H. R. Gross. 1989. Interaction of inherited sterility and diapause in the corn earworm (Lepidoptera: Noctuidae). J. Econ. Entomol. 82: 1354-1357.

Carpenter, J. E., and H. R. Gross. 1993. Suppression of feral *Helicoverpa zea* (Lepidoptera: Noctidae) populations following the infusion of inherited sterility from released substerile males. Environ. Entomol. 22: 1084-1091.

Carpenter, J. E., A. N. Sparks, and J. R. Raulston. 1979. Competitiveness of *Heliothis* hybrid vs.*H. virescens* females for *H. virescens* males in Georgia tobacco. J. Ga. Entomol. Soc. 14: 65-69.

Carpenter, J. E., A. N. Sparks, and H. L. Cromroy. 1987a. Corn earworm (Lepidoptera: Noctuidae): influence of irradiation and mating history on the mating propensity of females. J. Econ. Entomol. 80: 1233-1237.

Carpenter, J. E., J. R. Young, H. L. Cromroy, and A. N. Sparks. 1987b. Corn earworm (Lepidoptera: Noctuidae): comparison of field survival of larvae from normal and irradiated parents. J. Econ. Entomol. 80: 883-886.

Carpenter, J. E., J. R. Young, A. N. Sparks, H. L. Cromroy, and M. A. Chowdhury. 1987c. Corn earworm (Lepidoptera: Noctuidae): effects of substerilizing doses of radiation and inherited sterility on reproduction. J. Econ. Entomol. 80: 483-489.

Carpenter, J. E., A. N. Sparks, S. D. Pair, and H. L. Cromroy. 1989. *Heliothis zea* (Lepidoptera: Noctuidae): effects of radiation and inherited sterility on mating competitiveness. J. Econ. Entomol. 82: 109-113.

Cibrian-Tovar, J., and E. R. Mitchell. 1991. Courtship behavior of *Heliothis subflexa* (Gn.) (Lepidoptera: Noctuidae) and associated backcross insects obtained from hybridization with *H. virescens* (F.). Environ. Entomol. 20: 419-426.

Degrugillier, M. E. 1989. Virus-like particles in testes of *Heliothis virescens, H. subflexa,* and backcross males derived from the hybridization of *H. virescens* males x *H. subflexa* females. J. Invert. Pathol. 54: 281-299.

Degrugillier, M. E., and S. M. Newman. 1993. Hereditary viruses of *Heliothis*? Chromatin-associated virus-like particles in testes of six species of *Heliothis* and *Helicoverpa*, F_1, and backcross males. J. Invert. Pathol. 61: 147-155.

Elzen, G. W., B. R. Leonard, J. B. Graves, E. Burris, and S. Micinski. 1992. Resistance to pyrethroid, carbamate, and organophosphate insecticides in field populations of tobacco budworm (Lepidoptera: Noctuidae) in 1990. J. Econ. Entomol. 85: 2064-2072.

Firko, M. J., and E. G. King. 1991. Pyrethroid resistance in the tobacco budworm (Lepidoptera: Noctuidae) sterile hybrid strain. J. Econ. Entomol. 84: 1140-1144.

Forbes, W. T. M. 1954. Lepidoptera of New York and neighboring states, Noctuidae, Part III. Cornell Univ. Agr. Exp. Stn. Mem. 329: 28-29.

Furr, R. E., R. N. Paul, Jr., and M. L. Laster. 1974. Morphological characteristics of the male genitalia for separating *Heliothis virescens, H. subflexa,* and their F_1 hybrid progeny. Ann. Entomol. Soc. Am. 67: 816-817.

Gassner, G., and F. I. Proshold. 1978. Immunological identification of lepidoptera hybrid progeny and parents. Genetics 88: 30-31.

Gassner, G., S. G. Sears, and F. I. Proshold. 1979. Serological approaches to establish relative frequencies of native and released insects. Misc. Publ. Entomol. Soc. Am. 11: 55-68.

Goodpasture, C., L. E. LaChance, and R. D. Richard. 1980a. Persistence of abnormal spermiogenesis in the backcross generations of interspecific hybrids between *Heliothis virescens x H. subflexa.* Ann. Entomol. Soc. Am. 73: 397-403.

Goodpasture, C., R. D. Richard, D. F. Martin, and M. L. Laster. 1980b. Sperm cell abnormalities in progeny from interspecific crosses between *Heliothis virescens* and *H. subflexa.* Ann. Entomol. Soc. Am. 73: 529-532.

Haile, D. G., J. W. Snow, and J. R. Young. 1975. Movement by adult *Heliothis* released on St. Croix to other islands. Environ. Entomol. 4: 225-226.

Hardee, D. D., and M. R. Bell. 1996. Six years of area-wide management of bollworm/ budworm with pathogens -- what does it mean and where do we go from here. Proc. Beltwide Cotton Prod. Res. Conf. In Press.

Hardee, D. D., and G. A. Herzog. 1995. 48th Annual Conference Report on Cotton Insect Research and Control. Proc. Beltwide Cotton Prod. Res. Conf., pp. 725-744.

Hardwick, D. F. 1965. The corn earworm complex. Mem. Entomol. Soc. Can. 40, 247 pp.

Hartley, G. G., E. G. King, F. D. Brewer, and C. W. Gantt. 1982. Rearing of the *Heliothis* sterile hybrid with a multicellular larval rearing container and pupal harvesting. J. Econ. Entomol. 75: 7-10.

Hartstack, A. W., and J. A. Witz. 1981. Estimating field populations of tobacco budworm moths from pheromone trap catches. Environ. Entomol. 10: 908-914.

Hartstack, A. W., J. A. Witz, and D. R. Buck. 1979. Moth traps for the tobacco budworm. J. Econ. Entomol. 72: 519-522.

Heath, R. R., E. R. Mitchell, and J. C. Tovar. 1990. Effect of release rate and ratio of (Z)-11-hexadecen-1-ol from synthetic pheromone blends on trap capture of *Heliothis subflexa* (Lepidoptera: Noctuidae). J. Chem. Ecol. 16: 1259-1268.

Heath, R. R., J. R. McLaughlin, F. Proshold, and P. E. A. Teal. 1991. Periodicity of female sex pheromone titer and release in *Heliothis subflexa* and *H. virescens* (Lepidoptera: Noctuidae). Ann. Entomol. Soc. Am. 84: 182-189.

Hendricks, D. E., H. M. Graham, and J. R. Raulston. 1973. Dispersal of sterile tobacco budworms from release points in northeastern Mexico and southern Texas. Environ. Entomol. 2: 1085-1088.

Ignoffo, C. M., A. H. McIntosh, C. Garcia, M. D. Huettel, and A. J. Hill. 1983. Relative resistance of *Heliothis subflexa* to a single-embedded nucleopolyhedrosis virus of *Heliothis* species. J. Invert. Pathol. 42: 282-284.

Ignoffo, C. M., M. D. Huettel, A. H. McIntosh, C. Garcia, and P. Wilkening. 1985a. Genetics of resistance of *Heliothis subflexa* (Lepidoptera: Noctuidae) to *Baculovirus heliothis*. Ann. Entomol. Soc. Am. 78: 468-473.

Ignoffo, C. M., A. H. McIntosh, M. D. Huettel, and C. Garcia. 1985b. Relative susceptibility of *Heliothis subflexa* (Guenée) (Lepidoptera: Noctuidae) and other species of *Heliothis* to nonoccluded *Baculovirus heliothis*. Ann. Entomol. Soc. Am. 78: 740-743.

Karpenko, C. P., and F. I. Proshold. 1977. Fertility and mating performance of interspecific crosses between *Heliothis virescens* and *H. subflexa* backcrossed for three generations to *H. subflexa*. Ann. Entomol. Soc. Am. 70: 737-740.

Kimball, C. P. 1965. The Lepidoptera of Florida: arthropods of Florida and neighboring land areas. Division of Plant Industry, State of Florida, Department of Agriculture, Gainesville, Florida. 1: 106-107.

King, E. G., G. G. Hartley, D. F. Martin, and M. L. Laster. 1985. Large-scale rearing of a sterile backcross of the tobacco budworm (Lepidoptera: Noctuidae). J. Econ. Entomol. 78: 1166-1172.

Klassen, W., E. F. Knipling, and J. U. McGuire, Jr. 1970. The potential for insect-population suppression by dominant conditional lethal traits. Ann. Entomol. Soc. Am. 63: 238-255.

Klun, J. A., J. R. Plimmer, B. A. Bierl-Leonhardt, A. N. Sparks, and O. L. Chapman. 1979. Trace chemicals: the essence of sexual communication systems in *Heliothis* species. Science 204: 1328-1330.

Klun, J. A., J. R. Plimmer, B. A. Bierl-Leonhardt, A. N. Sparks, M. Primiani, O. L. Chapman, G. H. Lee, and G. Lepone. 1980a. Sex pheromone chemistry of female corn earworm moth, *Heliothis zea*. J. Chem. Ecol. 6: 165-175.

Klun, J. A., B. A. Bierl-Leonhardt, J. R. Plimmer, M. Primiani, O. L. Chapman, G. Lepone, G. H. Lee, and A. N. Sparks. 1980b. Sex pheromone chemistry of the female tobacco budworm moth, *Heliothis virescens*. J. Chem. Ecol. 6: 177-183.

Klun, J. A., B. A. Leonhardt, J. D. Lopez, Jr., and L. E. LaChance. 1982. Female *Heliothis subflexa* (Lepidoptera: Noctuidae) sex pheromone: chemistry and congeneric comparisons. Environ. Entomol. 11: 1084-1090.

Knipling, E. F. 1960. Use of insects for their own destruction. J. Econ. Entomol. 53: 415-420.

Knipling, E. F. 1966. Some basic principles in insect population suppression. Bull. Entomol. Soc. Am. 12: 7-15.

Knipling, E. F. 1970. Suppression of pest Lepidoptera by releasing partially sterile males: a theoretical appraisal. BioScience 20: 465-470.

Knipling, E. F., and W. Klassen. 1976. Relative efficiency of various genetic mechanisms for suppression of insect populations. U.S. Dep. Agric. Tech. Bull. 1533. 56 pp.

Knipling, E. F., and E. A. Stadelbacher. 1983. The rationale for area-wide management of *Heliothis* (Lepidoptera: Noctuidae) populations. Bull. Entomol. Soc. Am. 29: 29-37.

LaChance, L. E. 1974. Status of the sterile-insect release method in the world, pp. 55-62. *In* The Sterile-Insect Technique and its Field Applications. Proc. Panel Vienna, 1972, IAEA, Vienna.

LaChance, L. E. 1984. Hybrid sterility: eupyrene sperm production and abnormalities in the backcross generations of interspecific hybrids between *Heliothis subflexa* and *H. virescens* (Lepidoptera: Noctuidae). Ann. Entomol. Soc. Am. 77: 93-101.

LaChance, L. E. 1985. Genetic methods for the control of lepidopteran species: status and potential. USDA-ARS Series 28, 35 pp.

LaChance, L. E. 1987. Identification of sterile backcross *Heliothis* males in field collections. J. Econ. Entomol. 80: 1348-1350.

LaChance, L. E., and C. P. Karpenko. 1981. Effect of seven antibiotics on the growth and reproduction of *Heliothis subflexa* x *H. virescens* interspecific hybrids and backcross males. Ann. Entomol. Soc. Am. 74: 493-497.

LaChance, L. E., and C. P. Karpenko. 1983. Hybrid sterility in *Heliothis subflexa* x *H. virescens* (Lepidoptera: Noctuidae) crosses: expression after injection with antiviral agents, heat shocks, and rearing at extreme temperatures. Ann. Entomol. Soc. Am. 76: 104-109.

LaChance, L. E., and E. F. Knipling. 1962. Control of insect populations through genetic manipulation. Ann. Entomol. Soc. Am. 55: 515-520.

LaChance, L. E., and G. Olstad. 1988a. Spermiogenesis of eupyrene sperm in prepupae, pupae, and adults of *Heliothis virescens* (Lepidoptera: Noctuidae): an ultrastructural study. Ann. Entomol. Soc. Am. 81: 292-300.

LaChance, L. E., and G. Olstad. 1988b. Spermiogenesis of *Heliothis virescens* (Lepidoptera: Noctuidae): an ultrastructure study of eupyrene sperm in sterile backcross males. Ann. Entomol. Soc. Am. 81: 301-307.

Laster, M. L. 1972. Interspecific hybridization of *Heliothis virescens* and *H. subflexa*. Environ. Entomol. 1: 682-687.

Laster, M. L. 1979. Coping with the tobacco budworm/bollworm problem: status of hybrid insect approach, pp. 41-42. Proc. Beltwide Cotton Prod. Conf.

Laster, M. L. 1995. Suppressing tobacco budworm populations. National Conservation Tillage Digest. Oct.Nov.: 18-20.

Laster, M. L., and D. D. Hardee. 1992. Emergence and distribution of *Heliothis* moths in an area-wide release program, pp. 802-803. Proc. Beltwide Cotton Prod. Res. Conf.

Laster, M. L., and D. D. Hardee. 1995. Intermating compatibility between the North American *Helicoverpa zea* and *H. armigera* (Lepidoptera: Noctuidae) from Russia. J. Econ. Entomol. 88: 77-80.

Laster, M. L., and J. L. Roberson. 1990. *Heliothis* pupal handling and placement methods for improved moth emergence in wide-area release programs, pp. 311-312. Proc. Beltwide Cotton Prod. Res. Conf.

Laster, M. L., and C. F. Sheng. 1995. Search for hybrid sterility for *Helicoverpa zea* in crosses between the North American *H. zea* and *H. armigera* (Lepidoptera: Noctuidae) from China. J. Econ. Entomol. 88: 1288-1291.

Laster, M. L., and J. W. Smith. 1982. Studies to estimate the density of the *Heliothis virescens* overwintered population and to determine the dispersal and response to pheromone traps of the natural and released insects, pp. 195-197. Proc. Beltwide Cotton Prod. Res. Conf.

Laster, M. L., and E. A. Stadelbacher. 1991. Effect of long term colonization on attractancy of females to wild males: *Heliothis virescens* and *Heliothis* hybrid backcross (Lepidoptera: Noctuidae). J. Entomol. Sci. 26: 281-286.

Laster, M. L., D. F. Martin, and D. W. Parvin, Jr. 1976. Potential for suppressing tobacco budworms (Lepidoptera: Noctuidae) by genetic sterilization. Miss. Agric. For. Exp. Stn. Tech. Bull. 82. 9 pp.

Laster, M. L., D. F. Martin, and S. D. Pair. 1977. Mating incidence of male *Heliothis virescens*, hybrid and backcross males from *H. subflexa* x *H. virescens* crosses. Annals Ent. Soc. Am. 70: 293-295.

Laster, M. L., D. F. Martin, and S. D. Pair. 1978a. The attraction of wild *Heliothis virescens* males to sex pheromone traps baited with *H. virescens* and backcross females. Environ. Entomol. 7: 19-20.

Laster, M. L., D. F. Martin, S. D. Pair, and R. E. Furr. 1978b. Infusion of hybrid *Heliothis* male sterility into *H. virescens* populations in field cages. Environ. Entomol. 7: 364-366.

Laster, M. L., S. D. Pair, and D. F. Martin. 1982. Acceptance and development of *Heliothis subflexa* and *H. virescens* (Lepidoptera: Noctuidae) and their hybrid and backcross progeny on several plant species. Environ. Entomol. 11: 979-980.

Laster, M. L., C. E. Goodpasture, E. G. King, and P. Twine. 1985. Results from crossing the bollworms, *Helicoverpa armigera x H. zea*, in search of backcross sterility, pp. 146-147. Proc. Beltwide Cotton Prod. Res. Conf.

Laster, M. L., W. F. Kitten, E. F. Knipling, D. F. Martin, J. C. Schneider, and J. W. Smith. 1987a. Estimates of overwintered population density and adult survival rates for *Heliothis virescens* (Lepidoptera: Noctuidae) in the Mississippi Delta. Environ. Entomol. 16: 1076-1081.

Laster, M. L., N. R. Spencer, E. G. King, and P. Twine. 1987b. Current status of crossing exotic *Heliothis* spp with *H. zea* in search of hybrid sterility, pp. 315-316. Proc. Beltwide Cotton Prod. Res. Conf.

Laster, M. L., J. E. Carpenter, M. D. Huettel, S. G. Miller, F. I. Proshold, R. T. Roush, J. C. Schneider, and A. N. Sparks. 1988a. Autocidal systems for *Heliothis* control. pp. 74-103 *In* [Eds.] Herzog, G. A., S. Ramaswamy, G. Lentz, J. L. Goodenough, and J. J. Hamm, "Theory and Tactics of *Heliothis* Population Management: III. Emerging Control Tactics and Techniques." Southern Coop. Ser. Bull. No. 337.

Laster, M. L., E. G. King, and R. E. Furr. 1988b. Inter-specific hybridization of *Heliothis subflexa* and *H. virescens* (Lepidoptera: Noctuidae) from Argentina. Environ. Entomol. 17: 1016-1018.

Laster, M. L., D. D. Hardee, and J. L. Roberson. 1993a. Emergence of male-sterile *Heliothis virescens* (Lepidoptera: Noctuidae) backcross moths at a central release point and their resulting spatial distribution. J. Entomol. Sci. 28: 241-248.

Laster, M. L., D. D. Hardee, and J. C. Schneider. 1993b. A pilot program to control the tobacco budworm, *Heliothis virescens* (F.) by releasing sterile backcross insects into the natural population, pp. 874-876. Proc. Beltwide Cotton Prod. Res. Conf.

Laster, M. L., D. D. Hardee, and J. C. Schneider. 1994a. Release of sterile backcross moths during 1993 in a pilot program to control the tobacco budworm, pp. 925-927. Proc. Beltwide Cotton Prod. Res. Conf.

Laster, M. L., E. G. King, N. R. Spencer, and R. E. Furr, Jr. 1994b. Response to hybridization of the North American *Helicoverpa zea* and *H. assulta* (Lepidoptera: Noctuidae) from Pakistan and Thailand. Trends in Agr. Sci. Entomol. 2: 135-140.

Laster, M. L., D. D. Hardee, and J. C. Schneider. 1996a. *Heliothis virescens* (Lepidoptera: Noctuidae) suppression by sterile backcross releases. J. Econ. Entomol. In Press.

Laster, M. L., E. G. King, N. R. Spencer, and R. E. Furr. 1996b. Reproductive compatibility of four *Helicoverpa* species with *H. zea* (Lepidoptera: Noctuidae). J. Econ. Entomol. In Press.

Levins, R. A., and M. W. Parker. 1983. Short run aspects of a genetic control program for *Heliothis virescens* (Lepidoptera: Noctuidae). Ecol. Modeling 19: 213-220.

Levins, R. A., M. W. Parker, and D. W. Parvin, Jr. 1981. User's Manual for a simulation model of genetic control of the tobacco budworm. Miss. Agr. For. Exp. Stn. AEC. Tech. Publ. No. 31. 24 pp.

Lingren, P. D., J. R. Raulston, and A. N. Sparks. 1977. Interception of native male tobacco budworm by barriers of released laboratory-reared sterile females. Environ. Entomol. 6: 217-221.

Makela, M. E., and M. D. Huettel. 1979. Model for genetic control of *Heliothis virescens*. Theor. Appl. Genet. 54: 225-233.

Makela, M. E., and M. D. Huettel. 1981. Potential effect of interdemic selection on control of *Heliothis virescens* by sterile hybrids. Presented at Annual Meeting of the Entomol. Soc. Am., No. 687. 1981. San Diego, CA.

Mannion, C. M., J. E. Carpenter, and H. R. Gross. 1994. Potential of the combined use of inherited sterility and a parasitoid, *Archytus marmoratus* (Diptera: Tachinidae), for managing *Helicoverpa zea* (Lepidoptera: Noctuidae). Environ. Entomol. 23: 41-46.

Martin, D. F., M. L. Laster, and S. D. Pair. 1981a. Backcross progeny from interspecific hybrids of *Heliothis subflexa* x *H. virescens*: physiological diapause, winter survival and infusion into natural *H. virescens* populations. Environ. Entomol. 10: 197-200.

Martin, D. F., F. I. Proshold, and M. L. Laster. 1981b. Status of *Heliothis* hybrid pilot test, pp. 150-151. Proc. Beltwide Cotton Prod. Res. Conf.

Martin, D. F., M. L. Laster, F. I. Proshold, P. D. Lingren, S. D. Pair, J. R. Raulston, J. W. Smith, A. N. Sparks, and E. A. Stadelbacher. 1984. Tobacco budworm: behavioral studies of the sterile hybrid backcross in field releases in Puerto Rico. Environ. Entomol. 13: 701-707.

McElvare, R. R. 1941. Validity of the species *Heliothis subflexa* (Gn.) (Lepidoptera). Bull. Brooklyn Entomol. Soc. 36: 29-30.

Miller, S. G., and M. D. Huettel. 1986. Mitochondrial biogenesis during spermatogenesis in *Heliothis virescens, H. subflexa,* and their male sterile backcross hybrids. Arch. Ins. Biochem. Physiol. 3: 363-380.

Miller, S. G., M. D. Huettel, M. B. Davis, E. H. Weber, and L. A. Weber. 1986. Male sterility in *Heliothis virescens* x *H. subflexa* backcross hybrids. Evidence for abnormal mitochondrial transcripts in testes. Mol. Gen. Genet. 203: 451-461.

Mitchell, E. R., and R. R. Heath. 1987. *Heliothis subflexa* (Gn.) (Lepidoptera: Noctuidae): demonstration of oviposition stimulant from groundcherry using novel bioassay. J. Chem. Ecol. 13: 1849-1858.

Mitchell, E. R., R. W. Hines, and W. W. Copeland. 1988. *Heliothis subflexa* (Lepidoptera: Noctuidae): establishment and maintenance of a laboratory colony. Fla. Entomol. 71: 212-214.

North, D. T. 1975. Inherited sterility in Lepidoptera. Annu. Rev. Entomol. 20: 167-182.

North, D. T., J. W. Snow, D. Haile, and F. I. Proshold. 1975. Corn earworms: quality of sperm in sterile males released for population suppression on St. Croix Island. J. Econ. Entomol. 68: 595-598.

Pair, S. D., M. L. Laster, and D. F. Martin. 1977a. Hybrid sterility: mating dynamics of backcross progeny from crosses of *Heliothis subflexa* and *H. virescens*. Ann. Entomol. Soc. Am. 70: 665-668.

Pair, S. D., M. L. Laster, and D. F. Martin. 1977b. Hybrid sterility of the tobacco budworm: effects of alternate sterile and normal matings on fecundity and fertility. Ann. Entomol. Soc. Am. 70: 952-954.

Parvin, D. W., Jr., M. L. Laster, and D. F. Martin. 1976. A computer program for simulating the theoretical suppression of the tobacco budworm by genetic sterilization. Miss. Agric. For. Exp. Stn. AEc Tech. Publ. No. 15, 18 p.

Perlak, F. J., R. W. Deaton, T. A. Armstrong, R. L. Fuchs, S. R. Sims, J. T. Greenplate, and D. A. Fischhoff. 1990. Insect resistant cotton plants. Biol. Tech. 8: 939-943.

Proshold, F. I. 1983. Release of backcross insects on St. Croix, U.S. Virgin Islands, to suppress the tobacco budworm (Lepidoptera: Noctuidae): infusion of sterility into a native population. J. Econ. Entomol. 76: 1353-1359.

Proshold, F. I., and J. A. Bartell. 1970. Inherited sterility in progeny of irradiated male tobacco budworms: effects on reproduction, developmental time, and sex ratio. J. Econ. Entomol. 63: 280-285.

Proshold, F. I., and L. E. LaChance. 1974. Analysis of sterility in hybrids from interspecific crosses between *Heliothis virescens* and *H. subflexa*. Ann. Entomol. Soc. Am. 67: 445-449.

Proshold, F. I., and J. W. Smith. 1982. The potential for hybrid sterility in *Heliothis virescens* management, pp. 182-184. Proc. Beltwide Cotton Prod. Res. Conf.

Proshold, F. I., and J. W. Smith. 1990. *Heliothis virescens* (Lepidoptera: Noctuidae): analysis of population responses following introduction of backcrossed insects against native populations on St. Croix, U.S. Virgin Islands. J. Econ. Entomol. 83: 405-411.

Proshold, F. I., L. E. LaChance, and R. D. Richard. 1975. Sperm production and transfer by *Heliothis virescens, H. subflexa*, and the sterile hybrid males. Ann. Entomol. Soc. Am. 68: 31-34.

Proshold, F. I., M. L. Laster, D. F. Martin, and E. G. King. 1982b. The potential for hybrid sterility in *Heliothis* management. pp. 329-339 *In* Proc. Int. Workshop *Heliothis* Management, 15-20 November 1981, ICRISAT, Patancheru, A.P., India.

Proshold, F. I., C. P. Karpenko, and J. R. Raulston. 1983a. Dominant black body mutant in *Heliothis virescens* (Lepidoptera: Noctuidae) and transfer to *Heliothis subflexa* (Lepidoptera: Noctuidae) through hybridization. Ann. Entomol. Soc. Am. 76: 498-502.

Proshold, F. I., D. F. Martin, M. L. Laster, J. R. Raulston, and A. N. Sparks. 1983b. Release of backcross insects on St. Croix to suppress the tobacco budworm (Lepidoptera: Noctuidae): methodology and dispersal of backcross insects. J. Econ. Entomol. 76: 885-891.

Proshold, F. I., J. R. Raulston, D. F. Martin, and M. L. Laster. 1983c. Release of backcross insects on St. Croix to suppress the tobacco budworm (Lepidoptera: Noctuidae): behavior and interaction with native insects. J. Econ. Entomol. 76: 626-631.

Proshold, F. I., E. G. King, and G. G. Hartley. 1986. Survival, emergence, and release of laboratory-reared, backcross tobacco buworm (Lepidoptera: Noctuidae) pupae shipped from Stoneville, Miss. to St. Croix, U.S. Virgin Islands. J. Econ. Entomol. 79: 541-544.

Raina, A. K., and E. A. Stadelbacher. 1990. Pheromone titer and calling in *Heliothis virescens* (Lepidoptera: Noctuidae): effect of mating with normal and sterile backcross males. Ann. Entomol. Soc. Am. 83: 987-990.

Raulston, J. R., P. D. Lingren, A. N. Sparks, and D. F. Martin. 1979. Mating interaction between native tobacco budworms and released backcross adults. Environ. Entomol. 8: 349-353.

Raulston, J. R., W. W. Wolf, P. D. Lingren, and A. N. Sparks. 1982. Migration as a factor in *Heliothis* management. pp. 61-73 *In* W. Reed [ed.], Proceedings of the International Workshop on *Heliothis* Management, 15-20 November 1981, ICRISAT (International Crops Research Institute for the Semi-Arid Tropics) Center, Patancheru, India.

Richard, R. D., L. E. LaChance, and F. I. Proshold. 1975. An ultrastructural study of sperm in sterile hybrids from crosses of *Heliothis virescens* and *Heliothis subflexa*. Ann. Entomol. Soc. Am. 68: 35-39.

Roehrdanz, R. L. 1990. Maternal inheritance of a Y chromosome in *Heliothis* could play a role in backcross sterility. J. Hered. 81: 66-68.

Roehrdanz, R. L. 1994. Simple method for monitoring dispersal of *Heliothis* (Lepidoptera: Noctuidae) backcross sterility genes. J. Econ. Entomol. 87: 676-679.

Roehrdanz, R. L., and L. E. LaChance. 1981. Hybrid and backcross sterility in *Heliothis* sp. (Lepidoptera). Genetics 97: S91.

Roelofs, W. L., A. S. Hill, R. T. Carde, and T. C. Baker. 1974. Two sex pheromone components of the tobacco budworm moth, *Heliothis virescens*. Life Sci. 14: 1555-1562.

Roush, R. T., and J. C. Schneider. 1985. An analytical model for genetic control of *Heliothis virescens* incorporating the effects of sterile males. Theor. Appl. Genet. 71: 472-477.

Scheck, A. L., and F. Gould. 1995. Genetic analyses of differences in oviposition preferences of *Heliothis virescens* and *H. subflexa* (Lepidoptera: Noctuidae). Environ. Entomol. 24: 341-347.

Schneider, J. C. 1989. Role of movement in evaluation of area-wide insect pest management tactics. Environ. Entomol. 18: 868-874.

Schneider, J. C., R. T. Roush, W. F. Kitten, and M. L. Laster. 1989. Movement of *Heliothis virescens* (Lepidoptera: Noctuidae) in Mississippi in the spring: implication for area-wide management. Environ. Entomol. 18: 438-446.

Simpson, E. H., III. 1983. An analysis of the costs and benefits associated with the rearing and release of hybrid *Heliothis* in the Delta area of Mississippi. Ph.D. Dissertation, Miss. State University, Mississippi State, MS. 83 pp.

Sitchawat, T., and R. Thurston. 1980. *Heliothis* species on tobacco cultivars and *Physalis* species in Kentucky. Ann. Entomol. Soc. Am. 73: 375-377.

Smith, J. W., E. G. King, and D. W. Parvin. 1980. Comparative development of *Heliothis virescens* and hybrid backcross progeny reared at five temperatures. Agric. Res. Results SEA/USDA, ARS-S-6. 11 pp.

Smith, J. W., M. L. Laster, W. F. Kitten, and J. C. Schneider. 1985. Estimates of the population density of overwintered *Heliothis virescens* in the Mississippi Delta, pp. 147-149. Proc. Beltwide Cotton Prod. Conf.

Snow, J. W., W. W. Cantelo, and M. C. Bowman. 1969. Distribution of the corn earworm on St. Croix, U.S. Virgin Islands, and its relation to suppression programs. J. Econ. Entomol. 62: 606-611.

Snow, J. W., W. W. Cantelo, A. H. Baumhover, J. L. Goodenough, H. M. Graham, and J. R. Raulston. 1974. The tobacco budworm on St. Croix, U.S. Virgin Islands: host plants, population survey and estimates. Fla. Entomol. 57: 297-301.

Stadelbacher, E. A., and D. F. Martin. 1981. Fall diapause and spring emergence of *Heliothis virescens, H. subflexa,* and backcrosses of their hybrid. Environ. Entomol. 10: 139-142.

Teal, P. E. A., and A. Oostendorp. 1993. Interspecific hybridization between *Heliothis virescens* and *H. subflexa* (Lepidoptera: Noctuidae) affects the presence and structure of hair-pencil glands of males. Ann. Entomol. Soc. Am. 86: 322-326.

Teal, P. E. A., and A. Oostendorp. 1995a. Production of pheromone by hairpencil glands of males obtained from interspecific hybridization between *Heliothis virescens* and *H. subflexa* (Lepidoptera: Noctuidae). J. Chem. Ecol. 21: 59-67.

Teal, P. E. A., and A. Oostendorp. 1995b. Effect of interspecific hybridization between *Heliothis virescens* and *H. subflexa* (Lepidoptera: Noctuidae) on sex pheromone production by females. J. Insect Physiol. 41: 519-525.

Teal, P. E. A., and J. H. Tumlinson. 1987. The role of alcohols in pheromone biosynthesis by two noctuid moths that use acetate pheromone components. Arch. Insect Biochem. Physiol. 4: 261-269.

Teal, P. E. A., and J. H. Tumlinson. 1989. Isolation, identification, and biosynthesis of compounds produced by male hairpencil glands of *Heliothis virescens* (F.) (Lepidoptera: Noctuidae). J. Chem. Ecol. 15: 413-427.

Teal, P. E. A., and J. H. Tumlinson. 1990. Effect of hybridization on sex pheromone biosynthesis in *Heliothis* moths. p. 370 *In* H. H. Hagedorn et al. [eds.], Molecular Insect Science, Plenum, New York.

Teal, P. E. A., R. R. Heath, J. H. Tumlinson, and J. R. McLaughlin. 1981. Identification of a sex pheromone of *Heliothis subflexa* (GN.) (Lepidoptera: Noctuidae) and field trapping studies using different blends of components. J. Chem. Ecol. 7: 1011-1022.

Teal, P. E. A., A. Oostendorp, and J. H. Tumlinson. 1995. Induction of pheromone production in females of *Heliothis virescens* (F.) and *H. subflexa* (GN.) (Lepidoptera: Noctuidae). Canad. Entomol. 125: 355-366.

Tillman, P. G., and M. L. Laster. 1995. Parasitization of *Heliothis virescens* and *H. virescens - H. subflexa* backcross (Lepidoptera: Noctuidae) by *Microplitis croceipes* (Hymenoptera: Braconidae). Environ. Entomol. 24: 409-411.

Tingle, F. C., E. R. Mitchell, and A. H. Baumhover. 1978. Sex pheromone specificity in *Heliothis*. J. Chem. Ecol. 4: 471-479.

Todd, E. L. 1978a. A checklist of species of *Heliothis* Ochsenheimer (Lepidoptera: Noctuidae). Proc. Entomol. Soc. Wash. 80: 1-14.

Todd, E. L. 1978b. Maculation characters for separation of the species of the *Heliothis virescens* complex. Fla. Entomol. 61: 197-199.

Tumlinson, J. H., D. E. Hendricks, E. R. Mitchell, R. E. Doolittle, and M. M. Brennan. 1975. Isolation, identification, and synthesis of the sex pheromone of the tobacco budworm. J. Chem. Ecol. 1: 203-214.

Williams, M. R. 1995. Cotton insect losses - 1994. Proc. Beltwide Cotton Prod. Res. Conf., pp. 746-756.

COST/BENEFIT ANALYSIS OF A SORGHUM HYBRID RESISTANT TO SORGHUM MIDGE (DIPTERA: CECIDOMYIIDAE)

R. Terry Ervin, Tieiso M. Khalema, Gary C. Peterson[1], and George L. Teetes[2]

Department of Agricultural Economics
Texas Tech University
Lubbock, TX 79409-2132

ABSTRACT

The economic benefits resulting from the use of sorghum cultivars resistant to sorghum midge, *Stenodiplosis sorghicola* (Coquillett), were compared with the research and development costs. In 1984 dollars, the cost to develop the technology was approximately $2,720,000. For each dollar invested in research and development of a sorghum midge-resistant sorghum hybrid, the value of benefits to increased crop yields from the use of the resistant hybrid ranged from $24.2 at a 5% discount rate (the interest rate used to determine the present value of annual benefits and/or costs collected over time) when no insecticide was used to $2.7 at a 20% discount rate when insecticide was applied five times. The average value of benefits to increased crop yields at zero, three, and five insecticide applications at discount rates of 5, 10, 15, and 20% was $9.9 for each dollar spent to develop the technology. The intangible benefits that accrue to society as a result of the development and use of insect-resistant sorghum were not estimated. Important benefits from the development and deployment of sorghum midge-resistant sorghums include reduced crop production costs and greater yield, as well as enhanced sustainability of the sorghum production system and the conservation of natural and biological resources.

INTRODUCTION

The economic and environmental advantages of components of integrated pest management are important to the justification of research and development costs and the adoption of integrated pest management technology. Successful integrated pest management programs require creative and reliable direct control tactics that result in tangible benefits. Knipling (1979) stated, "No method of insect control offers greater advantages than growing plant varieties that resist insect attack." However, he concluded that the use of such technology depends on the costs of development and the benefits that result from the use of such technology. The ultimate measure

[1]Texas A&M University Agricultural Research and Extension Center, Lubbock, TX 79401-9757.
[2]Department of Entomology, Texas A&M University, College Station, TX 77843-2475.

of success of any integrated pest management direct control tactic should be based on a thorough evaluation of program costs and the benefits that result.

An example of research conducted to assess the economic benefit of components of integrated pest management is that of Gardner et al. (1983) who presented a cost efficiency analysis of the research resulting in the development of a synthetic sex pheromone monitoring program used by citrus producers for the detection of California red scale, *Aonidiella aurantii* (Maskell). Results indicated that for each dollar spent to develop the program, a return of between $2.5 and $3.6 was realized by citrus producers. Ervin et al. (1983) compared levels of damage caused by Comstock mealybug, *Pseudococcus comstocki* (Kuwana), in California to the biological control program developed to control this pest. They found that the cost of the developed control program would return as little as $14 or as much as $135 in benefits for each dollar invested. Results varied according to the crop considered, the expected level of crop loss, and the discount rate used (the interest rate used to determine the present value of a string of benefits and/or costs collected over time). Norgaard (1988) conducted a benefit-cost analysis of the research program which had previously identified and had established a parasitic wasp used to control cassava mealybug, *Phenacoccus manihoti* (Matile-Ferrero), in Africa. His results indicated that each dollar spent to develop the pest management program would return a minimum of $149 in benefits.

From a study conducted to assess the economic impact of the use of sorghum hybrids resistant to greenbug, *Schizaphis graminum* (Rondani), Dharmaratne et al. (1986) reported that resistant hybrids yielded 660 kg/ha more than susceptible sorghums when not treated with insecticide -- an increase in net profit of $33.94 per hectare. In another study of the impact of grain sorghum variety improvements, Eddleman et al. (1991) reported that development of greenbug-resistant sorghum hybrids resulted in a $389 million net benefit gain to the US.

The sorghum midge, *Stenodiplosis sorghicola* (Coquillett), is the most ubiquitous and damaging insect pest of sorghum, *Sorghum bicolor* (L.) Moench, worldwide (Davies 1982). This key insect pest governs sorghum agronomic practices and crop protection needs in much of the southern US, where most of the 4.5 million ha annually planted to sorghum in the US are grown. The largest concentration of hectares planted to sorghum is in drier areas such as Kansas and Texas (USDA 1980-1992). In the US, sorghum grain is produced primarily for use as livestock and poultry feed. Sorghum provides the advantages of drought tolerance, recovery from moisture stress, and relatively low production inputs compared with other crops, but provides returns slightly less per bushel than corn. However, low production inputs to sorghum are lost as an advantage when insecticides are used to control such insect pests as the sorghum midge.

During the adult life of <24 h, a female sorghum midge emerges from an infested sorghum spikelet, mates, and disperses to oviposit about 50 eggs between the glumes of flowering spikelets of sorghum or johnsongrass, *Sorghum halepense* (L.) Pers. (Walter 1941; Hallman et al. 1984; Teetes 1985, 1988). Larval feeding on the developing ovary inside the spikelet prevents normal kernel formation and results in direct grain loss (Walter 1941, 1959; Harding 1965). Sorghum and johnsongrass ecosystems provide a suitable habitat for the insect to reach economically damaging population levels.

Non-chemical tactics to manage the sorghum midge include early, uniform planting of sorghum to avoid the insect, destruction of johnsongrass, abiotic and biotic natural controls including indigenous parasites and predators, and the use of resistant

sorghum cultivars. When these tactics fail to be used or are ineffective, insecticide use is the only action available to control the sorghum midge. Insecticide use reduces profits and often results in less than adequate or desired control because of the short residual properties of insecticides, the flowering characteristics of sorghum, daily infestation by new adult sorghum midges, and that only sorghum midge adults, not damaging larvae, are affected by insecticides.

Sources of resistance in sorghum to sorghum midge had been reported but not exploited until TAM2566, derived from a zera-zera sorghum from Ethiopia, was discovered (Johnson et al. 1973, 1982a). This sorghum line has been the major source of resistance used in the Texas A&M University sorghum midge resistance-development program and has been the most widely used source of resistance (Johnson et al. 1982b; Peterson et al. 1985, 1991). AF28, an African sorghum identified in Brazil, has been the second most used source of resistance and has a resistance mode similar to that of TAM2566 (Peterson et al. 1984). Development of agronomically improved sorghum midge-resistant hybrids has been difficult because resistance is inherited as recessive quantitative traits (Widstrom et al. 1972; Berquist et al. 1974, Teetes and Johnson 1978).

Peterson et al. (1992) investigated the yield performance of susceptible and resistant sorghum hybrids in the presence and absence of sorghum midges. They found no significant yield difference between susceptible and resistant hybrids in the absence of the sorghum midge. However, when sorghum midges were present, resistant hybrids yielded significantly more grain than did susceptible hybrids.

Teetes et al. (1986) assessed differences in yield of sorghum midge-resistant and susceptible sorghum hybrids after insecticides had been applied at different intervals during the sorghum flowering period. They showed that sorghum midge-resistant sorghums yielded more than susceptible sorghums regardless of the insecticide treatment made to either kind of sorghum hybrid.

The development and adoption of sorghum midge-resistant sorghum hybrids would help reduce insecticide applications and result in greater economic returns to sorghum producers. The objective of this study was to evaluate the overall costs and benefits from the development and adoption of sorghum midge-resistant sorghum hybrids.

MATERIALS AND METHODS

The economic assessment of the development of sorghum midge-resistant sorghum hybrids was achieved through benefit-cost analyses. This study involved both a hindsight view of what has occurred (from 1980 to 1993) and a prospective view of what could occur (from 1994 to 2004). The procedure for collecting much of the data and related information was accomplished through personal interviews and discussions with sorghum breeders, entomologists, and seed company personnel. The experts consulted for this study included Mr. Jack Eberspacher, Executive Director of the National Grain Sorghum Producers Board; Mr. Y. F. Snodgrass, Field Representative of the Texas Grain Sorghum Board; Dr. Gary C. Peterson, Associate Professor and sorghum breeder, Texas A&M University Agricultural Research and Extension Center, Lubbock, TX; Dr. George L. Teetes, Professor, Department of Entomology, Texas A&M University, College Station, TX; Dr. A. Bruce Maunder, Senior Vice President, DeKalb Sorghum and Tropical Maize Research; Dr. Bulji Nukal, Manager, ICI Seed Company, Plainview, TX; Mr. Nathan Boardman, President, Crosbyton Seed Company, Crosbyton, TX; and the International Sorghum

and Millet Collaborative Research Support Program (INTSORMIL) management office based in Lincoln, NE. The documentation and related information sought from the persons mentioned above provided the bases for estimating the costs and benefits.

The standard benefit-cost formula was used to calculate the efficiency of hybrid development. A resulting benefit-cost ratio greater (less) than one implies that the returns from the research are greater (less) than the cost of the research. The equation used to discount expected future benefits and costs was:

$$\frac{B}{C}=\frac{\sum_{t=1}^{T}\frac{B_t}{(1+i)^t}}{\sum_{t=1}^{T}\frac{C_t}{(1+i)^t}} \qquad (1)$$

where B and C = present discounted values of the benefits and the costs, respectively, B_t = benefits accruing in year t, C_t = costs accruing in year t, i = discount rate, and T = the time period from 1993-2004. Costs and benefits incurred in the past were compounded to the present by modifying Equation 1:

$$\frac{B}{C}=\frac{\sum_{t=1}^{T}B_t(1+i)^t}{\sum_{t=1}^{T}C_t(1+i)^t} \qquad (2)$$

where B and C = present compounded values of the benefits and costs, respectively, and T = the time period from 1980-1992.

The following assumptions were made in this study: (1) sorghum midge-resistant sorghum hybrids produce the same weight-measured yields as susceptible hybrids (Peterson et al. 1992); (2) current resistant hybrids are the final product of the previous research; (3) 25 yr (1980-2004) is the time period being considered; (4) all Texas sorghum producers with sorghum potentially affected by sorghum midge are using only the resistant or only the susceptible hybrid, depending on the given scenario; and (5) only areas where sorghum midges are abundant enough to cause economic damage will benefit from the use of the resistant hybrid.

The scenarios referred to in this study consider varying insecticide treatment applications and the above stated assumptions. These assumptions are made using the benefit-cost equations when considering the following scenarios: (1) only the benefits of increased crop returns are considered from 1994 through 2004, and (2) only the value of the benefits of decreased applications of insecticides are considered from 1994 through 2004.

Costs and Benefits. Costs. The important costs on which an analysis such as this depend include the research operating costs and the scientist-year costs. The two primary scientists involved in the development of the resistant hybrids are Drs. Gary C. Peterson (plant breeder) and George L. Teetes (entomologist). In 1980, the US Agency for International Development began providing funds through INTSORMIL to promote this research. The Texas Grain Sorghum Board began funding this

research in 1990. Because both scientists are employed by the Texas A&M University System, the university has allocated funds in the form of scientist-man years, staff, and facilities support to continue this research. The total cost (in 1984 constant dollars) of annual appropriations to this research is presented in Table 1. These values represent the research operating costs and scientist-year costs for both researchers (Khalema 1993).

TABLE 1. Costs and Benefits in $1,000s (Constant 1984 Dollars[a]) of Developing and Using a Sorghum Midge-Resistant Sorghum Cultivar Based on Crop Returns and Reduced Cost of Insecticide Use.

		Crop returns			Value of decreased applications of insecticides	
Year	Research costs[b]	0 Applic.[c] CR_{r0}-CR_{s0}	3 Applic.[d] CR_{r3}-CR_{s3}	5 Applic.[e] CR_{r5}-CR_{s5}	Three Applic.[f]	Five Applic.[g]
1980	233	0	0	0	0	0
1981	217	0	0	0	0	0
1982	209	0	0	0	0	0
1983	205	0	0	0	0	0
1984	200	0	0	0	0	0
1985	196	0	0	0	0	0
1986	171	0	0	0	0	0
1987	169	0	0	0	0	0
1988	166	0	0	0	0	0
1989	163	0	0	0	0	0
1990	198	0	0	0	0	0
1991	197	0	0	0	0	0
1992	204	0	0	0	0	0
1993	192	0	0	0	0	0
1994	0	19,333	14,263	11,011	5,073	8,454
1995	0	17,223	12,706	9,809	4,519	7,532
1996	0	15,219	11,227	8,668	3,993	6,655
1997	0	13,312	9,821	7,582	3,493	5,821
1998	0	11,496	8,481	6,547	3,016	5,027
1999	0	9,764	7,203	5,561	2,562	4,270
2000	0	8,111	5,984	4,620	2,128	3,547
2001	0	6,532	4,819	3,720	1,714	2,856
2002	0	5,021	3,704	2,859	1,317	2,195
2003	0	3,574	2,636	2,035	938	1,563
2004	0	2,187	1,614	1,246	574	956
Total	2,720	114,592	84,538	65,263	30,066	50,110

[a] Consumer Price Index for all items 1982-1984, USDA Agricultural Statistics, 1992.
[b] Source: Dr. Gary C. Peterson, sorghum breeder, Texas A&M University Agricultural Research and Extension Center, Lubbock, TX; Dr. George L. Teetes, entomologist, Texas A&M University, College Station, TX.
[c] Values obtained as differences between net crop returns of the resistant and susceptible hybrids with zero applications of insecticide.
[d] Values obtained as differences between net crop returns of the resistant and susceptible hybrids with three applications of insecticide.
[e] Values obtained as differences between net crop returns of resistant and susceptible hybrids with five applications of insecticide.
[f] Value of the decreased applications of insecticide for the three applications scenario.
[g] Value of the decreased applications of insecticide for the five applications scenario.

Benefits. Benefits of developing the sorghum midge resistant hybrids are considered from 1994 through 2004. The number of hectares planted to grain sorghum in Texas decreased between 1980 and 1991. Thus, to predict the number of hectares in Texas planted to this crop from 1994-2004, a simple regression line where planted hectares were defined as a function of time was fitted using data from 1980-1991. The resulting model was:

$$\text{Planted hectares} = \underset{(8.51)}{1{,}944{,}534} - \underset{(-2.781)}{86{,}352}\ (\text{number of years})$$

where the values in parentheses represent the Student *t* statistics. The estimated model had an R^2 value of 0.436.

The benefits generated as a result of using resistant, rather than susceptible hybrids were recognized in two forms: first, the value of increased yields because of grain saved from sorghum midge damage when resistant rather than susceptible hybrids were used; and second, the savings in decreased applications of insecticides needed for sorghum midge control. To estimate grain saved from damage by sorghum midge, a comparison of yields from resistant hybrids (Yr) and yields from susceptible hybrids (Ys) was made. The estimated yields were based on the results of Teetes et al. (1986). Yields for both susceptible and resistant hybrids were estimated when different insecticide treatments were considered. The effects of using zero, three, or five applications of insecticide were determined. The following yields for the various scenarios were used, where $Yr_{(i)}$ = the yield of resistant plants with i number of insecticide applications and $Ys_{(i)}$ = the yield of susceptible plants with i number of insecticide applications:

$Yr_{(0)}$ = 2,517 kg/ha = 2,246.5 lb/ac = 40.12 bu/ac
$Yr_{(3)}$ = 3,997 kg/ha = 3,567.5 lb/ac = 63.71 bu/ac
$Yr_{(5)}$ = 4,131 kg/ha = 3,687.1 lb/ac = 65.84 bu/ac
$Ys_{(0)}$ = 377 kg/ha = 336.5 lb/ac = 6.00 bu/ac
$Ys_{(3)}$ = 2,418 kg/ha = 2,158.2 lb/ac = 38.54 bu/ac
$Ys_{(5)}$ = 2,912 kg/ha = 2,599.1 lb/ac = 46.41 bu/ac

Not all hectares planted to sorghum in Texas are sprayed to control sorghum midge. To investigate the advantage of using resistant instead of susceptible hybrids, it is important to estimate the land area planted to sorghum that has a sorghum midge problem. Dr. George L. Teetes (personal communication) estimated that 15% of the sorghum hectarage in Texas is sprayed for sorghum midge. Thus, it is assumed that 15% of all the hectares planted to grain sorghum in Texas will use the resistant hybrids.

Value of Grain Saved from Damage. The value of additional yield resulting from the use of resistant rather than susceptible hybrids in the presence of the pest is the difference in crop returns between the two kinds of hybrids when comparing insecticide application scenarios. When estimating the crop returns, it was assumed that all production costs were equal for both resistant and susceptible hybrids. However, the costs to purchase and apply insecticides and the costs to harvest and transport additional amounts of grain produced by resistant hybrids are considered in this analysis.

The annual crop returns reported for each scenario resulted from use of the following model:

$$CR_i = (AP_i * TP_i * Y_i) - (CI_i + CHT_i) \quad (3)$$

where CR_i = crop returns in year i; AP_i = the 15% of the hectares in Texas planted to sorghum that is affected in year i by sorghum midges; TP_i = target price (dollars per kilogram) of sorghum in year i (proxy for market price) (USDA 1993); Y_i = estimated yield per hectare (Teetes et al. 1986); CI_i = cost of insecticide and application on a per-hectare basis (average annual value from the Texas Crop and Livestock Enterprise Budgets for all state Extension Service districts for all counties that produce dryland grain sorghum in Texas). The 1990 CI estimated value was used for the period 1994-2004. This annual value is the product of the nominal CI per hectare, the number of hectares planted to sorghum in a particular year, and the number of insecticide applications. This value is adjusted using the Consumer Price Index to represent 1984 constant dollars. In the scenario where there is no application of insecticide, the cost of insecticide is considered to be zero; CHT_i = cost of harvesting and transporting estimated to be $0.014/kg for additional yield resulting from the use of the resistant hybrid (this value is the product of multiplying the number of hectares planted by the yield difference in grain produced for a particular insecticide scenario by the average cost per kilogram of harvesting and transporting).

Decreased Insecticide Applications. In addition to crop returns, another benefit realized from using resistant sorghum hybrids is the value of decreased applications of insecticides. Teetes et al. (1986) reported yields of susceptible and resistant hybrids after insecticide had been applied zero, three, or five times to the sorghum. Following their findings, data were generated to consider zero applications of insecticide on resistant hybrids and to compare these data to data on three and five applications of insecticide to susceptible hybrids. The difference in costs between zero applications of insecticide on resistant and three and five applications of insecticide on susceptible sorghum, respectively, is the value of the money saved by not having to apply insecticide. The annual cost of insecticide was computed as previously, where CI is the average cost of both the purchase and application of insecticide on a per-hectare basis (TAEX 1990). These values are presented in Table 1.

RESULTS AND DISCUSSION

The quantifiable benefits of the development and use of sorghum midge-resistant hybrids were compared to the costs in common monetary units (e.g., 1984 dollars). The benefits of the results of this ongoing research considered in this analysis are the net crop returns or the decreased costs because insecticides were not needed. The costs are research operating costs and scientist-year costs. Total costs and total benefits (value of crop returns and value of decreased chemical applications) presented in Table 1 were compounded from 1980-1994 and discounted from 1994-2004 to make equivalent to 1993 dollar amounts. The choice of discount rate used to compute present values may have an important effect in determining whether funding for a project should be continued or if results of the project should be implemented. In this analysis, discount rates ranging from 5-20% at five-point intervals were used to incorporate sensitivity analysis. Another means of incorporating sensitivity is the inclusion of different insecticide treatments (zero, three, and five applications). In addition to the alternative discount rates used, the

following scenarios were considered when testing for sensitivity: (1) compute benefit-cost ratios considering only the benefits of increased crop returns, and (2) compute benefit-cost ratios considering only the benefits of reduced insecticide applications.

The benefit-cost ratios discussed above are presented in Table 2. The ratios of benefits and costs under different scenarios indicated high levels of benefits returned for the research dollars invested. Under the scenario of zero applications of insecticide, when benefits of increased crop returns were considered at a 5% discount rate, a return of $24.2 in benefits was received for each dollar invested in the development of resistant sorghum hybrids. With the same level of benefits, under the scenario of three and five applications of insecticide at a 20% interest rate, returns of $3.5 and $2.7 in benefits were received for every dollar spent, respectively.

TABLE 2. Benefit-Cost Ratios of Developing and Using a Sorghum Midge-Resistant Sorghum Hybrid.

	Discount rate (percent)			
Variables	5	10	15	20
	Benefits of increased crop returns			
B_0/C	24.2	14.0	8.2	4.8
B_3/C	17.9	10.3	6.0	3.5
B_5/C	13.8	8.0	4.6	2.7
$\bar{X}$	18.6	10.8	6.3	3.7
	Benefits of decreased applications of insecticides			
B_3/C	6.4	3.7	2.1	1.3
B_5/C	10.6	6.1	3.6	2.1
$\bar{X}$	8.5	4.9	2.9	1.7

B_0 represents benefits for zero applications of insecticide.
C represents costs and remains the same throughout.
B_3 represents benefits for three applications of insecticide.
B_5 represents benefits for five applications of insecticide.

In another scenario, when considering only the benefits of decreased applications of insecticides for purposes of sensitivity, a return of $6.4 in benefits was received for every dollar spent at the 5% discount rate when three applications of insecticide were applied. In the same category, considering the scenarios of three and five applications of insecticide at a 20% interest rate, returns of $1.3 and $2.1 in benefits were received for every dollar invested in the research project, respectively.

Considering the scenarios and cases presented above, returns for each dollar spent on research are greater than one. The highest level of return on every dollar spent on research was $24.2 and the lowest was $1.3. Considering the worst situation, a return of $1.3 in benefits was obtained for each dollar invested in the development of grain sorghum hybrids resistant to sorghum midge.

The development of a resistant hybrid as a component of integrated pest management does not necessarily mean that no pesticides will be needed to produce

a crop. The need is, rather, for a program providing the optimal outcome in terms of agricultural output, resource savings, good public health, and a clean environment.

The benefit-cost analyses performed in this study indicate that public money invested in the development of sorghum midge-resistant sorghum hybrids has been well spent. There are both quantifiable economic benefits and intangible benefits associated with the development and adaptation of resistant hybrids. The quantifiable benefits are in monetary units which primarily are delivered as crop returns or revenues to sorghum producers, whereas intangible benefits (e.g., reduced numbers of chemical applications, reduced ecological and environmental detrimental effects, reduced development of resistance by the insect pest to chemical insecticides, advancement of scientific knowledge, etc.) accrue to society and can only be described, not quantitatively measured.

The greater yields of resistant over susceptible sorghum hybrids suggests that a greater supply of sorghum in the market will occur, which may result in decreasing consumer prices. Benefits of the sorghum midge resistance project may flow more to other regions of the world, especially to semi-arid regions and to less-developed countries where grain sorghum is consumed by humans. The increased yields of resistant hybrids grown in areas where sorghum midges are abundant will increase food security. In less-developed countries, facilities and regulations for pesticides are not well developed; therefore, the use and adaptation of resistant hybrids will reduce the frequency of handling hazardous chemicals and thus increase protection of human lives and wildlife.

Commercial sorghum producers have the potential of receiving additional benefits through money saved by not having to apply insecticides. It is presumed that once producers have recognized the costs saved by not having to apply insecticides, these savings will be handed down in the form of lower prices to sorghum consumers and lower prices of sorghum products. Fewer chemicals in the environment will result in increased protection of human health and wildlife.

ACKNOWLEDGMENT

This research was supported in part by grant A.I.D./DAN-1254-G-00-0021-00, from the Agency for International Development through the Sorghum/Millet Collaborative Research Support Program (INTSORMIL). The authors appreciate the constructive review of the manuscript by Emmitt Elam, Phillip N. Johnson, Sujit K. Roy, and Bonnie B. Pendleton.

LITERATURE CITED

Berquist, R. R., P. Rotar, and W. C. Mitchell. 1974. Midge and anthracnose head blight resistance in sorghum. Trop. Agric. 51: 431-435.

Davies, J. C. 1982. Pest losses and control of damage on sorghum in developing countries - the realities and the myths, pp. 215-223. *In* Proceedings of the International Symposium on Sorghum, Patancheru, Andhra Pradesh, India.

Dharmaratne, G., J. R. Stool, R. D. Lacewell, and G. Teetes. 1986. Economic impact of greenbug resistant grain sorghum varieties: Texas Blacklands. Tex. Agric. Exp. Stn. MP-1585.

Eddleman, B. R., C. C. Chang, and B. A. McCarl. 1991. Economic benefits from INTSORMIL grain sorghum variety improvements in the United States. Tex. Agric. Exp. Stn. Report TAMRF 9045.

Ervin, R. T., L. J. Moffitt, and D. E. Meyerdirk. 1983. Comstock mealybug (Homoptera: Pseudococcidae): cost analysis of a biological control program in California. J. Econ. Entomol. 76: 605-609.

Gardner, P. D., R. T. Ervin, D. S. Moreno, and J. L. Baritelle. 1983. California red scale (Homoptera: Diaspididae): cost analysis of a pheromone monitoring program. J. Econ. Entomol. 76: 601-604.

Hallman, G. J., G. L. Teetes, and J. W. Johnson. 1984. Relationship of sorghum midge (Diptera: Cecidomyiidae) density to damage to resistant and susceptible sorghum hybrids. J. Econ. Entomol. 77: 83-87.

Harding, J. A. 1965. Ecological and biological factors concerning the sorghum midge during 1964. Tex. Agric. Exp. Stn. MP-773.

Johnson, J. W., D. T. Rosenow, and G. L. Teetes. 1973. Resistance to the sorghum midge in converted exotic sorghum cultivars. Crop Sci. 13: 754-755.

Johnson, J. W., D. T. Rosenow, G. L. Teetes, and F. R. Miller. 1982a. Registration of TAM2566 sorghum germplasm line. Crop Sci. 22: 1271.

Johnson, J. W., G. L. Teetes, D. T. Rosenow, B. R. Wiseman, and J. M. Phillips. 1982b. Registration of 28 midge resistant sorghum germplasm lines. Crop Sci. 22: 1273.

Khalema, T. M. 1993. An estimation of the costs and benefits of developing a midge resistant sorghum hybrid. M.S. Thesis, Texas Tech University, Lubbock.

Knipling, E. F. 1979. The basic principles of insect population suppression and management. USDA Agric. Handbook 512.

Norgaard, R. B. 1988. The biological control of cassava mealybug in Africa. Amer. J. Agric. Econ. 70: 366-371.

Peterson, G. C., J. W. Johnson, G. L. Teetes, D. T. Rosenow, and R. E. Schaffert. 1984. Registration of Tx2782 midge resistant sorghum germplasm line. Crop Sci. 24: 389-390.

Peterson, G. C., J. W. Johnson, G. L. Teetes, and D. T. Rosenow. 1985. Registration of midge resistant sorghum germplasm. Crop Sci. 25: 372.

Peterson, G. C., G. L. Teetes, B. R. Wiseman, A. Sotomayor-Rios, J. W. Jones, and K. Schaefer. 1991. Registration of 22 midge-resistant sorghum germplasm lines. Crop Sci. 31: 498-499.

Peterson, G. C., G. L. Teetes, J. W. Jones, and R. M. Anderson. 1992. Yield and selected traits of sorghum midge resistant hybrids in the presence and absence of the pest insect. Tex. Agric. Exp. Stn. PR-4922.

TAEX. 1990. Texas crop and livestock enterprise budgets. Tex. Agric. Ext. Serv., College Station.

Teetes, G. L. 1985. Insect resistant sorghums in pest management. Insect Sci. Applic. 6: 443-451.

Teetes, G. L. 1988. Entomology of johnsongrass/sorghum/sorghum midge and agriculture. *In* M. K. Harris and C. E. Rogers [eds.] The Entomology of Indigenous and Naturalized Systems in Agriculture. Westview Press, Boulder, CO.

Teetes, G. L., and J. W. Johnson. 1978. Insect resistance in sorghum, pp. 167-189. *In* Proceedings of the 33rd Annual Corn and Sorghum Research Conference, Chicago, IL.

Teetes, G. L., M. I. Becerra, and G. C. Peterson. 1986. Sorghum midge (Diptera: Cecidomyiidae) management with resistant sorghum and insecticide. J. Econ. Entomol. 79: 1091-1095.

USDA. 1980-1992 (various issues). Agricultural statistics. US Government Printing Office, Washington, DC.
USDA. 1993. Feed situation and outlook yearbook. US Government Printing Office, Washington, DC.
Walter, E. V. 1941. The biology and control of the sorghum midge. USDA Tech. Bull. 778.
Walter, E. V. 1959. The sorghum midge with suggestions for control. USDA Farmers' Bull. 1566.
Widstrom, N. W., B. R. Wiseman, and W. W. McMillian. 1972. Some gene effects conditioning resistance to midge and webworm injury in sorghum. Sorghum Newsl. 15: 22-23.

THE BIOLOGY OF THE SNAKEWEED LEAFHOPPER, *EMPOASCA BITUBERA* (HOMOPTERA: CICADELLIDAE), IN NEW MEXICO.

Daniel E. Gandolfo[1] and David B. Richman

Department of Entomology, Plant Pathology and Weed Science
New Mexico State University
Las Cruces, NM 88003-0003

ABSTRACT

The life history of the snakeweed leafhopper, *Empoasca bitubera* DeLong, was studied at four sites in southern and central New Mexico. This apparently monophagous leafhopper was found to live on woody snakeweeds, *Gutierrezia* spp., throughout its life cycle. At least three apparent population peaks were observed during the year. Ten other leafhopper species were collected, of which only one, *Gyponana delta* Ball, was observed feeding on snakeweed in the field. Three other species were observed to feed on snakeweed in the laboratory. *Empoasca bitubera* was found to be attacked by a dryinid wasp, *Aphelopus* sp. *Gyponana delta* and *Balclutha neglecta* (DeLong and Davidson) were also found to have dryinid parasitoids (genus unidentified). Two other species of leafhopper, *Ceratagallia bigeloviae* (Baker) and *Aceratagallia uhleri* (Van Duzee) were parasitized by a strepsipteran, *Halictophagus* sp.

INTRODUCTION

Broom snakeweed, *Gutierrezia sarothrae* (Pursh) Britton and Rusby, and the closely related threadleaf snakeweed, *G. microcephala* (DC.) Gray, are widespread noxious weeds in southwestern rangelands of the United States and northern Mexico. These weeds cause losses of about $16.8 million in western Texas and nearly twice as much in the United States as a whole (McGinty and Welsh 1987, Cordo and DeLoach 1992). Several articles dealing with insects associated with snakeweed have been published, mostly during the last twenty years. Most of these were studies on defoliators (Parker 1984, 1985; Parker and Salzman 1985; Powell 1976; Edwards and Wangberg 1985; Richman et al. 1992; Sterling and Thompson 1992), and wood borers (Campbell and Bomberger 1934, Falkenhagen 1978, Richman and Huddleston 1981). In addition, two root borers that attack species of *Gutierrezia* endemic to Argentina were studied by Cordo (1985) and Cordo and DeLoach (1992).

1/ South American Biological Control Laboratory, ARS-USDA, Bolivar 1559, (1686) Hurlingham, ARGENTINA.

Foster et al. (1981) published a list of 338 species of insects associated with broom and threadleaf snakeweeds in western Texas and New Mexico. Thompson and Richman (1989) noted that about one-third of the insects listed in Foster's report were sap feeders, mainly leafhoppers, scales and mealybugs, but no research has been conducted on these insects. A total of 39 species of leafhoppers was listed by Foster et al. (1981), and nine of these were indicated as being "common". No information, however, was included as to whether these were incidental or if they actually fed on snakeweed, at least during part of their life cycles. Oman (1949) indicated that adults and nymphs of leafhoppers frequently do not feed on the same host plant. Also, Poos and Wheeler (1943) stated that plants upon which adults of *Empoasca* are collected may be of no great significance as hosts unless eggs are also deposited on that plant.

We present here the results of a study on leafhoppers associated with snakeweed conducted in southern and central New Mexico. The primary objectives were to determine the species of leafhoppers associated with snakeweeds that occur in the area, determine which of them feed on snakeweed and in which stage, as well as their seasonal occurrence and relative abundance.

METHODS AND MATERIALS

Initial general sweep net samples were collected from 1989 to 1992 at two sites on the Jornada Experimental Range, Doña Ana County, and at two sites on the Youngblood Ranch, 15 miles east of San Antonio, Socorro, New Mexico. Headquarters site at the Jornada Experimental Range was abandonded in 1992 because almost all of the snakeweed there died, aparently from drought stress. Samples consisted of 100 sweeps with a standard sweep net (38-cm diameter); samples were collected approximately every two weeks during the spring-fall, with a few collections made during the winter of 1990-91. Only woody snakeweeds were sampled, with both *G. sarothrae* and *G. microcephala* being sampled at South Well on the Jornada Experimental Range and only *Gutierrezia sarothrae* being sampled at Jornada Headquarters, Jornada Experimental Range, and at Old Stock Tank and South Edge sites on the Youngblood Ranch. As leafhopper density on the two species of snakeweed at the Jornada Experimental Range was not different, no distinction was made in the collections. Leafhoppers in this general survey were sorted and counted in each sample, but were not sexed, separated by age, or examined for parasites.

Samples for analysis of life history, including sex ratios and parasitism, especially for *Empoasca bitubera* DeLong, were collected from July 1991 to July 1992 at South Well, Jornada Experimental Range, Doña Ana Co. NM, from a mixed stand of *G. sarothrae* and *G. microcephala*. These samples also consisted of 100 sweeps with a standard sweep net (38 cm diameter). Four samples were taken each month except from December through February when just two samples were taken each month. Each sample was placed in 75% alcohol. Adult leafhoppers were sorted by species and sex, counted and the percentage of parasitism by drynids or strepsipterans recorded; parasitoids were evident as "tumors" on the abdominal or thoracic segments. Nymphs were not counted in this case, but the presence or absence of this developmental stage was registered and some specimens were taken alive to the laboratory where they were put on cuttings of snakeweed and reared to adults to observe feeding. The proportion of males and females of *E. bitubera* was tested with a likelihood ratio test (G test) (Sokal and Rohlf 1981). Some further statistical evaluation was attempted, using precipitation and temperature against numbers of leafhoppers in the samples in a multiple regression, but as none of these data, with the possible exception of temperature alone, turned out to be statistically significant, the data were not analysed further.

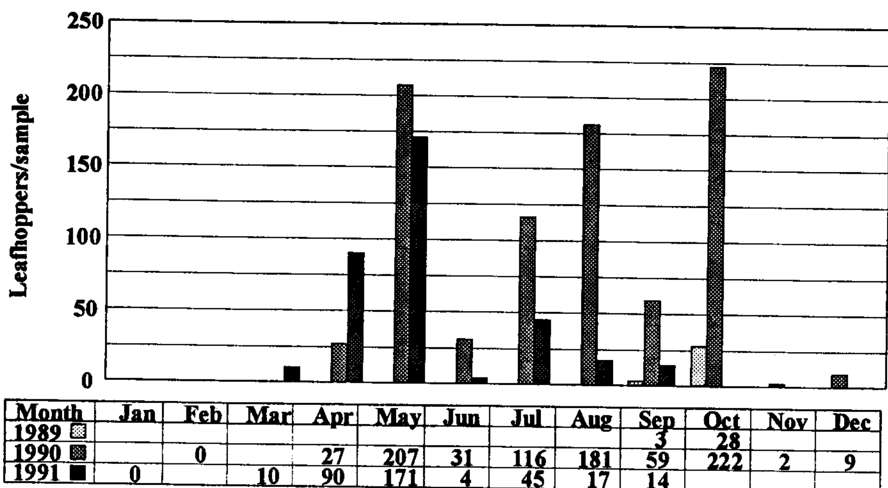
RESULTS AND DISCUSSION

The most abundant leafhopper collected was *Empoasca bitubera*. In the original description of the species, DeLong (1932) briefly indicated the host plant of *E. bitubera* as being *Gutierrezia*. In the original series collected at Tucson, Arizona, Yarnell Mts. Arizona, and Cedar, Utah, the food plant was listed as "Guterh." (probably *Gutierrezia)*. Delong also collected specimens at Santa Clara, Utah, and Las Vegas, Nevada, on *Gutierrezia*. To our knowledge, no other host plant genus has been listed for *E. bitubera*.

The incidence of *Empoasca bitubera* at the four sites is summarized in Figs. 1-4. Populations appear to have increased compared to earlier samples, during 1992 and large numbers were collected during May, June, July and August at all sites except the headquarters site on the Jornada Experimental Range and, during the month of July, at South Well. It should be noted that the rise of numbers at the Socorro County and the Doña Ana sites were roughly similar and that there was more variation in one site data between years than between sites in a given year.

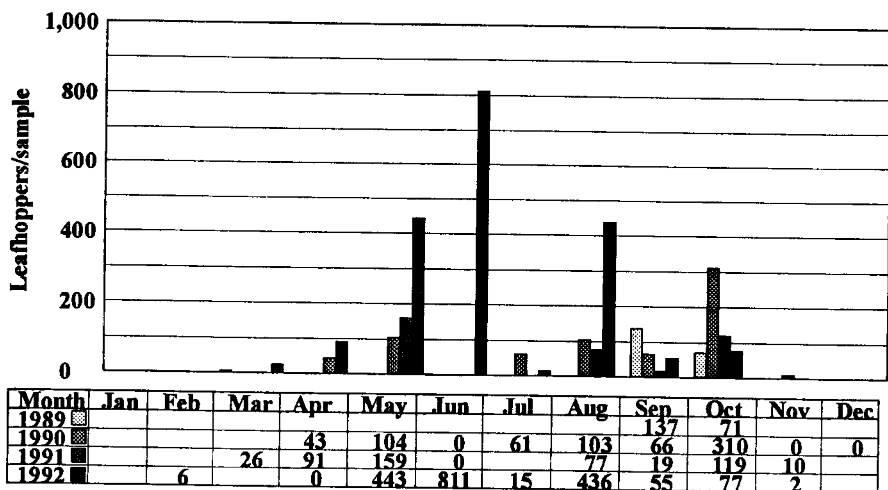
Adults of eight species of leafhoppers were collected at South Well during the 1991-92 period. Information on seasonal occurrence, stages collected, and parasitoids is summarized in Table 1. Two cicadellid species, *E. bitubera* and *Gyponana delta* Ball, were observed feeding on woody snakeweed plants. Adults of *Ceratagallia bigeloviae* (Baker), *Aceratagallia uhleri* (Van Duzee), and *Driotura* sp. [possibly *gammaroides* (Van Duzee)] fed on snakeweed under laboratory conditions; however, it is unlikely that this last species normally feeds on woody snakeweeds as it appears to feed on grasses, as do most of the Deltocephalinae (Oman 1949). In addition to the abundant nymphs of *E. bitubera*, we found nymphs of the last two instars of *G. delta*. Some of the latter were taken alive to the laboratory and completed development feeding on snakeweed. We occasionally found nymphs that might have been *C. bigeloviae*, but they did not complete development on snakeweed in the laboratory. Other leafhoppers collected that appeared to be incidental, because they were never observed to feed on woody snakeweeds, included *Balclutha neglecta* (DeLong and Davidson), *Cuerna arida* Oman and Beamer, *Driotura* sp., *Empoasca neaspersa* Oman and Wheeler, *Exitianus exitiosus* (Uhler), and *Xerophloea viridis* (Fabricius).

Nymphs and adults of *E. bitubera* feed on leaves and green photosynthetic stems of *G. sarothrae* and *G. microcephala* and are very active during the daytime. Plants with heavy infestations of *E. bitubera* have tiny yellowish spots on the leaves as the result of feeding. Adults of *E. bitubera* were collected throughout the year (Fig. 5). From August 1991 to July 1992, we collected 7,354 adults of this species. Females (4,156) were significantly more numerous than males (3,198), ($G=24$, $p<0.001$). Adults were extremely common on the plants from spring to fall with two peaks in August and June. The numbers of specimens collected decreased suddenly during July of both 1991 and 1992. This decrease seems to be correlated with high temperatures and low relative humidities, but no general conclusion can be made because of difficulties noted in the last section. In general, the size of adult populations seemed to rise after periods of highest rainfall during the summer, and decrease during winter, even when preciptation was high (Fig. 5). The data, however, were not statistically significant, and no statement can be made with any degree of certainty. A possible third peak of abundance, which may have been related to the amount of soil moisture but was not directly to precipitation per se, was recorded in October. Adults were still fairly abundant in November, but few adults were collected at the beginning of December, when most of the aerial part of the plants had dried out. They were completely inactive during December to March. When overwintering adults were taken to the laboratory, they recovered activity and started to feed immediately indicating that they were



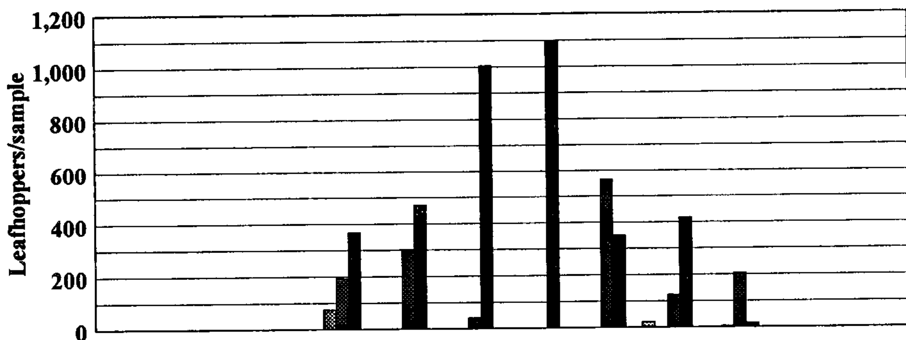
Month	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
1989									3	28		
1990		0		27	207	31	116	181	59	222	2	9
1991	0		10	90	171	4	45	17	14			

FIG. 1. Number of individuals of *Empoasca bitubera* in 100 sweep samples at Headquarters, Jornada Experimental Range,Doña Ana County, New Mexico, 1989-92. Blank cells indicate no sample was collected for that month.



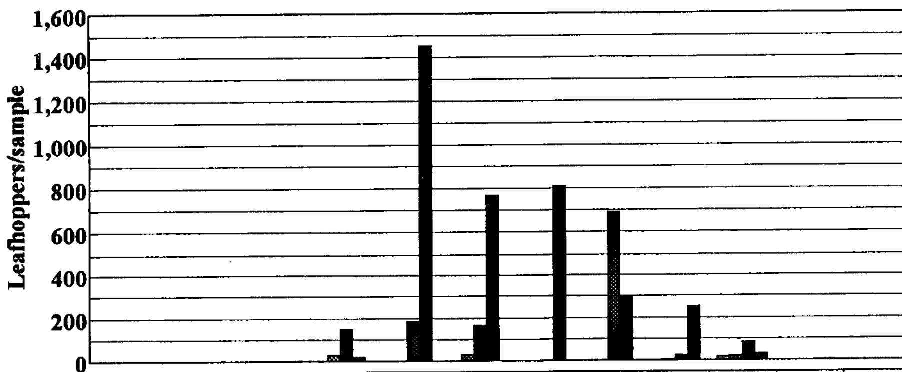
Month	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
1989									137	71		
1990				43	104	0	61	103	66	310	0	0
1991			26	91	159	0		77	19	119	10	
1992		6		0	443	811	15	436	55	77	2	

FIG. 2. Number of individuals of *Empoasca bitubera* in 100 sweep samples at South Well, Jornada Experimental Range, Doña Ana County, New Mexico, 1989-1992. Blank cells indicate no sample was collected for that month.



Month	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
1989									21			
1990				73	0		0	0	0	3		
1991			0	192	300	41	0	565	122	203		
1992				366	470	1,004	1,096	348	417	14		

FIG. 3. Number of individuals of *Empoasca bitubera* in 100 sweep samples at Old Stock Tank, Youngblood Ranch, 15 mi. East of San Antonio, Socorro Co., New Mexico, 1989-1992. Blank cells indicate no sample was collected for that month.



Month	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
1989										15		
1990			0	28	0	29	0	0	2	18	0	0
1991	0	0	0	150	186	164	0	690	21	84		
1992				18	1,453	767	810	299	250	29	1	

FIG. 4. Number of individuals of *Empoasca bitubera* in 100 sweep samples at South Edge, Youngblood Ranch, 15 mi. East of San Antonio, Socorro Co., New Mexico, 1989-1992. Blank cells indicate no sample was collected for that month.

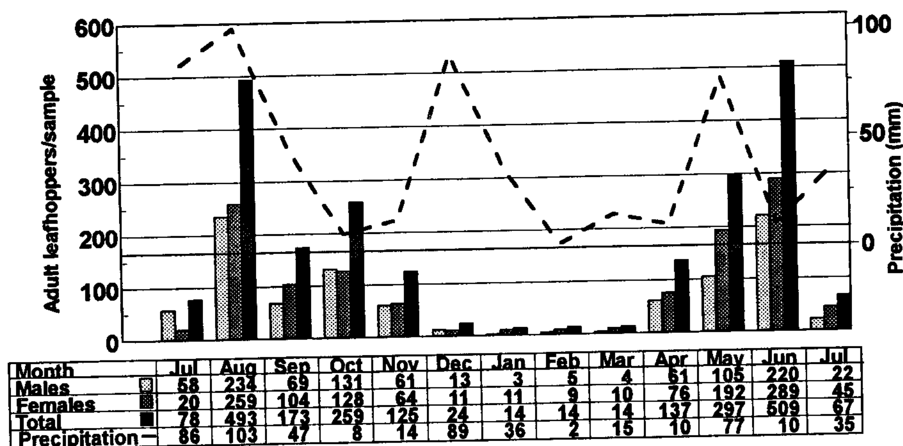
Table 1. Leafhoppers from snakeweed on the Jornada Experimental Range, Doña Ana County, New Mexico.

Species	Adult Seasonal Occurance	Stage Collected	Feed on Snakeweed *	Parasitoids
Empoasca bitubera DeLong	All year	Adults & nymphs	ff	*Aphelopus* on 1st and 2nd abdominal pleura
Empoasca neaspersa Oman & Wheeler	Summer	Adults	nf?	Not known
Balclutha neglecta (DeLong & Davidson)	April-September	Adults	nf	Dryinid on 2nd abdominal pleura
Gyponana delta Ball	August & April-May	Adults and last instar nymphs	ff	Dryinid on presternum of nymphs and adults
Driotura prob. *gammaroides* (Van Duzee)	Februrary-October	Adults	fl	Not known
Driotura sp.	April	Adults	nf	Not known
Ceratagallia bigeloviae (Baker)	June-October	Adults	ff?	*Halictophagus* sp. on 4th and 5th abdominal pleura
Aceratagallia uhleri (Van Duzee)	Summer	Adults	fl	*Halictophagus* sp. on 4th and 5th abdominal pleura
Exitianus excitiosus (Uhler)	Summer	Adults	nf	Not known
Cuerna arida Oman & Beamer	August-September	Adults	nf	Not known
Xerophloea viridis (Fabricius)	Summer	Adults	nf	Not known

* ff = fed on snakeweed in field, fl = fed on snakeweed in lab, nf = probably just incidental on snakeweed

in a stage of quiescence rather than diapause. Four samples were taken on other plants in the same area during the winter and no *Empoasca* were collected, so it is unlikely that there was any populations existing apart from the snakeweed.

Nymphs were abundant from August to October 1991, and were still present, all in the last instar, during November when adults were still numerous. No nymphs were found during winter. Their numbers increased during the last two weeks of March 1992, leading to an increase in adults starting in April (Fig. 5). Nymphs were as scarce as adults during July.



Month	Jul	Aug	Sep	Oct	Nov	Dec	Jan	Feb	Mar	Apr	May	Jun	Jul
Males	58	234	69	131	61	13	3	5	4	61	105	220	22
Females	20	259	104	128	64	11	11	9	10	76	192	289	45
Total	78	493	173	259	125	24	14	14	14	137	297	509	67
Precipitation	86	103	47	8	14	89	36	2	15	10	77	10	35

FIG. 5. Sex ratios and number of individuals of *Empoasca bitubera* in 100 sweep samples at Jornada Experimental Range, New Mexico, 1991-1992, compared with precipitation in mm.

Adults of *E. bitubera* were parasitized by a dryinid wasp (*Aphelopus* sp.). The first-instar larvae of this parasitoid developed internally and could not be detected; however, subsequent instars presented as tumor-like processes attached to the body wall in a laterodorsal position between the first and second abdominal segment. This process is the larval sac formed by the cast skin of the previous instars and inside which are found the U-shaped larvae. Each sac was characterized by a light to dark brown coloration and, when fully developed,was about 2/3 the length of the leafhopper host abdomen (Fig. 6).

The dryinid has two generations per year, one in the spring and another in the fall. Adults attacked by the parasitoid were found from September to November, reaching a peak abundance during October, when 19% of the collected *Empoasca* were parasitized (Fig. 7). A second generation of the parasitoid was found from April to May, but parasitized adults of the leafhopper were not found in June or August when the peak abundance of *E. bitubera* was recorded. We seldom found parasitized overwintering adults.

The observed differences in numbers of males and females of *E. bitubera* may be at least partially due to the fact that significantly more males than females were parasitized (chi square = (6.520, $p<0.025$). Nymphs were not parasitized by the drynids, but we did

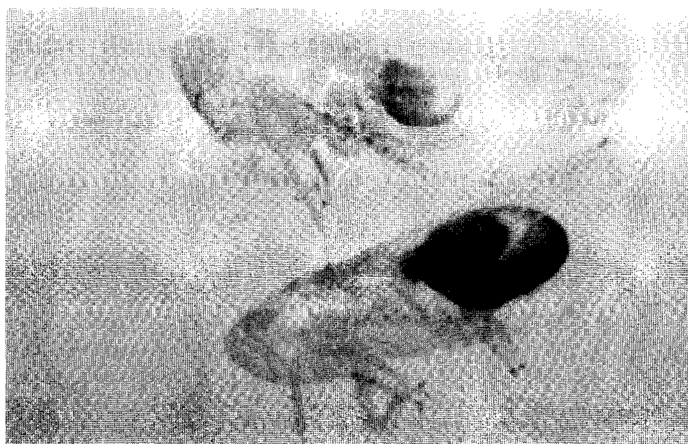
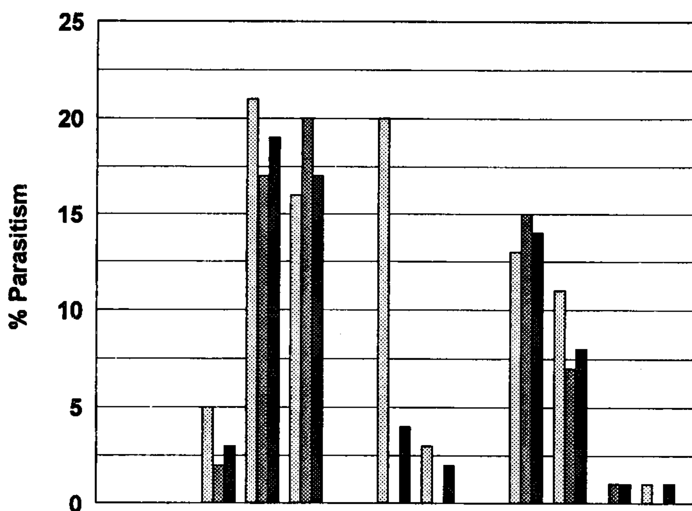


Figure 6. *E. bitubera* parasitized by the dryinid *Aphelopus* sp.



Month	Jul	Aug	Sep	Oct	Nov	Dec	Jan	Feb	Mar	Apr	May	Jun	Jul
% Males parasitized	0	0	5	21	16	0	20	3	0	13	11	0	1
% Females parasitized	0	0	2	17	20	0	0	0	0	15	7	1	0
Total % parasitized	0	0	3	19	17	0	4	2	0	14	8	1	1

Figure 7. Parasitization of *Empoasca bitubera* by a drynid wasp (*Aphelopus* sp.) from July 1991 to July 1992. The figure for % parasitism in male *Empoasca* during the month of January is somewhat misleading because only 5 males were collected, whereas of 245 overwintering specimens of both sexas collected from December to mid April, only 2 were parasitized.

find tiny unidentified mites clinging by their mouthparts to the dorsal area of the neck region. Two other species of leafhoppers were found to have similar dryinid parasitoids, but these were not identified to genus (Table 1). Both *Ceratagallia bigeloviae* and *Aceratagallia uhleri* were found to have strepsipteran parasitoids in the genus *Halictophagus*, but no strepsipteran was found that parasitized *E. bitubera*.

Members of the genus *Empoasca* are known vectors of several plant diseases (Nielson 1968), but there was no indication that this leafhopper, or any other, was transmitting any serious pathogen to the snakeweed plants, based on the fact that plants exhibited no symptoms of virus diseases, even in decline, at either the sites on the Jornada Experimental Range or the Youngblood Ranch. In addition, no diseases were observed at sites which were surveyed for snakeweed insects around New Mexico, Arizona or Texas (Richman, unpublished observation).

In summary, the data obtained during the current study indicates that *Empoasca bitubera* is the only leafhopper known to be complete its entire life cycle on woody snakeweeds, and that this leafhopper has at least two or three generations each year, based on the rise and fall of adult populations (Fig. 5). It is attacked by a dryinid wasp parasitoid, *Aphelophus* sp. In addition, several other leafhoppers will, at some time during their life cycle, feed on woody snakeweeds. One of these, *Gyponana delta*, also had a dryinid parasitoid and two others, *Ceratagallia bigeloviae* and *Aceratagallia uhleri*, have strepsipteran parasitoids. Other leafhoppers, which are occasionally found on the plants, are apparently incidental and do not actually feed on snakeweed. One of these, *Balclutha neglecta*, had a dryinid parasitoid, while the others were not found to have parasitoids in the material collected. While *E. bitubera* apparently does cause some damage to the photosynthetic tissues, we have found no evidence that it transmits any pathological organism to its host. Its function as a biological control agent may thus be limited to weakening the plant and making it more susceptible to attack by other natural enemies.

ACKNOWLEDGMENT

We thank Chris Dietrich (Systematic Entomology Laboratory, United States Department of Agriculture, Beltsville, MD) for the identification of *Empoasca* spp., P. H. Freytag (Department of Entomology, University of Kentucky, Lexington, KY) for the identification of *Gyponana delta*, and H. Derrick Blocker (Department of Entomology, Kansas State University, Manhattan, KS) for the identification of other leafhoppers. Leigh Murray of the Department of Experimental Statistics, New Mexico State University, helped with the statistical analysis. We also thank Ana Brussa for her assistance in sorting samples. The late T. J. Youngblood allowed access to his ranch near San Antonio and the United States Department of Agriculture Jornada Experimental Range permitted the more detailed work in Doña Ana County.

LITERATURE CITED

Campbell, R. S. and E. H. Bomberger. 1934. The occurrence of *Gutierrezia sarothrae* on *Bouteluoa eripoda* ranges in southern New Mexico. Ecol. 15: 49-61.

Cordo, H. A. 1985. Host specificity of *Heilipodus ventralis*, for the biological control of snakeweed (*Gutierrezia* spp.) in the U.S. pp. 709-720. *In* E. S. DelFosse [ed.] Proc. VI Internat. Symp. Biol. Control Weeds. 19-25 August 1984, Vancouver, Canada.

Cordo, H. A., and C. J. DeLoach. 1992. Ocurrence of snakeweeds (*Gutierrezia*: Compositae) and their natural enemies in Argentina: implications for biological control in the United States. Biol. Control 2: 143-158.

DeLong, D. M. 1932. Further studies on the genus *Empoasca* (Homoptera: Cicadellidae). Part I. Nine new species of *Empoasca*. Ohio J. Sci. 32: 393-401.

Edwards, D. R., and J. K. Wangberg. 1985. Life history of *Synalocha gutierreziae* Powell (Lepidoptera: Tortricidae) on snakeweed (Asteraceae: *Gutierrezia* spp.). Pan-Pac. Entomol. 61: 358-365.

Falkenhagen, T. J. 1978. Root boring insects in *Gutierrezia sarothrae*. M. S. Thesis, Univ. of Arizona, Tucson, 60 pp.

Foster, D. E., D. N. Ueckert, and C. J. DeLoach. 1981. Insects associated with broom snakeweed (*Xanthocephalum sarothtrae*) and threadleaf snakeweed (*Xanthocephalum microcephala*) in west Texas and eastern New Mexico. J. Range Manage. 34:446-454.

McGinty, A., and T. G. Welch. 1987. Perennial broomweed and Texas ranching. Rangelands 9: 246-249.

Nielson, M. W. 1968. The leafhopper vectors of phytopathogenic viruses (Homoptera, Cicadellidae) taxonomy, biology, and virus transmission. U.S.D.A. Tech. Bull. 1382:1-386.

Oman, P. W. 1949. The Neartic leafhoppers (Homoptera: Cicadellidae), a generic classification and check list. Ent. Soc. Wash. Mem. No.3.

Parker, M. A. 1984. Local depletion and the foraging behavior of a specialist grasshopper, *Hesperotettix viridis*. Ecol. 66: 824-835.

Parker, M. A. 1985. Grasshopper attack and water balance of the shrub *Gutierrezia microcephala*. Amer. Mid. Nat. 113: 193-197.

Parker, M. A., and A. G. Salzman. 1985. Herbivore exclosure and competitor removal: Effects on juvenile survivorship an growth in the shrub *Gutierrezia microcephala*. J. Ecol. 73: 903-913.

Poos, F. W. and N. H. Wheeler. 1943. Studies on host plants of the leafhoppers of the genus *Empoasca*. U. S. D. A. Tech. Bull. 850: 1-51.

Powell, J. A. 1976. Host plant preference, mating and egg development in *Synnoma lynosyrana* (Lepidoptera: Tortricidae). PanPac. Entomol. 52: 1-12.

Richman, D. B., and E. W. Huddleston. 1981. Root feeding by the beetle, *Crossidius pulchellus* LeConte and other insects on broom snakeweed (*Gutierrezia* spp.) in eastern and central New Mexico. Environ. Entomol. 10: 53-57.

Richman, D. B., D. C. Thompson and J. O'Mara. 1992. Effects of leaftiers on broom snakeweed in central New Mexico. Southwest. Entomol. 17:187-189.

Sokol, R. R., and F. J. Rohlf. 1981. Biometry: the principles and practice of statistics in biological research, 2nd ed. Freeman, San Francisco.

Sterling, T. M., and D. C. Thompson. 1992. Variable response of a potential biocontrol agent to snakeweed species. Proc. Western Soc. Weed Sci. 45: 40. Salt Lake City, UT, March 10-12, 1992.

Thompson, D. C., and D. B. Richman. 1989. The role of native insects as snakeweed biological control agents. pp. 179-187. *In*: Huddleston, E. W. and R. D. Pieper [eds.] Snakeweed: Problems and Perspectives, November 9-10, 1989 Las Cruces, NM.

EVALUATION OF BEET ARMYWORM (LEPIDOPTERA: NOCTUIDAE) TOLERANCE TO INSECTICIDES AND RESPONSE TO IGR's

G. W. Elzen

USDA, ARS, Southern Insect Management Laboratory,
Stoneville, Mississippi 38776

ASTRACT

Various insecticides were tested for their effect on beet armyworm, *Spodoptera exigua* Hübner, populations collected in 1988 and 1993 using a spray chamber bioassay. A new insecticidal pyrrole was the most effective material tested and produced 100% mortality of *S. exigua* larvae at 72 h post-treatment. Less effective treatments for *S. exigua* were organophosphorus and carbamate insecticides. Pyrethroids tested provided poor control. Interactions with insect growth regulators were also examined. Application of fenoxycarb and diflubenzuron produced low larval mortality. However, these materials, particularly in combination with *Bacillus thuringiensis* Berliner, resulted in mortality throughout the life cycle which approached the level obtained with the organophosphorus insecticide chlorpyrifos alone.

INTRODUCTION

Beet armyworm, *Spodoptera exigua* (Hübner), is an important pest in a wide variety of agriculturally important plants (Gelernter et al. 1986, Oatman and Platner 1972, Smits et al. 1988, Trumble and Baker 1984, Metcalf and Metcalf 1992). It usually causes sporadic damage to cotton but has also been documented to cause severe economic damage to this commodity (Wilson 1982), and has been recently reported as an important secondary pest in cotton causing severe yield losses (Headley 1988). During the past decade, *S. exigua* has become a more consistent pest of cotton in the southeast, particularly in states east of the Mississippi River, where it may be more adapted to feeding on cotton (Smith 1994).

High infestations of *S. exigua* occurred in the southern U.S. and California in 1988 (Anonymous 1989, Headley 1988) and again in the Southeast and mid-South in 1993 (Layton 1994, Smith 1994). In 1995, *S. exigua* infested 44.4% of the U.S. cotton acreage and treatments were applied on 2.6 million acres. Severe infestations of *S. exigua* occurred in several areas inside and outside the Boll Weevil Eradication Program areas. In Texas, 2.9 of the 5.2 million acres were infested (Carter 1996). *Spodoptera exigua* has been shown to be tolerant to many insecticides which are frequently recommended for control (Meinke and Ware 1978, Brewer and Trumble 1989, 1994, Wolfenbarger and Brewer 1993). Despite this, published insecticide evaluations for control of *S. exigua* in cotton have been few and only recently reported (Graves et al. 1993, 1995; Klein et al. 1994a, b; Reed et al. 1994; Leonard et al. 1995).

Because of high tolerance to insecticides in populations of *S. exigua*, alternative measures which may provide suppression are needed. Insect growth regulators (IGR's), such as the benzoylphenylurea diflubenzuron, may be effective alernatives to chemical insecticides for managing *S. exigua* populations (Chandler 1995). For the same reasons, juvenile hormone analogs, such as fenoxycarb, which interfere with metamorphosis, reproduction, embryogenesis, and diapause (Miyamoto et al. 1993) may prove useful.

As Smith (1995) noted, we do not have the chemical tools at present to control this insect in cotton. ·Because of the tenuous status of control of *S. exigua* with conventional insecticides, the efficacy of currently recommended insecticides was determined. In addition, efficacy of selected insecticides against a newly collected population was compared with results obtained on a population collected five years earlier. IGR's and an insecticidal pyrrole were also evaluated.

MATERIALS AND METHODS

The *S. exigua* strains tested were collected in similar locations near Stoneville, MS (Washington County) in August of 1988 and 1993. Larvae (minimum 400 per strain) were collected by whole plant visual search. The F_1 generation was tested in 1988 and the F_1 and F_2 were tested in 1993. Both strains were reared in the same manner. Adults were maintained in 3.8-liter cardboard cartons covered with cotton gauze as an ovipositonal substrate and fed a 5% sugar-water solution. Larvae were reared on a soybean flour-wheat germ diet (King and Hartley 1985).

Formulated insecticides tested in a spray chamber bioassay were thiodicarb [Larvin 3.2 flowable (F.); Rhone-Poulenc Agric. Co., Research Triangle Park, NC], profenofos [Curacron 8 emulsifiable concentrate (EC); CIBA, Greensboro, NC], sulprofos [Bolstar 6 EC; Miles, Inc., Kansas City, MO], chlorpyrifos [Lorsban 4 EC; DowElanco, Midland, MI], bifenthrin [Capture 2 EC; FMC Corp., Philadelphia, PA], acephate [Orthene 90 soluble powder (SP); Valent USA Corp., Walnut Creek, CA], methomyl [Lannate 1.8 liquid (L); E. I. Du Pont de Nemours & Co., Wilmington, DE], tralomethrin [Scout X-tra 0.9 EC; Hoechst-Roussel Agri-Vet Co., Somerville, NJ], pyrrole [Pirate 3 flowable suspension (FS); American Cyanamid, Wayne, NJ], *Bacillus thuringiensis* var. *kurstaki* [Javelin wettable granule (WG); Sandoz Crop Protection, Des Plaines, IL], diflubenzuron [Dimilin 25 wettable powder (WP); Uniroyal Chemical Co., Inc., Middlebury, CT], amitraz [Ovasyn 1.5 EC; NOR-AM Chemical Co., Wilmington, DE], and fenoxycarb [25 WP; Ciba-Geigy Corp., Greensboro, NC].

The methods and materials used in the spray chamber bioassay were previously described in detail (Elzen et al. 1990, 1992). This bioassay has been used to document changes in tolerance of tobacco budworm, *Heliothis virescens* (F.), during the cotton growing season, within generations, and over several years (Elzen et al. 1992; Elzen 1994, 1995, 1996). Briefly, cotton terminals (10-14 cm stem with three to four small leaves and buds) clipped from greenhouse grown plants were placed in florist's water picks. Each treatment consisted of three replicates of 20 terminals each. Controls were treated with water only. The spray chamber was calibrated to deliver 56 liters per ha at 2,109 g/cm pressure with one TX-6 hollow-cone nozzle at 3.2 km/h. The spray nozzle was positioned 30.5 cm above the spray surface. One third instar *S. exigua* (17 ± 3 mg) was placed on each terminal 30 min after spraying and confined by covering each plant with a 590-ml ventilated paper cup. Rates of formulated insecticides applied were selected by referring to an appropriate control guide (e.g., Layton 1995).

All treatments were held at 29 ± 3°C, 55-60% RH, and a 14L:10D photoperiod. Treatment efficacy was determined after 72 h, and numbers of dead and moribund larvae were used to calculate total mortality. Control mortality was never greater than 5%; data were corrected for control mortality using Abbott's (1925) formula. Percent mortalities were transformed to arcsine and analyzed by analysis of variance; means were separated by least significant difference [$P \geq 0.05$ (SAS Institute 1988)].

The IGR tests were initiated using the spray chamber bioassay described above and evaluated by the previously described criteria.
In addition, after percentage mortality was determined at 72 h post-treatment, surviving larvae were moved to 30 ml plastic cups containing soybean flour-wheat germ diet (King and Hartley 1985), covered with plastic tops and retained in an incubator at 29 ± 3°C, 55-60% RH, and a 14L:10D photoperiod. Cumulative percentage larval mortality was then determined at 144 h post-treatment after which most larvae that survived had pupated. Cumulative percentage mortality was then followed daily and recorded as mortality at pupation and at emergence. From these data, percentage survival throughout the life cycle was calculated. Control mortality throughout the life cycle averaged 11.1%; data were corrected for control mortality using Abbott's (1925) formula and analyzed as above.

RESULTS AND DISCUSSION

Several numerical differences in percentage mortality were detected among the strains collected and tested in 1988 and in 1993 in spray chamber bioassays. Mortalities in response to the plus/minus isomer organophosphorus insecticides acephate, sulprofos, and profenofos were numerically lower in bioassays on the 1993 strain than on the 1988 strain (Table 1). For the 1988 strain, profenofos, chlorpyrifos, and sulprofos were significantly more effective than other treatments ($P \leq 0.05$). For the 1993 strain, thiodicarb, chlorpyrifos, and methomyl were significantly more effective than other treatments ($P \leq 0.05$). These changes may indicate that the efficacy of some currently recommended insecticides for *S. exigua* control has diminished. The most effective of these insecticides, chlorpyrifos, is no longer available for use on cotton in Mississippi (Layton 1994).

TABLE 1. Efficacy of Selected Insecticides in a Spray Chamber Bioassay Against Strains of *S. exigua* Collected and Tested in 1988 and 1993.

Treatment	kg(AI)/ha	% Mortality[a] (72 h)	
		Strain	
		1988	1993
Methomyl	0.50	63.3bc	62.2b
Acephate	1.12	40.0b	22.2a
Sulprofos	1.12	83.3d	24.5a
Bifenthrin	0.11	16.7a	37.8a
Thiodicarb	0.67	63.3bc	68.9b
Profenofos	1.12	83.3d	33.3a
Chlorpyrifos	1.12	91.7d	82.2b
F; df		13.52; 6, 14	9.67; 6, 14

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

Significant differences ($P \leq 0.05$) in mortality were found among insecticides tested on the 1993 strain (Table 2). For example, chlorpyrifos was significantly more effective ($P \leq 0.05$) at the rates tested than the pyrethroid tralomethrin or *B. thuringiensis*. No synergism was found when the formamidine amitraz was combined with chlorpyrifos. Formamidines are known to synergize several classes of insecticides in insects, including Lepidoptera (Plapp 1979, Knowles 1982, Plapp and Campanhola 1986, Campanhola and Plapp 1989, Bagwell and Plapp 1992). Amitraz is recommended as a tank-mix with thiodicarb in the Mississippi Cotton Insect Control Guide (Layton 1995), presumably because of the synergistic activity of the mixture (Reed et al. 1994). This mixture produced only intermediate mortality at the rates tested and would not be acceptable for control in cotton. However, thiodicarb was not tested alone at the low rate. There was no synergism detected in mixtures of tralomethrin plus chlorpyrifos or amitraz plus chlorpyrifos. These tank-mixes are recommended for control of heliothines and are often used when concomitant populations of *S. exigua* are present. In contrast to other treatments, no *S. exigua* larvae survived treatment with the insecticidal pyrrole (Table 2) and this treatment was significantly more effective ($P \leq 0.05$) than any other treatment. This material is currently in development by American Cyanamid as a broad spectrum insecticide/miticide with the name Pirate (Kunh et al. 1993). It has been previously shown to have high activity against *S. exigua* (Farlow et al, 1992, Whitehead et al. 1993).

TABLE 2. Efficacy of Selected Insecticides in a Spray Chamber Bioassay Against a Strain of *S. exigua* Collected in 1993.

Treatment	kg(AI)/ha	% Mortality[a] (72 h)
Chlorpyrifos	0.56	80.0cd
Chlorpyrifos	0.56	84.5d
+ Amitraz	0.20	
Thiodicarb	0.34	57.8bc
+ Amitraz	0.20	
Amitraz	0.20	6.7a
Tralomethrin	0.024	2.2a
Tralomethrin	0.024	86.7d
+ Chlorpyrifos	0.56	
B. thuringiensis	1.12	37.8b
B. thuringiensis	1.12	57.8bc
+ Thiodicarb	0.34	
Pyrrole (Pirate)	0.22	100.0e
F; df		27.60; 8, 18

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

IGR bioassays with the 1993 strain are shown in Table 3. Very low percentage mortalities were found in larvae treated with the IGR's or *B. thuringiensis* (at lower rates than previously applied). Chlorpyrifos was significantly more effective ($P \leq 0.05$) than the IGR's at 72 h after larvae were exposed. Mortality increased by 144 h post-treatment, and significant differences ($P \leq 0.05$) were found between fenoxycarb and diflubenzuron. Chandler (1994) found that diflubenzuron was more effective than fenoxycarb on *S. exigua*. The data herein are in agreement with this finding; however, differences are not discernible until later in the life cycle (Table 3). In contrast, chlorpyrifos remained significantly more effective ($P \leq 0.05$) on larvae at 144 h post-treatment. Fewer differences are seen in later stages. Mortality observed at the pupal stage showed that *B. thuringiensis* was more effective

when combined with fenoxycarb, diflubenzuron was more effective than *B. thuringiensis* alone, and diflubenzuron plus chlorpyrifos was more effective than any treatment except chlorpyrifos alone ($P \leq 0.05$). Trends in mortality were similar for the entire life cycle.

TABLE 3. Effect of Selected Insecticides Applied in a Spray Chamber on an *S. exigua* Strain Collected in 1993.

Treatment	kg(AI)/ha	Cumulative % Mortality[a]			
		72 h	144 h	Pupa	Adults
Fenoxycarb	0.034	4.4a	6.7a	6.7a	15.5a
B. thuringiensis	0.56	4.5a	15.5ab	20.0ab	24.4ab
Fenoxycarb	0.034	8.7a	13.3a	35.5bc	55.5bc
+ *B. thuringiensis*	0.56				
Diflubenzuron	0.070	13.3a	44.3c	55.5c	57.8c
Diflubenzuron	0.070	22.2a	37.8bc	62.2cd	60.0c
+ *B. thuringiensis*	0.56				
Chlorpyrifos	0.56	80.0b	82.2de	82.2de	73.3cd
Diflubenzuron	0.070	84.5b	91.1d	93.3e	86.6d
+ Chlorpyrifos	0.56				
F; df		14.19; 6, 14	20.20; 6, 14	19.32; 6, 14	8.90; 6, 14

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

Alternative control strategies involving IGR's may provide suppression of *S. exigua* populations that are resistant to conventional insecticides and may be an effective measure for conserving beneficial species that are highly susceptible to organophosphorus insecticides.

ACKNOWLEDGMENT

Larry Adams (Southern Insect Management Laboratory, Stoneville, MS) reared insects, made field collections, and performed bioassays.

LITERATURE CITED

Abbott, W. S. 1925. A method of computing the effectiveness of an insecticide. J. Econ. Entomol. 18: 265-267.

Anonymous. 1989. Fighting early armyworms in SJV. Cotton Grower 25: 20.

Bagwell, R. D., and F. W. Plapp, Jr. 1992. Synergism of insecticides against susceptible and pyrethroid resistant tobacco budworms (Lepidoptera: Noctuidae) by amitraz. J. Econ. Entomol. 85: 658-663.

Brewer, M. J., and J. T. Trumble. 1989. Field monitoring for insecticide resistance in beet armyworm (Lepidoptera: Noctuidae). J. Econ. Entomol. 82: 1520-1526.

Brewer, M. J., and J. T. Trumble. 1994. Beet armyworm resistance to fenvalerate and methomyl: Resistance variation and insecticide synergism. J. Agric. Entomol. 11: 291-300.

Campanhola, C., and F. W. Plapp, Jr. 1989. Toxicity and synergism of insecticides against susceptible and pyrethroid-resistant neonate larvae and adults of the tobacco budworm (Lepidoptera: Noctuidae). J. Econ. Entomol. 82: 1527-1533.

Carter, F. L. 1996. The 1995 insect year. Cotton Grower. 32: 52-53.

Chandler, L. D. 1994. Comparative effects of insect growth regulators on longevity and mortality of beet armyworm (Lepidoptera: Noctuidae) larvae. J. Entomol. Sci. 29: 357-366.

Chandler, L. D. 1995. Compatability of insect growth regulators and bacterial insecticides for management of *Spodoptera* spp., pp. 1043-1045. *In* Proceedings Beltwide Cotton Prod. Res. Conf. National Cotton Council, Memphis, TN.

Elzen, G. W., P. J. O'Brien, and G. L. Snodgrass. 1990. Toxicity of various classes of insecticides to pyrethroid resistant *Heliothis virescens* larvae. Southwest. Entomol. 15: 33-38.

Elzen, G. W., B. R. Leonard, J. B. Graves, E. Burris, and S. Micinski. 1992. Resistance to pyrethroid, carbamate, and organophosphate insecticides in field populations of tobacco budworm (Lepidoptera: Noctuidae) in 1990. J. Econ. Entomol. 85: 2064-2072.

Elzen, G. W. 1994. Characterization of *Heliothis virescens* (F.) Resistance to insecticides in Mississippi in 1992: Multiple vs cross-resistance. Southwest. Entomol. 19: 219-227.

Elzen, G. W. 1995. Trends in *Heliothis* resistance, 1994, pp. 964-967. *In* Proceedings Beltwide Cotton Prod. Res. Conf. National Cotton Council, Memphis, TN.

Elzen, G. W. 1996. Changes in tolerance to insecticides in tobacco budworm populations, 1995. *In* Proceedings Beltwide Cotton Prod. Res. Conf. National Cotton Council, Memphis, TN. In press.

Farlow, R. A., G. Goddard, R. Kepner, K. Umeda, and J. R. Whitehead. 1992. Efficacy of Pirate insectidide-miticide against insect and mite pests of U.S. cotton, pp. 877-880. *In* Proceedings Beltwide Cotton Prod. Res. Conf. National Cotton Council, Memphis, TN.

Gelernter, W. D., N. C. Toscano, K. Kido, and B. A. Federici. 1986. Comparison of a nuclear polyhedrosis virus and chemical insecticides for control of the beet armyworm (Lepidoptera: Noctuidae) on head lettuce. J. Econ. Entomol. 79: 714-717.

Graves, J. B., B. R. Leonard, and P. A. Clay. 1993. Evaluation of selected insecticide mixtures against late season populations of bollworm, tobacco budworm, and beet armyworm, 1992. Arthropod Management Tests. 18: 223.

Graves, J. B., B. R. Leonard, and C. A. White. 1995. Efficacy of selected commercial and experimental insecticides against late season populations of beet armyworm, soybean looper, and tobacco budworm, 1994. Arthropod Management Tests. 20:197-198.

Headley, C. 1988. Beet armyworm sneak attack. Cotton Grower 24: 13-15.

King, E. G., and G. G. Hartley. 1985. *Heliothis virescens*, pp. 323-328. *In* P. Singh and R. F. Moore [eds.], Handbook of Insect Rearing, vol. 2. Elsevier, Amsterdam.

Klein, C. D., P. H. Slaymaker, N. P. Tugwell, and M. L. Wall. 1994a. Efficacy of Pirate against bollworm, tobacco budworm, and beet armyworm, 1993. Arthropod Management Tests. 19: 226-227.

Klein, C. D., P. H. Slaymaker, N. P. Tugwell, and M. L. Wall. 1994b. Control of bollworm, tobacco budworm, and beet armyworm in cotton with selected insecticides, 1993. Arthropod Management Tests. 19: 227.

Knowles, C. O. 1982. Structure-activity relationships among amidine acaricides and insecticides, pp. 243-277. *In* J. R. Coats [ed.], Insecticide mode of action. Academic Press, New York.

Kuhn, D. G., R. W. Addor, R. E. Diehl, J. A. Gurch, V. M. Kanhi, K. E. Henegar, D. A. Kremer, G. T. Lowen, B. C. Black, T. P. Miller, and M. F. Treacy. 1993. Insecticidal pyrroles, pp. 219-232. *In* S. O. Duke, J. J. Menn, and J. R. Plimmer, Jr. [eds.] Pest Control with Enhanced Environmental Safety. Am Chem. Soc. Symp. Ser. 524.

Layton, M. B. 1994. The 1993 beet armyworm outbreak in Mississippi and future management guidelines, pp. 854-856. *In* Proceedings Beltwide Cotton Prod. Res. Conf. National Cotton Council, Memphis, TN.

Layton, M. B. 1995. Cotton insect control guide, 1995. Ms. Coop Ext. Serv. Bull. 343.

Leonard, B. R., C. A. White, E. Burris, and J. B. Graves. 1995. Evaluation of Pirate 3SC against cotton insect pests in Louisiana, 1994. Arthropod Management Tests. 19: 205.

Meinke, L. J., and G. W. Ware. 1978. Tolerance of three beet armyworm strains in Arizona to methomyl. J. Econ. Entomol. 71: 645-646.

Metcalf, R. L. and R. A. Metcalf. 1992. Destructive and Useful Insects, 5th ed. McGraw-Hill, New York. 1104 pp.

Miyamoto, J., M. Hirano, Y. Takimoto, and M. Hatakoshi. 1993. Insect growth regulators for pest control with emphasis on juvenile hormone analogs - present status and future prospects, pp. 144-168. *In* S. O. Duke, J. J. Menn, and J. R. Plimmer, Jr. [eds.] Pest Control with Enhanced Environmental Safety, ACS Symp. Ser. 524.

Oatman, E. R., and G. R. Platner. 1972. An ecological study of lepidopterous pests affecting lettuce in coastal southern California. Environ. Entomol. 1: 202-204.

Plapp, F. W., Jr. 1979. Synergism of pyrethroid insecticides by formamidines against *Heliothis* pests of cotton. J. Econ. Entomol. 72: 667-670.

Plapp, F. W., Jr., and C. Campanhola. 1986. Synergism of pyrethroids by chlordimeform against susceptible and resistant *Heliothis*, pp. 167-169. *In* Proceedings Beltwide Cotton Prod. Res. Conf. National Cotton Council, Memphis, TN.

Reed, J. T., M. B. Layton, and E. Flint. 1994. Evaluation of insecticides and insecticide combinations for beet armyworm control, 1993. Arthropod Management Tests. 19: 237-238. SAS Institute. 1988. SAS/STAT user's guide, version 6.03 ed. SAS Institute, Cary, NC.

Smith. R. H. 1994. Beet armyworm: A costly caterpillar, pp. 13-14. *In* Proceedings Beltwide Cotton Prod. Res. Conf. National Cotton Council, Memphis, TN.

Smith, R. H. 1995. Beet armyworm update, pp. 144-145. *In* Proceedings Beltwide Cotton Prod. Res. Conf. National Cotton Council, Memphis, TN.

Smits, P. H., I. P. Rietstra, and J. M. Vlak. 1988. Influence of application techniques on the control of beet armyworm larvae (Lepidoptera: Noctuidae) with nuclear polyhedrosis virus. J. Econ. Entomol. 81: 470-475.

Trumble, J. T., and T. C. Baker. 1984. Flight phenology and pheromone trapping of *Spodoptera exigua* (Hübner) (Lepidoptera: Noctuidae) in southeastern coastal California. Environ. Entomol. 13: 1278-1282.

Whitehead, J. R., P. David, B. Watkins, P. Nester, and R. Lecense. 1993. Performance of Pirate, insecticide mixture, against cotton pests in the mid-south in 1992, pp. 832-834. *In* Proceedings Beltwide Cotton Prod. Res. Conf. National Cotton Council, Memphis, TN.

Wilson, L. T. 1982. Growth and development of normal and terminal-damaged cotton plants. Environ. Entomol. 11: 301-305.

Wolfenbarger, D. A., and Michael J. Brewer. 1993. Toxicity of selected pesticides to field collected beet armyworm populations, pp.1034-1037. *In* Proceedings Beltwide Cotton Prod. Res. Conf. National Cotton Council, Memphis, TN.

ALUMINIZED FABRIC DETERS THRIPS[1] FROM ENTERING GREENHOUSES

Julie A. McIntyre[2], Douglas A. Hopper[2], and Whitney S. Cranshaw[3]

ABSTRACT

Experiments were conducted utilizing aluminized shade fabric and other media to determine if Western flower thrips (WFT), *Franklinella occidentalis* Pergande, could be deterred from entering greenhouses. Experiments testing different patterns and amount of surface area covered by different patterns around the entrance revealed a significant number of thrips could be repelled from entering structures. An aluminum tape appeared to be more successful in reducing thrips entry than either Aluminet fabric or whitewash. Decreased entry levels, as much as 55%, occurred the closer the fabric was placed around the entrance, regardless of surface area covered. Utilization of reflective fabric around greenhouse vents could augment an integrated pest management (IPM) based thrips control program.

INTRODUCTION

Over the past decade, Western flower thrips (WFT), *Franklinella occidentalis* Pergande, has become one of the most pervasive pests in greenhouses. Their feeding behavior damages flowers and can cause bloom abortion in greenhouse vegetable production. In addition, WFT vector serious plant viruses, notably impatiens necrotic ringspot and tomato spotted wilt. Traditional means of control consist of repeated chemical applications; however, population reduction is difficult to achieve because WFT tend to be concealed in plant parts and they have developed resistance to some of the traditionally used chemicals (Immaraju 1992).

An integrated pest management (IPM) strategy is an effective means of achieving control and can include a wide array of possibilities, one of which is the use of aluminized fabrics. By interfering with long and short-wave radiation perception, aluminum foil mulch backgrounds have been shown to reduce landing of alate aphids (Kring 1964), reducing aphid and aphid-vectored virus diseases on cucurbits (Adlerz and Everett 1968), peppers (Loebenstein et al. 1975) and lettuce (Nawrocka et al. 1975). Aluminized reflective fabrics also have been demonstrated to repel thrips in outdoor tomato production (Brown 1992); however, these mulches have had limited use in greenhouse insect control.Thrips-proof screening also has been implemented commercially to impede physical entrance into the greenhouse. Modification to the

---

[1]Thysanoptera:Thripidae

[2] Department of Horticulture and Landscape Architecture, Colorado State University, Ft. Collins, CO 80523

[3] Department of Entomology, Colorado State University, Ft. Collins, CO 80523

greenhouse in the form of additional fans, increased fan power, or increased vent opening area are required to compensate for reduced air flow. Our objective was to explore different methods, compatible with normal greenhouse operation, to restrict thrips' entry into greenhouses.

MATERIALS AND METHODS

Initial experiments utilized small scale structures to represent area around greenhouse vents. Cages were constructed of wood, covered with plastic, and placed outside in a location with uniform background and no vegetation during the summer (July-September). Cages measured 1.22 x 1.22 x 0.6-m and were covered with 4-mil plastic sheets from the ground over the top, forming a skirting around each cage to simulate a miniature greenhouse. A 30.5 x 30.5-cm opening was cut in the top center of the plastic and a yellow sticky card attached to monitor thrips' populations. Aluminum fabric, covering different surface areas and in varying patterns, was placed directly on top of the plastic. All experiments were conducted in a completely randomized design and were analyzed by PROC GLM of SAS (SAS, Version 6.10, Cary, NC). Means were separated according to the Student-Newman-Keuls test.

The first experiment consisted of using varying patterns of Aluminet (GreenTek, Edgerton, WI), a coarsely woven aluminum fabric intended to provide 50% shade. The different patterns consisted of a solid area of the fabric covering the entire top of the structure except for the area that held the yellow card (AT1), a checker board pattern with strips of fabric (0.23 x 1.2-m) placed in strips of four vertically across the box and four horizontally across with the yellow card uncovered (CB1), a border trim consisting of 23-cm wide strips placed only around the outside perimeter of the cage (BT1), and a control with no fabric, plastic sheeting only (C1). The cages were arranged in a randomized design consisting of eight cages, two for each treatment. Sticky traps were replaced every three days and the number of thrips were counted on each card. The experiment was repeated three times for a total of six traps per treatment.

A second set of experiments was conducted utilizing the same Aluminet fabric as well as other media. One treatment was solid Aluminet, similar to AT1; however, the entire surface, including the area over the yellow card was covered (T2). The second treatment consisted of white latex painted over the entire top surface of the plastic sheet (WW2). The third treatment utilized 30.5-cm strips of 2.5-cm wide reflective tape similar to aluminum foil placed around the perimeter of the hole directly adjacent to the trap (P2). The plastic sheeting was left uncovered in the control (C2). Experimental design and replication counts were similar to that in the first experiment.

A third experiment utilized only reflective aluminum tape. We tested different patterns of tape on the surface, but all with the same surface area. Treatments consisted of a border trim with 15.2-cm tape strips just on the outside edge of the cage (BT3), a center trim with the same area covered as in the border test, but just around the center hole (P3), and a striped pattern with strips of tape running vertically across the cage, again with the same area as the other treatments (ST3), and an uncovered control (C3). Experimental design and replication counts were similar to that in the first experiment.

A fourth experiment tested reflective differences between aluminum tape and Aluminet. Treatments consisted of a center trim with 30.5-cm tape strips placed on the inside perimeter of the cage around the center hole (P4), a center trim with the same area covered as the tape, but utilizing the Aluminet (AP4), a pattern with Aluminet covering twice the surface area (TA4), and an uncovered control (C4). Experimental design and replication counts were similar to that in the first experiment.

An additional large-scale greenhouse experiment was conducted at a commercial facility, Colorado Greenhouse (Ft. Lupton, CO), a hydroponics producer of greenhouse tomatoes. This 8-ha greenhouse is passively cooled. Aluminum fabric was tested on west roof vents during August. The 2.8-m long roof vents were treated with 0.305 x 2.8-m strips of Aluminet. Patterns included a hanging piece of Aluminet secured to the top, but not at the bottom, of the open vent, so that it was open along the sides (HS5), a base strip that was secured along the bottom adjacent to the open vent (BS5), a pattern that included both a hanging and a base strip (B5), and a control where the vent was left untreated (C5). Yellow sticky cards were hung on the inside of the greenhouse, approximately 0.5-m from the vent with the sticky side facing toward the open vents and treatments. A completely randomized design was used, consisting of four replications. Traps were counted twice at one-week intervals.

RESULTS AND DISCUSSION

Results indicated a reduction in numbers of thrips migrating into a greenhouse when a reflective material was used. All experiments indicated population reductions (Table 1). These results are consistent with previous studies in which the numbers of thrips captured were reduced when aluminum-painted mulches were used in monitoring populations (Scott 1989).

In the first experiment of the farm trials, there appeared to be a direct relationship between the proximity of the reflective material to the entrance area and the ability to deter entry. The treatment applied on the exterior edges of the cage (BT1) did not provide significant reduction compared to the control, regardless of the material used (tape or fabric). Treatments made directly adjacent to the monitoring holes deterred a greater number of thrips from entering, as evidenced by AT1 and CB1. Increased interference with long and short wave radiation reflected at these areas could be interfering with alighting (Kring 1972).

The second experiment contained the treatment that produced the largest reduction in thrips populations. When the treatment actually covered the entrance (i.e., T2), an 80% reduction in the number of thrips caught occurred. This indicates that the fabric may have provided screening qualities as well as reflective abilities, as there was a reduced incidence of thrips passing through the material. The whitewash (WW2) treatment had no repelling effect. Previous studies found thrips populations were higher in the tomato blooms of plants grown on white plastic mulch than they were on plants grown on bare ground, black plastic, and aluminum plastic mulches (Brown 1992). Previous work with colored backgrounds and aphid landing showed different results with fewer aphids caught over light colored treatments (Liewehr 1991) and (Adlerz 1968). Possible explanations may again involve long- and short-wave interactions interfering with aphid landing, but with the white color not necessarily causing the same interference with thrips. In addition, treatment P2 again showed similar results as with the first experiment (i.e., closer the material, the better the deterrence).

In the third experiment, results were similar to those of the first experiment. Treatments placed closest to the monitoring area were more effective in deterring thrips from landing than those treatments placed further away. Treatment P3 caught significantly less thrips than BT3. Also, results from this experiment indicated that patterns did not provide better reflective capabilities than other types of treatments. Treatment ST3 did not differ significantly from the control nor did the checkerboard treatment (CB1), although deterrence was greater with this type of pattern. Again, this may be because CB1 consisted of material directly around the center hole with the trap,

TABLE 1. Number of Thrips Captured in Yellow Traps in Experiments with Different Aluminum Fabric Patterns, Ft. Collins, Colorado, 1995.

Farm trials	Number of thrips/trap[a]
Experiment #1	
Control (C1)	81.3 a
Border trim (BT1)	58.4 ab
Solid Aluminet (AT1)	37.2 b
Checkerboard (CB1)	36.0 b
Experiment #2	
Control (C2)	100.5 a
Whitewash (WW2)	106.9 a
Center tape (P2)	63.5 b
Solid Aluminet (T2)	18.5 c
Experiment #3	
Control (C3)	123.7 a
Border tape (BT3)	121.5 a
Strips of tape (ST3)	82.3 ab
Center tape (P3)	50.7 b
Experiment #4	
Control (C4)	87.3 a
Center Aluminet (AP4)	58.8 b
2x Aluminet (TA4)	43.5 bc
Center tape (P4)	34.8 c

[a]Mean from six traps per treatment. Numbers within each experiment followed by the same letter were not significantly different (P=0.05) by SNK.

whereas ST3 was arranged in vertical strips without being directly around the center hole.

Results from the fourth experiment suggest that the foil material may deter thrips more effectively than Aluminet. Treatment P4 significantly reduced thrips more than the Aluminet with the same area covered (AP4). Also, it appears the amount of surface area covered may not be as much of a deterrent factor as the reflective capacity of the material. Treatment TA4 did not reduce thrips' numbers caught significantly compared with the center foil treatment, P4. Increased interference with long- and short- wave radiation reflected by the aluminum foil again may be interfering with alighting (Kring 1972).

In the greenhouse trial, there appeared to be no significant differences among the treatments (Table 2). This indicates, unlike the farm trial of T2, that the treatment that

directly blocked access to the trap (HS5) and that might have provided some screening effect did not reduce populations more effectively than those adjacent to the trap. However, this may be attributable to the heat exiting from the greenhouse through the vents causing the fabric to rise so that it was horizontal above the vent as opposed to vertically hanging from the vent. This would then make this treatment similar to the strip placed along the base of the vent (BS5). Increased reduction, however, was not seen when both the base and hanging strips were used together (B5), suggesting that less material provides similar results. Again, proximity to the vent seemed most influential in discouraging landing.

TABLE 2. Thrips Captured in Experiments with Different Aluminum Fabric Around Roof Vents, Colorado Greenhouse, Ft. Lupton, Colorado, 1995.

Greenhouse trail	Number of thrips/trap[a]	
	Week 1	Week 2
Control (C5)	31.3 a	39.3 a
Hanging strip (HS5)	17.8 b	19.3 b
Base strip (BS5)	12.5 b	13.0 b
Base & hanging (B5)	13.8 b	17.3 b

[a] Mean from four traps. Numbers followed by the same letter within each week were not significantly different (P=0.05) by SNK.

ACKNOWLEDGMENT

We would like to thank Colorado Greenhouse for their help and allowing us the use of their facility, as well as Frank Stonaker, Casey Sclar, Aaron Spriggs, and Jason Bishop for assisting with field work. This study was funded, in part, by Colorado Agricultural Experiment Station Project 1-57471, and Colorado Greenhouse Growers Association.

LITERATURE CITED

Aldlerz, W.C., and P.H. Everett. 1968. Aluminum foil and white polyethylene mulches to repel aphids and control watermelon mosaic. J. Econ. Entomol. 61:1276-79.

Brown, S.L., and J.E. Brown. 1992. Effect of plastic mulch color and insecticides on thrips populations and damage to tomato. HortTech. Apr./June:208-211.

Immaraju, J.A., T.D. Paine, J.A. Bethke, K.L. Robb, and J.P. Newman, 1992. Western flower thrips resistance to insecticides in coastal California greenhouses. J. Econ. Entomol. 85: 9-14.

Kring, J.B. 1964. New ways to repel aphids. Frontiers of Plant Sci. 17: 6-7.

Kring, J.B. 1972. Flight behavior of aphids. Ann. Rev. Entomol. 17: 461-492.

Loebenstein, G., M. Alper, S. Levy, D. Palevitch, and E. Menagem. 1975. Protecting peppers from aphid-borne viruses with aluminum foil or plastic mulch. Phytoparasitica. 3:41-53.

Liewehr, D.J., and W.S. Cranshaw. 1991. Alate aphid trap capture over different background colors and different background patterns. Southwest. Entomol. 16:13-18.

Newrocka, B.Z., C.J. Eckenrode, J.K. Uyemoto, and D.H. Young. 1975. Reflective mulches and foliar sprays for suppression of aphid-borne viruses in lettuce. J.Econ. Entomol. 68:694-98.

Scott, S.J., P.J. Mcleod, F.W. Montgomery, and C.A. Handler. 1989. Influence of reflective mulch on incidence of thrips in staked tomatoes. J. Entomol. Sci. 24: 422-427.

OVIPOSITION OF THREE *ERYTHRONEURA* SPECIES[1] ON GRAPE LEAVES IN WESTERN TEXAS

Douglas W. Paxton[2] and Harlan G. Thorvilson

Department of Plant and Soil Science, Texas Tech University
Lubbock, Texas 79409-2122

ABSTRACT

Ovipositional sites and patterns of three species of *Erythroneura* (Homoptera: Cicadellidae) leafhoppers that infest grape vineyards on the Texas High Plains were studied. The three species, *Erythroneura coloradensis* (Gillette), *Erythroneura vulnerata* Fitch, and *Erythroneura ziczac* (Walsh), significantly oviposit greater numbers of eggs in top surfaces, in the mesophyll portion near vascular bundles, and in bottom surfaces of leaves under the epidermis, respectively. Ovipositional sites may be used to identify the leafhopper species that infest vineyards on the Texas High Plains.

INTRODUCTION

Large populations of leafhoppers may defoliate grape vineyards on the High Plains of Texas. The consequences of severe defoliation are reduced fruit quality and seasonal yield and greater susceptibility of vines and buds to winter-kill or frost damage. Adult leafhoppers oviposit on grape leaves, and nymphs and adults feed on leaves. Three species of leafhoppers (Homoptera: Cicadellidae) have been identified in vineyards on the Texas High Plains: *Erythroneura coloradensis* (Gillette), *E. vulnerata* Fitch, and *E. ziczac* (Walsh) (Paxton 1990).

In California, the western grape leafhopper, *Erythroneura elegantula* Osborn, lays eggs singly and at random locations on the top and bottom surfaces of grape leaves just under the epidermis (Cate 1975, Jensen and Flaherty 1981). Eggs cannot be seen with the naked eye; even when numerous, eggs are difficult to find with a hand lens. Leafhopper eggs appear as bean-shaped blisters after oviposition into leaf tissue and are approximately 0.8 mm long (Jensen and Flaherty 1981). Settle et al. (1986) reported that *Erythroneura varibilis* Beamer oviposited deep into the mesophyll layer of leaves or within tissues surrounding vascular bundles, and eggs were only seen under magnification with light transmitted from the underside of the leaf.

At least six *Erythroneura* spp. feed on grapes in New York: *E. comes* (Say), the eastern grape leafhopper; *E. bistrata* McAtee; *E. vitifex* Fitch; *E. vitis* (Harris); *E. tricinta* Fitch, the threebanded leafhopper; and *E. vulnerata* Fitch (Martinson and Dennehy 1995). *Erythroneura comes* inserts eggs shallowly in tissue between leaf veins on abaxial leaf surfaces and prefers to oviposit on pubescent leaves, such as cultivars with *Vitis labrusca* parentage. In contrast, *E. bistrata* prefers cultivars with glabrous leaves and deposits eggs under secondary leaf veins.

Leafhoppers overwinter as adults in a state of reproductive diapause in California (Cate 1975). A reliable indicator of *E. elegantula* female reproductive diapause is entirely darkened dorsa of all abdominal segments. In contrast, reproductive females are darkened only on the dorsa of the first three abdominal segments. *Erythroneura elegantula* remain in reproductive diapause until a photoperiod of ca. 11.6 h is reached in the spring, at which time reproductive organs begin to mature. Oogenesis and ovary maturation continue as leafhoppers feed on grape foliage (Jensen and Flaherty 1981). After diapause is broken, populations

[1]Homoptera: Cicadellidae

[2]Texas Agri. Ext. Ser., P.O. Box 1090, Stanton, TX 79782

rapidly increase, resulting in two to four overlapping generations per year. In California, diapausing populations develop late in the season when the photoperiod becomes approximately 13.6 h and shorter (Jensen and Flaherty 1981).

The objective of the present study was to determine the ovipositional site and pattern for each of the three species of leafhoppers attacking grape vines on the Texas High Plains.

MATERIALS AND METHODS

Grape plants used for this test were *Vitis vinifera* of the cultivar Muscat Canelli, propagated from cuttings provided by the Texas A&M Experiment Station near Lubbock, Texas. Grape plants were transplanted into 18.9-liter pots in a potting mixture of 60% sand, 20% soil, 10% potting soil, and 10% perlite. Grape plants were maintained in a greenhouse at temperatures from 22.0 to 29.5°C with seasonal (27 July to 1 September) daylight. Humidity in the greenhouse ranged between 27 and 87%.

Leaf cages to enclose individual grape leaves consisted of chiffon cloth (0.3 mm x 0.3 mm mesh) approximately 40 cm long x 20 cm wide. The cloth was folded in half lengthwise and fastened along two edges so that one end remained open. A leaf cage was secured to an individual grape leaf petiole to enclose the entire leaf and the introduced leafhoppers. During periods between experimental trials, the potted plants were enclosed in chiffon cloth, whole-plant cages to ensure that no rogue leafhoppers oviposited on the leaves. Each leaf was inspected with 20X to 45X magnification to ensure that no eggs were present prior to each trial.

Adults of the three leafhopper species (*E. coloradensis*, *E. vulnerata*, and *E. ziczac*) were collected in the Cox Family Vineyard (Lubbock Co.) and the Texas Tech University vineyard (Lubbock Co.) for oviposition trials. Leafhoppers were field-collected on 26 July, 10 August, and 16 August 1989 for the first, second and third, and fourth and fifth replications, respectively. Leafhoppers collected on 10 August and 16 August had been exposed naturally to approximately 13.8 and 13.6 daylight hours, respectively, which was near the critical photoperiod required to induce reproductive diapause in leafhoppers in California (Jensen and Flaherty 1981).

For the first replication, groups of five adult females of each leafhopper species were placed into separate leaf cages and allowed to feed and oviposit for 12 d (27 July to 7 August). Ten females of each species were placed in separate leaf cages for replications two and three (14 August to 25 August) and for replications four and five (21 August to 1 September). Eggs and nymphs on leaves after 12-d periods for each replication were counted using 10-40X magnification, and numbers were recorded. Data analysis tested the hypothesis that leafhoppers had no preferences for oviposition sites on grape leaves, i.e., top versus bottom surfaces. Data were compared using a Chi-square distribution analysis.

The amounts of leafhopper feeding damage to leaves were determined by placing a 0.25-cm^2 transparent grid over each leaf. The number of damaged, yellowed squares were counted on each leaf. The total surface area of each leaf was measured using an electronic area meter (model LI-3100, Li-Cor Inc., Lincoln, Nebr.). The damaged area (cm^2) of each leaf was divided by the total leaf area (cm^2) to determine percentage damage. Differences in percentage feeding damage were detected by analysis of variance (ANOVA).

RESULTS AND DISCUSSION

Eggs were found in three sites in grapes leaves: under the epidermis of top surfaces or on bottom surfaces and in tissues surrounding the vascular bundles on the midrib of leaves. These three sites were evidence of species oviposition differentiation (Table 1). The greatest numbers (44.8%) of leafhopper eggs were laid during the first replication. Leafhoppers collected on 16 August and used in the fifth trial did not oviposit which suggests that leafhoppers were in reproductive diapause.

Erythroneura ziczac eggs were oviposited in the lower surfaces of leaves (97.2%), and the preferred site was consistent throughout the experiment. Eggs were laid in rows of two to nine eggs. The 12 eggs located in the upper surface were in two rows of six eggs each. All eggs within rows were aligned with heads pointing in the same direction. Red eyespots were visible at the anterior end of developing embryos just before egg eclosion. Fifty-two eggs with eyespots were counted, and 19 nymphs emerged during 12-d trials. Chi-square analysis

detected significant differences between numbers of eggs in the top and bottom surfaces of leaves ($\chi^2_{t\alpha 0.05}$=3.84, df=1, χ^2=194.16).

Eggs laid by *E. coloradensis* were placed singly and randomly in the top surfaces (98.7%) of leaves and in no other apparent pattern. Significantly more eggs were laid on upper leaf surfaces than on lower leaf surfaces ($\chi^2_{t\alpha 0.05}$ = 3.84, df = 1, χ^2 = 73.55). Thirteen eggs with eyespots were noted, and three nymphs emerged during the trials. Only three *E. vulnerata* eggs were found, and they were laid in the vascular bundles on the leaf midrib.

TABLE 1. Oviposition by *Erythroneura* spp. Leafhoppers on *Vitis vinifera* (cultivar Muscat Canelli).

Site on leaves	Numbers of eggs		
	E. ziczac	*E. coloradensis*	*E. vulnerata*
Upper surface	12	153	0
Lower surface	423	3	0
Vascular bundles	0	0	3
Total	435	156	3

Feces were present in all cages during the trials which indicated active feeding by adult leafhoppers. Mean percentage feeding damage to grape leaves caused by leafhoppers during the trials were 7.2, 5.9, and 5.0% for *E. ziczac*, *E. coloradensis*, and *E. vulnerata*, respectively, which were not significantly different (F = 0.13; df = 2,12; P > 0.10).

Leafhopper generations overlap during the growing season; therefore, leafhoppers used for ovipositional trials may have been from at least two different generations. Leafhoppers were collected late in the growing season, and some females used in all trials had darker abdomens which is a sign of the diapausing population and may explain the small number of eggs laid by *E. vulnerata*.

Significant differences in ovipositional sites among leafhopper species may be helpful to researchers and consultants when sampling grape vineyards for data on leafhopper activity and population dynamics. For example, ovipositional data may assist identification of pestiferous leafhopper species and in the timing and application of insecticides targeting leafhopper nymphs. Also, biological control tactics, such as egg parasitism, may be evaluated more easily with this knowledge.

ACKNOWLEDGMENT

Access to the Cox Family Vineyard for leafhopper sampling and collecting was greatly appreciated. This research was supported by funds from the Food and Fiber Production account, a Texas Legislature State Line Item, and from the Enology and Viticulture Research program, Department of Plant and Soil Sciences, Texas Tech University. This manuscript is contribution no. T-4-401, College of Agricultural Sciences and Natural Resources, Texas Tech University.

LITERATURE CITED

Cate, J. R., Jr. 1975. Ecology of *Erythroneura elegantula* Osborn (Homoptera: Cicadellidae) in grape agroecosystems in California. Ph.D. Dissertation, University of California, Berkeley. 349 pp.

Jensen, F. L., and D. Flaherty. 1981. Grape leafhopper. pp. 98-110. *In* D. L. Flaherty, F. L. Jensen, A. N. Kasimatis, H. Kido, and W. J. Moller (eds.). Grape Pest Management. California Ext. Publ. No. 4105. Division of Agriculture and Natural Resources, Univ. of California, Berkeley. 312 pp.

Martinson, T. E., and T. J. Dennehy. 1995. Varietal preferences of *Erythroneura* leafhoppers (Homoptera: Cicadellidae) feeding on grapes in New York. Environ. Entomol. 24: 550-558.

Paxton, D. W. 1990. Population dynamics of leafhoppers, *Erythroneura* spp. (Homoptera: Cicadellidae), on Texas High Plains grapes. M.S. Thesis, Texas Tech University, Lubbock. 63 pp.

Settle, W. H., L. T. Wilson, D. L. Flaherty, and G. M. English-Leob. 1986. The variegated leafhopper, an increasing pest of grapes. Calif. Agri. 40: 30-32.

EFFECTS OF THE APHICIDES 'GAUCHO' AND CGA-215944 ON FEEDING BEHAVIOR AND TRITROPHIC INTERACTIONS[2] OF RUSSIAN WHEAT APHIDS[1]

John D. Burd, Norman C. Elliott and David K. Reed[3]

USDA-ARS, Plant Science and Water Conservation Research Laboratory,
1301 N. Western Street, Stillwater, OK 74075

ABSTRACT

The effect of low concentrations of the systemic aphicides CGA 215944 and Gaucho® (imidacloprid) on the feeding behavior of the Russian wheat aphid, *Diuraphis noxia* (Mordvilko), on wheat was evaluated using electronic feeding monitors. Aphids began surviving 15 days after treatment with CGA 215944 (soil drench, 10 g AI/liter) and 45 days after planting on plants grown from seed treated with Gaucho (1 fluid oz/cwt). Following these periods of acute toxicity, CGA 215944 exhibited antifeedant effects on *D. noxia* that were expressed by changes in the frequency and duration of different feeding behaviors. Aphids on plants treated with CGA 215944 spent significantly more time in nonprobing activities and had a much higher frequency of leaf penetration, followed by significantly shorter periods of phloem ingestion. Immediately after the period of acute aphid toxicity, there were no substantial carryover effects from CGA 215944 or Gaucho on the tritrophic relationship between host plant, *D. noxia* , and *Diaeretiella rapae* McIntosh.

INTRODUCTION

The Russian wheat aphid, *Diuraphis noxia* (Mordvilko), is a recent introduction into the United States and Canada (Stoetzel 1987). Estimated economic losses to the U.S. cereal industry due to *D. noxia* from 1987 through 1993 averaged over $127 million per year, with a cumulative total of nearly $900 million (Webster and Amosson 1994).

Modified cultural practices and control of volunteer wheat and barley can be important in minimizing *D. noxia* infestations (Peairs 1990); however, chemical control is the primary method used. For many growers the expense of pesticide applications is prohibitive, and the cost to the environment is a concern.

'CGA 215944' (4,5-dihydro-6-methyl-4-(3-pyridylmethyleneamino)-1,2,4-triazin-3(2H)-one) and 'Gaucho' (1-[(6-Chloro-3-pyridinyl)methyl]-N-nitro-2-imidazolidinimine) are newly developed insecticides with primary activity on sucking insects (Fluckiger et al. 1992, Ishii et al. 1994) and will likely become available for use against aphids in cereal agroecosystems (Pike et al. 1993). Both CGA 215944 and Gaucho have low mammalian toxicities (Elbert et al. 1990, Fluckiger et al. 1992), and when applied in low concentrations as a soil drench or as a seed dressing they have been reputed to have antifeedant effects on

[1] Homoptera: Aphididae

[2] *Diaeretiella rapae* McIntosh

[3] Current address: 4820 Royal Oak, San Angelo, TX 76904

aphids (Fluckiger et al. 1992, Nauen 1995) and, excluding host mortality, are touted as being innocuous to natural enemies (Balsdon et al. 1993, Fluckiger et al. 1992, Mizell and Sconyers 1992).

Aphid feeding behavior can be visualized using direct-coupled electronic systems that collect data on specific probing activities by measuring temporal fluctuations in voltage caused by subtle changes in stylet physical properties, position within the plant tissue, and membrane potentials of adjacent and penetrated plant cells (Kimsey and McLean 1987, Tjallingii 1990). To accomplish this, a primary circuit is established by applying voltage to the host media which during probing is conducted through the aphid via a gold wire attached to the dorsum, which is then connected to signal amplifier and various types of analog recorders. Two basic types of feeding monitors have been developed and are distinguished primarily by their type of voltage supply. The first system developed used alternating current (AC monitor) to measure aphid-induced fluctuations in electrical resistance that occur during feeding (McLean 1977). Tjallingii (1978) modified this system to use direct current (DC monitor) so that voltages that originate from electromotive forces during the aphid-plant interaction can also be measured. Both systems are in use today, and although the output differs between the two, several basic consistencies occur that make transient waveform comparisons possible.

Girma et al. (1992) identified six distinct waveforms associated with *D. noxia* feeding activities which were determined histologically to represent nonprobing, penetration spike, salivation, X-wave, phloem ingestion, and nonphloem ingestion. Analyses of frequency and duration of these waveform transients have yielded some insight toward the characterization of *D. noxia* host plant resistance modalities (Girma et al. 1992, Kindler et al. 1992, Webster et al. 1993). Moreover, Nisbet et al. (1993) used electronic feeding monitors to evaluate systemic effects of the insect antifeedant azadirachtin on aphid feeding behavior. They measured aphid feeding response to varying levels of azadirachtin and found a significant correlation between increases in azadirachtin concentrations and decreases in duration of non-penetration (feeding) periods.

Objectives of the present study were to assess quantitatively, by using electronic feeding monitors, the systemic effects of low concentrations of CGA 215944 and Gaucho on *D. noxia* feeding behavior and to determine if there were subsequent carryover effects through surviving aphids on *Diaeretiella rapae* McIntosh, a hymenopterous (Braconidae) parasitoid of *D. noxia*.

MATERIALS AND METHODS

D. noxia used were from greenhouse colonies cultured on 'TAM W-101' hard red winter wheat. Aphids were maintained on greenhouse benches under a 14-h photophase. TAM W-101 seed that had been dressed with Gaucho at a rate of 1 fluid oz/cwt were obtained from Gustafson, Inc., Dallas, TX. CGA 215944 WP25 was obtained from CIBA-GEIGY Corp., Charlotte, NC, and was applied as a soil drench at a rate of 10 g AI/liter.

Test plants were grown individually in plastic pots (7.6 cm diameter) on greenhouse benches in a fritted clay medium (Burd et al. 1993). Greenhouse temperatures were maintained at 21 ± 5°C with a photoperiod of 14:10 (L:D) provided by halide lamps. Fourteen days after planting, pots containing CGA 215944 TAM W-101 test plants were drenched with 100 ml of the insecticide mixture.

Treated plants were arranged into two separate paired tests; Gaucho-treated plus untreated controls, and CGA 215944-treated plus untreated controls. Five plants from each insecticide treatment were infested with ten *D. noxia* apterous adults. Infested plants were monitored daily to determine the end of the period of acute aphid toxicity. Each subsequent

week, different test plants from the same group were used. Aphids began to survive on the CGA 215944-treated plants 15 days after treatment (about 30 days after planting), and aphid survival on Gaucho seed-treated plants began at 45 days after planting. At this time, the test plants were used for feeding behavior studies and tritrophic evaluations.

Feeding monitor tests were conducted using four AC electronic feeding monitors (25 Hz) adapted from Brown and Holbrook (1976) (Oklahoma Engineering and Technical Services, Perry, OK), with an OE&TS Model PS-8 power supply. We used the experimental procedures for wiring aphids and electrical connections described by Webster et al. (1993).

Two randomly selected insecticide-treated and untreated plants were randomly assigned to one of four feeding monitors for each test. New adult apterous aphids and plants were used for all tests. Each feeding monitor test was conducted for 720 min for each individual aphid, and each treatment was evaluated 12 times. The two insecticide treatments were tested separately. In our analysis, we followed the interpretation of the six distinct waveform patterns used by Girma et al. (1992) as follows: (1) nonprobing, (2) penetration spikes (high amplitude salivation), (3) salivation, (4) X-wave, (5) phloem ingestion, and (6) nonphloem ingestion. For the mean duration analysis, however, we combined the X-wave and low amplitude salivation waveforms. We did not combine the high and low amplitude salivation waveforms.

Immediately following the period of acute aphid toxicity, ten plants of each insecticide treatment and ten untreated controls were infested with ten adult apterous *D. noxia* and then caged. Seventy-two h later, one mated adult *D. rapae* female was placed onto each of the caged test plants. Seven days after introduction of the parasitoids, all mummies were removed and placed individually into size 00 gelatin capsules and stored under test conditions. After emergence, individuals were weighed and sexed, and the relative percentage of emergence was calculated.

All data were analyzed using PROC GLM (SAS Institute 1988), and mean comparisons were made using DMRT ($P = 0.05$).

RESULTS AND DISCUSSION

All six waveform transients and the typical patterned sequences reported by Girma et al. (1992) for *D. noxia* were observed during aphid feeding tests on each of the plant treatments used in this study. In all cases, phloem ingestion was preceded by one to several X-waves. The mean frequencies per aphid for each waveform measured during the 720-min feeding behavior tests are shown in Figs. 1 and 2. During the feeding tests, the total number of distinct behavioral events exhibited by *D. noxia* was substantially greater on CGA 215944-treated ($x = 53$) versus untreated plants ($x = 28$). Nonprobing (NP), which is the combined total of those aphid activities during which the stylet is not inserted into the plant, and penetration spikes (PR), which are characterized by high amplitude waveforms associated with the initial insertion of the stylets into the plant (Niassy et al. 1987), were the most common feeding behaviors recorded for aphids on both plant treatments; however, the mean frequencies for NP and PR were significantly higher for aphids on CGA 215944-treated plants (Fig. 1). Frequencies for low amplitude salivation (SAL), nonphloem ingestion (NPI), and phloem ingestion (PI) events did not differ significantly for *D. noxia* feeding on CGA 215944-treated and untreated plants. Although the total number of feeding behavior events were generally greater for the Gaucho-treated plants, mean frequencies did not differ significantly for aphids on treated and untreated plants for any of the feeding behaviors (Fig. 2). Overall, NP and PR were the most frequently occurring feeding behaviors during the tests.

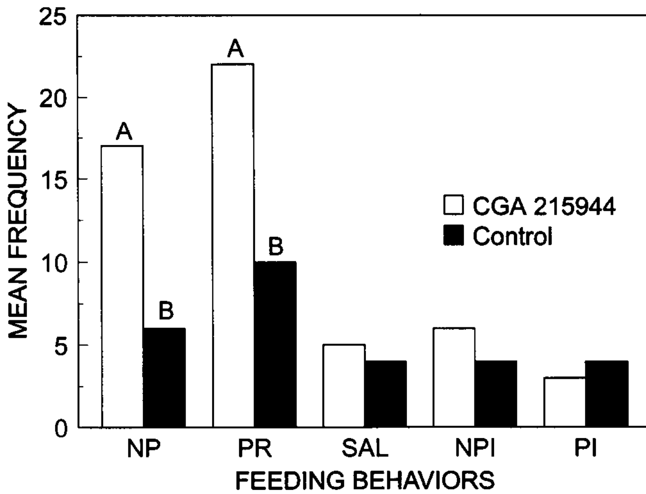


FIG. 1. Mean frequency of feeding behaviors of individual *Diuraphis noxia* on CGA 215944-treated and untreated wheat. NP, nonprobing; PR, penetration spikes; SAL, low amplitude salivation; NPI, nonphloem ingestion; PI, phloem ingestion. Histograms designated with different letters are significantly different ($P \leq 0.05$; DMRT).

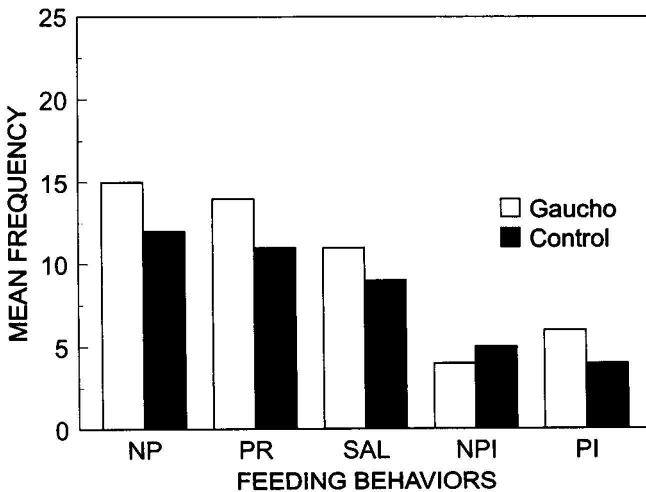


FIG. 2. Mean frequency of feeding behaviors of individual *Diuraphis noxia* on Gaucho-treated and untreated wheat. NP, nonprobing; PR, penetration spikes; SAL, low amplitude salivation; NPI, nonphloem ingestion; PI, phloem ingestion.

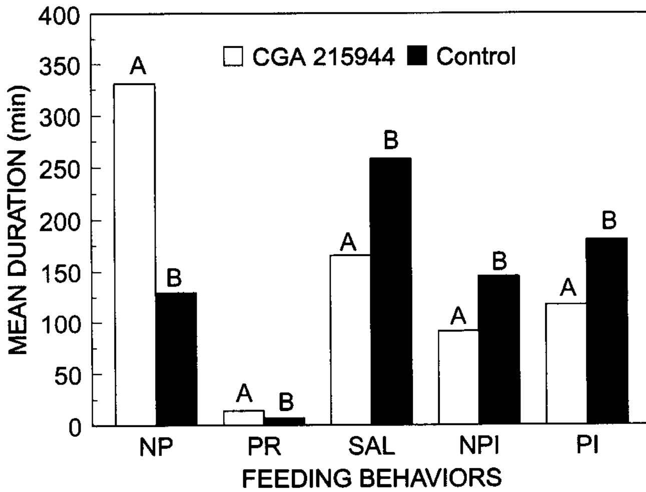


FIG. 3. Mean duration of feeding behaviors of individual *Diuraphis noxia* on CGA 215944-treated and untreated wheat. NP, nonprobing; PR, penetration spikes; SAL, low amplitude salivation; NPI, nonphloem ingestion; PI, phloem ingestion. Histograms designated with different letters are significantly different ($P \leq 0.05$; DMRT).

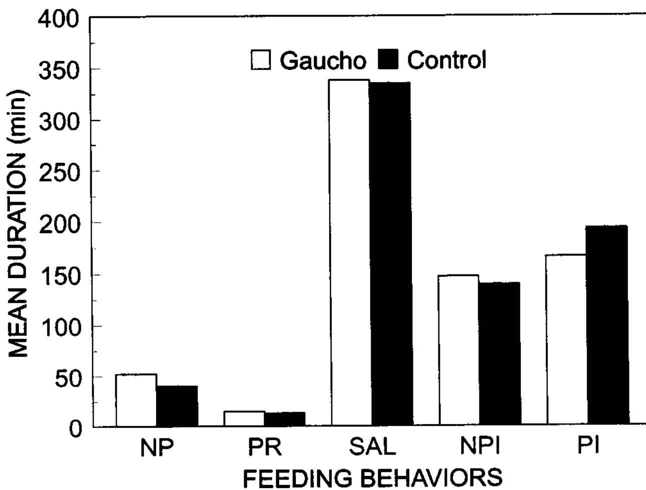


FIG. 4. Mean frequency of feeding behaviors of individual *Diuraphis noxia* on Gaucho-treated and untreated wheat. NP, nonprobing; PR, penetration spikes; SAL, low amplitude salivation; NPI, nonphloem ingestion; PI, phloem ingestion.

Although PR was the most frequent aphid behavior on the CGA 215944-treated plants, it had the shortest mean duration time per aphid (Fig. 3). Mean duration was greatest for NP and constituted ≈ 45% of the total duration time. In contrast, *D. noxia* on the untreated control plants spent the most time engaged in SAL activity (≈ 35% total duration). Mean duration for SAL, NPI, and PI activities were significantly increased, while NP and PR durations were significantly decreased for *D. noxia* feeding on plants treated with CGA 215944. In contrast, there were no significant differences between Gaucho-treated and untreated plants in mean duration for any of the feeding behaviors (Fig. 4).

Applied systemically in low concentrations, both CGA 215944 and Gaucho were effective in controlling *D. noxia*. However, following a period of acute aphid toxicity, only CGA 215944 exhibited antifeedant effects on *D. noxia* which were expressed by changes in the frequency and duration of different feeding behaviors.

Previous feeding behavior studies have suggested that Gaucho (≡imidacloprid) applied systemically at moderate concentrations acts as an insecticide with no antifeedant activity (Woodford and Mann 1992), whereas, when applied at lower concentrations, a reversible antifeedant effect occurs (Nauen 1995). Nonetheless, we found no differences in the frequency or duration of feeding behavior that would suggest an antifeedant effect on *D. noxia*.

Aphids on plants treated with CGA 215944 spent significantly more time in nonprobing activities and had a much higher frequency of leaf penetration spikes followed by significantly shorter periods of NPI and PI activity. Similar changes in aphid feeding behavior caused by the antifeedant azadirachtin has been described by Nisbet et al. (1993). They found that azadirachtin caused increases in the number of stylet probes coinciding with premature termination of sustained phloem feeding and suggested that this was due to the absence of external chemoreceptors on aphid mouthparts, and therefore gustation of phloem constituents can only occur after penetration and sap uptake.

Data on the effect of CGA 215944 and Gaucho on the tritrophic relationship between host plant, *D. noxia*, and *D. rapae* are summarized in Table 1. Except for a significant increase in the number of days to *D. rapae* emergence on aphid hosts that had fed on CGA 215944-treated plants, there were no apparent detrimental carryover effects to the aphid parasitoid. The increased developmental period may result from the observed antifeedant effect of CGA 215944 on *D. noxia*. Nonetheless, mean adult weights, relative percentage emergence, and male:female ratios of *D. rapae* that emerged from aphid hosts reared on the insecticide-treated plants did not differ significantly from those of the untreated control plants.

TABLE 1. Means[a] for Adult Weight, Days to Emergence, Percentage Emergence, and Sex Ratios for *Diaeretiella rapae* Reared on *Diuraphis noxia* Cultured on Insecticide-Treated and Untreated Wheat.

Insecticide treatment	Weight (mg) Male	Weight (mg) Female	Emergence Days	Emergence Percentage	Sex Ratio Male/Female
CGA 215944	0.182a	0.198a	11.4a	0.912a	0.550
Gaucho	0.190a	0.205a	10.1b	0.902a	0.586
Control	0.196a	0.217a	10.2b	0.928a	0.529

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

Results from these studies indicate that treatment of wheat with either the systemic CGA 215944 or Gaucho can effectively control *D. noxia*. Immediately following the period of irreversible acute toxicity, CGA 215944 appears to have a residual antifeedant effect on *D. noxia*, whereas no effects on aphid feeding behavior were observed with Gaucho-treated plants. The first surviving aphids from plants treated with either CGA 215944 or Gaucho were suitable hosts for the aphid parasitoid *D. rapae*.

ACKNOWLEDGMENT

We thank Drs. Aithel McMahon, CIBA-GEIGY Corporation, and Terry Pitts, Gustafson, Inc., for help in providing the test chemicals CGA 215944 and Gaucho, respectively. We gratefully acknowledge Brian Jones and Shonda Welch for technical assistance; Ruth Treat for editorial assistance; and Drs. Raymond Eikenbary and Gary Puterka for helpful reviews of the initial draft of the manuscript. This article reports the results of research only, and mention of a proprietary product does not constitute endorsement or recommendation for its use by the USDA.

LITERATURE CITED

Balsdon, J. A., S. K. Braman, A. F. Pendley, and K. E. Espelie. 1993. Potential for integration of chemical and natural enemy suppression of azalea lace bug (Heteroptera: Tingidae). J. Environ. Hort. 11: 153-156.

Brown, C. M., and F. R. Holbrook. 1976. An improved electronic system for monitoring feeding of aphids. Am. Potato J. 53: 457-462.

Burd, J. D., R. L. Burton, and J. A. Webster. 1993. Evaluation of Russian wheat aphid (Homoptera: Aphididae) damage on resistant and susceptible hosts with comparisons of damage ratings to quantitative plant measurements. J. Econ. Entomol. 86: 974-980.

Elbert, A., H. Overbeck, K. Iwaya, and S. Tsuboi. 1990. Imidacloprid, a novel systemic nitromethylene analogue insecticide for crop protection, pp. 21-28. Proc. Brighton Crop Prot. Conf.

Fluckiger, C. R., H. Kristinsson, R. Senn, A. Rindlisbacher, H. Buholzer, and G. Voss. 1992. CGA 215944 - a novel agent to control aphids and whiteflies, pp. 43-50. Proc. Brighton Crop Prot. Conf.

Girma, M., G. E. Wilde, and J. C. Reese. 1992. Russian wheat aphid (Homoptera: Aphididae) feeding behavior on host and nonhost plants. J. Econ. Entomol. 85: 395-401.

Ishii, Y., I. Kobori, Y. Araki, S. Kurogochi, K. Iwaya, and S. Kagabu. 1994. HPLC determination of the new insecticide imidacloprid and its behavior in rice and cucumber. J. Agric. Food Chem. 42: 2917-2921.

Kimsey, R. B., and D. L. McLean. 1987. Versatile electronic measurement system for studying probing and feeding of piercing and sucking insects. Ann. Entomol. Soc. Am. 80: 118-129.

Kindler, S. D., L. G. Greer, and T. L. Springer. 1992. Feeding behavior of the Russian wheat aphid (Homoptera: Aphididae) on wheat and resistant and susceptible slender wheatgrass. J. Econ. Entomol. 85: 2012-2016.

McLean, D. L. 1977. An electrical measurement system for studying aphid probing behavior, pp. 277-290. *In* K. F. Harris and K. Maramorosch [eds.] Aphids as Virus Vectors. Academic, New York.

Mizell, R. F., and M. C. Sconyers. 1992. Toxicity of imidacloprid to selected arthropod predators in the laboratory. Fla. Entomol. 75: 277- 280.

Nauen, R. 1995. Behaviour modifying effects of low systemic concentrations of imidaclopric on *Myzus persicae* with special reference to an antifeeding response. Pestic. Sci. 44: 145-153.

Niassy, A., J. D. Ryan, and D. C. Peters. 1987. Variations in feeding behavior, fecundity, and damage of biotype B and E of *Schizaphis graminum* (Homoptera: Aphididae) on three wheat genotypes. Environ. Entomol. 16: 1163-1168.

Nisbet, A. J., J. A. T. Woodford, R. H. C. Strang, and J. D. Connolly. 1993. Systemic antifeedant effects of azadirachtin on the peach-potato aphid *Myzus persicae*. Entomol. Exp. Appl. 68: 87-98.

Peairs, F. B. 1990. Russian wheat aphid management, pp. 233-241. *In* D. C. Peters, J. A. Webster, and C. S. Chlouber [eds.] Proceedings, Aphid-Plant Interactions: Populations to Molecules, August 12-17, 1990. Okla. Agric. Exp. Stn. MP-132. Stillwater, OK.

Pike, K. S., G. L. Reed, G. T. Graf, and D. Allison. 1993. Compatibility of imidacloprid with fungicides as a seed-treatment control of Russian wheat aphid (Homoptera: Aphididae) and effect on germination, growth, and yield of wheat and barley. J. Econ. Entomol. 86: 586-593.

SAS Institute. 1988. SAS User's Guide: Statistics, version 6. SAS Institute, Cary, NC.

Stoetzel, M. B. 1987. Information on and identification of *Diuraphis noxia* (Homoptera: Aphididae) and other aphid species colonizing leaves of wheat and barley in the United States. J. Econ. Entomol. 80: 696-704.

Tjallingii, W. F. 1978. Electronic recording of penetration behavior by aphids. Entomol. Exp. Appl. 24: 721-730.

Tjallingii, W. F. 1990. Stylet penetration parameters from aphids in relation to host-plant resistance. Symp. Biol. Hung. 39: 411-419.

Webster, J. A., and S. Amosson. 1994. Economic impact of the Russian wheat aphid in the western United States: 1992-1993. Great Plains Agric. Counc. Publ. 152.

Webster, J. A., D. R. Porter, C. A. Baker, and D. W. Mornhinweg. 1993. Resistance to Russian wheat aphid (Homoptera: Aphididae) in barley: effects on aphid feeding. J. Econ. Entomol. 86: 1603-1608.

Woodford, J. A. T., and J. A. Mann. 1992. Systemic effects of imidacloprid on aphid feeding behaviour and virus transmission on potatoes, pp. 557-562. Proc. Brighton Crop Prot. Conf.

AN INTEGRATED PEST MANAGEMENT APPROACH, EMPHASIZING BIOLOGICAL CONTROL, FOR PECAN APHIDS

D.R. LaRock and J.J. Ellington
New Mexico State University
P.O. Box 30003, Dept. 3BE
Las Cruces, New Mexico 88003-0003

ABSTRACT

An Integrated Pest Management program to control the yellow pecan aphid *Moneliopsis pecanis* (Bissel), the black-margined pecan aphid *Monellia caryella* (Fitch), and the black pecan aphid *Melanocallis caryaefolia* (Davis) was adopted by Stahmann Farms of Las Cruces, New Mexico, in 1987. This program relied on improved cultural management practices, compound leaf counts of pest and predator densities, inoculative releases of the lacewing *Chrysoperla rufilabris* (Burmeister), two species of lady beetles *Hippodamia convergens* (Guerin-Meneville), and *Harmonia axyridis* (Pallas), and reduced pesticide inputs. The population dynamics of aphids and predators are presented for years 1987 through 1993. Release and insecticide application rates declined during this seven-year interval, as did the financial expense associated with each. Yield and quality, however, remained high.

INTRODUCTION

Stahmann Farms Inc., founded in 1925 by William J. and Deane F. Stahmann, is a 4,000-acre orchard bordered by the Rio Grande River on the east and a naturally occurring mesa on the west; 3,615 acres are under cultivation. The working orchard is divided into 124 blocks which range in size from 8 to 48.8 acres each. Within each block, pecan trees are planted at approximate 30-foot spacings with 48 trees per acre. In some areas, spacing has been increased to 60 feet in order to permit greater light penetration into the canopy. A variety of cultivars have been planted over the years. Because of proven adaptability and apparent resistance to aphid pressure, 72% of the pecans are 'Western Schley', 18% are 'Ideal', and the final 10% is a mixture of 'Burkett', 'Wichita', 'San Saba', 'E-1', 'Texas-60', 'Stuart', and 'Desirable'.

This orchard receives between four and six acre feet of water each year with an irrigation frequency of every two weeks during the summer. Granular and liquid preparations of nitrogen and granular formulations of potassium and sulfur are applied annually. The total yearly nitrogen application rate is 280 pounds per acre. Fifteen pounds of zinc sulfate are applied annually in split applications as a foliar spray. Monoammonium phosphate is broadcast in one, 100 pound per acre application every other year.

Prior to 1988, indigenous vegetation under the pecan trees was controlled by frequent mowing. Beneficial insects were released in an Integrated Pest Management (IPM) program, beginning in 1987, to control pecan aphids. In an effort to provide refuge and other resources for these organisms, the management of Stahmann Farms allowed weeds to grow. Since 1988, annual and perennial vegetation has been allowed to grow approximately two feet tall before either being chemically mowed with glyphosate or mowed with a conventional mechanical mower.

Information presented in this paper is not the result of a single scientific study but is derived from a mixture of various research projects conducted by graduate and post-graduate students at NMSU. It is an effort to chronicle the IPM and biological control approach to pecan aphid control used between 1987 and 1993 at Stahmann Farms and the effect on yield and quality of the pecans produced there.

MATERIALS AND METHODS

Of the 124 blocks of land within the orchard, blocks C-2, C-12, E-2, L-8, N-9, and S-11 were most frequently used to evaluate aphid and predator populations. These areas are relatively well distributed throughout the orchard and reflected a reasonable estimate of pest pressure in the orchard. In some years, however, as few as one or as many as 16 blocks were monitored. Trees were selected randomly within each block.

Compound Leaf Counts. Compound leaves of approximately the same size were chosen from each quadrant of each tree. Leaves were handled carefully and all nymphal and adult pests and predators on both upper and lower leaf surfaces were counted. During 1987, compound leaf counts were conducted in blocks C-2, E-2, L-8, N-9, and S-11. Five trees per block, and three compound leaves per tree were assessed. Fourteen sampling dates, from 12 May through 12 October were included in this study. In 1988, twelve trees in each of blocks C-12, E-2, and N-9 were evaluated. Because L-8 is a smaller block, nine trees in this block were surveyed. Five leaves per tree were inspected. Sampling was performed on eight dates, from 8 June through 4 October. There is no specific information available concerning compound leaf count methodology used in 1989. Only an annual summary statement of aphid density remains. During 1990 and 1991, blocks C-12, E-2, L-8, and N-9 were monitored. Ten trees per block and five leaves per tree were inspected in both of these years. There were 20 sampling dates between 8 May and 24 September 1990. Sampling was conducted on 22 dates, between 8 May and 8 October in 1991. Block S-11 was evaluated in 1992. Twelve trees within this block and five leaves per tree were counted. This study included nine sampling dates between 2 June and 22 October. Fifteen and on occasion 16 blocks were monitored during 1993. Ten trees per block and five leaves per tree were examined. Twenty-one sampling dates ranging from 1 May to 10 October were included in this work.

Predator Releases. The most formidable aphid predators in southern New Mexico are the lacewings *Chrysoperla carneae* (Stevens), *Chrysoperla rufilabris* (Burmeister), *Chrysopa nigricornis* (Burmeister), and the lady beetles *Hippodamia convergens* (Guerin-Meneville), and *Olla v-nigrum* (Mulsant). Release data for 1987 through 1990 are excerpted from Stahmann (1991). Information concerning releases from 1991 through 1993 is via personal interview with Stahmann.

In 1987 through 1991, the lacewing, *C. rufilabris,* was released as either fully mature eggs or as newly eclosed larvae. During the first three years, eggs were dispensed into the tree canopy by aircraft. Release methods were changed during 1990 and 1991 so that first-instar larvae were manually placed in the crotch of randomly selected trees. Adult *C. rufilabris* were released manually during 1992 and 1993. Adult lady beetles, *H. convergens,* were released by hand into orchard ground cover or into the crotch of randomly selected trees from 1987 through 1991. *Harmonia axyridis* lady beetles were released by hand in 1993 only.

In 1987, *C. rufilabris* were released on three occasions during the growing season (mid-May, mid to late July, and mid-August). On the first two dates, the release rate was 10,000 per acre; 5,000 *C. rufilabris* were released per acre in mid-August. *H. convergens* were released twice during this time, first in early June, and again in late July. On both dates the application rate was one gallon per 20 acres. In 1988, *C. rufilabris* were released in mid-

May at a rate of 10,000 per acre and in mid-August at 5,000 per acre. During that year, *H. convergens* were released in late May at the rate of one gallon per 20 acres. The orchard was treated with 5,000 *C. rufilabris* per acre during September 1989. *H. convergens* were released at the same time and rate as in 1988. In 1990, only 90 acres were treated in mid-August with *C. rufilabris* at a rate of 10,000 per acre. *H. convergens* were dispensed in early June at a rate of one gallon per 60 acres. In 1991, 2,600 acres were treated with 5,000 *C. rufilabris* larvae per acre during mid-September. *H. convergens* were released at a rate of one gallon per 40 acres in early June. Five hundred adult *C. rufilabris* per 10 acres were released during mid-August 1992. No lady beetles were released in that year. In mid-July of 1993, adult *C. rufilabris* were dispensed at a rate of 500 per 10 acres throughout 1,200 acres. In June, three hundred adult *H. axyridis* were randomly released into the orchard ground cover in blocks L-12 and L-13, a 68-acre area.

Insecticide Applications. Thiodan® 3EC, FMC Corporation and Lorsban® 4E, DOW Elanco Chemical Company were applied in 1987, 1988, 1992, and 1993 for black pecan aphid control. Only one application of either Thiodan® or Lorsban® was used per year. No chemicals were used to treat the yellow aphid complex specifically during this seven-year period.

Between 1987 and 1993, insecticides were applied via aircraft to the orchard canopy, using standard application techniques. Table 1 is a summary of insecticide use by year.

TABLE 1. Insecticides Used at Stahmann Farms, Las Cruces, New Mexico 1987-1993[a].

Year	Insecticide	Month Of Application	Rate Per Acre	Number Of Acres Treated
1987	Thiodan®	August	2 pints	3,615
1988	Thiodan®	August	1 pint	3,615
1989	None	____	____	____
1990	None	____	____	____
1991	None	____	____	____
1992	Lorsban®	September	2 pints	1,808[b]
1993	Lorsban®	August	1 pint	1,808[b]

[a] Information supplied by Randy Stahmann
[b] Every other acre treated.

Yield and Quality. Nuts and leaf debris were harvested from the orchard floor and loaded into large, truck-mounted trailers. After the trailers from each harvested block were weighed, the yield per acre was estimated, given the number of acres per block.

Leaf litter removed, pecans were sorted by size, using a large cage tumbler, and loaded into bins. Nuts were not separated according to cultivar. A handful-sample from each bin was withdrawn and accumulated by nut size until 20 bins were represented. This sample constituted one lot. From each lot, a one-pound representative sample was extracted and sent to an on-site laboratory for further analysis. Criteria for evaluation included: (1) nut count per pound, (2) gross weight per sample, (3) net weight of nut meats, and (4) weight of shells and waste. From these weight parameters, the "meat yield" was calculated as percentage net weight of meat to the total sample weight. The moisture content of the meat was quantified, and the nut meats were graded subjectively using meat color as the criterion.

RESULTS AND DISCUSSION

Compound Leaf Counts. The data accrued between 1987 and 1993 suggests both

trends and discrepancies in the seasonal abundance pattern of pecan aphids, lacewings, and lady beetles. Information concerning the population dynamics of these organisms is illustrated graphically in Figs. 1-7.

In 1987, the mean density of yellow pecan aphids rose sharply after 15 May to a high of 34.00 aphids per compound leaf on 12 June. A secondary peak was documented on 2 October with the mean reaching 11.78 aphids per compound leaf. The black pecan aphid population began to escalate between 24 and 31 July. The mean peaked at 4.55 blacks per compound leaf on 21 August. Lacewings attained mean numbers of 0.63 per compound leaf on 29 May, one week before the maximum yellow aphid density was reached. Later in the season, it appears that lacewings responded to the buildup of black pecan aphids. On 7 August, lacewing concentrations reached a mean density of 0.2 per compound leaf. A concurrent spike in both the lady beetle and yellow pecan aphid populations occurred on 12 June. Lady beetle numbers reached 0.58 per compound leaf on this date. Only a slight fluctuation in mean lady beetle density was recorded between 7 August and 21 August, corresponding to the proliferation of black pecan aphids (Fig. 1).

In 1988, a maximum mean density of 8.20 yellow pecan aphids per compound leaf was recorded on 8 June (Fig. 2). Also, a very slight resurgence of this pest was observed on 20 September when numbers reached 1.00 per compound leaf. The black pecan aphid population was ostensibly stable on most sampling dates throughout the season. The highest mean density of black pecan aphids per compound leaf was 1.20 on 22 June, and the overall mean for this growing season was 0.60. The maximum mean density of lacewings lagged behind that of the yellow aphid complex by approximately two weeks. On 22 June, lacewing numbers were reported to reach 8.00 per compound leaf. An acute decline in lacewing numbers was observed after 22 June. No data were collected on the dynamics of the lady beetle population in 1988.

Only a summary statement of aphid numbers remains for the growing season of 1989 (Fig. 3). The overall mean density of the yellow aphid complex was approximately 42.00 aphids per compound leaf and that for the black pecan aphid was approximately 15.00. No information on either lacewing or lady beetle populations is available.

In 1990, the concentration of yellow pecan aphids climbed steadily between 8 May and 9 June. The maximum mean density of 38.50 yellow aphids per compound leaf was reached on 9 June. A slight increase in the number of yellow aphids was noted between 19 August and 10 September. On 10 September, an average of 2.00 yellow aphids per compound leaf was documented. According to the data, the black pecan aphid population was negligible until after 23 July. From that date through 19 August, the tally increased to a mean of 6.20 black aphids per compound leaf on 19 August. Numbers waned after this date. The highest density of lacewings was recorded on the first two sampling dates in June when means reached 0.63 lacewings per compound leaf. The escalation of lacewings occurred simultaneously with the rise in the yellow aphid complex. Another slight peak in lacewing numbers followed the build up of the black pecan aphid, near the end of the sampling period. Lady beetles responded more slowly, by approximately one week, to the buildup of the yellow aphid complex compared to the lacewing response. However, the number of lady beetles was substantially higher, reaching a mean density of 1.10 per compound leaf on 15 June (Fig. 4). The mean number rose to 0.50 and 0.54 lady beetles per compound leaf on 24 August and 17 September, respectively, perhaps in response to the black pecan aphid buildup.

In 1991, the yellow aphid complex began to increase in number from the first sampling date on 8 May through 18 June (Fig. 5). On 18 June, the greatest mean density equaled 15.00 yellow pecans aphids per compound leaf. The population fell sharply after 26 June to rebound modestly between 25 August and 23 September. On 23 September, yellow

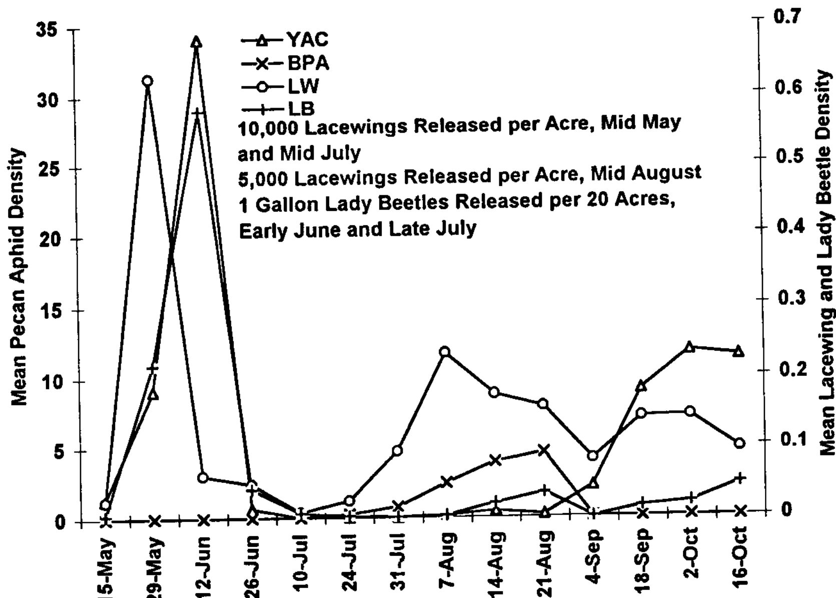


FIG 1. Mean Pecan Aphid (YAC, BPA), Lacewing (LW), and Lady Beetle (LB) Densities per Compound Leaf, Stahmann Farms, Las Cruces, New Mexico, 1987.

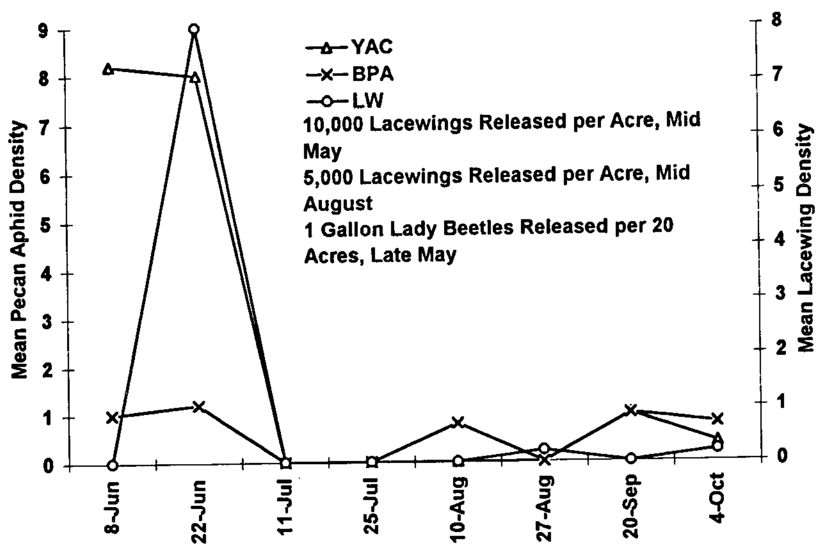


FIG 2. Mean Pecan Aphid (YAC, BPA) and Lacewing (LW) Densities per Compound Leaf (Lady Beetle Data Not Available), Stahmann Farms, Las Cruces, New Mexico, 1988.

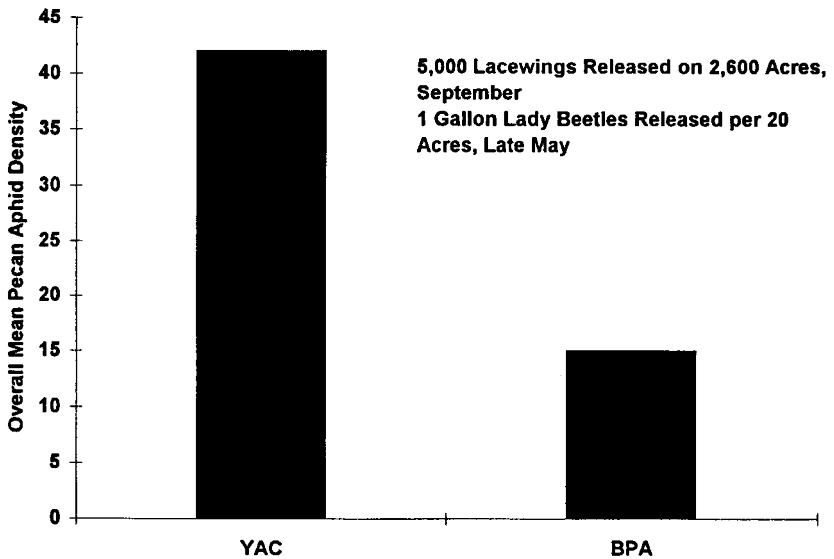


FIG 3. Overall Mean Pecan Aphid Density per Compound Leaf, Stahmann Farms, Las Cruces, New Mexico, 1989.

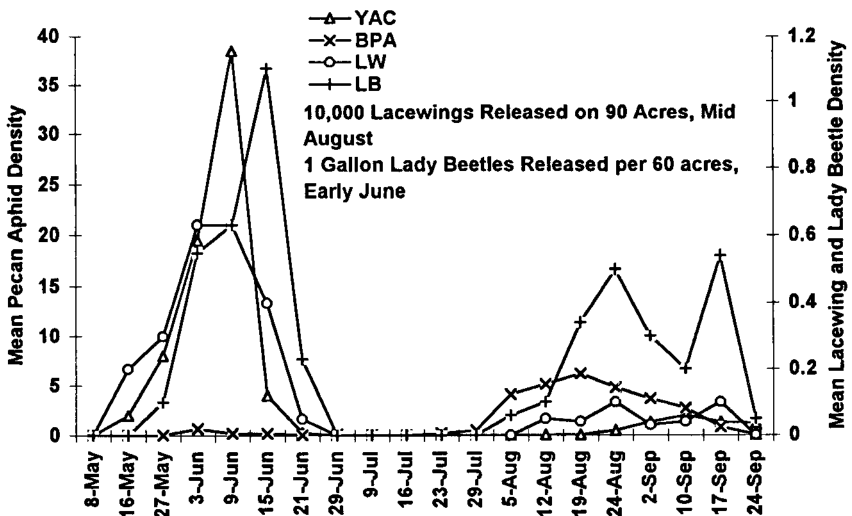


FIG 4. Mean Pecan Aphid (YAC, BPA), Lacewing (LW), and Lady Beetle (LB) Densities per Compound Leaf, Stahmann Farms, Las Cruces, New Mexico, 1990.

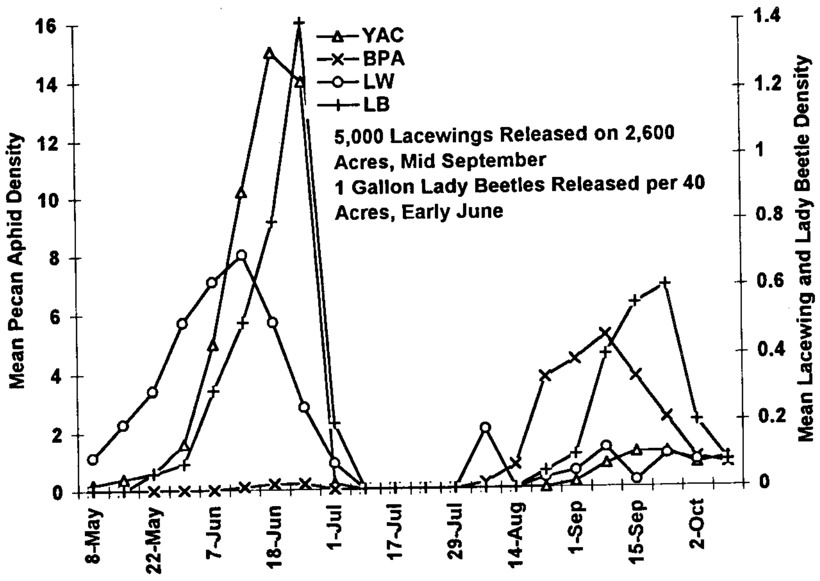


FIG 5. Mean Pecan Aphid (YAC, BPA), Lacewing (LW), and Lady Beetle (LB) Densities per Compound Leaf, Stahmann Farms, Las Cruces, New Mexico, 1991.

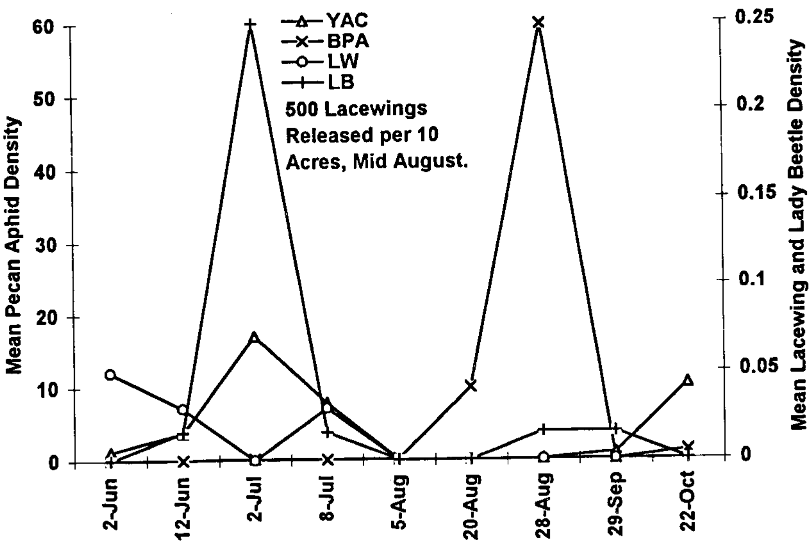


FIG 6. Mean Pecan Aphid (YAC, BPA), Lacewing (LW), and Lady Beetle (LB) Densities per Compound Leaf, Stahmann Farms, Las Cruces, New Mexico, 1992.

aphid density reached 1.20 aphid per compound leaf. The black pecan aphid was relatively quiescent until after 29 July. During the next five weeks, the density of this pest increased to a maximum mean density of 5.20 per compound leaf on 7 September. A linear decline occurred after this date. Again in 1991, lacewing response proceeded the climax of yellow pecan aphids with a peak density of 0.70 lacewings per compound leaf occurring on 11 June. Several smaller fluctuations in lacewing density were documented between 29 July and the end of the study. The growth of the lady beetle population paralleled that of the yellow aphid complex but reached its highest point approximately one week after that of the yellow aphid. The greatest mean density of lady beetles peaked at 1.40 per compound leaf on 26 June 1991. A secondary increase in lady beetle activity was documented on 23 September when the density reached 0.60 per compound leaf. After this date, the density fell, apparently marking the decline of the black pecan aphid population.

During 1992, an increase in yellow pecan aphid pressure appeared to begin on or about 2 June (Fig. 6). A maximum density of 17.02 yellow aphids per compound leaf was reported on 2 July. At the end of the season, on 22 October, the density of yellow aphids per compound leaf increased to 10.32. According to the data, numbers of black pecan aphids remained low until after 5 August. Between 5 and 28 August, an outbreak of black aphids occurred; mean densities of 0.22 black pecan aphids per compound leaf on 5 August exploded to 59.76 on 28 August. Premature defoliation and even death of several trees was attributed to black aphid damage (personal communication, Randy Stahmann). Some other orchards in the area also incurred serious damage. A cool, wet spring coupled with other factors, yet unknown, may have contributed to the fulmination of the black pecan aphid population in 1992. It is uncertain when the lacewing population began to increase in 1992. The greatest mean density of lacewings per compound leaf only reached 0.05 on 2 June. A maximum mean number of 0.25 lady beetles per compound leaf occurred concomitantly with the peak of the yellow aphid complex, on 2 July. A small response between 20 August and 29 September was also observed.

In 1993, the density of the yellow aphid complex increased from 2.22 yellow aphids per compound leaf on 8 May to 8.29 on 30 May. A comparable density of 7.37 documented on 12 September escalated to 14.19 aphids per compound leaf on the last sampling date of the season, 17 October. Black pecan aphid numbers were low in 1993, with mean densities never exceeding 0.65 aphids per compound leaf. This maximum density was reached on 15 August. A secondary peak of activity was reported on 17 October coinciding with peak yellow aphid concentrations. On that date, black aphid numbers reached 0.53 per compound leaf. A maximum density of 0.04 lacewings per compound leaf was not reached until 10 October. Except for two minor fluctuations in mid-June and early July, lacewings seemed virtually non-existent until after 12 September. Their response did not precede the development of the yellow aphid complex in either early or late season. Increases in lacewing numbers did precede the second increase in the black pecan aphid population. The culmination of early season lady beetle response followed that of the yellow aphid complex by about two weeks. On 13 June, the number of lady beetles per compound leaf averaged 0.15. As with the yellow pecan aphid buildup, the greatest number of lady beetles was recorded on 17 October. On that date, lady beetle density reached 0.27 per compound leaf (Fig. 7).

Although slight in some years, the expected bimodal seasonal abundance pattern of the yellow aphid complex was observed in all years for which monthly data were available. In general, lacewings and lady beetles appeared to respond numerically to increases in both spring and fall aphid populations. Frequently, lacewing proliferation precede aphid development by one to two weeks. The increase in predator numbers, typically observed yearly, in early June, may be in response to habitat destruction, as neighboring alfalfa fields

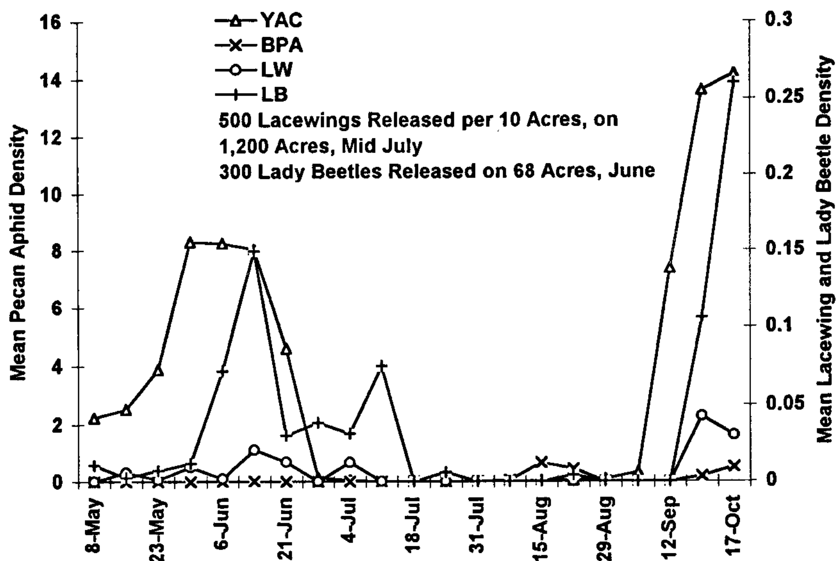


FIG 7. Mean Pecan Aphid (YAC, BPA), Lacewing (LW), and Lady Beetle (LB) Densities per Compound Leaf, Stahmann Farms, Las Cruces, New Mexico, 1993..

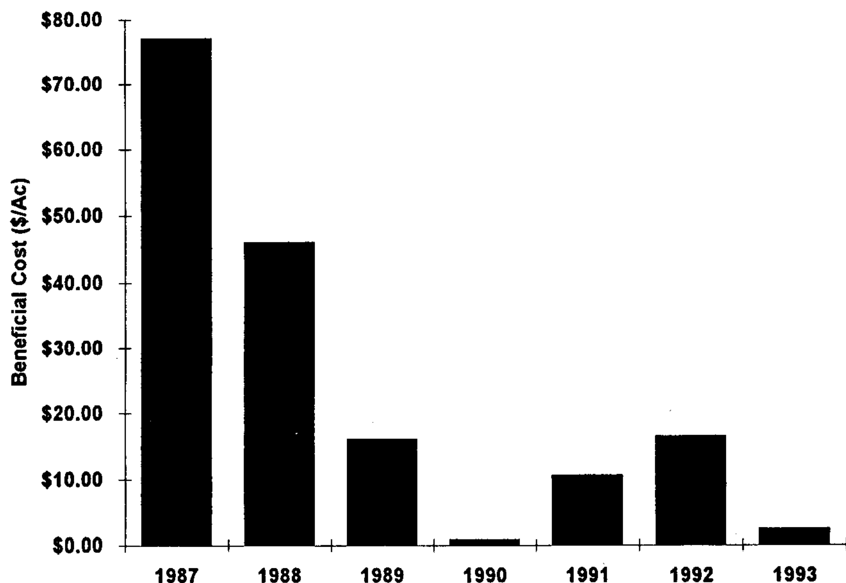


FIG 8. Cost of Beneficial Insects in Dollars per Acre, Stahmann Farms, Las Cruces, New Mexico, 1987-1993.

are commonly mowed in mid May. Lady beetles typically reacted more slowly, but their mean densities were greater than those of lacewings.

Orchard ground cover may play an integral role in pecan biological control by providing nectar for adult lacewings prior to oviposition and alternate prey for lady beetles. These resources along with the provision of refuge may help sustain predators between fluctuations in aphid density.

Predator Releases. Since 1987, aphidophagous insects have been released yearly at Stahmann Farms. Although a recommended predator release rate per acre has yet to be established for deciduous orchards, an optimal ratio of 10 prey per predator has been suggested by Metcalf and Luckman (1982). Seasonal peak predator-prey ratios for all years in which monthly data were collected are summarized in Table 2. Eight of the twelve entries reflect ratios of less than 1:30. Ratios of this magnitude may be acceptable given the other predators and parasitic Hymenoptera associated with an orchard ecosystem. In the fall of 1992, however, this apparent equilibrium was upset as the predator-prey ratio reached 1:4,384. Aside from a mean black aphid density of 59.76 aphids per compound leaf, predator density was also extremely low that fall.

TABLE 2. Bimodal Peak Predator[a]-Prey[b] Ratios Stahmann Farms, Las Cruces, New Mexico 1987-1993.

Year	Season	Predator-Prey Ratio
1987	Spring	1:28
	Fall	1:58
1988[c]	Spring	1:1
	Fall	1:10
1989[d]	———	———
1990	Spring	1:23
	Fall	1:13
1991	Spring	1:7
	Fall	1:8
1992	Spring	1:57
	Fall	1:4384
1993	Spring	1:23
	Fall	1:49

[a] Lacewings and Lady Beetles
[b] Yellow Pecan Aphid Complex and Black Pecan Aphids
[c] Lady Beetle Data Unavailable
[d] No Monthly Data Available

The price of lady beetles and lacewings obtained from Buena Biosystems, the insectory supplying Stahmann Farms, remained constant between 1987 and 1993. During this time, one gallon or approximately 72,000 lady beetles cost $42.00. Lacewing larvae sold for $3.50 per 1000; the charge for adult lacewings was $125.00 for 500. In theory, adult lacewings may be less expensive than larvae when the egg laying potential of about 250 females is considered. The timing of adult releases, however, may be extremely important because mating, oviposition, and egg development must all occur immediately prior to increases in aphid density. The nectar requirement of these adults again underscores the value of a flowering understory in the orchard to assist their survival and fecundity.

Biocontrol is considered to be permanent once the natural enemy has been

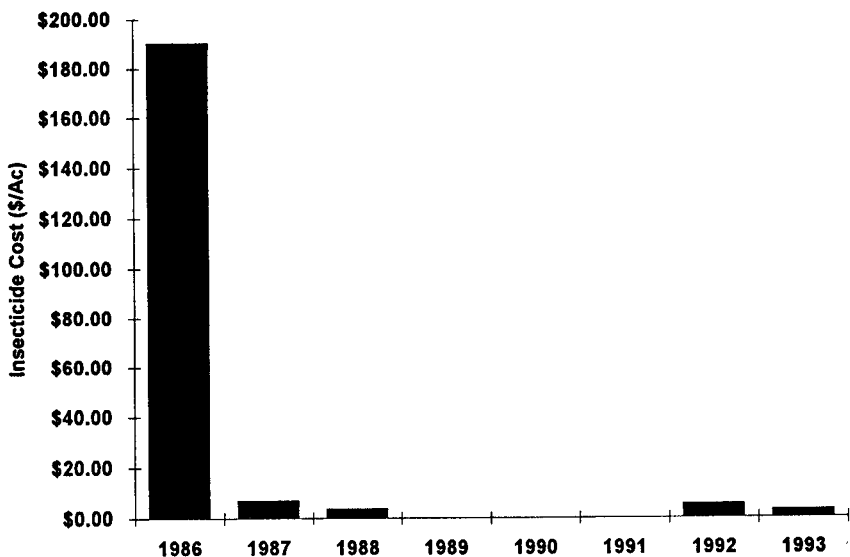


FIG 9. Cost of Insecticides in Dollars per Acre, Stahmann Farms, Las Cruces, New Mexico, 1986-1993.

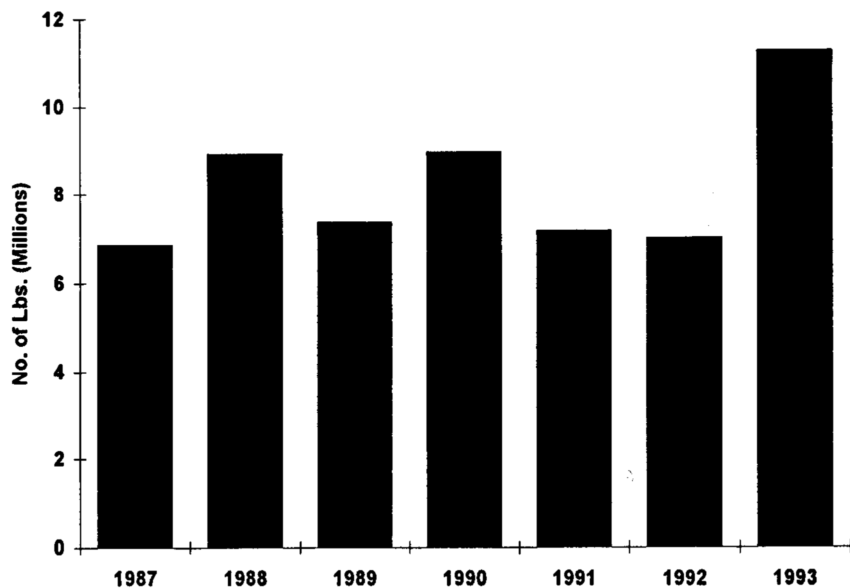


FIG 10. Pecan Nut Yield in Millions of Pounds, Stahmann Farms, Las Cruces, New Mexico, 1987-1993.

established. Therefore, costs associated with predator releases may decrease over time. Fig. 8 illustrates a general decline in expenses between 1987 and 1993. The upward fluctuations observed in 1991 and 1992 reflect increases in the numbers of acres treated and the expense of adult lacewings which were released in 1992. Overall, significant reductions in the cost of predators have been realized by Stahmann Farms. Expenses fell from $77.00 per acre in 1987 to $2.31 per acre in 1993.

Insecticide Applications. Between the late 1940's and late 1970's, the numbers and rates of insecticide applications increased steadily. In the late 1970's, pecan aphid resistance to insecticides was first recognized at Stahmann Farms. By 1986, six different insecticides (Pydrin®, Ammo®, Disyston®, Cymbush®, Thiodan®, and Lorsban®) were each applied one time during the growing season with little effect on the aphid population. The cost of these products in 1986 totaled $190.00 per acre.

In both 1987 and 1988, Thiodan® 3EC was applied one time. The Thiodan® 3EC label limits the total application rate to four quarts per acre per year. Two applications are allowed per year at a rate of two quarts per acre. In 1987, Stahmann Farms used two pints per acre, or 25% of that permitted by law. The amount applied in 1988 was reduced to one pint per acre. The insecticide cost in 1987 was $6.75 per acre and $3.35 in 1988. No insecticides were applied in 1989, 1990, or 1991.

In 1992 and 1993, Lorsban® 4E was used once in each year. Lorsban® may be applied to pecans up to five times a year at a rate of two quarts per acre per application. In 1992, Stahmann Farms used two pints per acre on every other acre, or 10% of that allowed. Because only one-half of the orchard was treated, the overall use rate was further lowered to 5% of the label tolerance. The cost of this product on a per acre basis was $5.25. Both the spray rate and therefore the expense was cut by one-half in 1993. Fig.9. diagrams the insecticide expenditures at Stahmann Farms from 1986 through 1993.

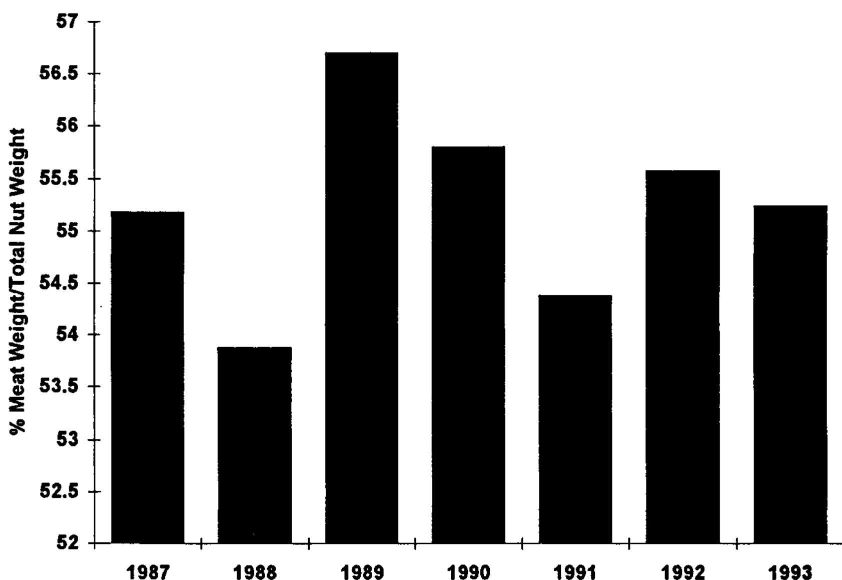


FIG 11. Pecan Nut Quality in Percent Meat Weight to Total Nut Weight, Stahmann Farms, Las Cruces, New Mexico, 1987-1993.

Yield and Quality. The yield data depicted in Fig. 10 reflects the biennial bearing nature of the pecan for years 1987 through 1990. With the exception of 1991, Figs. 10 and 11 document the reciprocal relationship between yield and quality. Both yield and quality may have been adversely affected by a series of night-time freezes occurring between 31 October and 5 November 1991 (National Weather Service, NMSU). Perhaps due to intense black pecan aphid pressure, yield was reduced in 1992. Yield rebounded in 1993 to approximately 11.2 million pounds from about 7 million pounds in both 1991 and 1992. The mean pecan yield between 1987 and 1993 was 8.17 million pounds. During this period, quality, expressed as the percent meat weight to total nut weight, averaged 55.26%.

CONCLUSION

The economic threshold is the point at which action must be taken to prevent economic loss (Metcalf and Luckman 1982). In the Southeast, the threshold for the yellow aphid complex is 10 to 20 per compound leaf and that for the black pecan aphid is one to three aphids per leaf (Harris 1983). Because biocontrol efforts are underway in the Mesilla Valley of New Mexico and because water, sunlight, and fertilizer may not be limited, the action level for these pests is higher. The economic threshold for the yellow aphid complex was arbitrarily set at 38 aphids per leaf. That for the black pecan aphids was arbitrarily set at 12 per compound leaf. The recommendation for black aphids was followed at Stahmann Farms until 1992 when the economic threshold was lowered to eight aphids per leaf. This tolerance was further reduced to three aphids per compound leaf in 1993.

Although predators were released each year at Stahmann Farms, compound leaf counts suggest that lacewing and lady beetle numbers were low compared to those of the pests. Research from greenhouse studies indicate that one lady beetle may consume 200 aphids per day (Tamaki and Weeks 1972). Similar studies documented the aphid consumption rate of *Chrysoperla carnea* at approximately 50 aphids per day (Scopes 1969). Aphid consumption studies in the field have yet to be finalized, however. The monetary investment associated with predator releases fell sharply during this seven-year interval. Expenses went from $77.00 per acre in 1987 to $2.34 per acre in 1993. In general, bimodal peak predator-prey ratios suggest some degree of stability. According to the compound leaf count data, for years where monthly mean densities are available, only in 1992 was the recommended economic threshold for black aphids exceeded.

A significant cash savings and perhaps environmental savings was realized by Stahmann Farms between 1987 and 1993 as insecticide expenditures dropped from $190.00 per acre in 1986 to a total of $2.63 per acre in 1993. This savings could be short-lived however, due to increased pressure from the hickory shuckworm *Cydia caryana* (Fitch) and the pecan nut casebearer *Acrobasis nuxvorella* (Nuenzig). The most valuable control agents of these pests appear to be parasitoids. As with many other natural enemies, adult parasitoids are dependent on nectar, pollen, and perhaps an alternate host for food. This again emphasizes the value of a flowering and aphid susceptible orchard understory.

In general, yearly yields and associated nut quality appears to have been very good. The reduction of both yield and quality observed in 1991 may be attributable to factors such as freezing temperatures but the yield deviation in 1992 appears to be the result of severe black pecan aphid damage.

Astute cultural management practices such as proper tree spacing, cover cropping, and the utilization of varietal resistance are important components of a successful IPM program. Other factors such as good sampling technique, natural enemy releases, limited insecticide inputs, and impeccable record keeping are vital.

ACKNOWLEDGMENT

We sincerely thank Bill and Randy Stahmann for granting NMSU researchers access to Stahmann Farms. We thank Karl Kiser, Gary Beverage, and Johnna Dines for collecting compound leaf count data. We are also grateful to Tracey Carrillo, Esther Frietze, Sherry Tomlinson, and Kurt Schneider for their technical assistance.

LITERATURE CITED

Harris, M. K. 1983. Integrated pest management of pecans. Ann. Rev. Entomol. 28:291-338.

Metcalf, R.L., and W.H. Luckman. 1982. Introduction to Insect Pest Management. John Wiley and Sons, New York. p. 15

Scopes, N.E.A. 1969. The potential of *Chrysopa carnea* as a biological control agent for *Mysus persica* on glasshouse chrysanthemum flowers. Ann. Appl. Biol. 64: 433-439.

Stahmann, R. 1991. Growers experience in using beneficials for aphid control, Proc. Western Pecan Growers Assoc. 25: 40-41.

Tamaki, G., and R.E. Weeks. 1972. Efficiency of three predators, *Geocoris bullatus, Nabis americaferus,* and *Coccinella transversoguttata,* used alone or in combination against three insect prey species, *Mysus persica, Ceramica picta,* and *Mamestra configurata* in a greenhouse study. Environ. Entomol. 1: 258-264.

EFFECT OF RUSSIAN WHEAT APHID[1] ON CONSTITUENT NONSTRUCTURAL CARBOHYDRATE CONTENT IN WHEAT SEEDLINGS

John D. Burd[2], James A. Webster, Gary J. Puterka[3], Robert P. Hoxie[4] and Stanley G. Wellso[5]

USDA-ARS, Plant Science and Water Conservation Laboratory, 1301 N. Western Street, Stillwater, OK 74075

ABSTRACT

The effect of Russian wheat aphid, *Diuraphis noxia* (Mordvilko), on nonstructural carbohydrate constituents and their partitioning patterns for 'TAM W-101' winter wheat, *Triticum aestivum* L., were studied using high-pressure liquid chromatography. Total nonstructural carbohydrate levels of aphid-infested plants were significantly decreased. The greatest reductions were observed in root and leaf tissue which were reduced ca. 58 and 65%, respectively, and resulted primarily from diminished fructan pools. Stem total nonstructural carbohydrate levels were reduced ca. 30%. Nonstructural carbohydrate partitioning patterns of infested plants were altered by Russian wheat aphid feeding, which indicated that the sink-to-source transition of infested leaves was influenced.

INTRODUCTION

It is generally accepted that phloem-feeding aphids create physiological sinks within their host plant by the localized mass removal of assimilates. With few exceptions, the nature of this plant response appears to be governed primarily by the mass removal of phloem constituents (Miles 1989, Wellings et al. 1989). Although recent studies of Russian wheat aphid, *Diuraphis noxia* (Mordvilko), indicate that feeding damage goes beyond the simple removal of phloem assimilates (Riedell 1989, Thomas and Butts 1990, Burd and Burton 1992, Burd and Todd 1992, Storlie et al. 1993), the effect of *D. noxia* on nonstructural carbohydrates should not be overlooked because of the key role they may play in observed plant physiological responses.

The present study was conducted to determine the effect of *D. noxia* on nonstructural carbohydrate constituents and their partitioning patterns in 'TAM W-101' winter wheat, *Triticum aestivum* L.

[1]Homoptera: Aphididae

[2]USDA-ARS, Plant Science Research Laboratory, 1301 N. Western St., Stillwater, OK 74075.

[3]USDA-ARS, Appalachian Fruit Research Laboratory, 45 Wiltshire, Kearnysville, WV 25430.

[4]USDA-ARS, Insect and Weed Control Research Laboratory, Entomology Hall, Purdue University, W. Lafayette, IN 47907.

[5]Current address: 511 Wilope Circle, Bastrop, TX 78602.

MATERIALS AND METHODS

Plant material used was a *D. noxia*-susceptible hard red winter wheat, 'TAM W-101' (Webster 1990). Pregerminated seeds were planted one seed per container (Ray Leach Supercell Cone-Tainer, Stuewe & Sons, Inc., Corvallis, OR), in a fritted clay medium (Absorb-N-Dry, Balcones Minerals, Flatonia, TX). Plants were grown on greenhouse benches under natural light conditions (March), and greenhouse temperatures were maintained at 21±5°C. Plants were watered daily. Beginning 7 days after emergence, they were fertilized biweekly with Peters' Peat-Lite Special (analysis 15-16-17) (Peters Fertilizer Products, Fogelsville, PA). Fourteen days after planting [at growth stage 13 (Zadocks et al. 1974)], ten plants were infested with 20 mature apterous *D. noxia*. The infested plants and ten noninfested control plants were then covered with ventilated clear plastic cages (Starks and Burton 1977). The aphids were allowed to feed and reproduce for 14 days, after which they were removed and counted. The experimental protocol followed a paired-plot design.

Plant damage was qualitatively evaluated by rating the relative amount of chlorosis, leaf rolling, and plant stunting (Burd et al. 1993). The amount of foliar chlorosis was measured using a scale of 1 to 9, where: 1 = plants appear healthy, may have small isolated chlorotic spots; 2 = isolated chlorotic spots prominent; 3 = chlorosis ≤ 15% of total leaf area, chlorotic lesions coalesced; 4 = chlorosis > 15% but ≤ 25% of total leaf area, streaky appearance; 5 = chlorosis > 25% but ≤ 40% of total leaf area, well-defined streaks; 6 = chlorosis > 40% but ≤ 55% of total leaf area; 7 = chlorosis > 55% but ≤ 70% of total leaf area; 8 = chlorosis > 70% but ≤ 85% of total leaf area; 9 = plant death, or damaged beyond recovery. Leaf rolling was rated using a 1 to 3 scale, where: 1 = no leaf rolling; 2 = one or more leaves conduplicately folded; 3 = one or more leaves convolutely rolled. Plant stunting was measured by comparing the height of aphid-treated plants with paired noninfested controls, where: 1 = plant height equal to control; 2 = plant height < 100% but ≥ 75% of control; 3 = plant height < 75% but ≥ 50% of control; 4 = plant height < 50% but ≥ 25% of control; 5 = plant height < 25% of control.

At the time of sampling, numbers of tillers and leaves of each plant were recorded. Next, 3-cm sections of leaf (basal portion of the third leaf of the primary tiller), stem (below the first leaf of the primary tiller), and root (seminal root below hypocotyl) tissue were collected from the same location on all test plants for soluble carbohydrate analysis. Plants were then partitioned into leaf, stem, and root components, oven-dried at 60°C for 24 h, and weighed. Dry weight of the excised sections was included in the determination of total dry weight of each plant component.

The leaf, stem, and root tissues were cut with a scalpel into small fragments and placed separately in 15 ml of 80% ethanol. Samples were then macerated to a pulp with a hand homogenizer and then placed in a shaker bath maintained at 60°C for 4 min. Next, samples were centrifuged at 700 *g* for 5 min; the supernatant was decanted into a 100-ml beaker and held at 60°C. The remaining pellet was extracted again with ethanol, then twice with deionized water (for 15 min), oven dried, and weighed. One-half gram of mixed bead resin was added to the supernatant, stirred for 5 min, filtered, and the solution evaporated to dryness with a rotoevaporator at 60°C. The sample extract was placed in a desiccator at 21°C until dried, usually after 48 h, and then weighed (dry weight was then calculated as the extract plus the pulp weight).

Nonstructural carbohydrates were separated by high-pressure liquid chromatography (HPLC) with a Bio-Rad HPX-87P Column (Bio-Rad Laboratories, Richmond, CA) heated to 85°C, with a mobile phase of degassed glass-distilled water at a flow rate of 0.4 ml per minute. Carbohydrates from the plant extract were detected by refractive index and quantified by co-chromatography with carbohydrates referenced from internal standards of

sucrose, glucose, and fructose. The amount of fructan was calculated by comparing the area under the curve to the sucrose standard. Retention times in minutes were fructan, 10.75; sucrose, 14.75; glucose, 17.5; and fructose, 23.5. Peak area was determined by triangulation.

RESULTS AND DISCUSSION

Fourteen days after infestation, *D. noxia* populations averaged 306.5 ± 31.4 aphids per plant and were aggregated primarily on the new growth. Chlorosis ratings ranged from 4.0 to 7.0, with a mean of 4.9 ± 0.35, indicating that all *D. noxia*-treated plants sustained significant damage. All infested plants had leaf-roll ratings of 3 (rolled convolutely) where the most recently developed leaves were rolled and the subsequent developing leaves were trapped. Plant stunting was not pronounced, and ratings for stunting ranged from 1 to 2, with a mean of 1.9 ± 0.1. Root, stem, and leaf biomass were reduced significantly by *D. noxia*; when compared with noninfested controls, mean dry weight for each plant partition was reduced by ca. 70% (Table 1).

TABLE 1. Summary of Mean Values for Plant Parameters Measured on *Diuraphis noxia* Infested and Noninfested 'TAM W-101' Winter Wheat.

TAM W-101	No. of tillers	No. of leaves	Root dry wt. (g)	Stem dry wt. (g)	Leaf dry wt. (g)	Total dry wt. (g)
Control	2.9*	8.7*	0.867*	0.376*	1.221*	2.464*
Infested	1.8	4.9	0.267	0.100	0.332	0.699

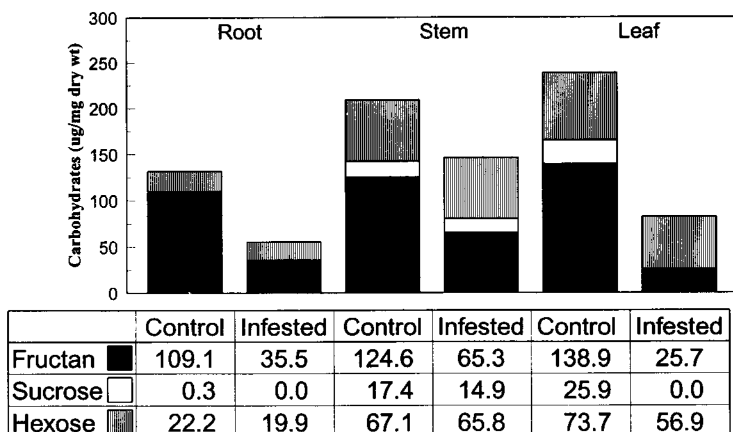
*, $P < 0.05$ (t test).

Whole-plant total nonstructural carbohydrate (TNC) profiles are presented in Table 2. Overall, percentage reduction of TNC was ca. 50% and was greatest for the root and leaf components which were reduced ca. 58 and 65%, respectively, while stem TNC was reduced ca. 30%. Changes in relative TNC partitioning patterns followed this trend having a 3 and 12% decrease in percentage TNC for the root and leaf partitions, respectively, while the percentage of stem TNC increased by 15%.

TABLE 2. Total Nonstructural Carbohydrate (TNC) Partitioning Patterns for *Diuraphis noxia* Infested and Noninfested 'TAM W-101' Winter Wheat.

	Control		Infested			
Plant component	TNC	% Total	TNC	% Total	% Change	% Reduction
Root	131.6	23	55.4	20	-3	57.9
Stem	209.1	36	146.0	51	+15	30.2
Leaf	238.5	41	82.6	29	-12	65.4
Total	579.2			284		51.2

Measurements of constituent nonstructural carbohydrate profiles for each plant partition are shown in Fig. 1. Fructan was the most abundant nonstructural carbohydrate (NSC) in each of the plant partitions of the noninfested controls. When compared with the control plants, there was a substantial reduction in the fructan pools for all partitions of the *D. noxia*-infested plants. Sucrose content of the control and *D. noxia*-infested plants were similar for the root and stem partitions, however, sucrose was absent in the leaf samples from the infested plants. Hexose sugars (glucose + fructose) were present in all plant partitions, and although the amount was generally greater for the control plants, hexose levels did not differ significantly between respective partition samples of the *D. noxia*-infested plants.



	Control	Infested	Control	Infested	Control	Infested
Fructan	109.1	35.5	124.6	65.3	138.9	25.7
Sucrose	0.3	0.0	17.4	14.9	25.9	0.0
Hexose	22.2	19.9	67.1	65.8	73.7	56.9

FIG. 1. Nonstructural carbohydrate profiles for *Diuraphis noxia* infested and noninfested 'TAM W-101' winter wheat.

D. noxia infestation greatly reduced TNC accumulation and altered the NSC partitioning pattern between root, stem, and leaf tissues of 'TAM W-101' wheat. The greatest reductions in NSC were observed in root and leaf tissue and resulted primarily from diminished fructan levels. A similar response was observed for mature cold-adapted winter wheat cultivars 'Froid' and 'Brawny', where fructan levels of crown tissue were significantly decreased following *D. noxia* infestation (Storlie et al. 1993). The reduction in fructan accumulation is significant because of the important role it plays in several physiological processes. Fructans have been associated with the conferment of winterhardiness and cell cryoprotection in cereals (Olien and Lester 1985, Suzuki and Nass 1988, Olien 1992). In addition, fructans constitute the primary energy reserve during vegetative growth, particularly during the initiation of spring growth (Pollock 1986), and can also contribute significantly to grain filling (Schnyder 1993).

The concomitant loss of sucrose in the leaves of the infested plants is consistent with the observed whole-plant decline in fructan levels. Fructan accumulation is a function of the vacuolar enzyme sucrose:sucrose fructosyl transferase (EC 2.4.1.99) whose activity is governed by sucrose concentration (Dubois et al. 1990). Moreover, fructan accumulation is generally increased during periods in which the plant is subjected to stresses that have a

more adverse affect on photosynthate utilization (i.e., plant growth) than on photosynthetic rates (Pontis and Campillo 1985, Jeong and Housley 1990, Olien 1992). *D. noxia* did reduce the growth and development rates of 'TAM W-101' wheat; however, the absence of detectable levels of sucrose in *D. noxia*-infested leaves suggests that the significant reduction of fructan levels results from decrease of the sucrose substrate. The depletion of leaf sucrose could be attributed to direct removal of photosynthates, a decline in chlorophyll content, the loss of photosynthetic function, or a combination of these factors (Burd and Todd 1992).

Young leaves are strong sinks for phloem mobile mineral nutrients, amino compounds, and carbohydrates (via solute mass flow) and remain so until the sink strength declines during leaf maturation. The transition from sink to source corresponds with development of a phloem loading capacity (assimilate export) that typically occurs when about one-half of the leaf's net photosynthetic capacity has been reached (Giaquinta 1983). Russian wheat aphids aggregate and feed primarily on new growth of their host and induce formation of a tubular-shaped leaf gall that is produced by preventing newly formed leaves from unfolding (Burd and Burton 1992). Removal of photosynthates (imported assimilates) combined with chlorophyll destruction at the feeding site could alter the sink-to-source transition of the rolled leaf, thereby providing a protracted assimilate sink status for the *D. noxia* leaf-gall niche. Our data indicate that *D. noxia* can alter assimilate partitioning patterns and are in agreement with this concept of *D. noxia* regulation of sink-source relationships.

ACKNOWLEDGMENT

We gratefully acknowledge Dallas Tomlinson and Mike Bell for technical assistance; Ruth Treat for editorial assistance; and Drs. Ray Eikenbary and Paul Richardson for helpful reviews of the initial draft of the manuscript. This article reports the results of research only, and mention of a proprietary product does not constitute endorsement or recommendation for its use by the USDA.

LITERATURE CITED

Burd, J. D., and R. L. Burton. 1992. Characterization of plant damage caused by Russian wheat aphid (Homoptera: Aphididae). J. Econ. Entomol. 85: 2017-2022.

Burd, J. D., R. L. Burton, and J.A. Webster. 1993. Evaluation of Russian wheat aphid (Homoptera: Aphididae) damage on resistant and susceptible hosts with comparisons of damage ratings to quantitative plant measurements. J. Econ. Entomol. 86: 974-980.

Burd, J. D., and G. W. Todd. 1992. Total chlorophyll and chlorophyll fluorescence profiles of Russian wheat aphid resistant and susceptible wheat, pp. 101-108. *In* W.P. Morrison, [comp] Proceedings of the fifth Russian wheat aphid conference. Great Plains Agric. Counc. Pub. 142.

Dubois, D., M. Winzeler, and J. Nosberger. 1990. Fructan accumulation and sucrose:sucrose fructosyltransferase activity in stems of spring wheat genotypes. Crop Sci. 30: 315-319.

Giaquinta, R. T. 1983. Phloem loading of sucrose. Ann. Rev. Plant Physiol. 34: 347-387.

Jeong, B. R., and T. L. Housley. 1990. Fructan metabolism in wheat in alternating warm and cold temperatures. Plant Physiol. 93: 902-906.

Miles, P. W. 1989. The responses of plants to the feeding of aphidoidea: principles, pp. 1-47. *In* A. K. Minks and P. Harrewijn, [eds.] Aphids: their biology, natural enemies and control, vol. C. Elsevier, New York.

Olien, R. 1992. Protective modification of freeze stress in plant tissue. Thermochimica Acta 212: 255-260.

Olien, C. R., and G. E. Lester. 1985. Freeze-induced changes in soluble carbohydrates of rye. Crop Sci. 25: 288-290.

Pollock, C. J. 1986. Fructans and the metabolism of sucrose in vascular plants. New Phytol. 104: 1-24.

Pontis, H. G., and E. Del Campillo. 1985. Fructans, pp. 205-227. *In* P. M. Dey and R. A. Dixon [eds] Biochemistry of storage carbohydrates in green plants. Academic, New York.

Riedell, W. E. 1989. Effects of Russian aphid infestation on barley plant response to drought stress. Physiol. Plant. 77: 587-592.

Schnyder, H. 1993. The role of carbohydrate storage and redistribution in the source-sink relations of wheat and barley during grain filling - a review. New Phytol. 123: 233-245.

Starks, K. J., and R. L. Burton. 1977. Greenbugs: determining biotypes, culturing, and screening for plant resistance, with notes on rearing parasitoids. USDA Tech. Bull. 1556.

Storlie, E. W., L. E. Talbert, G. A. Taylor, H. A. Ferguson, and J. H. Brown. 1993. Effects of the Russian wheat aphid on osmotic potential and fructan content of winter wheat seedlings. Euphytica 65: 9-14.

Suzuki, M., and H. G. Nass. 1988. Fructan in winter wheat, triticale, and fall rye cultivars of varying cold hardiness. Can. J. Bot. 66: 1723-1728.

Thomas, J. B., and R. A. Butts. 1990. Effect of Russian wheat aphid on cold hardiness and winterkill of overwintering winter wheat. Can. J. Plant Sci. 70: 1033-1041.

Webster, J. A. 1990. Resistance in triticale to the Russian wheat aphid (Homoptera: Aphididae). J. Econ. Entomol. 83: 1091-1095.

Wellings, P. W., S. A. Ward, A. F. G. Dixon, and R. Rabbinge. 1989. Crop Loss Assessment, pp. 49-64. *In* A. K. Minks and P. Harrewijn [eds] Aphids: their biology, natural enemies and control, vol. C. Elsevier, New York.

Zadoks, J. C., T. T. Chang, and C. F. Konzak. 1974. A decimal code for the growth stages of cereals. Weed Res. 14:415-421.

COMPARISON OF DESICCATION RATES AMONG THREE SPECIES OF FIRE ANTS[1]

Paul D. Munroe, Harlan G. Thorvilson, and Sherman A. Phillips, Jr.

Department of Plant and Soil Science, Texas Tech University, Lubbock, Texas 79409-2122

ABSTRACT

Effects of desiccation on survival times and percentage weight loss of the fire ant, *Solenopsis geminata* (F.), the red imported fire ant, *Solenopsis invicta* Buren, and the southern fire ant, *Solenopsis xyloni* McCook were investigated. LT_{50} and percentage weight loss were determined for three worker sizes (minor, media, and major workers) and alate males and females of the three species. *Solenopsis invicta* had smaller LT_{50}-values than did *S. geminata* and *S. xyloni*. Also, minor workers of *S. invicta* had greater percentage weight loss after 10 h of desiccation than did *S. xyloni* minor workers. Major workers of *S. invicta* lost a higher percentage of weight than did major workers of *S. xyloni* after both 8 and 40 h of desiccation. *Solenopsis invicta* female alates were as desiccation resistant as were *S. xyloni* female alates. Predictions of range expansion of *S. invicta* to the west of its present range are discussed.

INTRODUCTION

The red imported fire ant, *Solenopsis invicta* Buren, was accidentally introduced into the United States between 1933 and 1945 and first established near Mobile, Ala. This species may have been imported from the headwaters of the Paraguay River in Brazil (Buren et al. 1974). Since its introduction, the red imported fire ant has spread throughout the southern and southeastern US and now occupies about 93-101 million ha (Lofgren 1986).

The northern edge of this species' range has changed little since 1965 (Pimm and Bartell 1980), and considerable mortality has been reported at the northern limits (Morrill et al. 1978). Most of the recent range expansion has been westward into Texas. This species was first detected in Texas in 1953 (Culpepper 1953); it spread to 100 eastern counties by 1977 (Summerlin and Green 1977) and infested about 290,500 square km in 128 counties by 1988 (Cokendolpher and Phillips 1989).

Because of similarities in ecology to two native species, the fire ant, *Solenopsis geminata* (F.) and the southern fire ant, *Solenopsis xyloni* McCook, the red imported fire ant may eventually establish in areas where native fire ants are found (Hung et al. 1977). Presently, *S. xyloni* occurs from South Carolina and Florida to Oklahoma and from southeastern Colorado to California (Gregg 1963, Young and Howell 1964, Krombein et al. 1979). The species occurs throughout Texas, is widespread and abundant in western Texas, and is the only native fire ant in the US that occurs north of the -17.8° C January isotherm (Francke et al. 1983). Because *S. xyloni* occurs in humid-to-xeric regions in Texas, humidity may not be an important factor in its distribution (Moody et al. 1981).

The range of *S. geminata* in the US is from South Carolina to Florida and west to Texas (Krombein et al. 1979). In Texas, this species has been collected in 78 southeastern counties (Francke et al. 1983) and in only six counties west of the 100th meridian (Moody et al. 1981). Apparently this species lacks the broad moisture tolerances of *S. xyloni*, and

[1] Hymenoptera: Formicidae: *Solenopsis*

moisture may be the limiting factor that has prevented *S. geminata* from spreading farther westward in the US (Moody et al. 1981).

Moisture availability and relative humidity may be limiting factors for red imported fire ant distribution in the US (Pimm and Bartell 1980). Worker ants of four *Solenopsis* spp. found in Texas did not exhibit preferences for relative humidities between 50 and 100% in a laboratory chamber unless brood was introduced (Potts et al. 1984). Then, attending workers and brood aggregated in chambers of highest relative humidities.

Percentage water loss by major and minor *S. invicta* workers at 30°C has a curvilinear response to increasing time, but cumulative percentage mortality has a linear relationship to time (Mack et al. 1988). In this study, mean LT_{50} was 10.7 h, and 100% mortality occurred in 24 h. They concluded that *S. invicta* foragers cannot survive extended periods in xeric environments without access to water.

Mortality caused by dehydration was directly proportional to relative humidity and temperature in a laboratory study of worker castes of four *Solenopsis* spp. (Braulick et al. 1988). No significant differences in LT_{50}'s were detected among minor workers of *S. invicta, S. geminata, S. xyloni*, and *S. aurea* Wheeler. Major workers of *S. geminata* survived longer than major workers of the other three species, probably because *S. geminata* major workers were significantly larger. However, when worker size was taken into account (Hood and Tschinkel 1990), *S. invicta* had LT_{50}'s approximately 1.3 times those of *S. geminata*. Braulick et al. (1988) concluded that the red imported fire ant should be able to survive in areas already inhabited by *S. aurea* and *S. xyloni*. However, alate fire ants were not tested, nor was desiccation based on body weight compared among species.

Although the westward spread of the red imported fire ant may stop in arid western Texas if unaided by human transport (Hung and Vinson 1978), additional studies to compare rates of *Solenopsis* spp. dehydration are warranted. Therefore, the objective of the research reported herein was to compare percentage weight losses and LT_{50}'s of minor, media, and major worker size classes and alates of *S. invicta, S. geminata*, and *S. xyloni* exposed to low relative humidity in the laboratory.

MATERIALS AND METHODS

Colonies of three fire ant species were collected from various sites in Texas. *Solenopsis xyloni* was collected from Wichita Falls (Wichita Co.), *S. geminata* was collected from Junction (Kimble Co.), and *S. invicta* was collected from Kerrville (Kerr Co.), Abilene (Taylor Co.), and Lubbock (Lubbock Co.). All colonies were collected between September 1986 and January 1987 in early morning or late evening hours to obtain the majority of worker ants in each colony.

Colonies were separated from soil following the methods of Banks et al. (1981). A styrene sandwich box (3.5 x 11.0 x 11.0 cm) was prepared to house each colony. An inverted, styrene petri dish (90 mm diameter) containing a moistened sponge was placed in each box, and holes (3 mm diameter) were melted in each petri dish with a soldering iron. The holes allowed moisture from a sponge to be absorbed by Castone dental plaster (Dentsply Internat., Inc., York, PA 17405) that covered each dish. A tight-fitting lid on each sandwich box aided in maintaining near 100% relative humidity in the colony houses. A 3-mm diameter hole, through which ants could pass onto a foraging tray (7 x 44 x 56 cm; Panel Controls Corp., Detroit, MI), was melted in a side of each sandwich box. Generic canned dog food and a 1:10 solution of honey and water were supplied once daily. Frozen cockroaches were supplied once each week, and water was available at all times. Colonies were maintained at 28 ±5° C and subjected to a 12:12 (L:D) photophase. All ant colonies were maintained in the laboratory for a minimum of 2 wk prior to testing.

Head capsule widths of ants were measured using a binocular microscope equipped with an ocular micrometer. Body surface areas of ants were calculated by measuring the length and diameter of the head, thorax, and gaster of ten ants of each caste of each species using an ocular micrometer. These measurements were converted into surface area values, and mean surface areas of each caste of each species were determined.

Ants were subjected to desiccation conditions in modified plastic 00 Beem capsules (Ted Pella Inc., Redding, CA). Techniques were similar to those of North (1991) when he studied *Formica rufa* L., a European wood ant. Each capsule was punctured 50 times with a no. 5 insect pin to permit an equilibrium of relative humidity between the inside of the capsule

and the surrounding atmosphere while still retaining the ants. Desiccation chambers were constructed by placing 250 g of the drying agent Drierite ($CaSO_4$; W. A. Hammond Drierite Co., Xenia, Ohio) into each of several 355-ml, glass canning jars with tight-fitting lids. To prevent direct contact of Beem capsules with the Drierite, filter paper was placed above the drying agent. A control chamber, in which greater than 80% relative humidity was maintained, was established by placing distilled water in a glass desiccator jar (25 cm ID).

Three worker size classes, as defined by head capsule width (Table 1), and male and female alates of each species were subjected to the experimental procedures. Ten worker ants of the same size class and species were randomly selected from colonies and placed into each of two modified Beem capsules. Because of their larger size, only two male or two female alates were placed in each Beem capsule. The combined weight of each Beem capsule and the ants contained therein was measured prior to treatment using a Cahn 29 automatic electrobalance (accuracy = 0.0012%; Cahn Instruments, Inc., Cerritos, CA.). One capsule of each caste and species was randomly selected for the desiccation treatment, and the second capsule of ants was subjected to control conditions. Ten replications of the three worker size classes and the alate reproductives were performed for each species in this study. The control chamber (> 80% relative humidity) and the desiccation chambers (approximately 0% relative humidity) were kept in total darkness during each trial.

TABLE 1. Mean Head Capsule Widths of Three Worker Sizes of Three *Solenopsis* spp.

Taxon	Mean Head Capsule Widths (mm) ± SD[a]		
	Minor	Media	Major
S. geminata	0.45±0.02	0.77±0.08	1.41±0.09
S. invicta	0.40±0.03	0.56±0.05	0.82±0.03
S. xyloni	0.43±0.02	0.63±0.04	0.87±0.09

[a] Mean of 100 specimens of each size class and species.

To measure treatment effects, ants were weighed after time intervals that depended on the caste being tested: minor workers were recorded at 2-h intervals, media workers at 3-h intervals, major workers at 8-h intervals, and alate males and alate females at 12-h intervals. Measurements of control ant weights were taken after the same time intervals as were ants subjected to desiccation treatments. Air temperature in the laboratory ranged between 18-26° C during data collection; however in each trial, temperatures were constant, and weights of ants in each treatment were concurrently measured. After each weighing period, the treatment capsules were placed into desiccation chambers with fresh Drierite, and control-treatment capsules were returned to the control chamber.

The weighing procedure was halted when an LT_{50} [after application of Abbott's (1925) formula] was reached for each caste and species. Ants were considered dead when no locomotor response was elicited by gentle rolling of capsules or by prodding the ants with forceps.

Probit analysis (Joyner 1985) was applied to data using treatment time intervals and numbers of dead ants observed during each time interval. Estimated LT_{50} values were obtained from this analysis for each replication. The mean times required to reach an LT_{50} were tested to determine the interaction of caste upon species response and vice versa (Joyner 1985). A protected least significant difference (LSD) mean separation procedure was used to evaluate differences between LT_{50} values obtained from the probit analysis.

Initial weights were included in multiple linear regression equations as variables for ant size because body weight was probably a more accurate measurement than was body surface area. Also, surface areas and initial body weights within a species and caste were highly correlated (r > 0.9, product-moment correlation coefficient; Sokal and Rohlf 1981). Time and initial weights were the independent variables used in multiple linear regression analysis, and percentage weight loss was the dependent variable. Percentage weight loss values obtained from the multiple linear regression were used in multiple covariance analysis and the

Johnson-Neyman Technique (Huitema 1980) to determine differences ($P < 0.05$) among species.

RESULTS AND DISCUSSION

Based on initial weights of the three species, *S. geminata* workers were heaviest (Table 2). *Solenopsis invicta* and *S. xyloni* were similar in weight.

TABLE 2. Mean Initial Weights of Castes of Three *Solenopsis* spp.

Caste[b]	Mean Weight (mg) ± SD[a]		
	S. geminata	*S. invicta*	*S. xyloni*
Minor	4.08 ± 0.05 a	2.98 ± 0.07 c	3.25 ± 0.04 b
Media	17.54 ± 0.57 a	10.51 ± 0.34 b	11.22 ± 0.29 b
Major	63.03 ± 1.19 a	28.38 ± 0.87 b	29.90 ± 0.76 b
Male alate	17.06 ± 0.20 a	13.84 ± 0.09 b	10.44 ± 0.08 c
Female alate	28.77 ± 0.24 a	18.94 ± 0.05 b	18.45 ± 0.23 b

[a] Mean weight (n = 10) of minor, media, and major workers; mean weight (n = 2) of male or female alates.
[b] Means followed by the same letter within worker sizes are not significantly different (protected LSD; df = 9, $P > 0.05$).

Because no worker size and species interactions were detected ($F = 0.69$; df = 8,135; $P = 0.70$), the effects of worker size were considered independent of the effects of species on LT_{50} values. Therefore, comparisons of LT_{50} values were made among species and among worker sizes, and differences were detected among all sizes (Table 3). Media workers survived desiccation approximately twice as long (31.6 h) as did minor workers (15.3 h); whereas, the mean LT_{50} of major workers was more than 52 h. Female alates were significantly less susceptible to desiccation than were male alates. Among species, *S. invicta* reached an LT_{50} significantly faster than did either *S. geminata* or *S. xyloni*.

TABLE 3. Mean LT_{50} (h) of Each Caste for Three *Solenopsis* spp. Subjected to Desiccation.

Caste	Mean LT_{50} (h) (±SD)			Caste means[a]
	S. geminata	*S. invicta*	*S. xyloni*	
Minor	15.4 (±4.4)	12.5 (±3.6)	18.0 (±5.9)	15.3 a
Media	35.9 (±11.5)	27.4 (±7.7)	31.5 (±5.2)	31.6 b
Major	58.6 (±16.1)	46.8 (±13.0)	51.0 (±7.8)	52.1 c
Male alate	84.0 (±17.0)	70.8 (±8.9)	87.6 (±21.2)	80.8 d
Female alate	114.0 (±22.8)	102.0 (±16.3)	103.2 (±31.6)	106.4 e
Species means[b]	61.6 a	51.9 b	58.3 a	

[a] Caste means followed by different letters are significantly different (protected LSD; df = 4, $P > 0.05$).
[b] Species means followed by the same letter are not significantly different (protected LSD; df = 2, $P > 0.05$).

Differences among percentage weight loss values indicated that minor workers of *S. geminata* lost more weight after 2 h than did *S. invicta* (Table 4). After 10 h, *S. geminata* and *S. invicta* lost higher percentages of weight than did *S. xyloni*. Apparently, the rate of

weight loss by *S. invicta* minors increased faster through time than did the rates of weight loss by *S. geminata* and *S. xyloni*.

Percentage weight loss values were not significantly different among the media size class of fire ants. *Solenopsis invicta* major workers lost more weight than did *S. xyloni* after both 8 and 40 h of desiccation treatment. No differences in weight loss values were detected among male alates of the three fire ant species. Also, no differences were detected among female alates of *S. invicta* and *S. xyloni* (Table 4).

TABLE 4. Percentage Weight Loss of Three *Solenopsis* spp. at the Minimum and Maximum Treatment Exposure Times.

Caste	Exposure Time (h)	Percentage Weight Loss[ab]		
		S. geminata	*S. invicta*	*S. xyloni*
Minor workers	2	8.6 a	5.5 b	7.1 ab
	10	22.3 a	20.8 a	16.7 b
Media workers	3	7.9 a	5.8 a	3.9 a
	15	16.4 a	15.0 a	13.6 a
Major workers	8	--	10.6 a	6.8 b
	40	--	29.4 a	24.7 b
Male alates	12	5.2 a	7.6 a	11.0 a
	60	22.9 a	27.9 a	29.1 a
Female alates	12	--	6.4 a	6.4 a
	60	--	19.9 a	18.9 a

[a] Percentage weight loss values followed by the same letter within a row (among species) are not significantly different (Multiple Covariance Analysis and the Johnson-Neyman Technique; df = 1,14, $P > 0.05$). Mean initial weights: (n = 10 per replication), minors = 3.43 mg, medias = 13.09 mg, majors = 29.14 mg; (n = 2 per replication), male alates = 13.78 mg, female alates = 18.70 mg.

[b] If the range of initial weights exceeded the ranges of the other two species, percentage weight loss values are not presented and initial weights are not included in the mean.

Because of greater surface area-to-weight ratios, workers that weighed less were expected to desiccate at faster rates than larger workers. In general, our results support the hypothesis and are in agreement with Edney (1977) who stated that the amount of water loss in terms of total body water initially present was greater in small animals than in large animals. In addition, Braulick et al. (1988) confirmed that dehydration in fire ants was size-dependent. However, surface and volume relationships in ants may not fully explain the effects of size on LT_{50}'s (Hood and Tschinkel 1990), and cuticular waterproofing lipids may play a crucial role in desiccation resistance.

Because alate fire ants exit the colony during nuptial flights when the relative humidity is greater than 80% (Rhoades and Davis 1967) or during flooding (Lofgren et al. 1975), they are less exposed to desiccating conditions than are worker ants. However, dry conditions after nuptial flights may limit colony establishment by newly mated queens. Elzen (1986) found that the percentage water loss by *S. invicta* female alates was less than that of male alates, and our study showed that desiccation rates of *S. invicta* and *S. xyloni* female alates were not significantly different. Therefore, *S. invicta* queens may survive in the present range of *S. xyloni*, but colonies may not develop successfully because *S. invicta* workers are more susceptible to desiccation than are *S. xyloni* workers.

Major workers of *S. invicta* may have greater foraging responsibilities for the colony during periods of high temperatures. As soil surface temperatures increased from 22 to 36° C, the average weight of *S. invicta* foragers collected in pitfall traps increased 25-30% in Porter and Tschinkel's (1987) study. *Solenopsis invicta* major workers, with smaller surface-to-mass ratio, foraged at higher temperatures than did smaller workers and were less prone to desiccation. Hood and Tschinkel (1990) found that *S. invicta* foragers were more desiccation resistant than were *S. geminata* workers and may forage for longer periods in areas of high insolation. However, our results indicated that *S. invicta* majors were less resistant to desiccation than were *S. xyloni* major workers and had a smaller LT_{50} than did *S. geminata* and *S. xyloni*. *Solenopsis invicta* foragers may have difficulty surviving drier environmental conditions than those that are common in its present range and may not be able to fully exploit the present range of *S. xyloni*.

Although extremes of temperature and low relative humidity may limit the range of *S. invicta* (Hung and Vinson 1978, Moody et al. 1981, Pimm and Bartell 1980), its range has increased each year. Most of the new infestations can be attributed to nursery stock movement, and isolated infestations west of the 100th meridian in Texas are thus far limited primarily to urban habitats (authors' personal observations) including those in Wichita Falls, Lubbock, San Angelo, and Midland, Texas. Also, introductions to Mesa, Arizona, and Santa Barbara, California, have been reported.

Even in marginal regions, *S. invicta* colonies may survive in favorable microhabitats that provide adequate moisture (as in irrigated lawns) and moderation of soil temperatures by proximity to natural or man-made structures (Thorvilson et al. 1992). Also, behavioral adaptations may contribute to fire ant success. For example, fire ants may forage at night to avoid extreme solar insolation (Francke and Cokendolpher 1986), and daily, vertical movement patterns of eggs, larvae, pupae, and adult fire ants within mounds (Pinson 1980) may optimize and regulate temperature environments of the ants. Therefore, the effect of ant behavioral modification on range expansion, possibly resulting from adverse environmental conditions, remains unknown.

ACKNOWLEDGMENT

We thank D. Wester for his valuable assistance with statistical analysis. We also thank all of our colleagues who aided in this research, especially J. C. Cokendolpher for his assistance with experimental procedures. We also thank P. Drury and R. Beiriger for their assistance with data collection. This is contribution no. T-4-377 College of Agricultural Sciences and Natural Resources, Texas Tech University. This research was supported Interagency Agreement # IAC (86-87) 0800, Texas Department of Agriculture, and by Texas State Line Item for Red Imported Fire Ant Research.

LITERATURE CITED

Abbott, W. S. 1925. Method of computing the effectiveness of an insecticide. J. Econ. Entomol. 18: 265-267.

Banks, W. A., C. S. Lofgren, D. P. Jouvenaz, C. E. Stringer, P. M. Bishop, D. F. Williams, D. P. Wojcik, and B. M. Glancey. 1981. Techniques for collecting, rearing, and handling imported fire ants. USDA Science and Ed. Admin. Adv. in Agric. Tech. AAT-S-21.

Braulick, L. S., J. C. Cokendolpher, and W. P. Morrison. 1988. Effect of acute exposure to relative humidity and temperature on four species of fire ants (*Solenopsis*: Formicidae: Hymenoptera). Tex. J. Sci. 40: 331-340.

Buren, W. F., G. E. Allen, W. H. Whitcomb, F. E. Lennartz, and R. N. Williams. 1974. Zoogeography of the imported fire ants. J. N. Y. Entomol. Soc. 82: 113-124.

Cokendolpher, J. C., and S. A. Phillips, Jr. 1989. Rate of spread of the red imported fire ant, *Solenopsis invicta* (Hymenoptera: Formicidae), in Texas. Southwest. Nat. 34: 443-449.

Culpepper, G. H. 1953. Status of the imported fire ant in the southern states in July 1953. USDA, Bureau Entomol. Plant Quar. Special Report E-867.

Edney, E. B. 1977. Water balance in land arthropods. Springer-Verlag. New York. 282 pp.

Elzen, G. W. 1986. Oxygen consumption and water loss in the imported fire ant *Solenopsis invicta* Buren. Comp. Biochem. Physiol. 84A: 13-17.
Francke, O. F., and J. C. Cokenodolpher. 1986. Temperature tolerances of the red imported fire ant. pp. 104-113. *In* Lofgren, C. S., and R. K. Vander Meer (eds.) Fire ants and leaf-cutting ants. Biology and management. Westview Press, Boulder. 435 pp.
Francke, O. F., J. C. Cokendolpher, A. H. Horton, S. A. Phillips, Jr., and L. R. Potts. 1983. Distribution of fire ants in Texas. Southwest. Entomol. 8: 32-41.
Gregg, R. E. 1963. The ants of Colorado. Univ. Colorado Press. Boulder. 792 pp.
Hood, W. G., and W. R. Tschinkel. 1990. Desiccation resistance in arboreal and terrestrial ants. Physiol. Entomol. 15: 23-35.
Huitema, B. E. 1980. The analysis of covariance and alternatives. John Wiley and Sons, New York. 445 pp.
Hung, A. C. F., and S. B. Vinson. 1978. Factors affecting the distribution of fire ants in Texas. Southwest. Nat. 23: 205-214.
Hung, A. C. F., M. R. Barlin, and S. B. Vinson. 1977. Identification, distribution and biology of fire ants in Texas. Misc. Publ., Texas Agric. Exp. Sta., B-1185: 1-24.
Joyner, S. P. (ed.) 1985. SAS User's Guide: Statistics, Version 5 Ed. Statistical Analysis System Institute Inc., Cary, N. C., U.S.A.
Krombein, K. V., P. D. Hurd, Jr., D. R. Smith, and B. D. Burks. 1979. Catalog of Hymenoptera north of Mexico. Smithsonian Inst. Press. Washington, D.C. 2: 1199-2209.
Lofgren, C. S. 1986. History of imported fire ants in the United States, pp. 36-47, *In* C. S. Lofgren and R. K. Vander Meer (eds.), Fire ants and leaf-cutting ants: Biology and management, Westview Press, Boulder, Colorado, 435 pp.
Lofgren, C. S., W. A. Banks, and B. M. Glancey. 1975. Biology and control of the imported fire ants. Ann. Rev. Entomol. 20: 1-28.
Mack, T. P., A. G. Appel, C. B. Backman, and P. J. Trichilo. 1988. Water relations of several arthropod predators in the peanut agroecosystem. Environ. Entomol. 17: 778-781.
Moody, J. V., O. F. Francke, and F. W. Merickel. 1981. The distribution of fire ants, *Solenopsis (Solenopsis)* in western Texas. J. Kansas Entomol. Soc. 54: 469-480.
Morrill, W. L., P. B. Martin and D. C. Sheppard. 1978. Overwinter survival of the red imported fire ant: effects of various habitats and food supply. Environ. Entomol. 7: 262-264.
North, R. D. 1991. Transpiration and humidity preference in a temperate wood ant *Formica rufa* L. J. Insect Physiol. 37: 279-286.
Pimm, S. L., and D. P. Bartell. 1980. Statistical model for predicting range expansion of the red imported fire ant, *Solenopsis invicta*, in Texas. Environ. Entomol. 9: 653-658.
Pinson, C. K. 1980. Temperature regime in the *Solenopsis invicta* mound and its effect on behavior. M.S. thesis, Texas Tech University. 74 pp.
Porter, S. D., and W. R. Tschinkel. 1987. Foraging in the fire ant, *Solenopsis invicta* (Hymenoptera: Formicidae): effects of weather and season. Environ. Entomol. 16: 802-808.
Potts, L. R., O. F. Francke, and J. C. Cokendolpher. 1984. Humidity preferences of four species of fire ants (Hymenoptera: Formicidae: *Solenopsis*). Insectes Soc. 31: 335-339.
Rhoades, W. C., and D. R. Davis. 1967. Effects of meteorological factors on the biology and control of the imported fire ant. J. Econ. Entomol. 60: 554-558.
Sokal, R. R., and F. J. Rohlf. 1981. Biometry. The principles and practices of statistics in biological research. W. H. Freeman and Co., New York. 859 pp.
Summerlin, J. W., and L. R. Green. 1977. Red imported fire ant: a review on invasion, distribution, and control in Texas. Southwestern Entomol. 2: 94-101.
Thorvilson, H. G., J. C. Cokendolpher, and S. A. Phillips, Jr. 1992. Survival of the red imported fire ant (Hymenoptera: Formicidae) on the Texas High Plains. Environ. Entomol. 21: 964-968.
Young, J., and D. E. Howell. 1964. Ants of Oklahoma. Oklahoma Agr. Exp. Sta. MP-71. 42 pp.

EFFECTS OF IMIDACLOPRID SEED TREATMENT ON GREENBUG (HOMOPTERA: APHIDIDAE) INFESTATIONS ON THREE SORGHUM HYBRIDS

Phillip E. Sloderbeck, Merle D. Witt, and Lawrent L. Buschman

Kansas State University, Southwest Research-Extension Center
1501 Fulton Terrace, Garden City, KS 67846

ABSTRACT

Seed of each of three sorghum hybrids was treated with two different rates of imidacloprid insecticide (1.25 and 2.5 g AI/kg seed) or left untreated. Both rates of imidacloprid reduced greenbug abundance when populations occurred on seedling plants within 2 wk of planting. During both years of the study, the 2.5 g rate of imidacloprid usually significantly suppressed greenbug populations for 60 to 80 days after planting on the greenbug-susceptible hybrid, but the differences were not as noticeable on the greenbug-resistant hybrids. Yields were improved significantly by using the 2.5 g rate of imidacloprid on the greenbug-susceptible hybrid in 1993 when greenbugs exceeded 1,000 per plant in the untreated plots, but not in 1994 when the highest level of greenbugs observed was about 300 per plant. The data show that the higher rate of imidacloprid will be needed to provide late-season greenbug suppression and suggest that imidacloprid can reduce yield losses from late season greenbugs on greenbug-susceptible hybrids.

INTRODUCTION

The greenbug, *Schizaphis graminum* (Rondani), has been a major pest of grain sorghum, *Sorghum bicolor* (L.) Moench, throughout the High Plains region of the United States since 1968 when it was first observed to damage sorghum in Texas, Oklahoma, and Kansas (Harvey and Hackerott 1969). Since that time, it has been managed by a combination of insecticide applications and plant resistance. However, the development of new biotypes and insecticide resistance (Peters et al. 1975, Teetes et al. 1975, Archer and Bynum 1978, Porter et al. 1982, Harvey et al. 1991, Sloderbeck et al. 1991, Archer et al. 1994) necessitates new methodologies.

Recently, a new insecticide, imidacloprid, in the chloronicotinyl group of insecticides which act on the nicotinergic acetylcholine receptor has been shown to control Russian wheat aphid on wheat and barley (Pike et al. 1993, van der Westhuizen et al. 1994) and the green peach aphid on lettuce (Palumbo and Kerns 1994). The purpose of this study was to test the effectiveness of the imidacloprid seed treatment (registered as Gaucho™ by Gustafson Inc.) for controlling greenbug populations and maintaining grain yield potential of greenbug-resistant and -susceptible sorghum hybrids.

MATERIALS AND METHODS

Three sorghum hybrids were used in the study: NC+ 271 (a hybrid susceptible to both biotype E and I greenbugs), DK 56 (a biotype E greenbug-resistant hybrid), and Cargill 607E (a biotype I greenbug-resistant hybrid). Some seed of each hybrid was left untreated while other seed was treated by Gustafson, Inc. with either 1.25 or 2.5 g AI of imidacloprid per kg of seed (2 or 4 oz AI per cwt). In addition, all seed was treated with Captan and Concept and the DK 56 also was treated with Apron.

The seed was planted in plots at the Southwest Research-Extension Center in Finney County, Kansas, on 2 June 1993 and 24 May 1994. Treatments were arranged in a modified Latin square design with three replications (the hybrids were arranged in a Latin square with imcimidacloprid treatments inserted as subplots). Each plot was two rows wide (1.5 m) and 6.7 m long. A cone planter was used to plant the seed at a rate of 5 g per row. The entire plot area was treated shortly after planting with 3.36 kg AI/ha of Ramrod and 1.12 kg AI/ha of Atrazine for weed control.

Greenbug abundance was assessed three times during 1993. On 15 June when plants were at the two-leaf stage, greenbugs were counted by visually searching ten consecutive plants in each row of each plot. On 10-12 August when the plants were just beginning to head, four plants per plot (two randomly selected plants from each row) were examined visually for greenbugs. During the flowering stage on 20 August, two plants per plot (one randomly selected plant from each row) were cut off at the base and examined visually to estimate greenbug numbers.

Greenbug abundance was assessed on 23 July and 4-5 August during 1994, when plants were in the late boot and early heading stages, respectively. Two plants per plot (one randomly selected plant from each row) were cut off at the base and examined visually to estimate greenbug numbers.

Grain from the plots was harvested by machine on 5 November 1993 and 27 October 1994. All data were analyzed based on the main effects of seed treatment and hybrid and as individual treatments or interaction means. Mean separation was based on Duncan's Multiple Range Test at a probabilty level of 0.05.

RESULTS AND DISCUSSION

Greenbugs were observed on the sorghum plants a few days after emergence during 1993. Counts made on 15 June (13 days after planting) showed that the imidacloprid seed treatment significantly reduced greenbug abundance (Table 1). The main effects of seed treatment were significant, fewer immigrating winged greenbugs and no wingless ones were observed on plants grown from seed treated with either the 1.25 or 2.5 g/kg rates of imidaclorprid. The interaction between hybrid and insecticide treatment was not significant. These early-season greenbugs soon disappeared and did not cause any noticeable damage. Greenbug numbers began to increase rapidly in mid August. At this time, the main effects of both hybrid and insecticide seed treatment were significant; however, a significant interaction also occurred between hybrid and seed treatment. Few greenbugs were found on the two greenbug-resistant hybrids, but high numbers were found on the untreated greenbug-susceptible hybrid; as a result, the imidacloprid treatment appeared to be more beneficial on the susceptible hybrid (Table 2, Main Effect Means). Both the 1.25 g/kg and the 2.5 g/kg rates of imidacloprid effectively reduced greenbug abundance at 70 days after treatment, but only the 2.5 g/kg rate resulted in decreased greenbug numbers 79 days after planting (Table 2, Interaction

TABLE 1. Main Effect and Interaction Means of Sorghum Hybrid and Imidacloprid Seed Treatment on Early-Season Greenbug Populations, Southwest Research-Extension Center, Finney Co., Kansas, 1993.

		Winged greenbugs per plant 13 days after planting[a]	Non-winged greenbugs per plant 13 days after planting[a]
Main Effect Means			
Hybrid			
Greenbug Susceptible		4.6 a	14.9 a
Biotype-E Resistant		3.8 a	15.4 a
Biotype-I Resistant		3.7 a	18.9 a
Seed Treatment			
None		11.2 a	49.2 a
1.25g Imidacloprid		0.2 b	0.0 b
2.5g Imidacloprid		0.7 b	0.0 b
ANOVA Table *P*-Values			
Hybrid		0.7814	0.7271
Seed Treatment		0.0001	0.0001
Interaction (Hybrid x Seed Treatment)		0.9784	0.8558
Interaction Means	Imidacloprid Rate (g ai/kg of seed)		
Greenbug Susceptible Hybrid	0	12.3 a	44.7 a
	1.25	0.3 b	0.0 b
	2.5	1.0 b	0.0 b
Biotype-E Resistant Hybrid	0	11.0 a	46.3 a
	1.25	0.3 b	0.0 b
	2.5	0.3 b	0.0 b
Biotype-I Resistant Hybrid	0	10.3 a	56.7 a
	1.25	0.0 b	0.0 b
	2.5	0.7 b	0.0 b
P-Values for Interaction Means		0.0001	0.0001

[a]Means based on visually searching 20 plants per plot in each of three replications.

Means, Greenbug Susceptible Hybrid). Grain yield was significantly affected by both hybrid and quantity of insecticide. The susceptible sorghum hybrid treated with 2.5 g of imidacloprid showed the largest difference in yield from the insecticide seed treatment by yielding over 1,000 kg/ha more than the untreated susceptible hybrid (Table 2, Interaction Means, Greenbug Susceptible Hybrid). Samples of greenbugs taken from the plots during August were determined to be biotype E by the diagnostic laboratory at Kansas State University. This would explain why the biotype I hybrid did not differ significantly from the biotype E hybrid in greenbug numbers or show much of a yield increase due to the insecticide seed treatment, since both the biotype E and I hybrids would have had resistance to the biotype E greenbugs.

An early infestation of greenbugs did not occur during 1994; thus, no data were obtained on early-season control. However, on 23 July, the main effects of both hybrid and insecticide seed treatment on greenbug abundance were significant, indicating that the seed treatment was still having a significant effect on greenbug abundance 60 days after planting (Table 3). At 72 days after planting, greenbug abundance differed significantly only by hybrid. However, the interaction means indicated that the greenbug-susceptible hybrid treated with 2.5 g of imidacloprid supported greenbug numbers similar to those of the greenbug-resistant hybrids, but significantly lower than those of the untreated greenbug-susceptible hybrid or of the greenbug-susceptible hybrid treated with 1.25 g/kg of imidacloprid. Grain yields in 1994 were not affected significantly by the seed treatment, but did vary significantly among hybrids (Table 3). Lack of a yield response to the insecticide seed treatment was not surprising given the relatively low number of greenbugs. As in 1993, greenbugs from the plots were identified as biotype E.

In conclusion, although both the 1.25 g and 2.5 g rates of imidacloprid reduced early-season greenbug abundance, only the 2.5 g rate suppressed late-season greenbug numbers. In one instance (60 days after planting in 1993), the 1.25 g rate caused a significant reduction in late-season greenbug numbers, but it failed to result in significant yield differences even for the greenbug-susceptible hybrid in either year of the study. These data suggest that imidacloprid would be utilized best on greenbug-susceptible hybrids. The most consistent reductions in greenbug abundance occurred on plants in the plots of the greenbug-susceptible hybrids, and the largest differences in yield between treated and untreated plots was observed in the greenbug-susceptible plots during 1993. This yield response was enough to maintain the yield of the susceptible hybrid to be nearly equivalent to that of the biotype E-resistant hybrid. Biotype E greenbug-resistant hybrids treated with 2.5 g/kg of imidacloprid yielded significantly more than the non-treated biotype E-resistant sorghum during 1993, but the yield difference was much less than that observed between the treated and nontreated susceptible hybrid. Yields of treated and nontreated biotype I-resistant sorghum did not differ significantly in either year of the study. Since only the yield difference between the treated and untreated seed for the susceptible hybrid was large enough to probably pay for the treatment, we feel that imidacloprid should probably be recommended only on greenbug-susceptible hybrids in areas where greenbugs are the only major pest problem. This probably would include treating biotype E-resistant hybrids in areas where biotype I greenbugs constitute a significant part of the population. Greenbug resistant hybrids should probably be the first choice for controlling the greenbug, but the fact that we can reduce the pressure for developing a new biotype by continuing to use some greenbug-susceptible hybrids and the fact that greenbugs are showing resistance to the currently used pesticides means that imidacloprid seed treatment gives us another option to help reduce losses due to greenbugs.

TABLE 2. Main Effect and Interaction Means of Sorghum Hybrid and Imidacloprid Seed Treatment on Late-Season Greenbug Populations and Grain Yield, Southwest Research-Extension Center, Finney Co., Kansas, 1993.

		Greenbugs per plant 70 days after planting[a]	Greenbugs per plant 79 days after planting[b]	Grain Yield kg/ha
Main Effect Means				
Hybrid				
Greenbug Susceptible		263 a	837 a	9678 b
Biotype-E Resistant		26 b	79 c	10282 a
Biotype-I Resistant		66 b	210 b	9403 b
Seed Treatment				
None		172 a	431 a	9490 b
1.25g Imidacloprid		131 a	446 a	9629 b
2.5g Imidacloprid		52 b	250 b	10244 a
ANOVA Table *P*-Values				
Hybrid		0.0001	0.0001	0.0005
Seed Treatment		0.0041	0.0023	0.0013
Interaction (Hybrid x Seed Treatment)		0.0059	0.0003	0.2091
Interaction Means	Imidacloprid Rate (g ai/kg of seed)			
Greenbug Susceptible Hybrid	0	409 a	1087 a	9378 de
	1.25	289 b	965 a	9215 e
	2.5	91 c	459 b	10445 ab
Biotype-E Resistant Hybrid	0	41 c	76 cd	9949 bcd
	1.25	20 c	114 cd	10269 abc
	2.5	18 c	47 d	10627 a
Biotype-I Resistant Hybrid	0	65 c	103 cd	9152 e
	1.25	85 c	258 c	9403 de
	2.5	49 c	242 cd	9654 cde
P-Values for Interaction Means		0.0001	0.0001	0.0008

[a]Means based on visually searching 4 plants per plot in each of three replications.
[b]Means based on visually searching 2 plants per plot in each of three replications.

TABLE 3. Main Effect and Interaction Means of Sorghum Hybrid and Imidacloprid Seed Treatment on Late-Season Greenbug Populations and Grain Yield, Southwest Research-Extension Center, Finney Co., Kansas, 1994.

		Greenbugs per plant 60 days after planting[a]	Greenbugs per plant 72 days after planting[a]	Grain Yield kg/ha
Main Effect Means				
Hybrid				
Greenbug Susceptible Hybrid		64 a	250 a	9792 b
Biotype-E Resistant Hybrid		16 b	49 b	10420 a
Biotype-I Resistant Hybrid		26 b	61 b	9416 c
Seed Treatment				
None		64 a	151 a	9855 a
1.25g Imidacloprid		21 b	147 a	9918 a
2.5g Imidacloprid		20 b	63 a	9918 a
ANOVA Table *P*-Values				
Hybrid		0.0033	0.0009	0.0001
Seed Treatment		0.0033	0.1319	0.9107
Interaction (Hybrid x Seed Treatment)		0.0597	0.3265	0.9243
Interaction Means	Imidacloprid Rate (g ai/kg of seed)			
Greenbug Susceptible Hybrid	0	119 a	296 a	9729 bc
	1.25	32 ab	339 a	9729 bc
	2.5	41 ab	115 b	9980 abc
Biotype-E Resistant Hybrid	0	14 ab	56 b	10357 ab
	1.25	18 ab	56 b	10483 a
	2.5	16 ab	34 b	10357 ab
Biotype-I Resistant Hybrid	0	60 b	100 b	9416 c
	1.25	13 ab	45 b	9416 c
	2.5	3 c	39 b	9353 c
P-Values for Interaction Means		0.0020	0.0090	0.0050

[a]Means based on visually searching 4 plants per plot in each of three replications.

Imidacloprid gave a longer period of control in these studies than in a trial conducted by Harvey et al. (in press). In that test, differences were observed between treated and nontreated hybrids at three weeks, but not at seven weeks after planting. This apparent difference in efficacy may have been a result of lower greenbug populations or the smaller plot size in their study. More work is needed to better determine the frequency of receiving a favorable economic return from using imidacloprid.

ACKNOWLEDGMENT

Thanks to Bob Bowling and Roxanne Shufran for making the biotype tests on the greenbugs observed in these studies and to Gerald Wilde and Tom Harvey for their review of this manuscript. This research was supported in part by funding from Gustafson Incorporated through research manager Terry Pitts. This publication is contribution No. 96-58-J from the Kansas Agricultural Experiment Station.

LITERATURE CITED

Archer, T. L., and E. D. Bynum, 1978. Pesticide resistance by arthropod pests on feed grains. Southwest. Entomol. 3: 251-259.

Archer, T. L., E. D. Bynum, and F. W. Plapp. 1994. Chlorpyrifos resistance in greenbugs (Homoptera: Aphididae): cross-resistance and synergism. J. Econ. Entomol. 87: 1437-1440.

Harvey, T. L., and H. L. Hackerott. 1969. Recognition of a greenbug biotype injurious to sorghum. J. Econ. Entomol. 62: 776-779.

Harvey, T. L., K. D. Kofoid, , T. J. Martin, and P. E. Sloderbeck. 1991. A new greenbug virulent to E-biotype-resistant sorghum. Crop Sci. 31: 1689-1691.

Harvey, T. L., D. L. Seifers, and K. D. Kofoid. Effect of sorghum hybrid and imidacloprid seed treatment on infestations by corn leaf aphid and greenbug (Homoptera: Aphididae) and the spread of sugarcane mosaic virus strain MDMV-B. J. Agri. Entomol. In press.

Palumbo, J. C., and D. L. Kerns. 1994. Effects of imidacloprid as a soil treatment on colonization of green peach aphid and marketability of lettuce. Southwest. Entomol. 19: 339-346.

Peters, D. C., E. A. Wood, Jr., and K. J. Starks. 1975. Insecticide resistance in selections of the greenbug. J. Econ. Entomol. 68: 339-340.

Pike, K. S., G. L. Reed, G. T. Graf, and D. Allison. 1993. Compatibility of imidacloprid with fungicides as a seed treatment control of Russian wheat aphid (Homoptera: Aphididae) and effect on germination, growth, and yield of wheat and barley. J. Econ. Entomol. 86: 586-593.

Porter, K. B., G. L. Peterson, and O. Vice. 1982. A new greenbug biotype. Crop Sci. 22: 847-850.

Sloderbeck, P. E., M. A. Chowdhury, L. J. DePew, and L. L. Buschman. 1991. Greenbug (Homoptera: Aphididae) resistance to parathion and chlorpyrifos-methyl. J. Kans. Entomol. Soc. 64: 1-4.

Teetes, G. L., C. A. Schaefer, J. L. Gipson, R. C. McIntyre, and E. E. Latham. 1975. Greenbug resistance to organophosphorous insecticides on the Texas High Plains. J. Econ. Entomol. 68: 214-216.

van der Westhuizen, M. C., J. de Jager, M. H. Mason, and M. W. Deall. 1994. Cost benefit analysis of Russian wheat aphid (*Diuraphis noxia*) control in the Republic of South Africa using a seed treatment. Pflanzenxchutz-Nachrichten Bayer 47: 81-90.

TOXICITY OF FOUR COMMON INSECTICIDES TO FIELD-COLLECTED BEET ARMYWORM LARVAE[1,2]

L. D. Chandler and J. R. Ruberson[3]
Northern Grain Insects Research Laboratory, USDA-ARS
Brookings, South Dakota 57006

ABSTRACT

Relative toxicity of chlorpyrifos, cypermethrin, diflubenzuron, and thiodicarb to seven consecutive generations of field collected beet armyworm, *Spodoptera exigua* (Hubner), from Alabama (Macon Co.), Georgia [Bartow Co., Ben Hill Co., Decatur Co., Tift Co. (Gibbs and Rigdon Farms)], and Mississippi (Yazoo Co.) was determined. Insecticide tolerance varied among locations, insecticides and generations tested. No single collection-site colony (F_1) was significantly more tolerant to all insecticides than the susceptible laboratory colony. Macon Co., AL, insects were significantly more tolerant to chlorpyrifos, cypermethrin, and diflubenzuron than the laboratory colony; and Bartow Co. and Yazoo Co. exhibited significant tolerance to more than one evaluated insecticide. In most instances, tolerance to insecticides was inversely related to time in culture with a majority of colonies losing 50% of their tolerance to tested insecticides within two generations following removal of insecticide pressure. However, some fluctuations in tolerance existed from generation to generation, indicating that there was some naturally occurring genetic variation in colony susceptibility.

INTRODUCTION

Beet armyworm, *Spodoptera exigua* (Hubner) (Lepidoptera: Noctuidae), are polyphagous insects found on many wild and cultivated host plants throughout tropical and subtropical regions of the world (Metcalf and Metcalf 1992). They are now recognized as an important cotton pest in the southeastern United States (Smith 1989). In Georgia, outbreaks occurred on cotton in 1977, 1980, 1981, 1988, and 1990 (Douse and McPherson 1991). These outbreaks were associated with below-normal rainfall during the growing season, and were somewhat difficult to manage with insecticides. Populations of beet armyworms were difficult to control with insecticides in Alabama during 1984 and 1985 (Smith 1989). In 1993, widespread reports of beet armyworm outbreaks were reported on cotton in Alabama, Mississippi, and parts of Georgia. Beet armyworm outbreaks in Louisiana have generally followed patterns observed in other major cotton producing states in the Southeast (Burris et al. 1994). As in earlier years, control of the pest with insecticides was difficult.

---

[1] Lepidoptera:Noctuidae

[2] Mention of a commercial product or vendor does not constitute a guarantee or warranty of the product by the United States Department of Agriculture or the University of Georgia and does not imply its approval to the exclusion of other products or vendors that may also be suitable.

[3] University of Georgia, Coastal Plain Experiment Station, P.O. Box 748, Tifton, GA 31793.

Difficulties in controlling beet armyworm populations with labeled insecticides may be related to differences among populations in their susceptibility to the product active ingredients. Variable levels of beet armyworm tolerance to chemical insecticides, including insect growth regulators, have been reported in numerous studies (Cobb and Bass 1976, Meinke and Ware 1978, Chaufaux and Ferron 1986, Yoshida and Parrella 1987, Delorme et al. 1988, Van Laecke 1988, Brewer and Trumble 1989, Brewer et al. 1990, Wolfenbarger and Brewer 1993, Brewer and Trumble 1994). Wolfenbarger and Brewer (1993) indicated that resistant beet armyworm populations were present in Florida and Georgia. Beet armyworms collected in 1990 from Tift County, GA were more resistant to fenvalerate and permethrin than beet armyworms from California (Wolfenbarger and Brewer 1993).

We initiated a study during the 1993 growing season to determine the relative toxicities of chlorpyrifos, cypermethrin, diflubenzuron, and thiodicarb to progeny of field-collected beet armyworm larvae from Alabama, Georgia, and Mississippi. We determined lethal concentrations of the selected insecticides to seven consecutive generations of larvae from each field collection and evaluated differences in lethal concentrations (LC_{50}'s) through time. The results obtained should be useful in development of management plans for beet armyworm larvae on cotton throughout the United States.

MATERIALS AND METHODS

Beet armyworm larvae were collected (a minimum of 30 per collection site) on cotton during the 1993 growing season (July - September) from the following locations: Bartow Co., GA; Ben Hill Co., GA; Decatur Co., GA; Macon Co., AL; Tift Co. (Gibbs Farm), GA; Tift Co. (Rigdon Farm), GA; and Yazoo Co., MS. All insects (with the exception of Tift Co., Rigdon Farm) were collected from fields where organophosphate, pyrethroid, carbamate, and/or benzoylphenylurea insecticides had been applied for management of lepidopterous pests (including beet armyworm). Collections were made once at each site, and collection dates varied dependent upon occurrence of sufficient numbers of larvae to establish colonies. Larvae were returned to the laboratory and reared to adulthood on standard bean diet in a manner similar to that described for fall armyworm, *Spodoptera frugiperda* (J. E. Smith) (Perkins 1979). Adults were placed in 3.8-liter round cardboard containers for oviposition. The container tops were covered with paper towels and the inside lined with brown paper. Both materials were used as oviposition substrates. Adults were fed a 10% sugar-water solution. Eggs were collected every other day, placed in plastic bags, and allowed to hatch. Eggs were held in an environmental cabinet maintained at 24± 2° C, a 12:12 (L:D) photoperiod, and 50 ± 5% RH. Hatched larvae were then collected and held in capped 30-ml plastic cups containing bean diet. Cups were held in environmental cabinets at the above conditions. Insects from all collection sites were maintained separately and reared for seven generations. A laboratory strain of beet armyworm, maintained as described above at the USDA-ARS Insect Biology and Population Management Research Laboratory in Tifton, GA, was used as a standard insecticide susceptible population. The laboratory strain was originally obtained in 1991 from USDA-ARS insect rearing facilities in Stoneville, MS.

Commercial formulations of chlorpyrifos [Lorsban 4 emulsifiable (E), DowElanco, Indianapolis, IN], cypermethrin (Ammo 2.5, FMC Corp., Philadelphia, PA), diflubenzuron [Dimilin 25 wettable powder (WP), Uniroyal Chemical Co., Inc., Raleigh, NC], and thiodicarb [Larvin 3.2 flowable (F), Rhone-Poulenc, Research Triangle Park, NC] were obtained for conducting laboratory bioassays. One percent active ingredient

(AI) dilutions (10,000 mg AI/liter) of each insecticide were prepared in the laboratory with distilled water. Six serial dilutions ranging from 0.00001 to 1.0% were then prepared for each insecticide on each treatment occasion.

Newly-hatched beet armyworm larvae were held for three days on bean diet as described above, removed from the environmental cabinet, and used for laboratory bioassays. All larvae were of uniform size and age at the time of bioassay initiation. A single larva was placed in a 30-ml cup containing 10 ml of fresh bean diet that had been surface treated with 0.10 ml of either distilled water or one of the previously prepared insecticide dilutions. This treatment method has previously been described and tested with beet armyworm by Chandler (1994), and is an effective method of evaluating the effects of formulated insecticides on lepidopterous insects in the family Noctuidae. The number of larvae per dilution exposed to treated diet varied depending upon the number of larvae available in each tested generation. A minimum of two replications was conducted for each colony in each generation tested. The diet was air-dried for 2 hours after the addition of the water or insecticide to the diet surface before a larva was placed in a cup of treated diet. Cups were then capped and returned to the environmental cabinet. Mortality of individuals placed on diet treated with chlorpyrifos, cypermethrin, or thiodicarb was checked after 48 hours, while mortality of larvae on diflubenzuron-treated diet was checked after 5 days. Diflubenzuron is a benzoylphenylurea insect growth regulator that requires between 3.4 and 6.2 days for mortality to occur for 1st-instar beet armyworm (Chandler 1994). Larvae were considered dead if they did not respond to touch by a dissecting needle within two probes.

Concentration-mortality regressions were estimated for larval mortality for each colony and each tested generation using probit analyses (POLO-PC, LeOra Software 1987). Significance of the differences in lethal concentrations of the tested insecticides was based on non-overlap of 95% confidence limits (CL). LC_{50} ratios of each tested generation per colony were calculated for each insecticide by dividing the LC_{50} of a field collected colony by the average LC_{50} of five separate generation evaluations of the laboratory colony. Correlations (Pearson product-moment correlation coefficient, SAS 1985) of LC_{50} ratios from each field collection site among all evaluated insecticides were conducted to determine if possible resistance mechanisms among insecticides are related. Additionally, the number of generations required to lose 50% of the original concentration needed to effect 50% mortality was calculated for each insecticide tested in each field beet armyworm colony.

RESULTS AND DISCUSSION

Toxicity of all tested insecticides against 3-day-old beet armyworm larvae varied dependent on collection location and the generation evaluated (Tables 1-4, Figs. 1-3). In many instances LC_{50} values decreased through time for each collection site (Figs. 1-3). However, some variability of LC_{50} values through time was noted for all insecticides. Toxicity fluctuated from generation to generation in all colonies, including the laboratory colony. This phenomenon may be due to natural variation in response to chemical insecticides in insect populations with similar genetic makeup (Robertson et al. 1995) and may not indicate a shift in the degree of insecticide tolerance. The results presented here differ somewhat from those presented in a preliminary presentation of tolerance of F_1 and F_3 generations (Chandler and Ruberson 1994).

TABLE 1. Toxicity of Chlorpyrifos Against 3-Day-Old F_1 Beet Armyworm Larvae 48 Hours After Exposure, 1993.

Collection Site	Test Date	N[a]	Slope ± SE	LC_{50} (% AI)	95% CL (% AI)
Bartow Co., GA	10 Sep	210	1.96 ± 0.39	0.0422	0.0098 - 0.1204
Ben Hill Co., GA	2 Aug	91	8.17 ± 1.66 x 10^6	0.0973	--[b]
Decatur Co., GA	2 Aug	126	0.68 ± 0.11	0.0151	--[b]
Macon Co., AL	9 Aug	420	1.62 ± 0.024	0.0735	0.0314 - 0.1426
Tift Co. (Gibbs), GA	27 Aug	210	0.64 ± 0.08	0.0124	0.0022 - 0.1095
Tift Co. (Rigdon), GA	21 Jul	210	1.41 ± 0.20	0.0063	0.0022 - 0.0201
Yazoo Co., MS	14 Sep	210	1.68 ± 0.27	0.0796	0.0266 - 0.2148
Laboratory Colony	16 Aug	420	1.70 ± 0.34	0.0031	0.0008 - 0.0060

[a]Number of individuals at start of bioassay.
[b]Unable to calculate CL due to index of significance for potency estimation (g) > 0.5 (LeOra Software 1987).

TABLE 2. Toxicity of Cypermethrin Against 3-Day-Old F_1 Beet Armyworm Larvae 48 Hours After Exposure, 1993.

Collection Site	Test Date	N[a]	Slope ± SE	LC_{50} (% AI)	95% CL (% AI)
Bartow Co., GA	10 Sep	210	1.10 ± 0.15	0.0091	0.0033 - 0.0231
Ben Hill Co., GA	2 Aug	91	2.85 ± 0.72	0.0316	0.0164 - 0.0612
Decatur Co., GA	2 Aug	126	0.82 ± 0.13	0.0139	0.0017 - 0.1854
Macon Co., AL	9 Aug	420	1.68 ± 0.21	0.0562	0.0386 - 0.0802
Tift Co. (Gibbs), GA	27 Aug	210	0.98 ± 0.12	0.0164	0.0026 - 0.1545[b]
Tift Co. (Rigdon),GA	21 Jul	210	1.04 ± 0.13	0.0071	0.0006 - 0.1115[b]
Yazoo Co., MS	14 Sep	210	1.66 ± 0.27	0.0298	--[c]
Laboratory Colony	16 Aug	420	1.10 ± 0.12	0.0047	0.0003 - 0.0241

[a]Number of individuals at start of bioassay.
[b]90% CL.
[c]Unable to calculate CL due to index of significance for potency estimation (g) > 0.5 (LeOra Software 1987).

TABLE 3. Toxicity of Diflubenzuron Against 3-Day-Old F_1 Beet Armyworm Larvae 120 Hours After Exposure, 1993.

Collection Site	Test Date	N[a]	Slope ± SE	LC_{50} (% AI)	95% CL (% AI)
Bartow Co., GA	10 Sep	210	1.63 ± 0.29	0.0136	0.0044 - 0.0340
Ben Hill Co., GA	2 Aug	91	1.60 ± 0.41	0.0031	0.0009 - 0.0090[b]
Decatur Co., GA	2 Aug	126	1.22 ± 0.20	0.0061	0.0022 - 0.0138
Macon Co., AL	9 Aug	420	2.35 ± 0.64	0.0084	0.0042 - 0.0149
Tift Co. (Gibbs), GA	27 Aug	210	2.24 ± 0.49	0.0048	0.0027 - 0.0075
Tift Co. (Rigdon),GA	21 Jul	210	1.34 ± 0.18	0.0028	0.0005 - 0.0125[b]
Yazoo Co., MS	14 Sep	210	0.99 ± 0.31	0.0114	--[c]
Laboratory Colony	19 Aug	420	1.99 ± 0.70	0.0008	0.0002 - 0.0013

[a]Number of individuals at start of bioassay.

[b]90% CL

[c]Unable to calculate CL due to index of significance for potency estimation (g) > 0.5 (LeOra Software 1987).

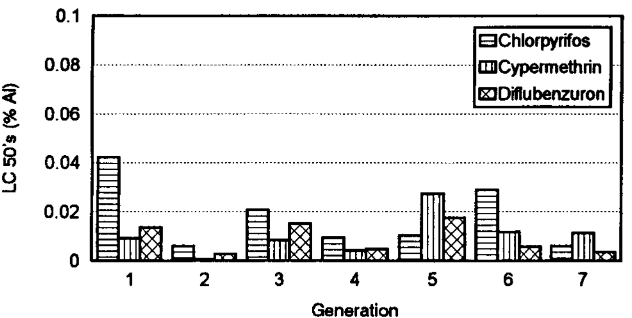
TABLE 4. Toxicity of Thiodicarb Against 3-Day-Old F_1 Beet Armyworm Larvae 48 Hours After Exposure, 1993.

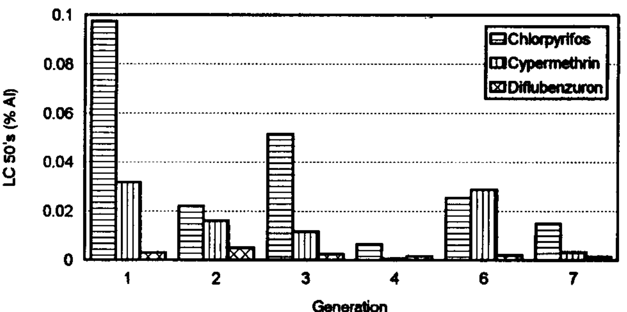
Collection Site	Test Date	N[a]	Slope ± SE	LC_{50} (% AI)	95% CL (% AI)
Bartow Co., GA	10 Sep	210	1.44 ± 0.28	0.2779	0.0818 - 1.2722
Ben Hill Co., GA	2 Aug	91	$9.33 \pm 4.84 \times 10^6$	0.7775	--[c]
Decatur Co., GA	2 Aug	126	0.64 ± 0.13	0.4346	--[c]
Macon Co., AL	9 Aug	420	3.69 ± 0.57	0.3594	0.1027 -0.6655
Tift Co. (Gibbs), GA	27 Aug	210	0.60 ± 0.08	0.0330	--[c]
Tift Co. (Rigdon),GA	21 Jul	210	0.61 ± 0.09	0.1798	0.0243 - 16.8049[b]
Yazoo Co., MS	14 Sep	210	1.00 ± 0.24	0.4066	0.1370 - 3.2119[b]
Laboratory Colony	16 Aug	420	1.74 ± 0.27	0.0316	0.0072 - 0.1289

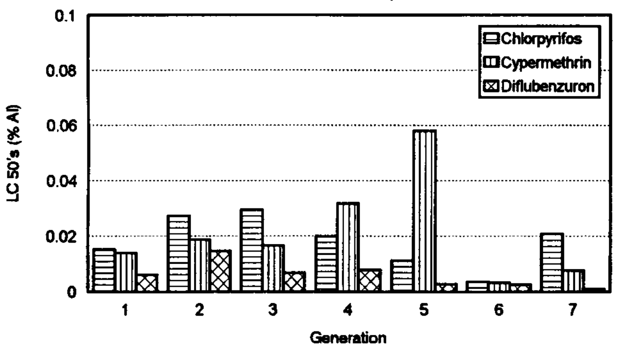
[a]Number of individuals at start of bioassay.

[b]90% CL

[c]Unable to calculate CL due to index of significance for potency estimation (g) > 0.5 (LeOra Software 1987).







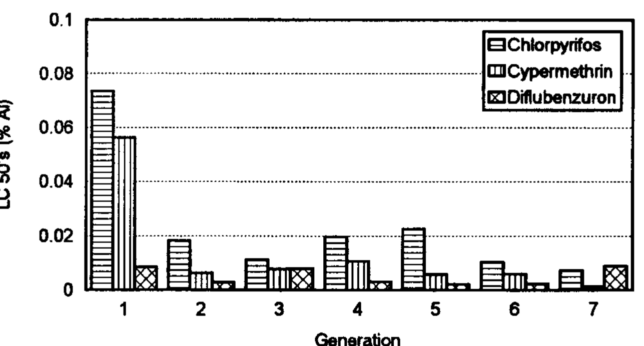
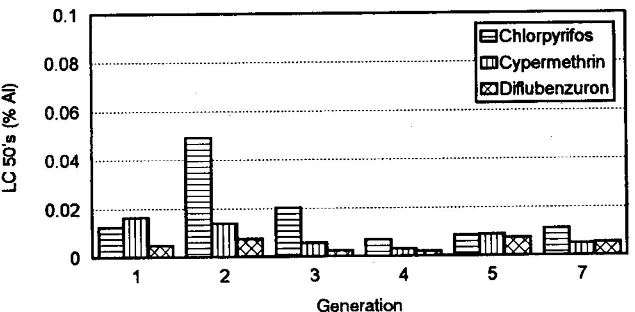
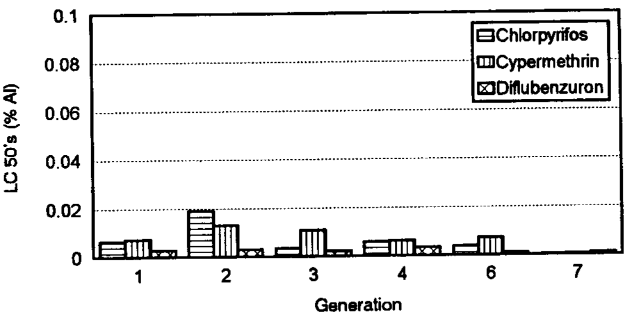
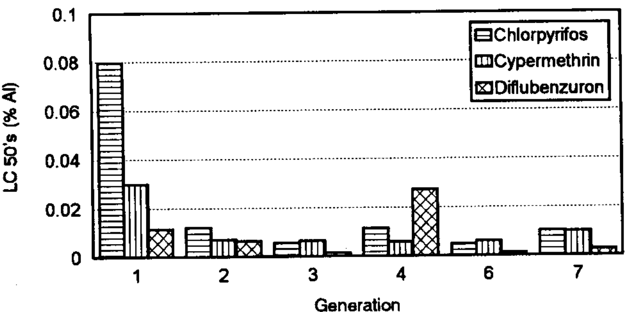


FIG. 1. Relative toxicity of chlorphyrifos, cypermethrin, and diflubenzuron to beet armyworm larvae from Bartow Co., GA, Ben Hill Co., GA, Decatur Co., GA and Macon Co., AL over 7 generations.







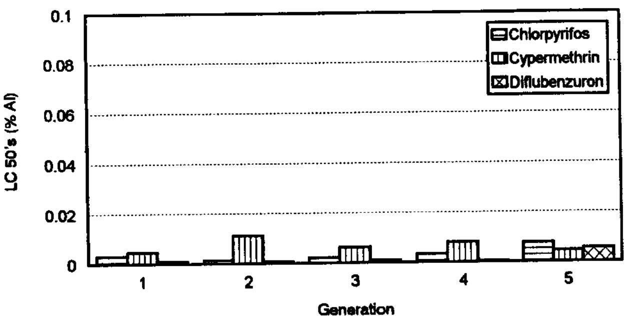


FIG. 2. Relative toxicity of chlorphyrifos, cypermethrin, and diflubenzuron to beet armyworm larvae from Tift Co. (Gibbs and Rigdon Farm), GA, Yazoo Co., MS over 7 generations, and a susceptible laboratory colony.

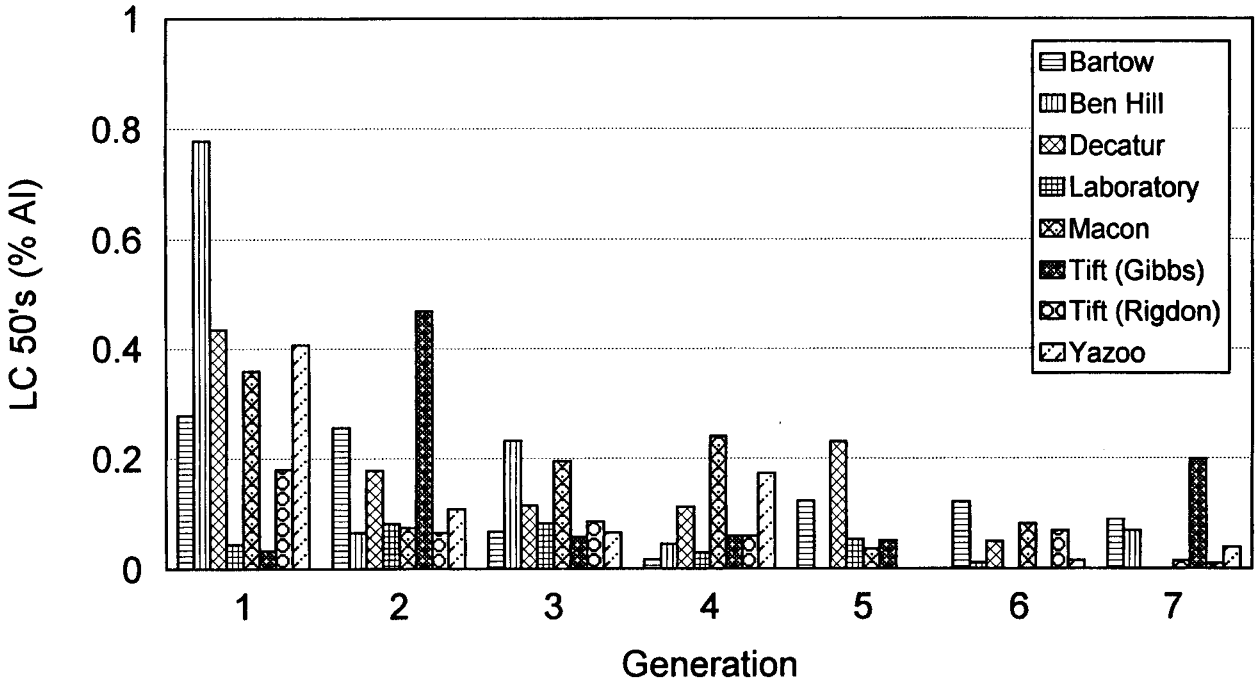


FIG. 3. Relative toxicity of thiodicarb to beet armyworm larvae.

Chlorpyrifos. LC_{50}'s calculated for F_1 beet armyworm response to chlorpyrifos varied significantly among tested colonies (Table 1). Larvae from Bartow Co., Macon Co., and Yazoo Co. were significantly more tolerant to chlorpyrifos compared to that of the laboratory-reared larvae. Larvae from Tift Co. (Rigdon Farm) were more susceptible to chlorpyrifos than larvae from Macon Co. and Yazoo Co. The Rigdon Farm colony was collected from cotton which had not been previously treated with chemical insecticides. The LC_{50} of F_1 larvae from Ben Hill Co. was numerically greater than the LC_{50}'s of any other tested colony. However, due to a large index of significance for potency estimation ($g > 0.5$), the 95% CL could not be calculated (Table 1), and thus statistical comparisons could not be made. It appears that the Ben Hill Co. colony was as difficult to kill with chlorpyrifos as the Bartow Co., Macon Co., and Yazoo Co. populations. LC_{50} ratios for F_1's ranged from 1.8 [Tift Co. (Rigdon Farm)] to 27.0 (Ben Hill Co.), and were numerically highest (> 5.0) in the Bartow Co., Ben Hill Co., Macon Co., and Yazoo Co. colonies (Table 5). LC_{50} ratios greater than 5.0 could indicate an increased level of tolerance to the tested insecticide.

LC_{50} values for chlorpyrifos were highly variable for generations 2-7 of all tested colonies (Figs. 1, 2). LC_{50} ratios ranged from 14.3 (Ben Hill Co., F_3) to 0.1 [Tift Co. (Rigdon Farm), F_7] (Table 5). Highest LC ratios for Decatur Co. occurred in the F_3 generation, while highest ratios for Tift Co. (Gibbs and Rigdon Farms) occurred in the F_2 generation. At various times during generations 2-7 the following colonies had LC_{50} values of \$ 5.0: Bartow Co. (F_3, F_6); Ben Hill Co.(F_2, F_3, F_6); Decatur Co. (F_2, F_3, F_4, F_7); Macon Co. (F_3, F_4, F_5); Tift Co. (Gibbs Farm) (F, F_3); and Tift Co. (Rigdon Farm) (F_2). Yazoo Co. LC_{50} ratios were < 5.0 for generations 2-7 (Table 5). It is apparent that natural fluctuation in chlorpyrifos toxicity occurred in most colonies and that a degree of tolerance (LC_{50} ratio \$5.0) to chlorpyrifos remained in at least the Decatur Co. population through seven generations.

Cypermethrin. The F_1 generation beet armyworm larvae from Macon Co. were the only tested insects significantly more tolerant to cypermethrin in comparison to larvae from the laboratory colony (Table 2), and the only ones with a LC_{50} ratio > 5.0 (Table 6). Larvae from Ben Hill Co. and Yazoo Co. were somewhat tolerant to cypermethrin with LC_{50} ratios of 4.6 and 4.3, respectively (Table 6). LC_{50} values ranged from 0.0047% AI (laboratory) to 0.0562% AI (Macon Co.).

The response of beet armyworm larvae from generations 2-7 varied among all tested colonies (Figs. 1, 2; Table 6). The Decatur Co. colony had a LC_{50} ratio of 8.4 in generation 5 (Table 6), the only instance where the LC_{50} ratio exceeded 5.0 in generations 2-7. As with chlorpyrifos, the LC_{50} values and ratios generally declined for each colony through time. However, generation to generation variability was observed and should be attributed to the natural variation within the populations as noted, for example, with western spruce budworm's, *Choristoneura occidentalis* Freeman, response to pyrethrins (Robertson et al. 1995).

Diflubenzuron. The relative toxicity of diflubenzuron to F_1 beet armyworm larvae varied significantly among tested populations (Table 3). LC_{50} values ranged from 0.0136% AI (Bartow Co.) to 0.0028% AI [Tift Co. (Rigdon Farm)], all of which were higher than the laboratory LC_{50} value of 0.0008% AI. Bartow Co., Decatur Co., Macon Co., and Tift Co.(Gibbs Farm) had LC_{50} values significantly greater compared to that of

TABLE 5. LC_{50} Ratios of Chlorpyrifos Tested Against 3-Day-Old Beet Armyworm Larvae From Indicated Generation.

Collection Site	LC_{50} Ratios[a]						
	F_1	F_2	F_3	F_4	F_5	F_6	F_7
Bartow Co., GA	11.7	1.7	5.8	2.6	2.9	8.0	1.7
Ben Hill Co., GA	27.0	6.1	14.3	1.8	--[b]	7.0	4.1
Decatur Co., GA	4.2	7.6	8.2	5.5	3.1	1.0	5.8
Macon Co., AL	20.4	5.1	3.1	5.4	6.3	2.8	2.0
Tift Co. (Gibbs), GA	3.4	13.7	5.6	1.9	2.3	--[b]	3.1
Tift Co. (Rigdon), GA	1.8	5.3	0.9	1.6	--[b]	1.0	0.1
Yazoo Co., MS	22.1	3.3	1.5	3.1	--[b]	1.3	2.8

[a]Ratios calculated by dividing LC_{50} values/collection site/generation by 5 generation average of laboratory colony LC_{50}. Chlorpyrifos laboratory average LC_{50} = 0.0036% AI (Range = 0.0015 - 0.0077T AI).

[b]No bioassay conducted on insects of indicated generation.

TABLE 6. LC_{50} Ratios of Cypermethrin Tested Against 3-Day-Old Beet Armyworm Larvae From Indicated Generation.

Collection Site	LC_{50} Ratios[a]						
	F_1	F_2	F_3	F_4	F_5	F_6	F_7
Bartow Co., GA	1.3	0.1	1.2	0.6	3.9	1.7	1.7
Ben Hill Co., GA	4.6	2.3	1.7	0.1	--[b]	4.2	0.5
Decatur Co., GA	2.0	2.7	2.4	4.6	8.4	0.5	1.1
Macon Co., AL	8.1	0.9	1.1	1.5	0.8	0.9	0.2
Tift Co. (Gibbs), GA	2.4	2.0	0.8	0.4	1.3	--[b]	0.7
Tift Co. (Rigdon), GA	1.0	1.9	1.6	0.9	--[b]	1.0	0.1
Yazoo Co., MS	4.3	1.0	0.9	0.8	--[b]	0.9	1.4

[a]Ratios calculated by dividing LC_{50} values/collection site/generation by 5 generation average of laboratory colony LC_{50}. Cypermethrin laboratory average LC_{50} = 0.0069% AI (Range = 0.0045 - 0.0112% AI).

[b]No bioassay conducted on insects of indicated generation.

the laboratory colony (Table 3). Larvae from Yazoo Co. appeared as difficult to kill with diflubenzuron as larvae from Bartow Co., but 95% CL could not be calculated for the colony due to a high index of significance for potency estimation (LeOra Software 1987). LC_{50} ratios ranged from 1.6 to 7.6, with Bartow Co. and Yazoo Co. having ratios > 5.0 (Table 7).

Beet armyworm larval response to diflubenzuron varied greatly in generations 2-7 for all colonies (Figs. 1, 2; Table 7). Calculated LC_{50} ratios exceeded 5.0 for the following colonies: Bartow Co. (F_3 and F_5), Decatur Co. (F_2), and Yazoo Co. (F_4). In general, most LC_{50} ratios declined through time. Variability, however, was present from generation to generation.

Thiodicarb. 95% CL could not be calculated for Ben Hill Co., Decatur Co., and Tift Co. (Gibbs Farm) colonies due to large indices of significance for potency estimation (Table 4). No differences in thiodicarb toxicity for the F_1 generation between the remainder of the tested field colonies and the lab colony were observed (Table 4). LC_{50} values ranged from 0.7775% AI (Ben Hill Co.) to 0.033% AI (Tift Co. [Gibbs Farm]). LC_{50} ratios ranged from 0.6 to 13.4, with Ben Hill Co., Decatur Co., Macon Co., and Yazoo Co. having LC_{50} ratios > 5.0 (Table 8). Despite the high degree of variability within tested colonies, it appears that high degrees of tolerance to thiodicarb were initially present in field populations.

Thiodicarb LC_{50} values and LC_{50} ratios were highly variable for generations 2-7 (Fig. 3, Table 8). However, most LC_{50} ratios decreased in value after the first generation. In only one instance [Tift Co. (Gibbs Farm) F_2] was the ratio greater than 5.0. Several colonies had LC_{50} ratios < 1.0 during the last four tested generations. It would appear that most tested colonies were equally susceptible to thiodicarb and were similar to the laboratory strain after the initial bioassays of the F_1 generation.

Correlation of F_1 LC_{50} ratios among tested insecticides indicated that beet armyworm response to chlorpyrifos and thiodicarb were similar (highly correlated), suggesting that the same mechanism conferred tolerance to both insecticides (Table 9). Since both chlorpyrifos and thiodicarb are ChE inhibitors target site resistance to both insecticides might be indicated. A strong correlation, thought not at the 95% level, is also indicated between chlorpyrifos and cypermethrin (Table 9). Other insecticide comparisons were not related.

Table 10 illustrates the number of generations needed for each field colony to lose 50% of their initial tolerance to the tested insecticides. In most instances, these reductions were accomplished with two generations following removal of insecticide pressure. Based on the assumption that target site resistance may be prevalent in beet armyworm for chlorpyrifos and thiodicarb it is not entirely surprising that the loss of tolerance occurs rapidly. F. W. Plapp (personal communication 1995) indicates that target site resistance tends to be lost faster than metabolic resistance. The Decatur Co. field collection required 5-6 generations to lose tolerance to chlorpyrifos, cypermethrin and diflubenzuron. Explanations for this slower loss of tolerance compared to other field colonies are unknown.

These data indicated that beet armyworm larvae had different responses to various tested insecticides and those responses were dependent upon the source of the colony. No single population was significantly more tolerant to all insecticides in comparison to the laboratory colony. However, Macon Co., AL, insects were significantly more tolerant to chlorpyrifos, cypermethrin, and diflubenzuron and had a

TABLE 7. LC_{50} Ratios of Diflubenzuron Tested Against 3-Day-Old Beet Armyworm Larvae From Indicated Generation.

Collection Site	LC_{50} Ratios[a]						
	F_1	F_2	F_3	F_4	F_5	F_6	F_7
Bartow Co., GA	7.6	1.5	8.4	2.6	9.7	3.2	1.9
Ben Hill Co., GA	1.7	2.8	1.3	0.9	--[b]	1.1	0.8
Decatur Co., GA	3.4	8.2	3.7	4.3	1.6	1.4	0.5
Macon Co., AL	4.7	1.6	4.4	1.7	1.2	1.3	4.9
Tift Co. (Gibbs), GA	2.7	4.1	1.4	1.1	4.1	--[b]	3.0
Tift Co. (Rigdon), GA	1.6	1.7	1.3	1.9	--[b]	0.6	0.6
Yazoo Co., MS	6.3	3.6	0.8	15.1	--[b]	0.7	1.4

[a]Ratios calculated by dividing LC_{50} values/collection site/generation by 5 generation average of laboratory colony LC_{50}. Diflubenzuron laboratory average LC_{50} = 0.0018% AI (Range = 0.0004 - 0.0056% AI).

[b]No bioassay conducted on insects of indicated generation.

TABLE 8. LC_{50} Ratios of Thiodicarb Tested Against 3-Day-Old Beet Armyworm Larvae From Indicated Generation.

Collection Site	LC_{50} Ratios[a]						
	F_1	F_2	F_3	F_4	F_5	F_6	F_7
Bartow Co., GA	4.8	4.4	1.2	0.3	2.1	2.1	1.5
Ben Hill Co., GA	13.4	1.1	4.0	0.8	--[b]	1.9	1.2
Decatur Co., GA	7.5	3.1	2.0	1.9	4.0	0.9	--[b]
Macon Co., AL	6.2	1.3	3.4	4.1	0.6	1.4	0.2
Tift Co. (Gibbs), GA	0.6	8.1	1.0	1.0	0.9	--[b]	3.4
Tift Co. (Rigdon), GA	3.1	1.1	1.5	1.0	--[b]	1.2	0.2
Yazoo Co., MS	7.0	1.9	1.1	3.0	--[b]	0.3	0.7

[a]Ratios calculated by dividing LC_{50} values/collection site/generation by 5 generation average of laboratory colony LC_{50}. Thiodicarb laboratory average LC_{50} = 0.0580% AI (Range - 0.0290 - 0.823% AI).

[b]No bioassay conducted on insects of indicated generation.

TABLE 9. Pearson Product-Moment Correlation Coefficients For All Possible Comparisons Among Insecticides Evaluated Against 3-Day-Old Beet Armyworm Larvae From Seven Field Colonies[a].

Insecticide Comparisons	R	Prob. > |R|
Chlorpyrifos vs. Thiodicarb	0.756	0.049
Chrlopyrifos vs. Diflubenzuron	0.239	0.606
Chlorpyrifos vs. Cypermethrin	0.724	0.066
Thiodicarb vs. Diflubenzuron	-0.105	0.823
Thiodicarb vs. Cypermethrin	0.390	0.387
Diflubenzuron vs. Cypermethrin	0.085	0.856

[a]Correlations calculated using LC_{50} ratios for F_1 generation of each field collection site.

TABLE 10. Number Of Generations Needed To Lower Initial LC_{50} Values Of Each Beet Armyworm Field Colony By 50%.

Collection Site	Generations Chlorpyrifos	Cypermethrin	Diflubenzuron	Thiodicarb
Barton Co., GA	2	2	2	2
Ben Hill Co., GA	2	2	--[a]	2
Decatur Co., GA	6	6	5	2
Macon Co., AL	2	2	2	2
Tift Co. (Gibbs), GA	--[a]	3	--[a]	--[a]
Tift Co. (Rigdon), GA	3	7	--[a]	2
Yazoo Co., MS	2	2	3	2

[a]LC_{50} values never reduced 50%.

LC_{50} ratio of 6.2 for thiodicarb indicating that this population could be difficult to control with all tested compounds. Other locations where significant tolerance to more than one insecticide occurred included Bartow Co., GA and Yazoo Co., MS (high numerical values for diflubenzuron LC_{50} values). All of these locations had previously been treated with numerous chemical insecticides, both during the 1994 season and in previous years, which may have contributed to the increased levels of tolerance noted. In most cases, the LC_{50} values decreased by 50% within a single generation after the initial F_1 bioassays indicating that some degree of susceptibility to the insecticides may quickly return to populations after continual insecticide pressure is removed. However, since fluctuations from generation to generation were observed for all insecticides in all tested colonies, the conclusion that return to insecticide susceptibility could occur in as few as two generations after removal of insecticide pressure must be viewed cautiously. The naturally-occurring genetic variation found in populations could account for much of the toxicity variation and may indicate that some degree of tolerance remains in the populations for long periods of time even without insecticide pressure. There is considerable uncertainty at present regarding the source(s) of beet armyworm populations. If populations persist year-to-year in a given locale, there will be a strong

opportunity for the development of localized resistance. If populations must recolonize from distant locations each year, the insecticide histories of the source locations will greatly influence the susceptibility of the colonizing individuals. Additional research is needed to more fully understand the genetics of beet armyworm populations in adapting to insecticide pressure.

ACKNOWLEDGMENT

The authors wish to thank Lenny Atkins, Steve Hooks, Larry Walker, Wendy Tyson, and Laura Abbott for assistance in conducting the laboratory bioassays. Denise Hovland is acknowledged for preparation of the graphic presentations. We also thank Eddie McGriff (Decatur Co., GA) for assistance in locating beet armyworm populations, and Russell J. Ottens (Dept. of Entomology, Univ. of GA, Tifton) and William R. Lambert (Univ. of GA Coop. Ext. Serv.) for collections of beet armyworm in Ben Hill Co., GA, Bartow Co., GA and Yazoo Co., MS.

LITERATURE CITED

Brewer, M. J., and J. T. Trumble. 1989. Field monitoring for insecticide resistance in beet armyworm (Lepidoptera: Noctuidae). J. Econ. Entomol. 82: 1520-1526.

Brewer, M. J., and J. T. Trumble. 1994. Beet armyworm resistance to fenvalerate and methomyl: Resistance variation and insecticide synergism. J. Agric. Entomol. 11: 291-300.

Brewer, M. J., J. T. Trumble, B. Alvarado-Rodriguez, and W. E. Chaney. 1990. Beet armyworm (Lepidoptera: Noctuidae) adult and larval susceptibility to three insecticides in managed habitats and relationship to laboratory selection for resistance. J. Econ. Entomol. 83: 2136-2146.

Burris, E., J. B. Graves, B. R. Leonard, and C. A. White. 1994. Beet armyworms (Lepidoptera: Noctuidae) in northeast Louisiana: Observations on an uncommon insect pest. Fla. Entomol. 77: 454-459.

Chandler, L. D. 1994. Comparative effects of insect growth regulators on longevity and mortality of beet armyworm (Lepidoptera: Noctuidae) larvae. J. Entomol. Sci. 29: 357-366.

Chandler, L. D., and J. R. Ruberson. 1994. Comparative toxicity of four commonly used insecticides to field-collected beet armyworm larvae from the southeastern United States. Proc. 47th Cotton Insect Res. and Control Conf. pp. 860-864.

Chaufaux, J., and P. Ferron. 1986. Sensibilite differente de deux populations de *Spodoptera exigua* Hub. (Lepid., Noctuidae) aux baculovirus et aux pyrethrinoides de synthese. Agronomie 6: 99-104.

Cobb, P. P., and M. H. Bass. 1976. Beet armyworm: Dosage mortality studies on California and Florida strains. J. Econ. Entomol. 68: 813-814.

Delorme, R., D. Fournier, J. Chaufaux, A. Cuany, J. McBride, D. Auge, and J. B. Berge. 1988. Esterase metabolism and reduced penetration are causes of resistance to deltamethrin in *Spodoptera exigua* Hub. (Noctuidae: Lepidoptera). Pesti. Biochem. Physiol. 32: 240-246.

Douse, G. K.., and R. M. McPherson (Eds.). 1991. Summary of losses from insect damage and costs of control in Georgia, 1989. Ga. Agric. Expt. Stn. Special Pub. 70. 47 pp.

LeOra Software. 1987. POLO-PC, a user's guide to probit or logit analysis. LeOra Software, Inc., Berkeley, CA, 15pp.

Meinke, L. J., and G. W. Ware. 1978. Tolerance of three beet armyworm strains in Arizona to methomyl. J. Econ. Entomol. 71: 645-646.

Metcalf, R. L., and R. A. Metcalf. 1992. Destructive and useful insects, 5th ed. McGraw-Hill, NewYork.

Perkins, W. D. 1979. Laboratory rearing of the fall armyworm. Fla. Entomol. 62: 87-91.

Robertson, J. L., H. K. Preisler, S. S. Ng, L. A. Hickle, and W. D. Gelernter. 1995. Natural variation: A complicating factor in bioassays with chemical and microbial pesticides. J. Econ. Entomol. 88: 1-10.

SAS Institute. 1985. SAS user's guide: statistics version 5 ed. SAS Institute, Cary, NC.

Smith, R. H. 1989. Beet armyworms on cotton. Alabama Coop. Ext. Serv. Circular ANR-538. 4 pp.

Van Laecke, K. 1988. Toxiciteit en werkingswijze van de chitinesynthese-inhibitoren chloorfluazuron, diflubenzuron en XRD-473 op *Spodoptera exigua* (Hubner). M.S. Thesis, Rijksuniversiteit, Gent.

Wolfenbarger, D. A., and M. J. Brewer. 1993. Toxicity of selected pesticides to field collected beet armyworm populations. Proc. 46th Ann. Cotton Insect Res. and Control Conf. pp. 1034-1037.

Yoshida, H. A., and M. P. Parrella. 1987. The beet armyworm in floricultural crops. Calif. Agric. 41(3,4): 13-15.

SCIENTIFIC NOTE

TIMULLA CENTROAMERICANA (DALLA TORRE) (HYMENOPTERA: MUTILLIDAE), A PARASITOID OF *LIRIS* (HYMENOPTERA: SPHECIDAE)

Diomedes Quintero A.[1] and Roberto A. Cambra T.

Museo de Invertebrados G. B. Fairchild
Universidad de Panamá, Estafeta Universitaria, Panamá

The genus *Timulla* Ashmead includes 161 nominal species and three subspecies in the Neotropical Region (Cambra and Quintero 1993). Very little is known about their biology and hosts (Mickel 1937, 1938; Nonveiller 1990). Fattig (1943) reared three nearctic species of *Timulla*, and reported their host records (Table 1); two species were reared from ground nesting eumenids and one was from a dynastine beetle. Unfortunately, host cocoons were not kept associated with the reared parasitoid mutillid specimens, now kept at the Entomology Department of the University of Georgia (Robert Matthews, personal communication). The only host reported for a neotropical species of *Timulla* is a larrid sphecid, *Tachysphex inconspicuus* (Kirby) (as *T. blatticidus* Williams); three females of *Timulla eriphyla* Mickel were reared in Trinidad from this host (Callan 1942, 1990).

Table 1. Hosts and nest sites of *Timulla*.

Timulla	Host	Nest site	References
eriphyla Mickel	*Tachysphex incospicuus* (Kirby) Sphecidae	open sandy areas gregarious	Callan 1942, 1990
ferrugata (Fabricius)	*Eumenes fraternus* Say Eumenidae	mud nests under leaves attached to wings	Fattig 1943
ocellaria ocellaria (Mickel)	*Ligurus gibbosus*[a] Scarabaeidae	not indicated	Fattig 1943
rufosignata (Bradley)	erroneous det. by Fattig[b]; undet. Eumenidae	ground nesting	Fattig 1943
centroamericana (D. Torre)	*Liris* sp. nr. *argenticauda*	open sandy area	new record

[a]Dubious record; probably a parasite of Scoliidae or Tiphiidae using scarabaeid larvae as hosts.
[b]Host misdet. as *Odynerus erinnys* [sic] = *Pachodynerus erynnis* (Lep.)

Here we report the second host record for a neotropical species of *Timulla* and the first report of parasitism in the neotropics on the large cosmopolitan sphecid genus *Liris*. Krombein and Shanks Gingras (1984) stated that no parasites had been reported for North American *Liris*. *Timulla centroamericana* (D. Torre) was the only parasitoid that we reared from eight pupae of the larrid sphecid *Liris* (*Leptolarra*) sp. near *argenticauda* (Cameron) collected from sandy ground nests in Capira, Panama Province, on 25-28 December 1990. On 10 January 1991, two

[1]Smithsonian Tropical Research Institute, Unit 0948, APO AA 34002-0948 USA

females of *T. centroamericana* emerged, one distinctly longer than the other (7.3 and 5.6 mm). The larger female emerged from a 10.6 mm long sphecid cocoon, the smaller from a 8.6 mm cocoon. Mutillid size is correlated with that of the host (Mickel 1924, Cambra 1994). The following body length measurements of the host, *L.* (*L.*) sp. nr. *argenticauda*, are from specimens reared from the Capira area (n= 6 for each sex): males 7-8.2 mm (pupae length 8.5-12.6 mm); females 9-10.9 mm (pupae length 13-16.7 mm). Size variation in *T. centroamericana* is: males, 12-16.5 mm (n= 20); females, 6-10.5 mm (n= 54) (Cambra and Quintero 1993). From the preceeding measurements, we infer that the mutillid females we reared eclosed from parasitized male host pupae (although it is possible that female host pupae are also used to rear females). In the Capira area we found a balanced sex ratio in the host *Liris* (*L.*) sp. nr. *argenticauda* (1:1 female:male), thus we infer that the female mutillid's selection of male pupae, for laying female eggs, was by choice and not the result of finding a host skewed sex ratio in the Capira area.

Species of *Timulla* are known to engage in phoretic matings, and males are distinctly larger than females (to be able to airborn the females). Thus, it appears that *Timulla* females choose the smaller, male pupae of *L.* (*L.*) sp. nr. *argenticauda* to lay female eggs. In most other mutillid genera, females develop in the larger host pupae. We consider *L.* (*L.*) sp. nr. *argenticauda* too small to be used as the host for males of *T. centroamericana* (the smaller known males of *centroamericana* are 12 mm long; the larger known adults of *argenticauda* are only 10.4 mm long). Thus at least one additional host must be used by *T. centroamericana* to rear males.

The following observations cannot be considered as host records but they suggest additional ground nesting aculeate hosts for the following three species of *Timulla*. *Timulla leona* (Blake) was seen entering burrows of the sphecid *Bembix troglodytes* Handl. (Evans 1957). One female of the same species emerged from a ground nest of the sphecid *Gorytes canaliculatus* (Packard), in Pottawatomie County, Kansas, at 17:00 h on 17 July 1952 (Evans 1966). Rozen (1964) observed a female *Timulla vagans rufinota* Mickel that had just emerged from a burrow of the anthophorid bee *Svastra obliqua obliqua* (Say), at Flamingo, Everglades National Park, Florida in April 1963. We observed one female *Timulla cordillera* Mickel entering an open ground burrow of the sphecid *Sphex ichneumoneus* (Lin.), nesting in hard clay soil at the Pakitza Field Station, Manu Reserved Zone, Madre de Dios Department, Perú, at 15:50 h on 7 March 1992.

ACKNOWLEDGMENT

We thank the Smithsonian Tropical Research Institute (STRI) for providing research facilities and the Biological Diversity in Latin America Project (BIOLAT) for providing funds, facilities and logistic support during our expeditions to Pakitza, Perú. We are specially thankful to James Coronado, for the rearing records, field observations, and much help in the field. Our thanks to Annette Aiello, STRI, and Karl V. Krombein, USNM-Smithsonian Institution, for valuable comments on how to improve the manuscript.

LITERATURE CITED

Callan, E. McC. 1942. A note on *Timulla* (*Timulla*) *eriphyla* Mickel (Hym., Mutillidae), a parasite of *Tachysphex blatticidus* F. X. Williams (Hym., Larridae), from Trinidad, B.W.I. Proc. Royal Entomol. Soc., London 17: 18.

Callan, E. McC. 1990. Mutillidae of Trinidad. Sphecos 19: 21-22.

Cambra T., R. A. 1994. Biología y sistemática de *Dasymutilla araneoides araneoides* (Hymenoptera: Mutillidae). Resúmenes, 12 Congreso Científico, Universidad de Panamá: 89.

Cambra T., R. A., and D. Quintero A. 1993. Studies on *Timulla* Ashmead (Hymenoptera: Mutillidae): New distribution records and synonymies, and descriptions of previously unkown allotype. Pan-Pacific Entomol. 69: 299-313.

Fattig, P. W. 1943. The Mutillidae or Velvet Ants of Georgia. Emory University Museum Bulletin 1: 1-23.

Evans, H. E. 1957. Studies on the comparative ethology of digger wasps of the genus *Bembix*. Cornell University Press, Ithaca. 248 pp.

Evans, H. E. 1966. The Comparative Ethology and Evolution of the Sand Wasps. Harvard University Press, Cambridge. 526 pp.

Krombein, K. V. 1979. Superfamily Scoloidea. Genus *Timulla* Ashmead, pp. 1281-1283. *In* K. V. Krombein, P. D. Hurd, D. R. Smith and B. D. Burks. Catalog of Hymenoptera in America North of Mexico, 2. Washington, D.C.: Smithsonian Inst. Press.

Krombein, K. V., and S. Shanks Gingras. 1984. Revision of North American *Liris* Fabricius (Hymenoptera: Sphecoidea: Larridae). Smithsonian Contributions to Zoology 404: 1-96.

Mickel, C. E. 1924. An analysis of a bimodal variation in size of the parasite *Dasymutilla bioculata* Cresson (Hymen.: Mutillidae). Entomol. News 35: 236-241.

Mickel, C. E. 1937. The mutillid wasps of the genus *Timulla* which occur in North America North of Mexico. Entomol. Americana 17: 1-119.

Mickel, C. E. 1938. The Neotropical mutillid wasps of the genus *Timulla* Ashmead (Hymenoptera: Mutillidae). Trans. Royal Entomol. Soc., London 87: 529-680.

Nonveiller, G. 1990. Catalogue of the Mutillidae, Myromosidae and Bradynobaenidae of the Neotropical region including Mexico (Insecta: Hymenoptera). SPB Academic Publishing bv, The Netherlands. 150 pp.

Rozen, J. G. 1964. The Biology of *Svastra obliqua obliqua* (Say), with a Taxonomic Description of its Larvae (Apoidea, Anthophoridae). American Museum Novitates 2170: 1-13.

SCIENTIFIC NOTE

SURVIVAL OF *HELICOVERPA ZEA* (LEPIDOPTERA: NOCTUIDAE) LARVAE AS AFFECTED BY SUBFREEZING TEMPERATURES IN THE SUBTROPICS

Luis A. Rodríguez-del-Bosque

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

Interest on the ecology of the corn earworm, *Helicoverpa zea* (Boddie), in northeastern Mexico and southern Texas has recently increased due to the relationship of this insect pest with aflatoxin contamination in preharvest corn (Rodríguez-del-Bosque et al. 1995), and the role of local population buildup as a source for migrating adults to northern latitudes (Raulston et al. 1992). The apparent northern limit for *H. zea* overwintering is near the 40° north latitude in the US central states (Snow and Copeland 1971). Yearly damaging populations of *H. zea* in northern latitudes are the result of adults migrating from southern latitudes (Snow and Copeland 1971, Raulston et al. 1992). *Helicoverpa zea* overwinters as pupae; therefore, winter survival and impact of low temperature studies for *H. zea* has been focused on this stage (Barber and Dicke 1939; Snow and Copeland 1971; Stadelbacher and Pfrimmer 1972; Kogan et al. 1978; Eger et al. 1982, 1983; Rummel and Neece 1989). However, *H. zea* develops continuously in southern latitudes (Snow and Copeland 1971), and all developmental stages are exposed to the infrequent freezing and subfreezing temperatures that occur in these regions.

More than 200,000 ha of field corn are planted annually during the spring growing season in the subtropical region of northern Tamaulipas, Mexico. A smaller proportion of the land (about 50,000 ha) is planted for a second growing period (fall season) during July-August, with harvest occurring during December or early January, the coldest months in this area. When corn is planted as late as September, plants may be exposed to freezing temperatures at ear development stage. Due to the continous reproduction cycle occurring in this area, these ears are likely to be infested with *H. zea*. According to the nearly 200-yr *H. zea* bibliography by Kogan et al. (1978) and a literature search in the AGRICOLA CD-ROM database (National Agricultural Library) from 1970 to 1995, no studies apparently have been reported on the impact of low temperatures on *H. zea* larvae, which prompted this investigation.

The study was conducted at the Campo Experimental Río Bravo (Rio Bravo Experiment Station), near Río Bravo, Tamaulipas, Mexico. A 0.5-ha plot was planted with the field corn hybrid 'H-433' on 21 September 1995. Forty-eight hours after the passage of a cold spell occurring on 7-9 January 1996, 400 plants were randomly selected, and ears (dough stage) were harvested, transported to the laboratory, and examined for *H. zea* larvae. Larvae were categorized by size (3rd-4th instars = medium; 5-6th instars = large); larvae not responding to gentle picking with a toothpick in the thoracic segments, and which appeared typically flaccid, were considered dead as the result of exposure to cold temperatures. Percentage mortality caused by low temperatures was calculated for each larval size by dividing the number of dead larvae by the number of total larvae collected. Dead larvae showing signs of predation or disease infection were not included in these calculations. Minimum temperatures were obtained daily from the weather station located at the Station.

To complement the field study, a laboratory experiment was conducted to test the susceptibility of *H. zea* larvae to subfreezing temperatures. Larvae were collected in the plot mentioned above at the beginning of the cold spell (7 January) when temperature was 5°C. Larvae were placed individually in 30-ml plastic cups with five fresh corn kernels and maintained at 25°C in the laboratory for 24 h. Larvae then were exposed to a constant -2, -5, and -10°C in different growth chambers for 1, 3, 6, 12, 24, 48, and 72 h, respectively. Twenty larvae of each size (medium and large) were subjected to each temperature and exposure time. After the exposure time expired, larvae were placed at 25°C for 1 h and then observed for survival. In addition, 20 larvae of each size were placed at 25°C (control) and observed for mortality after 24, 48, and 72 h. Larval mortality was determined as above. Multiple regression analyses using PROC REG (stepwise procedure, SAS Institute 1988) were conducted to determine the association between temperature (x_1) and exposure time (x_2) with percentage of survivorship (y) for each medium and large larvae. Quadratic effects ($x^2{}_1$, $x^2{}_2$) and the interaction ($x_1 * x_2$) were included also in the regression analyses. The stepwise procedure found the best-fit multiple regression model when no other independent variable (x_n) met the 0.15 significance level for entry into the model.

A total of 50 medium and 153 large *H. zea* larvae were collected from the ears of 400 corn plants. Mortality attributed to the cold spell was only 8.0 and 6.5% for medium and large larvae, respectively. The cold spell lasted 3 d with minimum temperatures ranging from 1 to -2°C. However, temperatures below 0°C lasted for only 4 h (8 January). In addition, larvae were probably not exposed to temperatures as cold as the ambient due to the insulation effects of the corn husk cover.

In the laboratory study, survival of control larvae (25°C) after 72 h was 95 and 100% for medium and large larvae, respectively. Table 1 shows survival of *H. zea* larvae subjected to subfreezing temperatures and exposure duration. Survival was highest at -2°C regardless of larval size and exposure duration. Exposure of larvae to -5°C increased mortality substantially, particularly after 3 h of exposure. Medium larvae appeared to survive longer than large larvae at $\leq$ -5°C. Mortality of *H. zea* larvae was substantial at -10°C, with only 20% of medium larvae surviving at 1 h exposure. The multiple regression analysis for testing the association between survivorship (%) of medium larvae (y) and temperature (x_1) and exposure duration (x_2) yielded the following best-fit model: $y = 144.6 + 26.9\ x_1 + 1.39\ x^2{}_1 + 0.26\ (x_1 * x_2)$ ($F = 62.4$; $df = 3, 11$); $P < 0.0001$; $r^2 = 0.94$). The best-fit model for survivorship of large larvae was $y = 128.9 + 10.8\ x_1 + 3.11\ x_2 + 2.09\ (x_1 * x_2)$ ($F = 27.0$; $df = 3, 8$); $P < 0.001$; $r^2 = 0.91$).

TABLE 1. Percentage of Survival of Medium and Large *H. zea* Larvae at Different Subfreezing Temperatures and Exposure Times.

		Exposure (h)						
Temp. (°C)	Larval size	1	3	6	12	24	48	72
-2	Medium	100	100	100	90	80	70	50
	Large	100	100	100	100	85	55	30
-5	Medium	50	45	40	10	5	0	0
	Large	95	45	5	0	0	0	0
-10	Medium	20	0	0	0	0	0	0
	Large	0	0	0	0	0	0	0

Both field and laboratory studies showed *H. zea* larvae were dramatically affected only when exposed for several hours at temperatures $\leq$ -5°C, which is very unlikely to occur in northern Tamaulipas. Temperatures below -5°C have occurred only twice in this region during the past three decades (1966-1995, unpublished data). In fact, the minimum temperature recorded in northern Tamaulipas was -11°C, nearly a century ago (Morales et al. 1980). In addition, cold spells usually last only a few hours or days in this area, further minimizing the effect of low temperatures on *H. zea* larvae. This study demonstrated that *H. zea* larval populations are not seriously affected by cold during the winter months in subtropical Mexico, a situation favoring local population buildup during the spring, which also represent an infestation source for northern latitudes through adult migration.

ACKNOWLEDGMENT

The field and laboratory assistance by Rosalío Navarro and Julian Fuentes is greatly appreciated. J. R. Raulston (USDA-ARS), J. Vargas Camplis (INIFAP), and two anonymous reviewers made helpful comments on an early draft of this manuscript. Approved by the Centro de Investigación Regional del Noreste as INIFAP-CIRNE-A022.

LITERATURE CITED

Barber, G. W., and F. F. Dicke. 1939. Effect of temperature and moisture on overwintering pupae of the corn earworm in the northeastern states. J. Agr. Res. 59: 711-723.

Eger. J. E., Jr., J. A. Witz, A. W. Hartstack, Jr., and W. L. Sterling. 1982. Survival of pupae of *Heliothis virescens* and *Heliothis zea* (Lepidoptera: Noctuidae) at low temperatures. Can. Entomol. 114: 289-301.

Eger, J. E., Jr., W. L. Sterling, and A. W. Hartstack, Jr. 1983. Winter survival of *Heliothis virescens* and *Heliothis zea* (Lepidoptera: Noctuidae) in College Station, Texas. Environ. Entomol. 12: 970-975.

Kogan, J., D. K. Sell, R. E. Stinner, J. R. Bradley, Jr., and M. Kogan. 1978. A bibliography of *Heliothis zea* (Boddie) and *H. virescens* (F.) (Lepidoptera: Noctuidae). International Soybean Program (INTSOY), College of Agriculture, University of Illinois. INTSOY Series No. 17. 242 pp.

Morales, A. O., F. Leal, H. Villarreal, J. A. González, and J. Valero. 1980. Marco de referencia del área de influencia del Campo Agrícola Experimental Río Bravo. SARH, INIA, CIAGON, Campo Experimental Río Bravo, Tamaulipas, México.

Raulston, J. R., S. D. Pair, J. Loera, A. N. Sparks, W. W. Wolf, J. K. Westbrook, G. P. Fitt, and C. E. Rogers. 1992. *Helicoverpa zea* (Lepidoptera: Noctuidae) pupa production in fruiting corn in northeast Mexico and south Texas. Environ. Entomol. 21: 1393-1397.

Rodríguez-del-Bosque, L. A., C. A. Reyes, S. Acosta, J. R. Girón, I. Garza, and R. García. 1995. Control de aflatoxinas en maíz en Tamaulipas. Campo Experimental Río Bravo, CIRNE, INIFAP, SAGAR. Folleto Técnico 17: 1-18.

Rummel, D. R., and K. C. Neece. 1989. Winter survival of *Heliothis zea* (Boddie) in cultivated and noncultivated soil in the southern Texas High Plains. Southwest. Entomol. 14: 117-125.

SAS Institute. 1988. STAT/SAS user's guide, release 6.03 ed. SAS Institute, Cary, NC.

Snow, J. W., and W. W. Copeland. 1971. Distribution and abundance of the corn earworm. U.S. Dep. Agr., Coop. Econ. Insect Rep. 21: 71-76.

Stadelbacher, E. A., and T. R. Pfrimmer. 1972. Winter survival of the bollworm at Stoneville, Mississippi. J. Econ. Entomol. 65: 1030-1034.

PREDATION ON THE HARVESTER ANT, *POGONOMYRMEX TENUISPINA* FOREL (HYMENOPTERA: FORMICIDAE), BY THE SPIDER, *STEATODA FULVA* (KEYSERLING) (ARANEAE: THERIDIIDAE) IN BAJA CALIFORNIA SUR, MÉXICO

William H. Clark

Orma J. Smith Museum of Natural History
Albertson College of Idaho
Caldwell, Idaho 83605 USA

Spider associations with ants are varied: predation on ants is known (Wuorenrinne 1956; Engelhardt 1970; Heller 1974, 1976; MacKay 1982; McIver 1989; to list a few) but specific instances of spider predation upon harvester ants of the genus *Pogonomyrmex* in the literature are less common (Moggridge 1873, 1874; McCook 1879; Hölldobler 1970; Edwards et al. 1974; Clark and Comanor 1975; MacKay 1982; Porter 1982; Wing 1983; Clark and Blom 1992) presumably because of the extremely hostile nature of these ants (Cole 1968, MacKay 1982). The spider family Theridiidae seems especially prone to predation upon ants (McCook 1879; Levi 1954, 1957; Hölldobler 1970; Snelling and George 1979; MacKay 1982; Porter 1982; Nyffeler et al. 1988; Clark and Blom 1992). Ants are also known to feed on spiders (Petal and Breymerer 1969, Cherix and Bourne 1980) and spiders may even be myrmecophilous (Donisthorpe 1927, Gertsch 1979, Wuorenrinne 1956). Some spiders mimic ants (Gertsch 1979, Jackson 1982, McIver 1989).

Little is known concerning the biology/natural history of *P. tenuispina* (Creighton 1950, Cole 1968, Wheeler and Wheeler 1973, Snelling and George 1979, Snelling 1981, and MacKay et al. 1985). The distribution of the species is southern California, USA, and Baja California, México (MacKay et al. 1985). The type locality for the species is unknown but may be in the Miraflores area of the Cape Region, Baja California Sur (Cole 1968). Blom and Clark (1980) reported the species from Baja California Norte, where it is uncommon compared to other seed harvester ant species in the Cataviña area.

Steatoda fulva is known from the Cape Region of Baja California Sur (Levi 1957, Jiménez 1988). Levi (1957) stated the following concerning the natural history of the *S. fulva* group of Theridiidae, "Virtually nothing is known of the natural history of these three species [*S. medialis* (Banks), *S. fulva*, and *S. Pulcher* (Keyserling)]. It is assumed that all three are found in arid situations under stones, logs and in debris." Hölldobler (1970) has provided the most detailed information. He found *S. fulva* constructing webs across the nest entrances of *P. badius* (Latreille) near Tampa, Florida, USA, during a period of ant inactivity (midafternoon with high temperatures). After a few ants were captured in the webs, the remaining ants closed their nest entrances. Hölldobler did not observe the spiders chasing ants. Jiménez (1988) briefly described the web of *S. fulva* and noted that the spider could be found below rocks and logs at 840 m in the the mountains in Baja California Sur, México.

Snelling and George (1979) note that spiders such as *Steatoda* are common ant predators in our local deserts: "The spider builds a fine web over the nest entrance of a colony, presumably during a period when it is inactive, and seizes unwary ants as they emerge to forage. It is cryptically colored and barely visible among the soil particles which

TABLE 1. Summary of Interactions Between *Pogonomyrmex tenuispina* and *Steatoda fulva* in Baja California, Sur, Mexico.

DATE/TIME	NEST #1	NEST #2
March 5, 1974		
1600	Spider attempted to capture an ant from behind, ant turned and faced spider and spider retreated, repeated three times. Ant retreated into nest entrance and plugged the hole with sand particles.	No spider activity.
1700	Nest entrance closed.	Nest entrance closed.
March 6		
0600		Spider web constructed over ant nest entrance.
1320	Spider on edge of nest,made three attempts to capture ants passing by with no success.	
1430		Spider near nest caught an ant but ant was able to struggle free, at this point the spider jumped back away from the ant.
1540	Spider made 2 attempts at ant capture from the rear near nest, each time the ant turned, faced the spider, the spider retreated.	
2100		Spider placing new web across entrance (spider collected).A second spider feeding on ant in web on blade of grass 25 cm east of the entrance (collected).
7 March		
0910	Spider at nest entrance, entrance closed and no ants active.	
1030	Another spider four m from nest.	
1035	Another spider two m S of nest made unsuccessful attempt on ant.	
1130	First ant activity at nest #1.	
8 March		
1115	Spider feeding on ant attached to small stick with web at edge of nest. Spider had a grip on ant head venter. Ants not active.	
1210	Spider followed ant (*P. californicus*) to different nest entrance Spider fled as ant neared nest entrance.	
1420	Spider following *P. californicus* one m from nest unable to capture ant.	

hang in the webbing. The colony members eventually become aware of the spider and close the nest entrance entirely, constructing a new one in the vicinity sometime later."

The objective of the present study was to add biological/natural history information concerning *Pogonomyrmex tenuispina* Forel and *Steatoda fulva* (Keyserling) in Baja California Sur, México.

Field observations were conducted on two nests of *P. tenuispina* and a nest of *P. californica* (Buckley) during late February and early March 1974 in Baja California Sur, México. The study site is located in the Cape Region, 13.6 km south of Miraflores along México Highway 1, at latitude 23°15'N and longitude 109°44'W, elevation 230 m. The area lies within the Cape Region phytogeographic area (Coyle and Roberts 1975, Gilmartin and Neighbours 1978, Wiggins 1960), an area of arid tropical forest. Voucher specimens are in the collections of the author (WHCC) and the Orma J. Smith Museum of Natural History, Albertson College of Idaho (ALBRCIDA).

Pogonomyrmex tenuispina and *P. californicus* (Buckley) were both found at this study site but inhabited different habitats. *P. californicus* was restricted to the sandy arroyo areas and two nests of *P. tenuispina* were found in a rocky cut into the hillside evidently resulting from highway construction about 1961 (Gerhard and Gulick 1970). Both species of *Pogonomyrmex* were actively harvesting plant seeds at this time and did not seem to interact with each other.

The spider, *Steatoda fulva* (Keyserling) was observed constructing webs across *P. tenuispina* entrances, chasing individual workers, and feeding on captured ants. I made observations of these ants and spiders during two weeks and summarize the results of a four day observation period (Table 1).

P. tenuispina made nests with an external appearance of an approximately 15 cm diameter, low (ca 1 cm) ring of gravel, around a large, 1-cm diameter entrance hole. The species nests in more rocky areas and not in the sandy arroyos as does *P. californicus*. Ants were observed foraging plant seeds at air temperatures of 35°C and ground surface temperatures of 42°C. *P. tenuispina* would aggregate around the nest entrance when disturbed and take up a defensive posture, consisting of the gaster being brought up under the ant, between its legs, and pointed at an intruder. Early morning activity seemed to be spent mostly in nestwork (moving sand and debris out of the nest). Foraging took place later in the morning and into the afternoon.

The observations reported here indicate that *S. fulva* has better success capturing prey (*Pogonomyrmex*) in its webs compared to direct prey capture. The spider was only observed preying on *Pogonomyrmex* during these observations. *Pogonomyrmex* appears to be able to fend off *S. fulva* in one-on-one confrontations. As Hölldobler (1970) found, spider activity alters the behavior of *Pogonomyrmex*. The ants seemed to close their entrance or simply remain inside their nest when *S. fulva* was hunting nearby.

ACKNOWLEDGEMENTS

Mary and Ellen Clark and Jane Clark Luther assisted with the field research. Dr. Arthur C. Cole, Jr. verified the *Pogonomyrmex* identifications and Dr. Norman Platnick determined *Steatoda fulva*. This research was assisted with a grant from Sigma Xi, The Scientific Research Society of North America.

LITERATURE CITED

Blom, P.E., and W.H. Clark. 1980. Observations of ants (Hymenoptera: Formicidae) visiting extrafloral nectaries of the barrel cactus, *Ferocactus gracilis* Gates (Cactaceae), in Baja California, Mexico. SW Natur. 25:181-196.

Cherix, D., and J.D. Bourne. 1980. A field study on a super-colony of the red wood ant *Formica lugubris* Zett. in relation to other predatory arthropodes (spiders, harvestmen and ants). Revue suisse Zool. 87: 955-973.

Clark, W.H., and P.E. Blom. 1992. Notes on spider (Theridiidae, Salticidae) predation of the harvester ant, *Pogonomyrmex salinus* Olsen (Hymenoptera: Formicidae: Myrmicinae), and a possible parasitoid fly (Chloropidae). Great Basin Natur. 52:385-386.

Clark, W.H., and P.L. Comanor. 1975. Removal of annual plants from the desert ecosystem by western harvester ants, *Pogonomyrmex occidentalis.* Environ. Entomol. 4:52-56.

Cole, A.C., Jr. 1968. *Pogonomyrmex* harvester ants: A study of the genus in North America. Univ. TN Press, Knoxville. 222 pp.

Coyle, J., and N.C. Roberts. 1975. A field guide to the common and interesting plants of Baja California. Natur. Hist. Publ. Co., La Jolla, CA. 206 pp.

Creighton, W. S. 1950. The ants of North America. Bull. Mus. Comp. Zool. 104: 1-585.

Donisthorpe, H.J.K. 1927. The guests of British ants. George Routledge and Sons, Ltd., London. 244 pp.

Edwards, G.B., J.F. Carroll, and W.H. Whitcomb. 1974. *Stoidis aurata* (Araneae: Salticidae), a spider predator of ants. FL Entomol. 57: 337-346.

Engelhardt, W. 1970. Gestalt und lebensweise der ameisenspinne *Synageles venator* (Lucas) zugleich ein beitrag zur ameisenmimikryforschung. Zool. Anz. 185:317-334.

Gerhard, P., and H.E. Gulick. 1970. Lower California guidebook. Arthur H. Clark Co., Glendale, CA. 243 pp.

Gertsch, W.J. 1979. American spiders. Van Nostrand Reinhold Co., NY. 274 pp.

Gilmartin, A.J., and M.L. Neighbours. 1978. Flora of the Cape Region, Baja California, Sur. Nat. Geog. Soc. Resear. Rpt., 1969 Projects. pp. 219-225.

Heller, G. 1974. Zur biologie der ameisenfressenden spinne *Callilepis noctura* Linnaeus 1758 (Aranea, Drassodidae). PhD. Diss. Johannes Gutenberg-Univ., Mainz, Germany. 119 pp.

Heller, G. 1976. Zum beutefangverhalten der ameisenfressenden spinne *Callilepis nocturna* (Arachnida: Araneae: Drassodidae). Entomol. Germ. 3:100-103.

Hölldobler, B. 1970. *Steatoda fulva* (Theridiidae), a spider that feeds on harvester ants. Psyche 77:202-208.

Jackson, R.R. 1982. The biology of ant-like jumping spiders: intraspecific interactions of *Myrmarachne lupata* (Araneae, Salticidae). Zool. Jour. Linn. Soc. 76:293-319.

Jiménez, M.L. 1988. Aspectos ecológicos de las arañas, pp. 149-164. *IN* L. Arriaga and A. Ortega [eds], La Sierra de La Laguna de Baja California Sur. Publ. 1, Cent. Invest. Biol. Baja CA Sur, La Paz.

Levi, H.W. 1954. Spiders of the genus *Euryopis* from North and Central America. Amer. Mus. Nov., 1666:1-48.

Levi, H.W. 1957. The spider genera *Crustulina* and *Steatoda* in North America, Central America, and the West Indies (Araneae, Theridiidae). Bull. Mus. Comp. Zool. 117:367-424.

McIver, J.D. 1989. Protective resemblance in a community of lupine arthropods. Nat. Geog. Resear. 5:191-204.

MacKay, W.P. 1982. The effect of predation of western widow spiders (Araneae: Theriudiidae) on harvester ants (Hymenoptera: Formicidae). Oecologia 53:406-411.

MacKay, W.P., E.E. MacKay, J.F. Perez-D., L.I. Valdez-S., and P. Vielma-O. 1985. Las hormigas del estado de Chihuahua Mexico: El genero *Pogonomyrmex* (Hymenoptera: Formicidae). Sociobiology 11:39-54.

McCook, H.C. 1879. The natural history of the agricultural ant of Texas. A monograph of the habits, architecture, and structure of *Pogonomyrmex barbatus*. J.B. Lippincott and Co., Philadelphia, PA. 311 pp.

Moggridge, J.T. 1873. Harvesting ants and trap-door spiders with notes and observations on their habits and dwellings. L. Reeve and Co., London. 156 pp.

Moggridge, J.T. 1874. Supplement to harvesting ants and trap-door spiders with notes and observations on their habits and dwellings. L. Reeve and Co., London. Pp. 157-304.

Nyffeler, M., D.A. Dean, and W.L. Sterling. 1988. The southern black widow spider, *Latrodectus mactans* (Araneae, Theridiidae), as a predator of the red imported fire ant, *Solenopsis invicta* (Hymenoptera, Formicidae), in Texas cotton fields. Jour. Appl. Entomol. 106:52-57.

Petal, J., and A. Breymeyer. 1969. Reduction of wandering spiders by ants in a Stellario-Deschampsietum meadow. Bull. Acad. Polonaise Sci. 17:239-244.

Porter, S.D. 1982. *Euryopis coki* (Theridiidae) a spider that preys on *Pogonomyrmex* ants. Jour. Arachnol. 10:275-277.

Snelling, R.R. 1981. The taxonomy and distribution of some North American *Pogonomyrmex* and descriptions of two new species (Hymenoptera: Formicidae). Bull. So. CA Acad. Sci. 80:97-112.

Snelling, R.R., and C.D. George. 1979. The taxonomy, distribution and ecology of California desert ants. Rpt. USDI, Bur. Land Mangt., Riverside, CA. 335 pp.

Wheeler, G.C., and J. Wheeler. 1973. Ants of Deep Canyon. Univ. CA Press, Riverside, xiii + 162 pp.

Wiggins, I.L. 1960. Symposium: The biogeography of Baja California and adjacent seas. Part III. Terrestrial and fresh-water biotas. The origin and relationships of the land flora. Syst. Zool. 9:148-165.

Wing, K. 1983. *Tutelina similis* (Araneae: Salticidae): an ant mimic that feeds on ants. Jour. KS Entomol. Soc. 56:55-58.

Wuorenrinne, H. 1956. *Thyreosthenius biovatus* Camb.- ja *Metopobactrus prominulus* Camb.- *hämähäkkien esiintymisestä* Suomessa. Arch. Soc. Zool. Bot. Fennicae Vanamo 10:1.

MINUTES OF THE 1996 ANNUAL MEETING OF THE EXECUTIVE COMMITTEE OF THE SOUTHWESTERN ENTOMOLOGICAL SOCIETY

The Executive Committee met at 9:00 a.m., January 15, 1996, at the Red Lion Inn in Austin, Texas, during the Annual Meeting of the Southwestern Branch of the Entomological Society of America. President Cocke called the meeting to order. Present were President Jesse Cocke, Past-President Pete Lingren, President-elect Joe Ellington, Editor Darrell Bay, Pete Teel, member of the Editorial Committee and Allen Knutson, Secretary-Treasurer.

Editor Bay reported that 70 manuscripts had been submitted for publication in 1995 which was the same number as last year. Supplement no. 18 was published and no. 19 is scheduled for publication in 1996. The quality of the manuscripts submitted in Spanish has improved and the efforts of Associate Editor Broce in this regard were recognized. Discussion followed on ways to expedite review and revision of Spanish manuscripts and President Cocke appointed Editors Bay and Broce to a committee to address this concern.

Secretary-Treasurer Knutson distributed the Treasurer's report. Membership in 1995 (400) was similar to that in 1995 (405) and income met expenses. The need to increase membership dues and subscription fees was discussed. Since the Society was meeting its financial obligations, no increase in fees was deemed necessary. A motion to support the American Association of Zoological Nomenclature with a one time $50 donation was approved. A motion was also made and seconded to exchange entomological journals, upon approval by the Executive Committee, and deposit such journals in the Library Museum of the Entomology Department at Texas A&M University. The Society is currently exchanging journals with the "Mitteilungen des Thuringer Entomologenverbandes" in Germany. A motion was also made and passed to provide a one year free subscription to the first place winner of the Student Paper Contest at the annual meetings of the ESA Southwestern Branch. The topic of electronically publishing the Southwestern Entomologist was discussed. The Committee then discussed electronic publication of the Southwestern Entomologist but decided to wait and learn from the experiences of other publications (i.e. Florida Entomologist) which are being provided electronically.

President Cocke reported that the Nominating Committee composed of D. Rummel, P. Morrison and W. Newton had nominated Miles Karner and Joe Ellington for President-elect. The need to fill the position of J. Michels who rotated off the Editorial Committee was discussed.

There being no further business, the meeting was adjourned at 10:15 a.m.

MINUTES OF THE 20TH ANNUAL MEETING OF THE SOUTHWESTERN ENTOMOLOGICAL SOCIETY

The 20th Annual Meeting of the Southwestern Entomological society was called to order by President Jesse Cocke, Jr. at 8:00 a.m., January 17, 1996, at the Red Lion Inn during the Annual Meeting of the Southwestern Branch Meeting of the Entomological

Society of America. Thirty-two members were present. President Cocke noted that the Minutes of the 19th Annual Meeting were published in the June issue of the Southwestern Entomologist and asked for any corrections or additions. There being none, a motion was made and seconded to approve the minutes as published. Motion passed. Secretary-Treasurer Allen Knutson presented the Editor's report for Darrell Bay who was unable to attend because of teaching responsibilities. Knutson also distributed and reviewed the Secretary-Treasurer's report.

President Cocke then reported on Executive Committee meeting, including the review of electronic publishing and the policy regarding late payment of membership dues. Currently, membership payment received before June 1 is applied to the current year and the March issue is sent to the members with the society bearing the single-piece postage cost. After further discussion, a motion was made and seconded that membership payments received after March 1 would be applied to the following year. Motion passed. Members renewing after March 1 and desiring to receive the current year volume can do so by purchasing those numbers and supplement(s) as back-issues at $5.00 each plus postage.

A motion was made and seconded to award a one year membership to the first place winner of the Student Paper Competition in both the oral and poster categories of the ESA Southwestern Branch Meeting. A motions was made and seconded to support the AASN with a $50 contribution which would be reviewed annually. Following a discussion on exchanging journals with other entomological societies, a motion was made and seconded to donate exchanged journals to the University Library at Texas A&M so they would be available to all members through interlibrary exchange. Motion passed.

President Cocke then took nominations from the floor to fill the vacancy on the Editorial Committee. There being no other nominations, Jesus Esquival was elected by majority vote. Greg Cronholm, Society Archivist, reported he had met with David Chapman, University Archivist at TAMU on January 9 to deliver the Society's historical records. documents deposited included letters and notes on the Society's history, and copies of the constitution, tax status, editorial policies, supplement editors, editor reports, correspondence, committee assignments, meeting agendas, membership lists and Secretary-Treasurer minutes. Greg thanked Horace Burke, Bill Chamberlain and Allen Knutson for providing materials.

President Cocke reported that 128 ballots for President-elect had been received and counted and that James Coppedge had received the most votes. President Cocke then asked for any new business. There being none, he recognized Joe Ellington as incoming President. Past-President Pete Lingren recognized Jesse Cocke for his service as President of the Society. President Ellington thanked the membership for their support and adjourned the meeting at 8:50 a.m.

Respectfully submitted,

Allen Knutson
Secretary-Treasurer

1995 SECRETARY-TREASURER'S REPORT

Balance on hand as of January 31, 1995	$13,519.84
Income: February 1, 1995 - January 31, 1996	
Memberships	3,463.00
Subscriptions	1,380.00
Page Charges	18,948.76
Back-issues, misc.	136.85
Total Income	$23,928.61
Expenses: February 1, 1995 - January 31, 1996	
Journal:	
Printing	12, 703.64
Secretary	1,782.50
Supplies	490.40
Supplies, editor	250.00
Postage	1,890.72
Editor	1,800.00
Society:	
Secretary	900.00
Bank Charges	5.00
Supplies	92.53
Postage	528.85
Plaque	0.00
Secretary-Treasurer	1,200.00
Total Expenses	$21,643.64
Balance on hand January 31, 1996	$15,804.81

As of January 31, 1996, there were 400 members paid for 1995 and 102 subscribers. There were 19 unpaid page charges, totaling $4,667.74. The December journal was shipped January, 1996, and represents 16 of the 19 pending invoices which total $4,091.00.

Respectfully submitted,
Allen Knutson
Secretary-Treasurer

AUDIT COMMITTEE REPORT:

On April 12, 1996, I examined the 1995 fiscal records of the Southwestern Entomological Society prepared by Secretary-Treasurer Allen Knutson and records of income and expenses and found these records to be in order.

Respectfully submitted,
Robert Crocker

EDITOR'S REPORT:

There were 58 manuscripts and a total of 529 pages in the four regular issues published in 1995 compared to 52 manuscripts and 428 pages in 1994. This is the second time that the four regular issues have exceeded 500 pages, and the current volume is the largest to date. One supplement, Number 18, also was completed during this time and a Number 19 remains in preparation.

I received 70 manuscripts, the same number as in 1994, for consideration. Three of these were rejected and a number are still in review; however, it appears that the rejection rate will be lower than the eight from last year.

Editor's Financial Report

Balance on hand January 1, 1995	$123.24	
Income		
From Treasurer	400.00	
Total Income	523.24	
Expenses:		
Stamps		280.00
Airborne Express		24.15
Total Expenses		304.15
Balance on hand January 1, 1996	$219.09	

Respectfully Submitted,
Darrell E. Bay, Editor

HICKORY SHUCKWORM, *CYDIA CARYANA* (FITCH)[1], LARVAL RESPONSE TO TWO LOW TEMPERATURES

Urbano Nava-Camberos, Marvin K. Harris, and Bill Ree
Department of Entomology, Texas A&M University,
College Station, TX 77843-2475

ABSTRACT

Pecan shucks infested by hickory shuckworm larvae *Cydia caryana* (Fitch) (Lepidoptera: Tortricidae), were exposed to temperatures of -2 and 9 °F for up to 336 hours (14 days). All larvae collected 22 October, 1992 were dead after 48 and 336 hours of exposure to -2 °F and 9 °F, respectively. More than 90% mortality was attained after 48 hours at 9 °F. Larvae collected 16 November, 1992 were affected less by the same temperatures. More than 90% mortality was eventually achieved, but only after 336 hours.

These data indicate that fall populations of hickory shuckworm larvae vary in their tolerance to cold. This variability may be due to the time when they enter diapause. Further studies are needed to fully characterize larval susceptibility through time and length of exposure at lower temperatures. Based on these data, quarantine requirements are premature, but temperatures below 0 °F would be necessary to eliminate hickory shuckworm from infested pecans.

INTRODUCTION

The hickory shuckworm (HSW), *Cydia caryana* (Fitch), is a major multivoltine pest of several nut crops including pecan, *Carya illinoensis* (Wang) K. Koch (Gunasena and Harris 1987). HSW larvae destroys nuts completely if attack occurs before the gel stage; however, nuts attacked after this point of pecan maturation have larval tunneling in the shuck during late summer and early fall. Depending on when attack occurs and the degree of tunneling damage at the post-gel stage, damage can range from zero to failure of the pecan kernel to develop. If nuts are tunneled severely during early formation of the kernel, nutrients will be lacking to complete nut development, because shuck damage interferes with nutrient flow from the twig to the developing kernel. Nuts attacked following kernel formation are indirectly affected, because they sometimes fail to dehisce their shuck properly. This causes problems in harvesting, handling, and marketing (McWhorter et al. 1979, Harris 1983, Gunasena and Harris 1987, Harris 1991).

The HSW overwinters as mature larvae in pecan shucks. They pupate in late winter and early spring, and adults emerge during spring and early summer (McWhorter et al. 1979, Harris 1983, Gunasena and Harris 1987).

Temperatures below freezing in the Bryan-College Station area are possible during fall and winter. Record low temperatures for January, February, March, October, November and December are -3, 5, 17, 29, 19 and 14 °F, respectively (pers. comm., Dr. Mickey Flynn, meteorologist National Weather Service, College Station, TX). The degree to which these

[1]Lepidoptera: Tortricidae

temperatures affect survival of mature larvae is important in understanding the basic biology of the HSW. In addition, control programs could be established against this species, using low temperature treatments on nuts with infested shucks. Gunasena and Harris (1987) determined that the longer the diapausing larvae were held in cold storage (5 ± 1 °C = 41 °F) during January-June, the shorter the time required for adult emergence. The time range varied from 2 to 5 weeks, and differences in mortality were not significant under different lengths of cold storage. On the other hand, Harris (1973) found 100 % mortality of pecan weevil larvae after 30 hours at -20 °C (-4 °F), after 3 days at -10 °C (14 °F) and after 6 days at 38 °C (100.4 °F). No differences in larval mortality were observed in temperatures ranging from -2 °C (28.4 °F) to 29 °C (84.2 °F). Therefore, the present study was initiated to evaluate the effects of two low temperatures on HSW larvae.

MATERIALS AND METHODS

Pecan shucks infested by HSW were collected 22 October 1992 and 16 November 1992 from the Royalty Pecans orchard in Burleson county, TX. Infested shucks were subjected to 14 treatments, representing two temperatures, -2 °F (± 2 °F) and 9 °F (± 3 °F), and seven exposure times, 12, 24, 48, 96, 120, 168 and 336 hours. Each treatment was replicated six times. For the first collection date, each treatment consisted of a 100 gram shuck sample. Nuts collected in November were sorted into goups of five, with infested shucks, to represent each treatment. Samples were placed in 500 ml-plastic cups. After exposure times, samples were removed from their respective temperature treatments and held under laboratory conditions (mean temperature 73 °F) for 24 hours. Afterward, samples were examined for the presence of live and/or dead larvae. This information was recorded for each treatment within each replication.

For both collection dates, percent mortality per treatment was transformed using arcsine of the square root and tested using analysis of variance (SAS Institute 1987). Treatment means were sorted for differences using a Tukey's studentized range test. Mean response was transformed back to percent mortality for the purpose of reporting results in this paper.

RESULTS AND DISCUSSION

The number of live and dead larvae was different among the two collection dates due to differences in sample size. For the first collection date high numbers of dead larvae were obtained from both temperature regimes. In contrast, larvae collected in November yielded lower and more variable numbers of dead larvae, depending on temperature and exposure time. Fig. 1 and 2 present the percent mortality values of HSW larvae after exposure to -2 and 9 °F for the various time periods. Significant differences in percent mortality were found between treatments for each collection date.

Fig. 1 indicates HSW larvae collected 22 October could not withstand -2 °F, but survived 168 hours of exposure to 9 °F. Complete larval mortality of this population was achieved only after 336 hours (14 days) of exposure to 9 °F.

HSW larvae collected 16 November, exposed to the two temperatures, were affected less (% mortality) than insects collected earlier in the year. Complete mortality of this larval population was not attained even after 336 hours at the lowest temperature (Fig. 2). This may be due to effects of diapause in late fall larval populations. Pecan weevil is apparently less tolerant to cold than HSW (Harris 1973). This may be related to differences in overwintering habitat between the two species, since the pecan weevil burrows about 10 cm into the soil during fall and early winter, whereas the HSW overwinters in shucks on the soil surface.

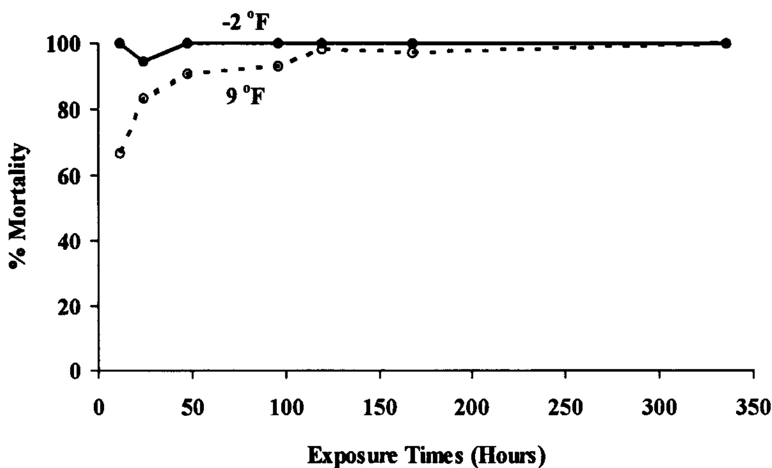


FIG. 1. Percentage mortality of hickory shuckworm larvae after infested shucks were subjected to two low temperatures. Collection date: 22 October, 1992.

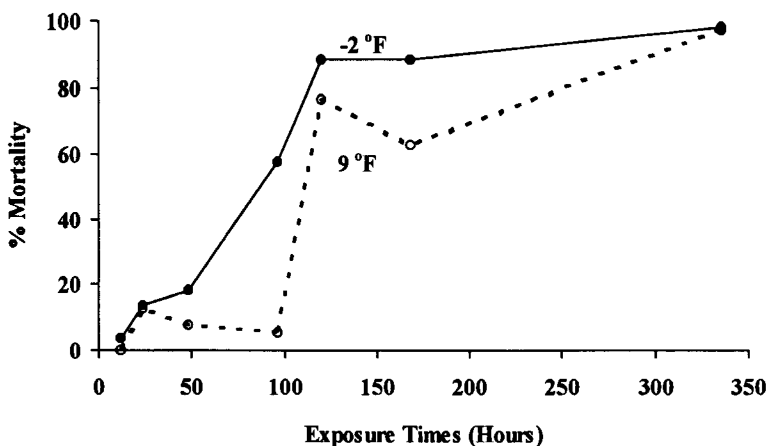


FIG. 2. Percentage mortality of hickory shuckworm larvae after infested shucks were subjected to two low temperatures. Collection date: 16 November, 1992.

Data show that HSW larvae collected 22 October can be completely killed by exposing them to temperatures of -2 °F for 48 hours and to 9 °F for 336 hours. More than 90% mortality was achieved at 9 °F after 48 hours. HSW larvae collected 16 November were less affected by the same temperatures. More than 90% mortality was achieved only after 336 hours of exposure to -2 °F or 9 °F. Populations of HSW from 16 November were more tolerant to cold than populations from 22 October, indicating the latter had not yet entered diapause.

Based on results of this study, record low temperatures in Central Texas could have an acute effect on survival of HSW larvae. Guidelines for establishing quarantine treatments on transport of infested shucks are premature based on these data, but apparently will require either a longer exposure time or lower temperatures than current quarantine for pecan weevil (0 °F for 7 days) to achieve complete control.

LITERATURE CITED

Gunasena, G. H. and M. K. Harris. 1987. Effect of humidity and cold storage on diapause termination of hickory shuckworm larvae. Southwest. Entomol. 12:25-31.

Harris, M. K. 1973. Pecan weevil larval response to some temperatures while in the nut. TAES PR-3176.

Harris, M. K. 1983. Integrated pest management of pecans. Ann. Rev. Entomol. 28:291-318.

Harris, M. K. 1991. Pecan IPM, pp.691-706. *In* D. Pimentel [ed.] Handbook of Pest Management in Agriculture, 2nd Ed., Vol. III. CRC Press, Boca Raton, Florida.

McWhorter, G. M., J. G. Thomas, M. K. Harris, and H. W. Van Cleave. 1979. Pecan insects of Texas. Texas Coop. Ext. Serv., Texas A&M Univ., College Station. Misc. Publ. 1270. 18 pp.

SAS Institute. 1987. SAS/STAT user's guide for personal computers, version 6 ed. SAS Institute, Cary, NC.

SEASONAL INFESTATION BY PINK BOLLWORM, *PECTINOPHORA GOSSYPIELLA* (SAUNDERS)[1] OF TRANSGENIC COTTON, CONTAINING THE BOLLGARD™ GENE, PLANTED IN COMMERCIAL FIELDS IN CENTRAL ARIZONA[2]

H. M. Flint, L. Antilla[3], J. E. Leggett, N. J. Parks

USDA-ARS Western Cotton Research Laboratory
4135 E. Broadway Rd., Phoenix, AZ 85040-8830

ABSTRACT

Bolls from transgenic cotton NuCOTN 33 (Delta and Pine Land Co.), containing the Bollgard™ gene (Monsanto Co.) and from the parental cultivar DPL-5415 were examined for fourth-instar larvae of the pink bollworm (78,240 total bolls). Bolls from five paired fields were collected in the Queen Creek, Buckeye, and Gila Bend, AZ, areas, and a composite of ten fields of each cultivar were collected in the Paloma Ranch area near Gila Bend, AZ. Collections of 100 or 80 bolls per field were made weekly from July through November 1995. Numbers of pink bollworm larvae were very low in all fields through August and thereafter increased steadily in the control fields. Numbers of fourth-instar larvae in transgenic cotton were extremely low or zero throughout the season, even in fields which were adjacent to heavily infested control fields. Observations of infestations of the cotton leafperforator, *Bucculatrix thurberiella* Busck, in the paired field study also indicated greatly reduced leaf damage in NuCOTN 33 compared to DPL-5415. These results show that NuCOTN 33 retained a high degree of efficacy for preventing the development of fourth-instar pink bollworm larvae late in the season into diapause. Most important, these data provide baseline information against which efficacy in subsequent years can be compared.

INTRODUCTION

Transgenic lines of cotton, *Gossypium hirsutum* L., that carry a gene for a toxin protein (Cry1A) produced by *Bacillus thuringiensis* var. kurstaki (Berliner) (Bollgard™ gene, Monsanto Co., St. Louis, MO) show a high level of resistance to infestation by the pink bollworm, *Pectinophora gossypiella* (Saunders) (Wilson et al. 1992, Flint et al. 1995). Heretofore, these lines had a common genetic background, Coker 312, the cultivar used to regenerate them in culture. The Bollgard™ gene was subsequently transferred by traditional breeding methods to locally adapted cultivars for commercial sale in 1996. The

---

[1] Lepidoptera: Gelechiidae.

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

[3] Arizona Cotton Research and Protection Council, 2803 W. Huntington Dr., Tempe, AZ 85282.

need for commercial seed led to seed-increase plantings in Arizona contract fields in 1995. One of the transgenic cultivars, NuCOTN 33 (Delta and Pine Land Co., Scott, MS), was grown under commercial management practices in several areas of central Arizona. These fields provided an opportunity to compare the extent of pink bollworm infestations in NuCOTN 33 with its parent cultivar Deltapine 5415 (DPL-5415) in the first year of bollworm infestations, particularly late in the season, and to establish baseline efficacy data. By establishing baseline efficacy data, subsequent measurements of resistance (Deaton 1995) may be made in subsequent years.

MATERIALS AND METHODS

Paired Field Studies. In this study, NuCOTN 33 was compared to DPL-5415 in five paired fields under the management of four growers. Paired fields were located at Queen Creek, Buckeye, and Gila Bend, AZ. One comparison (field pair A) consisted of 2 ha of NuCOTN 33 grown in 35 rows through the center of a single full-size field (>16 ha) of DPL-5415. Two other comparisons (field pairs B and C) consisted of separate full-size fields of NuCOTN 33 with a single adjoining larger field of DPL-5415 divided in half to provide two controls. Each half was immediately adjacent to its respective field of NuCOTN 33. Two of the comparisons (field pairs D and E) were between adjacent full-size fields. Planting and termination dates, fertilization, insect control, and other management practices were considered proprietary by the growers and are not provided. However, all practices were standard for the areas and were uniformly applied to the paired cultivars. Most insecticide applications were for control of the silverleaf whitefly, *Bemisia argentifolii* Bellows and Perring, with some applications for lygus bugs, *Lygus hesperus* Knight, or beet armyworm, *Spodoptera exigua* (Hübner). Insecticide applications for these insects were made on a scale sufficient to suppress pink bollworm in both cultivars during much of the season.

Samples of 100 firm green bolls (14-21 d old) were collected weekly from each field from mid-July until the end of the season in November by walking directly through one half of the field and returning through the other half while collecting 25 bolls per quadrant. Additional bolls were collected during and after September to augment late season infestation data. We also took greater numbers of bolls from paired fields found to have greater pink bollworm infestations. Bolls were incubated in ventilated plastic boxes at ca. 27°C and ambient humidity for 2 weeks before cut-out larvae (fourth-instar) were counted (July to mid-September). After mid-September, most fourth-instar larvae are in diapause and are found only by dissection of bolls. Therefore, all bolls collected after mid-September were dissected for larvae. The Bollgard™ gene does not reduce infestations of first-instar larvae, but very few of these larvae survive to the fourth-instar (Watson 1995). For this study, only fourth-instar larvae were counted in the transgenic and parent cultivars.

Five Delta traps baited with rubber septa baits (Scentry Inc., Buckeye, AZ) containing 4 mg of gossyplure (1:1 mixture of Z, Z- and Z, E-7, 11 hexadecadienyl acetate, Shin Etsu Chemical Industries, Tokyo, Japan) were placed in a single row (40 m between traps) through the center of each field on 8 May. Traps were serviced weekly, replaced as needed, and baits changed monthly. Traps were adjusted to be at the tops of cotton plants throughout the season and were removed from all fields on 2 October.

Infestations of the cotton leafperforator, *Bucculatrix thurberiella* Busck, were observed in the control fields in August. We decided to quantify damage by this insect in the five paired fields. Six weekly samples of 60 fifth main-stem node leaves per field (15

from each quadrant) were collected during the period 21 August to 29 September. Leaves were placed in paper bags and transported to the laboratory where the numbers of damaged leaves, mines (tunneling by first- to third-instar larvae) and windows (feeding on the lower leaf surface by late-instar larvae) per leaf were determined. Adults were also surveyed to determine if equal numbers were found in the two cultivars. Four Delta traps baited with 1 mg of the pheromone of the cotton leafperforator on rubber septa baits [100:2 mixture of (Z)-9-tetradecenyl nitrate and (Z)-8-tridecenyl nitrate, Natural Resources Institute, Kent, United Kingdom] were placed ca. 5 m outside and 30 cm above soil surface on the borders of each field and four traps were placed at plant height 30 m into the field from each border (Leggett et al. 1994). These traps were in service 3 days each week from 6 to 25 September, and male cotton leafperforators were counted each week. The data for leaf damage were compared by analysis of variance using MSTAT-C statistics program (Michigan State University 1990). Where a significant F value was obtained, we partitioned means into significant ranges using a Tukey test ($P = 0.05$). The data for trap catch were compared using t tests.

Composite Field Study. Ten fields each of NuCOTN 33 and DPL-5415 were selected in the Paloma Ranch area near Gila Bend, AZ. This area contains about 16,000 acres of cotton in a matrix of mixed agricultural crops, primarily alfalfa. Test fields were selected and the study conducted by the Arizona Cotton Research and Protection Council (ACRPC) with the objective of area coverage (fields not paired) and consistent planting dates. All cotton fields in this test (>16 ha) were under an insect control program supported by grower assessments and directed by ACRPC. The fields of DPL-5415 were treated with pink bollworm pheromone at pin-square (first flower bud) and thereafter as needed based upon trap catches of pink bollworm males in in-field traps through 30 September. The pheromone was applied at 25 gm of hollow fibers (Ecogen, Buckeye, AZ) per ha containing 1.85 gm of gossyplure. Conventional insecticides were required to control whiteflies or other insect pests as listed in the paired-field studies. The fields of NuCOTN 33 did not receive pheromone treatments, but did receive comparable applications of conventional insecticides. Fields were divided into quadrants and 40 bolls from each of two diagonal quadrants were collected weekly (80 bolls per field) beginning in mid-July. The other pair of diagonal quadrants were sampled the following week and alternately thereafter until the final samples were collected from available fields in early November. All bolls were dissected either after 2 week incubation or dissected without incubation. Five delta traps were placed in each of the 20 fields by the methods used in the paired-field studies.

RESULTS AND DISCUSSION

Paired Field Studies. Very few pink bollworm larvae were found in any of the fields until mid-September and then only in some fields (Table 1). In paired fields D and E, where a combined total of 4166 pink bollworm larvae were found in 7200 bolls of DPL-5415 during October, only two larvae were found in the same number of bolls of NuCOTN 33. The greater infestations in paired fields D and E were due to termination of insecticidal control after 12 and 16 September, respectively (our records). In paired fields B and C, no pink bollworm larvae were found in NuCOTN 33 throughout the season and only 51 larvae were found in paired control fields. Paired fields A, D, and E had no bolls developing on plants during November due to earlier termination of irrigation than in paired fields B and C.

TABLE 1. Infestation of Pink Bollworm in NuCOTN 33 and DPL-5415 in Five Paired Fields in Central Arizona and in a Composite of 10 Fields Each of NuCOTN 33 and DPL-5415 at Paloma Ranch, Gila Bend, AZ, 1995.

Field Pair	Cultivar	Jul		Aug		Sep		Oct		Nov	
		Bolls	PBW	Bolls	PBW	Bolls	PBW	Bolls	PBW	Bolls	PBW
A	NuCOTN 33	200	0	400	0	900	2	2200	9 [b]		
	DPL-5415	200	0	400	0	900	4	2200	7		
B	NuCOTN 33	200	0	400	0	900	0	2000	0	650	0
	DPL-5415	200	0	400	0	900	1	2000	5	650	10
C	NuCOTN 33	200	0	400	0	900	0	2000	0	650	0
	DPL-5415	200	0	400	0	900	5	2000	23	650	7
D	NuCOTN 33	200	0	400	0	2100	0	4800	1		
	DPL-5415	200	1	400	5	2100	256	4800	1591		
E	NuCOTN 33	200	0	400	0	1100	0	2400	1		
	DPL-5415	200	0	400	1	1100	81	2400	2575		
Composite [a]	NuCOTN 33	1400	0	3680	0	4480	0	3840	0	1320	0
	DPL-5415	2360	0	5000	1	4160	12	3200	45	1600	81

[a] Data from dissected bolls collected by the Arizona Cotton Research and Protection Council.
[b] These larvae may have inadvertently come from bolls of DPL-5415, see text.

In the NuCOTN 33 field of pair A, nine pink bollworm larvae were found during October, more than in the control field during the same period. These larvae were in five bags of 50 bolls each, collected over three dates. We suspect that the boll collectors accidentally wandered outside of the rows of transgenic cotton and sampled these bolls from the control area. This is the field where NuCOTN 33 was planted through the center of a larger field of DPL-5415 with no distinguishing boundaries between the two cultivars.

Trap catches indicated comparable numbers of adult pink bollworm moths in fields of NuCOTN 33 and DPL-5415 (Fig. 1). In these fields, the growers routinely sprayed both cultivars, primarily for whiteflies, and trap catches of pink bollworm occurred at comparable levels during the season.

In the paired field study, the mean number of leaves per 15-leaf sample showing damage by the cotton leafperforator was significantly greater in DPL-5415 than in NuCOTN 33 (6.55 and 0.88 leaves, respectively; F = 150.6; df = 1, 188; $P \leq 0.01$). Similarly, the numbers of mines per leaf (DPL-5415 = 3.79, NuCOTN 33 = 0.47; F = 42.7, df = 1, 188; $P \leq 0.01$) and windows per infested leaf (DPL-5415 = 12.24, NuCOTN 33 = 1.57; F = 27.3, df = 1, 188; $P \leq 0.01$) indicated significantly greater damage for DPL-5415. The numbers of cotton leafperforators caught in pheromone baited traps were comparable within (DPL-5415 = 260, NuCOTN 33 = 238 moths per trap; t = 0.42, df = 8; $P \leq 0.34$) and without (DPL-5415 = 299, NuCOTN 33 = 284 moths per trap; t = 0.14, df = 8; $P \leq 0.45$) the paired test fields. These results indicate that some infestation and feeding by mature larvae (windows) occurred in NuCOTN 33, but damage was greatly reduced compared to DPL-5415.

Composite Field Study. No mature pink bollworm larvae were found in 14,720 bolls of NuCOTN 33 throughout the season in the Paloma Ranch area (Table 1). Fields of DPL-5415 at Paloma Ranch had 139 pink bollworm larvae in 16,320 bolls collected throughout the season. However, DPL-5415 bolls collected during November had ca. 5 larvae per 100 bolls, suggesting that the transgenic cotton was exposed to potential infestation.

Trap catches indicated greater numbers of adult moths in fields of NuCOTN 33 early and late in the season (Fig. 2). The reduced catches in DPL-5415 early in the season were due to applications of pink bollworm pheromone, which was applied only to control fields. The greater catches in NuCOTN 33 at the end of the season were unexplained, but may have been due to earlier suspension of insecticide applications in these fields, due to confidence in NuCOTN 33 to control pink bollworm.

Control of the cotton leafperforator was anticipated with NuCOTN 33. Previous observations of cotton lines containing the Bollgard™ gene indicated 94-100% reductions of cotton leafperforator larvae compared to parental Coker 312 (Wilson et al. 1992, Flint et al. 1995). Watson (1995) found a high degree of control of cotton leafperforator based on leaf damage assessments in cotton lines containing the Bollgard™ gene. In our present test, we obtained an 87% reduction in leaf windows in NuCOTN 33 compared with DPL-5415. Windows are the result of late-instar larval feeding, but the fate of these larvae is unknown. The level of damage to NuCOTN 33 would not be considered of economic importance.

Previous tests of transformed cotton lines containing various insertions of the Bollgard™ gene in a Coker 312 background provided 95-99% reductions in bloom and boll infestations of pink bollworm (Wilson et al. 1992). Recent tests of similar lines gave 93-99.8% reductions in fourth-instar larvae of pink bollworm (Flint et al. 1995). Small plot tests, conducted during September 1994, comparing DPL-5415 and transgenic DPL-5415 containing the Bollgard™ gene, revealed 30% of the DPL-5415 bolls had late-instar

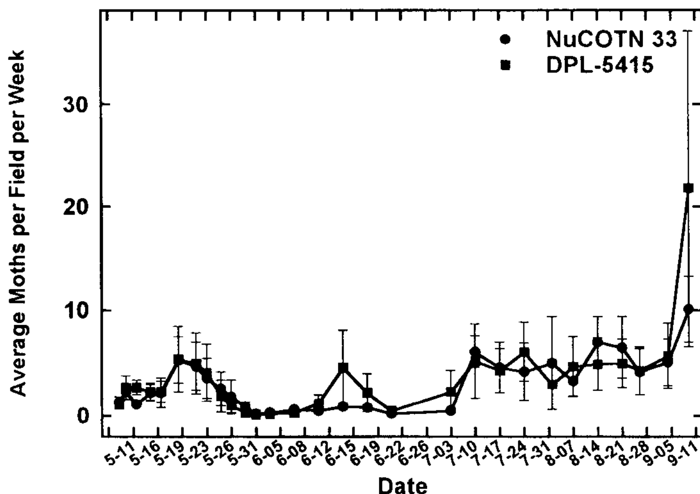


FIG. 1 Average catch per field per week for 5 traps in each of five fields of NuCOTN 33 and DPL-5415 in central Arizona, 1995.

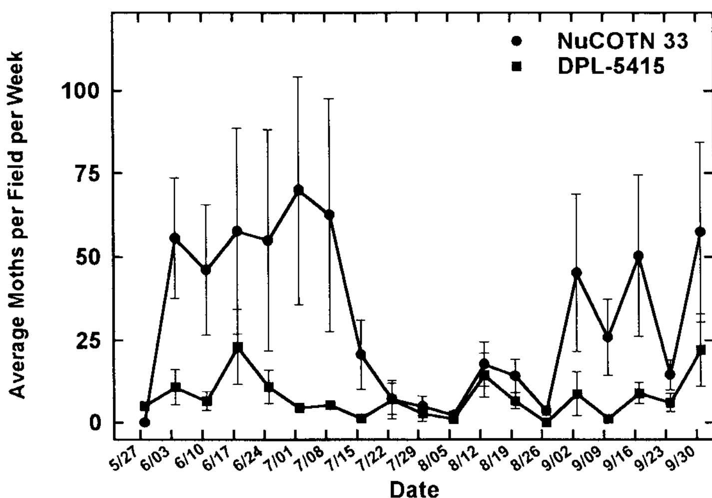


FIG. 2 Average catch per field per week for 5 traps in each of ten fields of NuCOTN 33 and DPL-5415 in the Paloma Ranch area of central Arizona, 1995.

larvae, whereas only 2% of the transgenic DPL-5415 bolls had late-instar larvae (Ellsworth et al. 1995). Our current results indicate that the Bollgard™ gene transferred to DPL-5415 (NuCOTN 33) provided nearly complete control of fourth-instar pink bollworm larvae in commercial fields in central Arizona. The capability of larvae found in NuCOTN 33 bolls to complete diapause, pupate, and yield a reproductive adult moth is unknown. Although the current results were obtained under conditions of heavy insecticide use to control whiteflies on both NuCOTN 33 and DPL-5415, reductions of larvae at the end of the season are particularly important. NuCOTN 33 provided virtual elimination of late season diapausing larvae in fields adjacent to two heavily infested control fields. Eliminating overwintering larvae is an important advantage in control programs with long term goals of area-wide eradication of pink bollworm.

Most importantly, our results provide baseline information on the efficacy of NuCOTN 33 in commercial plantings against which subsequent years can be compared.

ACKNOWLEDGMENT

The authors thank LeAnne Elhoff, Cindy Higuera, Jim Holmes, Jason Jones and Missy Webster who collected bolls and leaves and counted larvae in the paired field study. We also thank Maria Evans, Angie Soto, Shannon Vega and Rick Webb who collected bolls and counted larvae in the composite field study. Thanks are also owed to the growers and PCAs who cooperated in these studies.

LITERATURE CITED

Deaton, W. R. 1995. Managing for resistance to the Bollgard™ gene, pp. 758. *In* D. J. Heber and D. A. Richter [eds.], Proc. Beltwide Cotton Prod. Res. Conf., Natl. Cotton Council, Memphis, TN.

Ellsworth, P., J. Silvertooth, and J. Diehl. 1995. Bt cotton in Arizona: 94 variety results. The University of Arizona, College of Agriculture, Cooperative Extension. 2 pp.

Flint, H. M., T. J. Henneberry, F. D. Wilson, E. Holguin, N. J. Parks, and R. E. Buehler. 1995. The effects of transgenic cotton, *Gossypium hirsutum* L., containing *Bacillus thuringiensis* toxin genes for the control of the pink bollworm, *Pectinophora gossypiella* (Saunders) and other arthropods. Southwest. Entomologist. 20: 281-292.

Leggett, J. E., T. J. Henneberry, and R. D. White. 1994. Cotton leafperforator (Lepidoptera: Lyonetiidae) pheromone studies. Southwest. Entomol. 19: 229-236.

Michigan State University. 1990. A microcomputer program for analysis of agronomic research experiments. Michigan State University, East Lansing, MI.

Watson, T. F. 1995. Impact of transgenic cotton on pink bollworm and other lepidopteran insects, pp. 759-763. *In* D. A. Richter and J. Armor [eds.], Proc. Beltwide Cotton Prod. Res. Conf., Natl. Cotton Council, Memphis, TN.

Wilson, F. D., H. M. Flint, W. R. Deaton, D. A. Fischoff, F. J. Perlak, T. A. Armstrong, R. L. Fuchs, S. A. Berberich, N. J. Parks, and B. R. Stapp. 1992. Cotton lines containing a *Bacillus thuringiensis* toxin to pink bollworm (Lepidoptera: Gelechiidae) and other insects. J. Econ. Entomol. 85: 1516-1521.

SAMPLING APHIDOPHAGOUS COCCINELLIDAE IN GRAIN SORGHUM

G. J. Michels, Jr., N. C. Elliott[1], R. L. Romero[2], and T. D. Johnson[1]

Texas A&M Agricultural Experiment Station
P.O. Drawer 10
Bushland, TX 79012

ABSTRACT

Five sampling methods were used for sampling lady beetles in grain sorghum fields in the Texas High Plains. Removal sampling provided accurate estimates of absolute population for adult coccinellids but not for larvae. Sweep net sampling was not correlated with absolute population for adults or larvae. Timed counts of coccinellids were correlated with absolute population for adults, but results for larvae were inconclusive. A regression model was developed to convert relative estimates of adult populations obtained by timed counts to estimates of absolute population. The model accounted for 80% of the variability in the relationship between the relative density estimated by timed counts and absolute density estimated by removal sampling.

INTRODUCTION

Coccinellids are important aphid predators in grain sorghum fields in the southern Great Plains (Kring et al. 1985, Rice and Wilde 1988). Kring et al. (1985) demonstrated that early season suppression of greenbugs in grain sorghum in the Texas High Plains is primarily due to predation by coccinellids, while Rice and Wilde (1988) demonstrated that coccinellids significantly reduce greenbug populations in grain sorghum in Kansas. Although economic thresholds exist for the greenbug in grain sorghum, they do not take into account the potential impact of coccinellids on greenbug populations. Efficient sampling methods for coccinellids in grain sorghum would be a useful step toward incorporating coccinellid impact on greenbug populations in the IPM decision-making process.

To incorporate the impact of aphidophagous coccinellids into an IPM program, one must be able to estimate their populations, determine their impact on pest aphid populations, and relate predator numbers to economic thresholds. An adequate sampling procedure for coccinellids should provide estimates of the absolute population (i.e., the number of coccinellids per unit area) or be transformable to absolute population (Morris 1955). Specifically, it would be desirable to obtain statistically accurate and precise estimates of coccinellid population density with reasonable expenditure of time and effort.

[1] USDA, ARS, SPA, Plant Science Research Laboratory, 1301 North Western Street, Stillwater, OK 74075

[2] Present address, Headquarters, Ft. Carson, Attn: AFZC-TMD (Romero), DECAM Bldg. 302, Ft. Carson, CO 80913

Michels and Behle (1992) compared five relative sampling methods for estimating adult and larval coccinellid populations in grain sorghum. They found that counting coccinellids on 50 sorghum plants within a row was statistically the most precise method of those tested, and sampling efficiency of the method did not appear to be influenced by factors such as weather or plant growth stage. Two other methods, sweep net sampling and drop cloth sampling, also had potential for estimating relative population. These methods were not as statistically precise or accurate as the visual counting method but took less time to perform (Michels and Behle 1992).

The purpose of this study was to expand upon that of Michels and Behle (1992). In particular, our objectives were: 1) to test additional methods for sampling coccinellid populations in sorghum [i.e., D-Vac® sampling, removal sampling (Seber and Le Cren 1967), and quadrat sampling]; 2) to determine the adequacy of removal sampling for estimating absolute population density of adult coccinellids; and 3) to develop statistical models for converting population estimates obtained by relative sampling methods to estimates of absolute population.

MATERIALS AND METHODS

Sampling Methods. The research was accomplished during the spring and summer of 1991 and 1992 in a grain sorghum field at the Texas Agricultural Experiment Station Research and Extension Center near Bushland, TX. The field was managed using typical agronomic practices for furrow-irrigated sorghum in the High Plains, except that no insecticides were applied. During June of each year, several 24- by 24-m plots were established in the fields (12 in 1991, 8 in 1992). Each plot was divided into 36, 4- by 4-m subplots (Fig. 1). Plots were sampled once per grain sorghum growth stage from stage 2 (collar of 5th leaf visible) through stage 6 (flowering) (Vanderlip 1972).

Five sampling methods were used to sample coccinellid densities: 1) timed visual counts while walking at a constant velocity; 2) removal sampling; 3) sweep net sampling; 4) D-Vac suction sampling; and 5) complete enumeration of coccinellids in 1-m^2 enclosed quadrats. Methods 1 through 4 were used in 1991, while quadrat sampling was used instead of D-Vac sampling in 1992.

Timed counts. Each time a plot was sampled, a row of six subplots was chosen at random (Fig. 1). An observer walked through each of the six subplots within that row at a velocity of 8 m per min. All adult coccinellids seen in an approximately 1-m wide path immediately in front and along the direction of movement of the observer (prior to disturbance by the observer's walking) were counted and recorded. Upon reaching the end of the plot, the observer moved over approximately 2 m and walked the length of the plot in the opposite direction. Thus, the same path was traversed only once by the observer and a total of 1 min was spent searching for coccinellids in each subplot. The observer repeated the same procedure in two additional rows of subplots chosen so that no two adjacent rows were used for visual count sampling.

Removal sampling. Immediately after the observer finished counting within a row, three subplots within the row were selected randomly for removal sampling. The removal sampler entered a subplot within the row and collected in an aspirator all coccinellids seen in a 12-min search of the entire area within the subplot; both the soil surface and plants were inspected for coccinellids. A second 12-min collection was made within the subplot immediately after the first. Two 12-min removal samples were then taken from two other subplots within the row. In total, removal samples were taken from nine subplots per plot on each sampling occasion (three subplots per row times three rows per plot).

1	7	13	19	25	31
2	8	14	20	26	32
3	9	15	21	27	33
4	10	16	22	28	34
5	11	17	23	29	35
6	12	18	24	30	36

Step 1. Three non-adjacent, 4-m wide rows of six subplots per row selected for timed counts, 18 subplots sampled.

1	7	13	19	25	31
2	8	14	20	26	32
3	9	15	21	27	33
4	10	16	22	28	34
5	11	17	23	29	35
6	12	18	24	30	36

Step 2. Three random 4- by 4-m subplots from each timed count row selected for removal sample, 9 subplots sampled.

1	7	13	19	25	31
2	8	14	20	26	32
3	9	15	21	27	33
4	10	16	22	28	34
5	11	17	23	29	35
6	12	18	24	30	36

Step 3. Remaining three subplots from each timed count sample row selected for quadrat sampling, 9 subplots sampled.

1	7	13	19	25	31
2	8	14	20	26	32
3	9	15	21	27	33
4	10	16	22	28	34
5	11	17	23	29	35
6	12	18	24	30	36

Step 4. Sweep net samples taken from remaining three rows of subplots. Six 25-sweep samples, one from the right and left half of each row of subplots, total of 150 sweeps.

FIG. 1. Example of subplot selection pattern for four sampling techniques in individual 24- by 24-m grain sorghum plots.

De Lury's method (Seber and Le Cren 1967) was used to estimate the number of beetles per m^2 in each plot on each sampling occasion. Using De Lury's method, a maximum likelihood estimate of the total population within a subplot is given by,

$$n = c_1^2 / (c_1 - c_2)$$

where c_1 and c_2 are the numbers of coccinellids collected in the first and second sample, respectively. The number of coccinellids per m^2 in the plot is then given by $n \div 16$ (the total area of a subplot was 16 m^2). The efficiency of removal sampling, defined as the proportion of the total population within a 4- by 4-m subplot caught in two 12-min searches, was estimated by the following equation:

$$E = (c_1 + c_2) / n$$

D-Vac sampling and quadrat. D-Vac sampling (in 1991) or quadrat sampling (in 1992) was conducted in the three subplots within the row not used for removal sampling. D-Vac sampling in a subplot was accomplished by beginning at an outside row of plants within the confines of the subplot. The 33-cm diameter sampling cone was placed at the base of the plant and moved slowly upward toward the top. The sampler was then moved down the row approximately one cone width and the process repeated until the entire 4 m of row were covered. Both sides of each row of plants within a subplot were sampled in this manner. When plants were small enough for the sampling cone to fit easily over a single plant, D-Vac sampling was accomplished by placing the cone over an entire plant and holding it there for 3 sec; each plant within a subplot was sampled in this manner. Quadrat sampling was accomplished within plywood enclosures (1.0 m by 1.0 by 0.4 m high). The enclosure was placed at three random locations within each subplot sampled, and all coccinellids trapped inside were counted.

Sweep net sampling. These samples consisted of 25 pendular, cross-row sweeps through each of the remaining three rows of six subplots (six 25-sweep samples per plot) with a standard 38-cm diameter beating net. One 25-sweep sample was taken from the left 2.0 m of each row of plots while walking in one direction, and a second 25-sweep sample was taken from the right 2.0 m of the row while walking in the opposite direction. After each 25-sweep sample, the insects were shaken down to the bottom of the sweep net and all coccinellids were counted and released. Adults were recorded by species and larvae were recorded as a group.

Converting Relative to Absolute Density. The relationship between absolute (m_d) and relative (m_r) density was modeled using the general formula of Ruesink and Haynes (1973):

$$m_d = f(x_1, x_2, \ldots, x_n)m_r \qquad \text{(Equation 1)}$$

where f is a function of the x_i's, and the x_i's are measured biotic and abiotic variables that influence capture using the relative sampling procedure. Partial correlation coefficients (after adjusting relative sampling data for absolute population density) were calculated to determine the degree of association between population estimates obtained from the relative method and each of the above mentioned variables. Partial correlations were used to determine variables to include in f. Because of their simplicity and general suitability for describing linear and nonlinear phenomena, we used polynomial functions of the x_i's to model f. Stepwise regression with the maximum R^2 method (SAS Institute 1988) was used to determine appropriate models for f. Variables were entered in a regression model until the incremental increase in R^2 failed to exceed 0.04. Only variables with significant partial correlations with sampling efficiency for a relative sampling method were included in regressions.

Several abiotic and biotic variables were measured concomitant with sampling. The following meteorological variables were measured at 1-min. intervals using Campbell CR-10 microloggers: wind velocity (m/sec), air temperature (°C), percent relative humidity, and solar irradiance (kw/m^2). The average of each variable was calculated for the hour nearest the mid-point of the time during which timed count and sweep net sampling were conducted. Sorghum plant growth stage (Vanderlip 1972) and the number of aphids per sorghum plant (absolute counts *in situ*) were also determined.

RESULTS

In total, 20 plots were sampled during the two years, 12 in 1991 and eight in 1992. Two species (*Hippodamia convergens* Guérin-Méneville and *H. sinuata* Mulsant) dominated the coccinellid fauna in the fields, comprising more than 90% of all coccinellid species encountered in samples (Table 1). *Coleomegilla maculata* DeGeer was consistently collected, but in much lower numbers than *H. convergens* or *H. sinuata*. *Olla v-nigrum* (Mulsant), *Coccinella septempunctata* (L.), and *Scymnus* sp. [*S. (Pullus) loweii* Mulsant, and *S. americanus* Mulsant] occurred sporadically in samples. Other species were rarely encountered.

In 1991, aphid density per plant ranged from 0 to 65 for greenbugs and 0 to 317 for corn leaf aphids. In 1992, the per plant density ranges were 0 to 999 for greenbugs and 0 to 368 for corn leaf aphids.

Our interest was in comparing sampling methods for estimating the density of aphidophagous coccinellids in grain sorghum that could be adapted for use in an IPM program. Thus, we pooled data for all species because *H. convergens* and *H. sinuata* are sampled with similar efficiency using a variety of sampling methods (Michels and Behle 1992), and these two species normally comprise 90% or more of the aphidophagous coccinellids in grain sorghum (see Table 1 and Michels and Behle 1992). Data for adults and larvae were treated separately because these life stages have very different morphologies and behaviors which may cause them to be sampled with markedly different efficiency using relative sampling methods. This might be particularly true for sampling methods that rely on the visual acuity of a human observer.

Removal, quadrat, and D-Vac sampling. An important assumption that must be satisfied in order for De Lury's method to be appropriate for estimating population size in a defined area is that the probability of capture remains constant among successive removal samples from the area (Seber and Le Cren 1967). Each time a plot was sampled, one subplot was sampled a third time. These 20 subplots which were sampled a third time provided the data required to test the hypothesis of constant probability of capture. Regressions of the number of coccinellids captured versus sample number (1, 2, or 3) were calculated for adult and larval coccinellids. Linear regressions were significant for adults ($F=28.8$; $df=1, 50$; $P<0.001$) and larvae ($F=5.11$; $df=1, 23$; $P<0.05$) and had negative slope parameters, indicating a decrease in capture with each successive sample. However, constant was not significant for adults ($F=0.43$; $df=1, 49$; $P>0.05$) or larvae ($F=0.22$; $df=1, 22$; $P>0.05$). Thus, there was no evidence that the probability of capture varied among successive removal samples.

A second important assumption is that the probability of capture is large enough so that repeated removal causes a significant reduction in the size of the population (Seber and Le Cren 1967). The average efficiency of removal sampling was 76.3% (SE=2.1) for adults and 80.8% (SE=4.1) for larvae. Thus, on an average we removed more than three-fourths of the coccinellids from a subplot during two 12-min searches.

In theory, quadrat sampling, in which all individuals in the area enclosed by the quadrat are counted, provides accurate (unbiased) estimates of population density. In practice, however, individuals occasionally are not detected or escape and, therefore, are not counted. Thus, quadrat sampling usually slightly underestimates density. This limitation not withstanding, we are confident that quadrat sampling provided nearly unbiased estimates of population density in this study because of the thoroughness with which quadrats were inspected; however, the method required a great deal of labor to obtain estimates with high precision, and removal and D-Vac sampling may provide estimates with similar precision

TABLE 1. Numbers (Per Unit Measure) and Percentages (of Total) of Adult Coccinellids Using Five Sampling Methods in Sorghum Fields in 1991 and 1992

Year/Species	Sampling Method									
	Quadrat		D-Vac		Removal		Sweep net		Timed Count	
	no./m^2	%	no./m^2	%	no./m^2	%	no./m^2	%	no./min	%
					1991 (n=12)					
H. convergens	--	--	97	32.1	304	46.5	54	42.2	97	43.5
H. sinuata	--	--	177	58.6	332	50.8	68	53.1	124	55.6
C. septempunctata	--	--	0	0.0	0	0.0	1	0.8	0	0.0
Scymnus sp.	--	--	7	2.3	2	0.3	1	0.8	0	0.0
O. v-nigrum	--	--	0	0.0	0	0.0	0	0.0	0	0.0
C. maculata	--	--	15	5.0	16	2.4	4	3.1	2	0.9
Other species	--	--	6	2.0	0	0.0	0	0.0	0	0.0
					1992 (n=8)					
H. convergens	173	58.2	--	--	506	57.2	53	63.1	160	53.5
H. sinuata	102	34.3	--	--	331	37.4	30	35.7	128	42.8
C. septempunctata	3	1.0	--	--	2	0.2	0	0.0	0	0.0
Scymnus sp.	8	2.7	--	--	5	0.6	0	0.0	0	0.0
O. v-nigrum	1	0.3	--	--	0	0.0	0	0.0	0	0.0
C. maculata	10	3.4	--	--	41	4.6	1	1.2	11	3.7
Other species	0	0.0	--	--	0	0.0	0	0.0	0	0.0

for less sampling effort if they can be shown to provide statistically accurate estimates of density.

If removal sampling provides accurate population estimates, then estimates of average density obtained by quadrat and removal sampling should not differ significantly and estimates should be highly correlated. Population density estimates for adult coccinellids obtained from removal and quadrat sampling in 1992 did not differ significantly ($t=0.48$; $df=7$, $P>0.05$) averaging 1.34 and 1.37 beetles per m^2 from removal and quadrat sampling, respectively (Table 2). Furthermore, density estimates obtained using the two methods were significantly correlated (Table 3). Removal sampling in 1991 provided significantly greater estimates of ladybird beetle density than did D-Vac sampling ($t=4.01$; $df=11$; $P<0.01$), and the two methods were not significantly correlated.

For larvae, removal sampling provided significantly ($t=-2.49$; $df=7$; $P<0.05$) lower estimates of density than quadrat sampling (Table 2), although estimates obtained by the two methods were highly correlated (Table 3). D-Vac sampling provided significantly ($t=-2.31$; $df=11$; $P<0.05$) lower estimates of larval density than removal sampling (Table 2); these two methods were significantly correlated (Table 3).

TABLE 2. Mean Numbers of Coccinellids (±SE) Obtained by Five Sampling Methods

Year/Sampling Method[a]	Larvae	Adult
	1991	
Removal (no./m^2)	1.70 ± 0.75	0.37 ± 0.077
D-Vac (no./m^2)	0.087 ± 0.023	0.059 ± 0.010
Sweep net (no./sweep)	0.042 ± 0.016	0.059 ± 0.017
Timed Count (no./min)	1.03 ± 0.49	0.81 ± 0.020
	1992	
Removal (no./m^2)	0.019 ± 0.011	1.34 ± 0.20
Enclosed 1-m^2 quadrat (no./m^2)	0.20 ± 0.087	1.37 ± 0.14
Sweep net (no./sweep)	0.0028 ± 0.0028	0.079 ± 0.020
Timed Count (no./min)	0.00	2.33 ± 0.55

[a] Note differences in units of measurement.

TABLE 3. Pearson Correlations Between Estimates of Adult (Above Diagonal) and Larval (Below Diagonal) Coccinellid Population Densities in 24- by 24-m Plots Using Five Sampling Methods

Sampling Method	Removal	D-Vac	Sweep net	Counting
Quadrat	0.79b (8)[a]	no data	0.40 (8)	0.75[b] (8)
Removal	--	0.31 (12)	0.39 (20)	0.67[b] (20)
D-Vac	0.64[b] (12)	--	0.11 (12)	0.13 (12)
Sweep net	0.65[b] (12)	0.60[b] (12)	--	0.49[b] (20)
Counting	0.94[b] (12)	0.63[b] (12)	0.81[b] (12)	--

[a] Number of plot samples used to calculate correlation;
[b] Differs significantly ($\alpha=0.05$) from zero.

Sweep net and timed count sampling. Relative estimates of density based on sweep net and timed count sampling have different units of measurement (i.e., number per sweep and number per minute of counting) than quadrat or removal sampling (Table 1). In

addition, the efficiency of these methods may be influenced by a variety of biotic and abiotic variables. For estimates obtained by relative methods to be converted to absolute population estimates, they should be highly correlated with absolute methods and it must be possible to adjust for the effects, if any, of environmental variability on sampling efficiency.

For adult coccinellids, timed counts were significantly correlated with the quadrat method (Table 3). Because no larvae were observed in timed counts and larval means were very low in removal and sweepnet samples for 1992, these data were not used for correlation analyses (Table 2).

For adults, estimates from sweep net sampling were not correlated with estimates of absolute population derived from quadrat or removal sampling.

Converting relative to absolute density. None of the relative methods we tested were acceptable for sampling larval coccinellids for the purpose of obtaining estimates of the absolute population. The timed visual count method was significantly correlated with absolute population for adults, while estimates obtained from sweep net sampling were not. Therefore, we developed a single model, the purpose of which was to convert estimates obtained from timed counts of adult coccinellids to estimates of absolute population.

Partial correlations between relative population estimates obtained by timed counts and meteorological, crop, and aphid variables give the degree of association between the efficiency of sampling using the relative method and the particular variable after accounting for the effect of population density on timed counts of adults. Only one partial correlation was significant ($r = -0.60$, $P<0.01$), that being between timed counts and plant growth stage.

A regression model was developed using Equation 1. The fitted model converting relative estimates of adult density obtained by timed counting sampling to absolute density was significant ($F=37.0$; $df=2,18$; $P<0.001$) and is given by,

$$m_d = (0.15 + 0.11 \cdot GS) \cdot m_r \qquad \text{(Equation 2)}$$

where m_d is absolute density from removal sampling, m_r is relative density from timed count sampling, and *GS* is plant growth stage. The model explains 80% of the variation in the relationship between absolute and relative density.

DISCUSSION

Michels and Behle (1992) showed that visual counting was a more statistically precise method of sampling coccinellids than sweep net, drop cloth, or pitfall trap sampling. However, if the time required to sample was taken into account, the visual counting method, in which 50 plants were sampled, was less efficient than drop cloth or sweep net sampling. Our counting method involved fixed time rather than a fixed number of plants, but the total time required to sample a plot (18 min) was about the same as the average time required to sample 50 plants (15 min) in their study. We have shown that sweep net sampling is a poor method for sampling coccinellids in sorghum because estimates are not correlated with absolute density. Thus, even though the variance per unit cost is lower for sweep net than for visual count sampling (Michels and Behle 1992), counting is a preferable technique because estimates obtained by the method can be directly converted to absolute density using Equation 2. Time spent counting can be reduced, at the expense of reduced precision, and Equation 2 can still be used to make the conversion from relative to absolute density.

None of the relative methods we employed were satisfactory for sampling coccinellid larvae. Removal sampling consistently underestimated larval populations. There was no evidence of a nonconstant probability of capture, which would cause estimates

to be biased. Sampling efficiency averaged greater than 80% which is generally sufficient for population estimates derived from removal sampling to be approximately unbiased (Seber and Le Cren 1967). We can speculate that larval coccinellid populations are composed of two classes of individuals from the standpoint of their availability for capture to a human observer: those visible actively searching or resting on plants or the soil surface, and those resting or searching in concealed locations (such as the whorl of the plant or cracks in the soil) where they are unlikely to be seen. Larvae in this second class would generally go undetected and would not be included in population estimates derived from removal sampling. Based on comparison of densities estimated from quadrat and removal sampling, a large proportion of larvae in a population may fall into this second category.

Because larvae were not identified to species in this study, there may be undetected, species-specific differences in microhabitat that account for a portion of the population of 'undetected' larvae. Differences in microhabitat use among coccinellid species have been reported for both adults and larvae (e.g., Ewert and Chiang 1966, Coderre and Tourneur 1986). Regardless of the explanation, we were not successful in developing a method for estimating larval coccinellid population density in sorghum using time-efficient relative methods such as sweep net and timed count sampling.

The results described in this paper cannot, in all probability, be used in every sorghum field in all regions because coccinellid and aphid species composition may change across regions. However, because of the fact that aphid density did not play a major role in our results, when coccinellid species are the same or similar to those we encountered, the use of timed counts as a measure of relative density do give a robust measure of absolute coccinellid adult density and, therefore, may be used as an efficient sampling method for adult coccinellid densities in grain sorghum. We would assume that the model described here would work best in the southern to central Great Plains region of the United States or in similar climates. Extrapolation outside this region would, at a minimum, necessitate an evaluation of the coccinellid complex.

ACKNOWLEDGMENT

We extend our thanks to J. B. Bible, T. D. Johnson, B. J. Thompson and T. M. McDonald for technical assistance. To G. B. Heidel, C. M. Rush and J. E. Slosser of the Texas Agricultural Experiment Station and C. D. Patrick of the Texas Agricultural Extension Service for critical reviews of the manuscript. A portion of this research was supported by a USDA-CSRS Southern Region Integrated Pest Management Program grant (Cooperative Agreement No. 91-34103-5839). Mention of a commercial product does not constitute endorsement for its use by the Texas Agricultural Experiment Station or the USDA.

LITERATURE CITED

Coderre, D., and J. C. Tourneur. 1986. Vertical distribution of aphids and aphidophagous insects on maize. pp. 291-296. *In* I. Hodek [ed.] Ecology of Aphidophaga. Academia Press, Prague.

Ewert, M. A., and H. C. Chiang. 1966. Dispersal of three species of coccinellids in corn fields. Can. Entomol. 98: 999-1001.

Kring, T. J., F. E. Gilstrap, and G. J. Michels, Jr. 1985. Role of indigenous coccinellids in regulating greenbugs (Homoptera: Aphididae) on Texas sorghum. J. Econ. Entomol. 78: 269-273.

Michels, G. J., Jr., and R. W. Behle. 1992. Evaluation of sampling methods for lady beetles (Coleoptera: Coccinellidae) in grain sorghum. J. Econ. Entomol. 85: 2251-2257.
Morris, R. F. 1955. The development of sampling techniques for forest insect defoliators, with particular reference to the spruce budworm. Can. J. Zool. 33: 225-294.
Rice, M. E., and G. E. Wilde. 1988. Experimental evaluation of predators and parasitoids in suppressing greenbugs (Homoptera: Aphididae) in sorghum and wheat. J. Econ. Entomol. 17: 836-841.
Ruesink, W. G., and D. L. Haynes. 1973. Sweep net sampling for the cereal leaf beetle, *Oulema melanopus*. Environ. Entomol. 2: 161-172.
SAS Institute. 1988. SAS/STAT User's Guide, Version 6.03. SAS Institute, Cary, NC.
Seber, G. A. F., and E. D. Le Cren. 1967. Estimating population parameters from catches large relative to the population. J. Anim. Ecol. 36: 631-643.
Vanderlip, R. L. 1972. How a sorghum plant grows. Kans. State Univ. Coop. Ext. Serv. Bull. C-447.

LOCALIZATION OF SUBCUTICULAR IRON-CONTAINING TISSUE IN THE RED IMPORTED FIRE ANT

T. J. Slowik and H. G. Thorvilson

Department of Plant and Soil Science, Texas Tech University,
Lubbock, Texas 79409-2122

ABSTRACT

Queens, alates, and three worker classes of the red imported fire ant, *Solenopsis invicta* Buren, were examined for the presence of internal iron concentrations using analytical and anatomical techniques. Iron-specific staining located subcuticular ferric material in the abdomens of major, media, and minor workers; however, tissues of worker heads and thoraces did not consistently contain areas of localized iron. Queens and alates also did not consistently stain for concentrated iron in body tissues. Gut and ommatidial tissues of all ants occasionally revealed iron-containing areas. Iron was confirmed by X-ray spectroscopy in positively stained tissues.

INTRODUCTION

Scientific literature is rife with examples of animals which are able to detect and use magnetic fields (MFs) and the directional information that fields provide. Organisms as diverse as bacteria (Blakemore 1975), cartilagenous fish (Kalmijn 1978), salamanders (Phillips and Adler 1978), woodmice (Mather and Baker 1981), and homing pigeons (Gould 1980) sense MFs and use magnetic information for navigation and orientation.

A wide array of insects has been tested for magnetic sensitivity. Insects such as the flour beetle, *Tenebrio molitor* L., (Arensde 1978), large yellow underwing moth, *Noctua pronuba* L., (Baker and Mather 1982), fruit fly, *Drosophila melanogaster*, (Picton 1966), and hornet, *Vespa orientalis*, (Kisliuk and Ishay 1977) respond to both artificial and natural MFs. Insect behavior associated with magnetism, however, has been most extensively studied in the honey bee, *Apis mellifera* L. For example, the effects of magnetism on worker mobility (Hepworth et al. 1980), local field detection (Walker and Bitterman 1989), spatial and temporal orientation (Lindauer and Martin 1972), and general activity and aging (Martin et al. 1989) are well-documented.

Additionally, the physiology and biology of MF sense have been studied using the honey bee as a model. Frankel et al. (1979) first discovered that an iron oxide in the form of intracytoplasmic magnetite (Fe_3O_4) imparted a magnetic dipole to the unicellular magnetotactic bacterium, *Aquaspirillium magnetotacticum*, and accounted for that organism's response to MFs. With the observation of magnetite in the honey bee abdomen (Gould et al. 1978) and the localization of iron oxide granules in trophocytes of the subcuticular fat body of the bee's abdominal segments (Kuterbach et al. 1982; Kuterbach and Walcott 1986a, b), a more complex system of magnetic sensitivity may exist in the honey bee. Although Hsu and Li (1993, 1994) discovered fat body trophocytes which contained superparamagnetic magnetite and were innervated and cytoskeletally attached, their results have been disputed. Kirschvink and Walker (1995) and Nesson (1995) doubted that Hsu and Li had found superparamagnetic magnetite. Nichol and Locke (1995) could not find innervation or cytoskeletal attachments associated with fat body cells and believed that Hsu and Li had overlooked the role of iron metabolism in their findings. Clearly, the physiology behind MF sense in honey bees needs to be elucidated.

The use of MF cues by other hymenopterans (i.e., ants) has been investigated as well. The red wood ant, *Formica rufa*, may use geomagnetic cues (Rosengren and Fortelius

1986). As part of a larger study of the ant's fidelity to routes and allegiance to nest sites, two Heimholtz coils redirected the horizontal MF component (by 60° counterclockwise) surrounding the foraging and nest arena. Ants showed no asymmetry in their movement responses, which indicated no tracking or cueing on the altered MF lines. The researchers concluded that ants did not use MF cues to preserve nest sites or foraging routes in the dark. However, Anderson and Vander Meer (1993) concluded that the red imported fire ant, *Solenopsis invicta* Buren, has geomagnetic orientation capabilities. In nocturnal experiments with ant colonies foraging in a Heimholtz coil-enclosed arena, the time required for trail development from a newly introduced bait to the nest was measured. Ants were acclimated to either a normal or artificially reversed MF (180° change), then forced to find new bait and establish a trail in an environment of opposite MF polarity. Ants in these circumstances always took significantly longer to create a return trail than in a control environment where the acclimation-stage MF did not change. Fire ants (Eagleson 1940; MacKay et al. 1989, 1990) and other ants (Jolivet 1986; Little 1984) also accumulate in and damage active electrical equipment with which electromagnetic fields are associated. This evidence suggests a possible MF response in ants, in general, and *S. invicta* in particular.

The objective of this research was to histologically examine the head, thoracic, and abdominal tissues of *S. invicta* Buren and to specifically look for iron and iron-containing cells similar to those confirmed in the honey bee.

MATERIALS AND METHODS

Several polygynous *S. invicta* colonies were collected during the summer and fall of 1994 and maintained in the laboratory. Workers from major (mean head capsule width = 1.29 mm, SE ± 0.01 mm), medium (0.98 mm, SE ± 0.13 mm), and minor (0.74 mm, SE ± 0.17 mm) size-classes (Porter and Tschinkel 1985), male alates, and dealate queens were removed from three separate but similarly populous colonies for examination.

Insects were killed using chloroform and immediately dismembered. Ants were subdivided by body region, separating head from thorax and thorax from abdomen in preparation for embedding. This separation was conducted to localize potential iron-containing tissues and to allow maximal penetration of fixative solution and stain. Dissection and handling of all tissue throughout the experiment were done with nonmetallic instruments, and chemical treatments were carried out in glassware thoroughly washed with distilled water. Ants were fixed within minutes after death.

The fixation agent was 4.0% paraformaldehyde buffered in 0.1 M phosphate buffer at 7.2 pH for 30 min at room temperature (16°C). One ml of wetting agent (Tween 80, Fisher Scientific, Fair Lawn, NJ; Triton-X, Fisher Scientific) was added to the fixative mixture to facilitate tissue penetration. In preparation for staining and to remove all paraformaldehyde, body regions were washed three times for 5 min in 0.1 M phosphate buffer.

Body regions were then dehydrated in a sequential series of seven 10-min washes in 50, 75, 85, 95, 95%, and two pure ethyl alcohol solutions. Final preparation for embedding involved clearing the alcohol from body regions with 100% xylene twice, for 10 min.

In the first step of the embedding process, specimen segments were placed in embedding plastic (LX-112 Embedding Medium, LADD Research Ind., Inc., Burlington, VT) and xylene mixture (1:2, V:V) for 3 hr. Specimens were next transferred to an embedding plastic and xylene mixture (2:1, V:V) for 3 hr. In the final step of the embedding process, tissue segments were placed in three successive volumes of 100% plastic for at least 3 hr., with the final volume left overnight just prior to the polymerization process.

Air bubbles were removed from the liquid plastic containing the specimens in a vacuum chamber for 45 min. at -90 kPa, and the plastic was allowed to polymerize slowly in an oven at 70°C. During the initial hardening, sections were manipulated and positioned in the slowly gelling plastic to best align them for either sagittal or medial cross-sectioning.

After complete hardening of the plastic matrix, embedded body regions were thin-sectioned by microtome (Sorvall MT2-B Ultra Microtome, Ivan Sorvall, Inc., Norwalk, CT). Cross-sections were cut completely through each body region (i.e., from anterior to posterior or vice-versa), and each 2-µ thin-section was mounted on a clean glass slide for every 10 µ of tissue sliced ("step-sections"). This procedure produced slides with three

rows of multiple, 2-µ sections, in sequence, through each body region. A total of 36 body regions from queens, male and female alates and worker castes were thin-sectioned.

The Prussian Blue reaction stains for the presence of free iron or its oxides in tissue (Kuterbach et al. 1982). In this reaction, tissue containing iron reacts with acidic potassium ferrocyanide to form a bright blue precipitate, ferric ferrocyanide. As a positive stain model, abdomens of freshly killed worker honey bees were fixed and stained, both whole *in situ* and embedded as sections. A steel tine and a drop of mounting medium (Permount, Fisher Scientific, Fair Lawn, NJ) served as positive and negative stain controls, respectively, during each segmental staining procedure.

Separate, fresh stock solutions of 0.5% hydrochloric acid and 0.5% potassium ferrocyanide were heated to 60°C and mixed (1:1, V:V). Slides with mounted ant tissue sections and honey bee tissues were then submerged in the mixture for 15 min, maintained in a 60°C water bath. After staining, preparations on slides were washed with distilled water in preparation for viewing.

Coverslips were added to slide sections, and slides were examined under light microscopy at 100, 200 and 400X magnifications for the presence of stained tissue. Entire, whole honey bee abdomens were examined under a light microscope (Olympus BHA Light Microscope, Olympus Optical Co., Ltd., Japan) at 20 and 40X.

Presence of iron in selected areas of blue-staining tissue was verified using x-ray spectroscopy (Noran TM5500 with Light Element Detector, Noran, Inc., USA). Freshly stained thin-sections were temporarily coverslipped with a water-based mounting medium (Gel/Mount, Biomeda, Foster City, CA), and positively stained tissues were marked. The mounting medium was removed with distilled water, and the intact sections mounted on slides were air-dried. Entire slides were scored with a diamond-tipped marker, and small portions of the slide were broken away. Each of these glass portions retained a marked thin-section melded to its top surface. These slide portions holding tissues were attached by carbon tape (STR Tape, Shinto Chemitron Co., Ltd., Japan) to standard metallic stubs for use in electron microscopy (EM). Each stub-mounted specimen was coated with a 20-nm layer of gold using a vacuum gold-coating device (Polaron SEM Coating System, Watford, England) in preparation for viewing and analysis by scanning transmission EM (H-600 Transmission Electron Microscope, Hitachi, Japan).

Tissues were viewed and oriented by EM using 100, 500, and 1000X magnification. Once located, the marked, positively stained areas were analyzed by x-ray spectroscopy for iron among the other component elements. X-ray spectra were obtained using 25 kV acceleration and a spot count of 100 s on stained tissue and granules at magnifications of 1000X.

RESULTS AND DISCUSSION

Honey bee abdomens stained *in situ* and examined by light microscopy revealed blue-stained tissue areas associated with subcuticular areas of the dorsal, anterior abdomen. Stained, 5-µ cross-sections examined under 400X confirmed these results and showed granular blue staining among fat body cells and trophocytes just under the cuticle, supporting the results of Kuterbach et al. (1982) and Kuterbach and Walcott (1986a).

Step-sections from the body segments of *S. invicta* male alates and queens did not consistently contain areas of localized staining (Fig. 1). Diffuse blue staining occurred in the digestive tract of the reproductives' abdomens. Internal portions of ommatidia in ants' eyes also stained, indicating the presence of iron (Fig. 2).

Step-sections from the head and thoracic regions of the three *S. invicta* worker size-classes did not show consistent iron staining. The ommatidial staining noted in reproductives also appeared in workers. Internal staining in gut areas of worker abdomens was less noticeable and consistent than in alates and queens.

Interpretation of the staining in the abdominal segments of the workers was more problematic. While some workers' abdomens showed clear, granular staining localized to subcuticular regions (Figs. 3, 4), other abdominal regions revealed inconsistent staining or more diffuse, nonspecific staining areas. Positive results ranged from highly-localized, blue granules interspersed with subcuticular cells in some major and media workers, to the somewhat random aggregations of iron near and under the cuticle of some minors. Locations

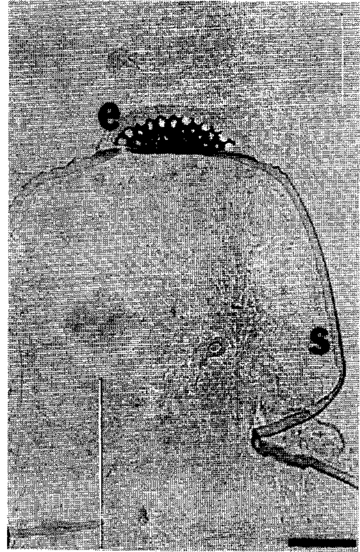
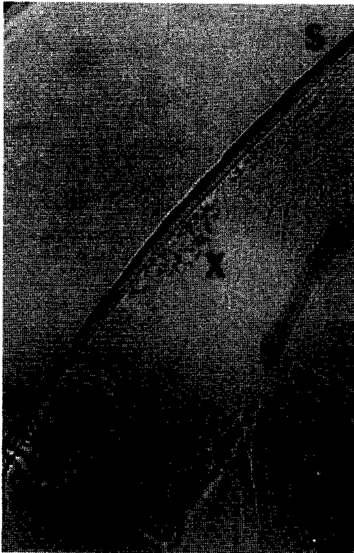
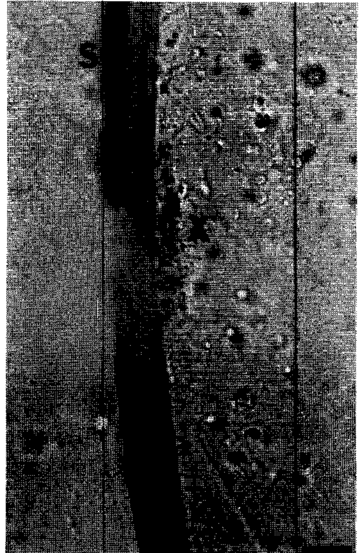


FIG. 1 and FIG. 2. *Solenopsis invicta* male alate, thorax cross-section showing negative Prussian Blue staining: (s) cuticle. *S. invicta* queen, head cross-section showing positive Prussian Blue staining: (e) compound eye, (s) cuticle; Scale bar 100 μ.

FIG. 3 and FIG. 4. *Solenopsis invicta* major worker, thorax cross-section showing positive, granular, subcuticular Prussian Blue staining: (s) cuticle, (x) iron granules; Scale bar 25 μ. *S. invicta* major worker, abdomen cross-section showing positive, granular Prussian Blue staining in subcuticle: (s) cuticle, (x) iron granules; Scale bar 10 μ.





of tissue staining in each segment also varied, but were seen most frequently in the more anterior regions, often ventrally and pleurally.

X-ray analysis of three selected, stained areas from minor, medium, and major worker abdomens confirmed the presence of elemental iron (Fig. 5). Calcium, oxygen, and sodium were also revealed as distinctive peaks on the spectra, as were silicon (most likely from slide glass) and gold (from the coating preparation). X-ray analysis of three nonstaining tissue areas from minor, medium, and major workers did not detect the presence of elemental iron.

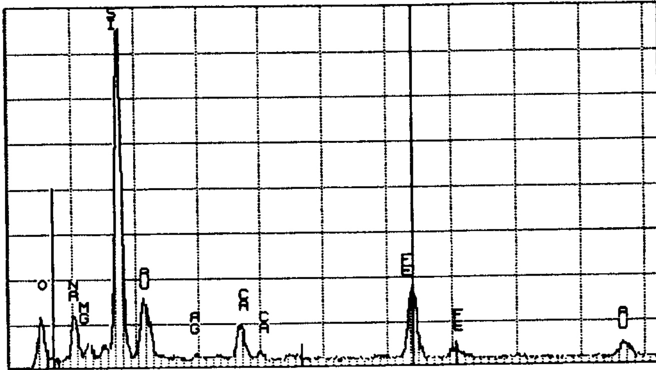


FIG. 5. X-ray spectrum analysis (STEM mode) of positively stained granules in *Solenopsis invicta* major worker. (FE) iron

Although the results of this study indicate that queen and alate fire ants probably do not possess concentrated ferric areas outside of the gut and ommatidial areas, workers of all size-classes show subcuticular ferric material. Minor and media worker size-classes generally showed staining spread over no more than two sequential step-sections, indicating that their concentrated ferric areas stretch for lengths laterally ranging from 1 to 20 µ in the abdomen. Major workers showed both shorter spreads or concentrations of staining (one and two step-sections) and longer regions of up to 50 µ in length (four to five subsequent sections). The most consistently localized and granular staining in worker abdomens suggests a "rod" or "tube" shaped concentration of ferric material running posteriorly, just beneath the abdominal cuticle. Whereas the short rods of iron-containing tissues were found towards the anterior abdomen, they were not consistently found in any one particular region (i.e., dorsal, pleural or ventral). Longer rods were located more anteriorly in majors, and one particularly definitive specimen revealed two pleural and one ventral rod of stained material.

The relative consistency and amount of stained material in each caste fits the profile of activities performed by queen, alate, and worker ants. Reproductives do not forage and, except for one mating flight, stay within the colony's nest boundaries. They would have the least need for magnetic orientation abilities afforded by an internal, ferromagnetic compass. Minor and medium workers perform duties both inside (brood grooming, larval care) and outside (nest maintenance, food relay) colonies and may forage short distances from the interior nest (Sorensen and Vinson 1985). They may employ magnetic information provided by localized ferric material, specifically in duties removing them from the immediate vicinity of the nest. Major workers, which predominantly perform tasks outside and away from the actual colony (i.e., foraging, Mirenda and Vinson 1979), may likely possess an MF-sensing device, perhaps as a back-up orientation system to their use of pheromonal cues and trails. Our results lend support to these hypotheses.

Similarities between our results in the fire ant, *S. invicta*, and those with the honey bee exist. Granular staining patterns were located just beneath the abdominal cuticle, as in Kuterbach et al. (1982) and Kuterbach and Walcott (1986a, b). Examination and analysis of these granules revealed them also to be composed of iron and closely located near other cells of the subcuticular fat body, but we did not determine their association with nerve cells. Honey bees studied by Kuterbach et al. (1982) and Kuterbach and Walcott (1986a, b) showed more anterior, positive-tissues organized within the "sheet" of fat body internally lining the abdomen. Our specimens revealed shorter "rods" of subcuticular tissue, running anterior to posterior, which seem to traverse the more anterior, abdominal segments. Thus, some similarity to the localization of ferric material exists between these two hymenopterans.

Kuterbach and Walcott (1986a, b) also determined that iron granules were present only in post-eclosion adult bees, with trophocytic granules increasing in number and size during bee aging. Accumulation of subcuticular iron related directly to the iron levels in the bees' pollen and honey diet (Kuterbach and Walcott 1986a, b), a trend previously noted in other insects fed artificially high iron diets (Lennox 1940, Waterhouse 1940). Iron levels peaked in aged adult bees precisely when they assumed duties as foragers, supporting behavioral evidence and hypotheses of bee orientation via MF information (Kuterbach and Walcott 1986a, b). More specifically, Raes et al. (1989) found iron-containing granules in the columnar cells of the bees' midgut. Our histology supports a similarity between foraging bees and foraging worker ants, which show positive gut-staining and could also have dietarily accumulated iron.

Locke and Nichol (1992) documented the diverse physiological roles of iron in the entire insect body, particularly in basic cell activity. Observations of diffuse iron-staining in ant tissues also support this.

Further description of this ferric material found in ants, such as completed by Kuterbach and Walcott (1986 a, b), Raes et al. (1989), and Hsu and Li (1993, 1994) with the honey bee, is needed before an internal compass hypothesis in ants is presented. Confirmation of the impact of MF information on ant behavior may determine whether ants also possess a magnetic sensory system that influences their navigation and orientation abilities.

ACKNOWLEDGMENT

Thanks to Chuck Butterick, Pattye Staub, and Mary-Catherine Hastert of the Electron Microscopy Center, University Medical Center, Texas Tech University, for their advice, support, and facility use. We thank manuscript reviewers Dr. P. A. Dotray, Dr. E. B. Peffley, and Dr. V. G. Allen for their comments. This research was supported by State of Texas Line Item for Red Imported Fire Ant Research. This is contribution number T-4-405, College of Agricultural Sciences and Natural Resources, Texas Tech University. Labeled tissues are on reserve at the Texas Tech University Insect Collection, Texas Tech University, Lubbock, Texas.

LITERATURE CITED

Anderson, J. B., and R. K. Vander Meer. 1993. Magnetic orientation in the fire ant, *Solenopsis invicta*. Naturwissenschaften 80: 568-570.

Arensde, M. C. 1978. Magnetic field detection is distinct from light detection in the invertebrates *Tenebrio* and *Talitrus*. Nature 274: 358-362.

Baker, R. R., and J. G. Mather. 1982. Magnetic compass sense in the large yellow underwing moth, *Noctua pronuba* L. Anim. Behav. 30: 543-548.

Blakemore, R. P. 1975. Magnetotactic bacteria. Sci. 190: 377-379.

Eagleson, C. 1940. Fire ants causing damage to telephone equipment. J. Econom. Entomol. 33: 700.

Frankel, R. B., R. P. Blakemore, and R. S. Wolfe. 1979. Magnetite in freshwater magnetic bacteria. Sci. 203: 1355-1357.

Gould, J. L., J. L. Kirschvink, and K. S. Deffeyes. 1978. Bees have magnetic remanence. Sci. 201: 1026-1028.

Gould, J. L. 1980. The case for magnetic sensitivity in birds and bees (such as it is). Amer. Sci. 68: 256-267.

Hepworth, D., R. S. Pickard, and K. J. Overschott. 1980. Effects of the periodically intermittent application of a constant magnetic field on the mobility in darkness of worker honey bees. J. Apic. Res. 19: 179-186.
Hsu, C. Y., and C. W. Li. 1993. The ultrastructure and formation of iron granules in the honey bee (*Apis mellifera*). J. Exp. Biol. 180: 1-13.
Hsu, C. Y. and C. W. Li. 1994. Magnetoreception in honey bees. Science 265: 95-96.
Jolivet, P. 1986. Les fourmis et la television. L'Entomologiste 42: 321-323.
Kalmijn, A. J. 1978. Experimental evidence of geomagnetic orientation in elasmobranch fishes. pp. 347-353. *In* K. Schmidt and W. T. Keeton (eds.), Animal Migration, Navigation and Homing. Springer, Heidelberg, Germany.
Kirschvink, J. L., and M. M. Walker. 1995. Technical comments. Science 269: 1889.
Kisliuk, M., and J. Ishay. 1977. Influence of an additional magnetic field on hornet nest architecture. Experientia 33: 885-887.
Kuterbach, D. A., B. Walcott, R. J. Reeder, and R. B. Frankel. 1982. Iron-containing cells in the honey bee. Science 218: 695-697.
Kuterbach, D. A., and B. Walcott. 1986a. Iron-containing cells in the honey bee (*Apis mellifera*). I. Adult morphology and physiology. J. Exp. Biol. 126: 375-387.
Kuterbach, D. A., and B. Walcott. 1986b. Iron-containing cells in the honey bee (*Apis mellifera*). II. Accumulation during development. J. Exp. Biol. 126: 389-401.
Lennox, F. G. 1940. A quantitative examination of the iron content of *Lucilia cuprina*. Coun. Sci. Ind. Res. (Aust.). 102: 51-67.
Lindauer, M., and H. Martin. 1972. Magnetic effects on dancing bees. pp. 559-567. *In* S. R. Galler, K. Koenig-Schmidt, and R. E. Belleville (eds.), Animal Orientation and Navigation. U. S. Government Printing Office, Washington, DC.
Little, E. C. 1984. Ants in electric switches: Note. N. Z. Entom. 8: 47.
Locke, M., and H. Nichol. 1992. Iron economy in insects: Transport, metabolism, and storage. Ann. Rev. Entomol. 37: 195-215.
Mackay, W. P., S. O. Majdi, S. B. Vinson, C. J. Messer, and J. P. Irving. 1989. Prevention of fire ant damage to signal control. Texas A&M Univ. Research Report 1135-2F, Texas Trans. Inst. 51 pp.
Mackay, W. P., D. Sparks, and S. B. Vinson. 1990. Destruction of electrical equipment by *Solenopsis xyloni* McCook (Hynmenoptera: Formicidae). Pan-Pac. Entom. 66: 174-175.
Martin, H., H. Horall, and B. Forster. 1989. Magnetic field effects on activity and aging in honey bees. J. Comp. Phys. A. 164: 423-431.
Mather, J. G., and R. R. Baker. 1981. Magnetic sense of direction in woodmice for route-based navigation. Nature 291: 152-155.
Mirenda, J. T., and S. B. Vinson. 1979. A marking technique for adults of the red imported fire ant. Fla. Entomol. 62: 279-280.
Nesson, M. H. 1995. Technical comments. Science 269: 1889-1890.
Nichol, H., and M. Locke. 1995. Technical comments. Science 269: 1889.
Phillips, J. B. and K. Adler. 1978. Directional and discriminatory responses of salamanders to weak magnetic fields. pp. 325-333. *In* K. Schmidt and W. T. Keeton (eds.), Animal Migration, Navigation and Homing. Springer, Heidelberg, Germany.
Picton, H. D. 1966. Some responses of *Drosophila* to weak magnetic and electrostatic fields. Nature 211: 303-304.
Porter, S. D., and W. R. Tschinkel. 1985. Fire ant polymorphism: The ergonomics of brood production. Behav. Ecol. and Soc. 16: 323-336.
Raes, H., W. Bohyn, P. H. De Rycke, and F. Jacobs. 1989. Membrane-bound iron-rich granules in fat cells and midgut cells of the adult honey bees (*Apis mellifera* L.). Apidologie 20: 327-337.
Rosengren, R., and W. Fortelius. 1986. Ortstreue in foraging ants of the *Formica rufa* group-hierarchy of orienting cues and long-term memory. Insectes Sociaux 33: 306-337.
Sorensen, A. A., and S. B. Vinson. 1985. Behavior of temporal subcastes of the fire ant, *Solenopsis invicta*, in response to oil. J. Kans. Entomol. Soc. 58: 586-596.

Walker, M. M., and M. E. Bitterman. 1989. Conditioning analysis of magnetoreception in honey bees. Bioelectromagnetics 10: 261-275.
Waterhouse, D. F. 1940. The absorption and distribution of iron. Coun. Sci. Ind. Res. (Aust.). 102: 28-50.

GUSTATORY AND OLFACTORY RESPONSES OF *CYLINDROCOPTURUS ADSPERSUS* (LECONTE)[1] TO VARIOUS PLANT SPECIES IN THE LABORATORY

L. K. Rieske[2] and F. B. Peairs
Department of Entomology
Colorado State University
Fort Collins Colorado 80523

ABSTRACT

Feeding preferences and olfactory responses of *Cylindrocopturus adspersus* (LeConte) (Coleoptera:Curculionidae) to nine plant species were compared with sunflower, *Helianthus annuus* L. (Asteraceae), for stem weevil feeding preference in the laboratory. Sunflower was strongly preferred in each assay. Six crop species were also assayed for stem weevil preference in the absence of sunflower. Although overall feeding was diminished, *C. adspersus* showed a measurable feeding response to several plant species unrelated to its host plant. In olfactory assays, stem weevils preferred sunflower leaf discs to blank controls, but showed no preference for sunflower stems to blank controls. However, when assayed together, sunflower stems elicited a greater olfactory response than did sunflower leaf discs. Possible explanations regarding the gustatory and olfactory responses of *C. adspersus* are discussed, as is the potential for employing phytochemical stimuli as baits for incorporation into a pest monitoring program.

INTRODUCTION

The sunflower stem weevil, *Cylindrocopturus adspersus* (LeConte) (Coleoptera:Curculionidae), is a major pest of cultivated sunflower, *Helianthus annuus* L. (Asteraceae), in the North American Great Plains. Sunflower stem weevils are univoltine and overwinter as mature larvae in the stalks and crowns of sunflowers. They pupate within larval galleries, and adults emerge throughout spring and early summer. Oviposition occurs in the epidermal tissue of host plant stems. Direct damage is caused by larval tunneling in the vascular tissue, disrupting water and nutrient flow (Casels-Bustos 1976, Rogers *et al.* 1983). In addition, extensive indirect damage can result from larval infestations due to premature ripening syndrome (Gulya and Charlet 1984); the opening of infection courts to potentially pathogenic microorganisms (Yang and Owen 1982, Yang *et al.* 1983, Yang 1984), and extensive lodging of plants which contributes to harvesting difficulties and yield losses (Rogers and Serda 1982, Charlet 1987). Although adult feeding on leaves, stalks and petioles causes negligible damage, adult female preferences are important in the selection of oviposition sites and subsequent larval infestation. In addition to cultivated sunflower, *C. adspersus* has been reared from other species of *Helianthus*, as well as species in other

[1] Coleoptera: Curculionidae
[2] Current address: Department of Entomology, University of Kentucky, Lexington, Kentucky 40546

genera within the Asteraceae, including *Xanthium* and *Ambrosia* (Mitchell and Pierce 1911, Pierce 1916, Goeden and Ricker 1976, Charlet 1983a).

A variety of management strategies, both chemical and cultural, have been employed to reduce losses to *C. adspersus* in cultivated sunflower, but these have proven to be of limited value (Rogers and Seiler 1985). Although systemic insecticides can be effective in reducing *C. adspersus* populations, they are costly for growers and difficult to time (Rogers *et al.* 1983, Charlet *et al.* 1985). Development of a successful sampling or monitoring scheme would enable growers to forecast damage, allowing for prompt use of chemicals only when warranted by high weevil populations. An understanding of the chemical stimuli governing adult host plant selection could facilitate the development of sampling methods, allowing for effective baiting of stem weevils and contributing to the development of effective integrated pest management tactics for this pest. Although this weevil has been studied extensively, little research has been conducted on its host seeking and acceptance behavior, specifically factors influencing gustatory and olfactory responses.

The objective of this study was to evaluate the gustatory and olfactory cues used by adult sunflower stem weevils in their host seeking behavior, and to determine whether previous exposure to non-host plants influences stem weevil preferences. Specifically, we assessed the response of *C. adspersus* cohorts to eight vegetable crops and one common weed species in relation to cultivated sunflower, *H. annuus cv.* 'Italian white', and to each other, to evaluate the presence of plant cues affecting host attraction and acceptance.

MATERIALS AND METHODS

Sunflower stalks were collected in early spring 1995 from a field located at the Central Great Plains Research Station, Akron, CO, that had been heavily infested with *C. adspersus* in 1994. Stalks were maintained in the laboratory in emergence cages (68 x 36 x 62 cm) at 28°C with a 15L:9D photoperiod and 50-60% rh. Adult weevils were collected from emergence cages daily and stored at 14°C for use in bioassays. Sex ratio was not determined in our studies, but based on previous research, was assumed to be 1 male:1 female (Casels-Bustos 1976).

Plant material for bioassays was obtained from seed provided by NK Lawn and Garden Co., Minneapolis, MN, and the Department of Weed Science, Colorado State University, Fort Collins, CO. Seeds were planted in 17.5 cm diameter pots containing a 3:2:1 soil:perlite:peat mix and grown in a lightly shaded glasshouse under fluorescent lighting (16L:8D) and watered daily.

C. adspersus feeding preference was assessed by allowing adult weevils to feed on leaf discs excised from host and non-host material in a petri dish arena. The plants assayed (Table 1) were chosen for their phytochemical and morphological diversity, and ease of propagation. Leaves were excised from plants at the V4 to V6 vegetative stage for use in feeding preference assays.

In each assay, one 10 mm diameter leaf disc (or an entire leaflet if discs were not possible) from each plant species was randomly placed around the perimeter of a 5.5 cm diameter petri dish (see Table 1). The leaf material was anchored with small pins to a paraffin wax bottom which was lined with Whatman #1 filter paper moistened with distilled water. Moistened filter paper was also placed in the top of the petri dishes, which were then inverted. Adult weevils were allowed to feed for 7 h. For each of the four feeding assays, weevils were divided into three cohorts. The first cohort consisted of "experienced" weevils >30 d old, which had been exposed to plant species in the families Brassicaceae, Leguminoseae, Poaceae, Solanaceae, and Umbellifereae. The second cohort of weevils were removed from cold storage approximately 7-14 d prior to the assays, and

had been exposed to a diet of only sunflowers. The last cohort was comprised of weevils removed from cold storage 7-14 d earlier, but which had never fed.

TABLE 1. Plant Species Assayed for Feeding Preferences of Adult *Cylindrocopturus adspersus.*

Common name	Plant family: name
a) assayed in conjunction with 'Italian white' sunflower:	
	Assay #1 (N = 30)
pea, snap	Leguminoseae: *Pisum sativum* 'Sugar Snap'
tomato	Solanaceae: *Lycopersicon esculentum* 'Beefsteak'
broccoli	Brassicaceae: *Brassica oleracea* 'Emperor Hybrid'
	Assay #2 (N = 26)
pepper, Hungarian	Solanaceae: *Capsicum annuum* 'Yellow wax
lettuce	Asteraceae: *Lactuca sativa* 'Red sails'
parsley	Umbelliferae: *Petroselinum crispum* 'Dark Moss Curled'
	Assay #3 (N = 29)
bean, bush	Leguminoseae: *Phaseolus vulgaris* 'Blue Lake 274'
carrot	Umbelliferae: *Daucus carota* 'Scarlet Nantes'
kochia	Chenopodiaceae: *Kochia scoparia*
b) assayed in the absence of sunflower:	
	Assay #4 (N = 28)
bean, bush	Leguminoseae: *Phaseolus vulgaris 'Blue* Lake 274'
broccoli	Brassicaceae: *Brassica oleracea 'Emperor* Hybrid'
carrot	Umbelliferae: *Daucus carota* 'Scarlet Nantes'
corn	Poaceae: *Zea mays* 'Northrup King 9540'
pea, snap	Leguminoseae: *Pisum sativum* 'Sugar Snap'
tomato	Solanaceae: *Lycopersicon esculentum* 'Beefsteak'

All weevils were starved for 24-36 h prior to initiation of the feeding assays. Assays were conducted at 25°C with a 15L:9D photoperiod and 50-60% rh. Each assay was conducted three times, with 10 replications per assay (N = 26-30, see Table 1). One weevil per assay arena was used, with the exception of the final assay involving no sunflower, where two weevils per arena were used. Feeding was quantified by counting feeding scars under a microscope.

Olfactory responses of *C. adspersus* adults were analyzed using a petri dish-pitfall cup arena. The arena consisted of the bottom of a 9 cm diameter petri dish, through which two 2.5 cm diameter holes were cut. Two 30 ml (3.8 x 4.5 cm) cups were then glued to the bottom of the petri dish. Insects were placed in the petri dish arena where they could make a choice between the two pitfall cups. The bottom of each pitfall cup contained 30 small holes

which acted as a screen through which olfactory cues could diffuse. Each screened cup was placed inside a second intact cup, in which the baits were placed. Weevils were assayed for olfactory responses to sunflower (cv. 'Italian white') stems and leaves. Each assay was conducted three times, with 12 to 15 replications per assay. Ten mm diameter leaf discs and 2.5 cm long sunflower stems were tested against blank controls (N = 38 and N = 34, respectively), as well as against each other (N = 45). Each pitfall cup also contained a distilled water moistened Whatman #1 filter paper disc. In assays containing sunflower stems, the ends of the stems were dipped in melted paraffin wax to reduce desiccation. All insects were from a single cohort that had been exposed to a diet of sunflowers. As with the feeding assay, the weevils were starved for 24-36 h prior to the initiation of the assays. Assays were conducted for 6 h, with one weevil per arena.

Analysis of variance was used to assess weevil feeding preferences according to plant species and weevil cohort, and olfactory responses to plant part. When necessary, a square root + 0.5 transformation was used to normalize data prior to analysis. Pairwise comparisons were determined by Least Squares Means; where significant effects were observed ($P < 0.05$), differences were determined by Fisher's Protected LSD (Abacus Concepts, SuperANOVA 1989).

RESULTS AND DISCUSSION

The feeding behavior of adult *C. adspersus* was affected by the presence or absence of sunflower. In the presence of sunflower, weevil cohort had no impact on feeding; there was no significant difference in *C. adspersus* response to any of the plant species tested based on weevil age or experience (Table 1: Assay #1, $F_{2,114} = 0.430$, $P = 0.6514$; Assay #2, $F_{2,98} = 0.511$, $P = 0.6012$; Assay #3, $F_{2,110} = 0.727$, $P = 0.4858$). However, when sunflower was eliminated from the choice assay, experienced weevils showed a trend toward consuming more than their less experienced counterparts (Assay #4, $F_{2,160} = 2.304$, $P= 0.1032$). Because there was no statistical difference in response based on weevil cohort, data were combined for all subsequent analyses.

In assays where sunflowers were present, adult weevils showed little response to other plant species (Fig. 1). Sunflower leaf discs consistently elicited a greater gustatory response than did the other species tested (Fig. la: $F_{3,114} = 80.278$, $P = 0.0001$, $N = 30$; Fig. lb: $F_{3,98} = 54.483$, $P = 0.0001$, $N = 26$; Fig. 1c: $F_{3,110} = 57.702$, $P = 0.0001$, $N = 29$). Of the plant species tested in conjunction with sunflower, only pea, broccoli, bean and carrot elicited a measurable response (Fig. la and 1c). Regurgitant was often present in the vicinity of the carrot leaflets.

When sunflower was eliminated from the choice bioassays, overall feeding activity significantly decreased by up to 80% (Fig. 1c and 2, $F_{5,160} = 10.352$, $P = 0.0001$, $N = 28$). In the absence of sunflower, *C. adspersus* fed most readily on carrot, followed by bean. Broccoli and pea also elicited a measurable feeding response in the absence of sunflower, but there was no significant difference in weevil response to broccoli, pea, corn and tomato (Fig. 2).

There was a significant difference in the proportion of adult *C. adspersus* responding to sunflower leaf discs, unbaited controls, and the number making no choice ($F_{2,111} = 6.770$, $P = 0.0017$, $N = 38$). Of those making a choice, weevils preferred sunflower leaf discs to the blank controls (Fig. 3a, $F_{1,74} = 8.621$, $P = 0.0044$, $N = 30$).

There was a significant difference in the proportion of *C. adspersus* adults responding to sunflower stems, unbaited controls, and the proportion of nonresponsive adults ($F_{2,99} = 3.045$, $P = 0.0521$, $N = 34$). Of those choosing, adult weevils showed no

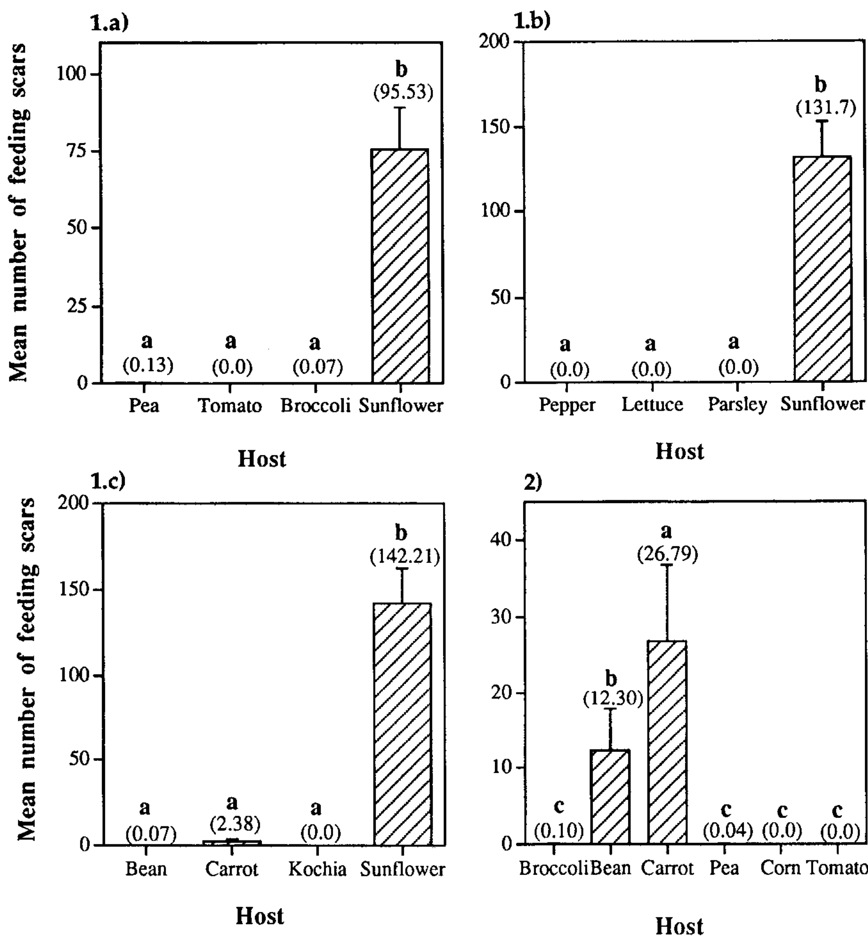


FIG. 1. Feeding preference of adult *C. adspersus* for non-host plants in the presence of 'Italian white' sunflower. Mean number of feeding scars (± s.e.) followed by the same letter are not significantly different ($P < 0.05$, Fisher's Protected LSD). Sunflower in conjunction with: a) pea, tomato and broccoli; b) pepper, lettuce and parsley; c) bean, carrot and kochia.

FIG. 2. Feeding preference of adult *C. adspersus* for non-host plants in the absence of sunflower. Mean number (± s.e.) of feeding scars followed by the same letter are not significantly different ($P < 0.05$, Fisher's Protected LSD).

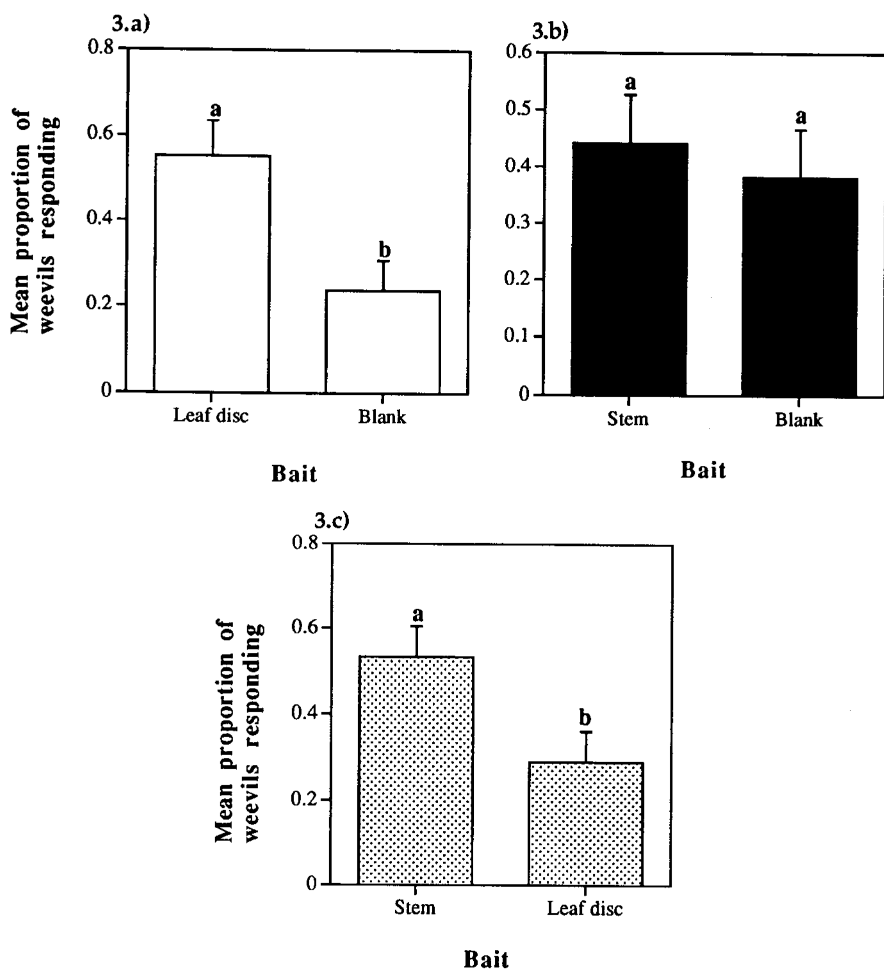


FIG. 3. Olfactory response of adult *C. adspersus* to certain parts in a petri dish-pitfall arena. Mean proportion of choices (± s.e.) followed by the same letter are not significantly different ($P < 0.05$, Fisher's Protected LSD). a) sunflower leaf disc versus blank control; b) sunflower stem versus blank control; c) sunflower stem versus sunflower leaf disc.

significant difference in response to the stem baits versus the unbaited controls (Fig. 3b, $F_{1,66}$ = 0.237, P = 0.6283, N = 28).

When comparing weevil response to sunflower stems versus sunflower leaf discs, there was a significant difference in *C. adspersus* response to each of the baits ($F_{2,132}$ = 7.273, P = 0.0010, N = 45). The weevils were more responsive to the stem baits than the leaf disc baits (Fig. 3c, F = 5.787, P = 0.0182, N = 37).

Although sunflower stem weevils have been reared from a variety of plant species, there are no records of adult feeding behavior on these alternate hosts (Mitchell and Pierce 1911, Pierce 1916, Goeden and Ricker 1976, Charlet 1983b).

In our feeding bioassays, adult *C. adspersus* were responsive to plants from a wide variety of taxa. However, there appeared to be no correlation between plant family and sunflower stem weevil host acceptance. For example, stem weevils consistently exhibited no response to two Solanaceaeous plants, peppers and tomatoes (Fig. la, lb, and 2), and a weakly positive response to the two Leguminoseae tested, beans and peas (Fig. la, 1c, and 2). However, weevil response to members of the Umbellifereae was inconsistent. In spite of producing regurgitant, sunflower stem weevils readily accepted carrot as a feeding host, but showed no gustatory response to closely related parsley (Fig. lb, 1c, and 2). Both species are in the family Umbelliferae, which is characterized by the presence of potentially toxic coumarins which act as feeding deterrents in some systems (Berenbaum 1990). It is possible that acceptance of carrot may be due to the fact that many of the toxins characteristic of wild type Umbelliferae are bred out of commercial cultivars intended for human consumption. Sesquiterpene lactones, common to both Umbelliferae and Asteraceae, may also negatively impact insect response (Spring *et al.* 1987, Gershenzon *et al.* 1985, Berenbaum 1990).

Our data show that host acceptance broadens as the availability of traditional host plants declines (Fig. 2), suggesting a trend toward relaxation of host acceptance parameters. Behavioral responses to host chemicals have been shown to be sensitive to the physiological state of the insect (Jaenike and Papaj 1992). The trend toward decreasing host discrimination with increased weevil experience might possibly be due to the build-up in the weevil mid-gut of mixed function oxidases, which are essential to the digestion of certain plant compounds in many insect species (Berenbaum and Seigler 1992).

The olfactory assays suggest a relative inability of *C. adspersus* to discriminate among certain sunflower host plant parts. It is possible that volatile emissions from sunflower stems and leaves form a complex of general green leaf volatiles ["green odour" *sensu* Visser and Avé (1978)] which is attractive to stem weevils. Host plant recognition requires that variations in plant volatiles occur within specific limits determined by the insect sensory system (Roseland *et al.* 1992). Sunflower leaves are a rich source of terpenoid compounds, which have been shown to have some biological activity (Spring *et al.* 1987, Gershenzon *et al.* 1985). Terpenoids have been shown to have both attractant and repellent properties (Raffa 1988, Nordlander 1990). Roseland *et al.* (1992) showed that a specific terpenoid blend is essential to the host location plume utilized by the red sunflower seed weevil, *Smicronyx fulvus* LeConte. Similar results have been shown with pine root weevils (Nordlander *et al.* 1986, Rieske and Raffa 1991) and the cotton boll weevil (Chang *et al.* 1987). Since female weevils oviposit in sunflower stems, it follows that females should be able to distinguish potential oviposition sites. Since the sex ratio of the weevils was not measured for our assays, it was not possible to determine how this influenced our results.

This study clearly shows that gustatory cues do influence host acceptance in *C. adspersus*. Previous studies have shown that physical aspects of the sunflower plant may affect sunflower stem weevil feeding behavior (Barker 1990, 1992), but have little influence on oviposition preferences (Charlet 1983b). Future work will concentrate on identifying

which phytochemical components of sunflower elicit the observed stimulatory response. The inconclusive results of the olfactory assays suggest that olfactory cues eliciting a response in *C. adspersus* may consist of more than host plant volatiles alone. The presence of an aggregation or sex pheromone cannot be dismissed.

Determining the phytochemical cues governing host location and acceptance could eventually lead to the deployment of a baited monitoring system for *C. adspersus*. This would allow for timely use of insecticides only when weevil damage is imminent, and ultimately enable the development of more effective control measures against the sunflower stem weevil in cultivated sunflowers.

ACKNOWLEDGMENT

The authors thank Scott Armstrong, Leanne Fehringer, Mark Sisterson, and Ellen Snizek (Colorado State University) for technical assistance, and Y. Li (Colorado State University) for statistical advice. Lou Bjostad and Karl Kinney (Colorado State University) offered insight and valuable advice; Karl Kinney reviewed an earlier version of this manuscript. The comments of two reviewers greatly strengthened this manuscript.

LITERATURE CITED

Abacus Concepts. 1989. SuperANOVA: Accessible General Linear Modeling. Abacus Concepts, Inc. Berkeley, California.

Barker, J. F. 1990. Sunflower trichome defenses avoided by a sunflower stem weevil, *Cylindrocopturus adspersus* LeConte (Coleoptera:Curculionidae). J. Kansas Entomol. Soc. 3:638-641

Barker, J. F. 1992. Plant age as a factor in the feeding preference of the sunflower stem weevil, *Cylindrocopturus adspersus* LeConte (Coleoptera: Curculionidae). J. Kansas Entomol. Soc. 65:453-455.

Berenbaum, M. R. 1990. Evolution of specialization in insect-umbellifer associations. Ann. Rev. Entomol. 35:319-343.

Berenbaum, M. R., and D. Seigler. 1992. Biochemicals: engineering problems for natural selection, pp. 89-121, *In* B. D. Roitberg and M. B. Isman [eds.] Insect Chemical Ecology; an Evolutionary Approach. Chapman and Hall, NY.

Casels-Bustos, P. 1976. Bionomics of *Cylindrocopturus adspersus* LeC. and *Baris strenua* (LeC.) (Coleoptera: Curculionidae). Ph.D. Dissertation, North Dakota State University, Fargo. 108 pp.

Chang, J. F., J. H. Benedict, T. L. Payne, and B. M. Camp. 1987. Attractiveness of cotton volatiles and grandlure to boll weevils in the field, pp. 102-104. *In T.* C. Nelson [ed.] Proc. of the Beltwide Cotton Prod. Res. Conf., National Cotton Council of America, Memphis.

Charlet, L. D. 1983a. Distribution and abundance of a stem weevil *Cylindrocopturus adspersus* (Coleoptera:Curculionidae), in cultivated sunflower in the northern plains. Environ. Entomol. 12:1526-1528.

Charlet, L. D. 1983b. Ovipositional behavior and site selection by a sunflower stem weevil, *Cylindrocopturus adspersus* (Coleoptera:Curculionidae). Environ. Entomol. 12:868-870.

Charlet, L. D., C. Y. Oseto, and T. J. Guyla. 1985. Application of systemic insecticides at planting: effects on sunflower stem weevil (Coleoptera:Curculionidae) larva numbers, plant lodging, and seed yield in North Dakota. J. Econ. Entomol. 78:1347-1349.

Charlet, L. D. 1987. Seasonal dynamics of the sunflower stem weevil, *Cylindrocopturus adspersus* (LeConte) (Coleoptera:Curculionidae), on cultivated sunflower in the northern great plains. Can. Entomol. 119:11311137.

Gershenzon, J., M. C. Rossiter, T. J. Mabry, C. E. Rogers, M. H. Blust, and T. L. Hopkins. 1985. Insect antifeedant terpenoids in wild sunflower; a possible source of resistance to the sunflower moth, pp. 433-446. *In P.* A. Hedin [ed.] Bioregulators for Pest Control. ACS Symposium Series 276. American Chemical Society, Washington, D.C.

Goeden, R. D., and D. W. Ricker. 1976. The phytophagous insect faunas of the ragweeds, *Ambrosia eriocentra* and *A. ilicifolia,* in southern California. Environ. Entomol. 5:923-930.

Gulya, T. J., and L. D. Charlet. 1984. Involvement of *Cylindrocopturus adspersus* in the premature ripening complex of sunflower. Phytopathol. 74:869.

Jaenike, J., and D. R. Papaj. 1992. Behavioral plasticity and patterns of host use by insects, pp. 245-264. *In* B. D. Roitberg and M. B. Isman [eds.] Insect Chemical Ecology; an Evolutionary Approach. Chapman and Hall, NY.

Mitchell, J. D., and W. D. Pierce. 1911. The weevils of Victoria county, Texas. Proc. Entomol. Soc. Washington 13:45-62.

Nordlander, G., H. H. Eidmann, U. Jacobsson, H. Nordenhom, and K. Sjodin. 1986. Orientation of the pine weevil, *Hylobius abietis,* to underground sources of host volatiles. Entomol. Exp. App. 41:91-100.

Nordlander, G. 1990. Limonene inhibits attraction to alpha-pinene in the weevils *Hylobius abietis* and *H. pinastri.* J. Chem. Ecol. 16:1307-1320.

Pierce, W. D. 1916. Notes on the habits of weevils. Proc. of the Entomol. Soc. Washington 18:6-10.

Raffa, K. F. 1988. Host orientation behavior of *Dendroctonus ponderosae:* integration of host token stimuli and defenses, pp. 368-390. *In W.* J. Mattson, J. Levieux, and C. Bernard-Dagan [eds.] Mechanisms of Woody Plant Defences Against Insects: Search for Pattern. Springer-Verlag, NY.

Rieske, L.K., and K.F. Raffa. 1991. Effects of varying ethanol and turpentine levels on attractions of two pine root weevil species, *Hylobius pales* and *Pachylobius picivorus* (Coleoptera:Curculionidae). Environ. Entomol. 20:48-52.

Rogers, C. E., and J. G. Serda. 1982. *Cylindrocopturus adspersus* in sunflowers: overwintering and emergence patterns on the Texas high plains. Environ. Entomol. 11:154-156.

Rogers, C. E., P. W. Unger, T. L. Archer, and E. D. Bynum, Jr. 1983. Management of a stem weevil, *Cylindrocopturus adspersus* (Coleoptera:Curculionidae), in sunflower in the southern great plains. J. Econ. Entomol. 76:952-956.

Rogers, C. E., and G. J. Seiler. 1985. Sunflower *(Helianthus)* resistance to a stem weevil, *Cylindrocopturus adspersus* (Coleoptera:Curculionidae). Environ. Entomol. 14:624-628.

Roseland, C. R., M. B. Bates, R. B. Carlson, and C. Y. Oseto. 1992. Discrimination of sunflower volatiles by the red sunflower seed weevil. Entomol. Exp. App. 62:99-106.

Spring, O., U. Bienert, V. Klemt. 1987. Sesquiterpene lactones in glandular trichomes of sunflower leaves. J. Plant Physiol. 130:433-439.

Visser, J. H., and D. A. Ave. 1978. General green leaf volatiles in the olfactory orientation of the Colorado beetle, *Leptinotarsa decemlineata.* Entomol. Exp. App. 24:538-549.

Yang, S. M. 1984. Etiology of atypical symptoms of charcoal rot in sunflower plants parasitized by larvae of *Cylindrocopturus adspersus.* Phytopathol. 74:479-481.
Yang, S. M., and D. F. Owen. 1982. Symptomatology and detection of *Macrophomina phaseolina* in sunflower plants parasitized by *Cylindrocopturus adspersus* larvae. Phytopathol. 72:810-821.
Yang, S. M., C. E. Rogers and N. D. Luciani. 1983. Transmission of *Macrophomina phaseolina* in sunflower by *Cylindrocopturus adspersus.* Phytopathol. 73: 1467-1469.

QUANTIFICATION AND GENETIC ANALYSIS OF BIFENTHRIN RESISTANCE IN THE SILVERLEAF WHITEFLY

Wei-Jia Tan[1], David G. Riley and Dan A. Wolfenbarger[2]

University of Georgia, Coastal Plain Experiment Station, P.O. Box 748, Tifton, GA 31793

ABSTRACT

Bifenthrin resistance in the silverleaf whitefly, *Bemisia argentifolii* Bellows and Perring [previously sweetpotato whitefly, *Bemisia tabaci* (Gennadius), Strain B], was inherited as an incompletely dominant factor. Backcrosses of bifenthrin resistant (R) and susceptible (S) parental colonies to their respective hybrids demonstrated significant segregation. Two backcrosses with F_1 males did not segregate; thus, these were consistent with the condition of facultative arrhenotoky (diploid females and haploid males) reported for *Bemisia*. However, segregation did occur with the two F_1 males backcrossed to their respective parent populations, complicating this mode of inheritance. Probit lines for the reciprocal F_1 crosses (RR♀xS♂, SS♀xR♂) were significantly different. Resistance was inherited to a greater degree from females than males. The estimates of degree of dominance (-1, recessive; +1, dominant) for RR♀xS♂ and SS♀xR♂ were 0.91 and 0.51, respectively. The resistant LC_{50} to susceptible LC_{50} ratios in the backcross populations for SS/SR and RR/RS were closest to 0.6:1 and 1: 0.9, respectively. Plateaus occurred in both F_1 backcrosses at 35-45% mortality. We suggest that multiple genes and ploidy incidences complicated observed resistance to susceptibility ratios in the progeny. Additive inheritance of multiple genes and/or parental extranuclear effects could have been involved. High, stable bifenthrin resistance (608 fold) was observed for six months in an isolated, unselected resistant colony derived from a bifenthrin-selected greenhouse colony. This could potentially threaten insecticide resistance management for the silverleaf whitefly for isolated populations, such as in an enclosed greenhouse.

INTRODUCTION

The emergence of *Bemisia tabaci* as a primary pest has been associated with insecticide resistance in many parts of the world (Dittrich *et al.* 1985, Dittrich *et al.* 1990, Perry 1985). Prabhaker *et al.* (1985) demonstrated broad spectrum resistance to organophosphorus insecticides in *B. tabaci* populations in California. Since 1990 *Bemisia* has become resistant not only to organophosphorous insecticides, but also to some pyrethroids and other classes of

---

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 USDA.

[1]Institute of Plant Protection, Chinese Academy of Agricultural Science, Beijing 100094, People' s Republic China.

[2]USDA-ARS, 2413E. Hwy.83, Weslaco, TX 78596

insecticides (Byrne *et al.* 1992). The silverleaf whitefly, *Bemisia argentifolii* Bellows and Perring (Bellows *et al.* 1994), has exhibited high levels of insecticide resistance (Wolfenbarger and Riley 1994). The rapid development of insecticide resistance in *Bemisia* populations around the world and associated increase in pest status signals the need for a better understanding of the inheritance of insecticide resistance in these insects.

Arrhenotokous parthenogenesis is reported for *B. tabaci* (Azab *et al.* 1971) and *B. argentifolii* (Perring *et al.* 1993). The sex determination of *Bemisia* is probably based on ploidy level as with other arrhenotokous insects (Kerr 1962). Facultative arrhenotoky is production of males from the unfertilized, haploid egg. Kerr (1962) suggested that a series of cumulative female-tendency genes (F) and static male-tendency genes (M) scattered through several chromosomes could cause this condition. Since the effect of female-tendency genes accumulates, the diploid condition would always result in a female (2F > M). The cytology of *Bemisia* spp. is not currently described, so inheritance studies for *Bemisia* should provide some insight into the mechanisms of this unique genetic system.

The objectives of these studies conducted at Weslaco, Texas were to (1) investigate inheritance characteristics of bifenthrin resistance, and (2) to demonstrate the stability of bifenthrin resistance in an isolated silverleaf whitefly population. The LC_{50s} for the resistant and susceptible populations were determined for females and males individually to investigate ploidy differences.

MATERIALS AND METHODS

The two populations of whitefly used in these tests were confirmed as Strain B sweetpotato whitefly using electrophoretic techniques (J. Brown, personal communications). The bifenthrin-susceptible colony (SS001) was obtained from Gary Leibee's Lab, University of Florida, Sanford, Florida in November 1993 and held under laboratory conditions. The bifenthrin-resistant colony (SR001) was collected from a cotton field at the Texas A&M University Experimental Station, Weslaco in July 1993 and held under greenhouse conditions. In the greenhouse, the field collection was exposed to monthly treatments of bifenthrin 0.1 kg AI/473 l/ha for six months (Riley, unpublished data) before moving to the laboratory. In the laboratory, both resistant and susceptible colonies were maintained separately on cotton plants in cages at 28 ± 5 °C, 80 ± 5% RH, and a 24:0 (L:D) photoperiod. No selection pressure for increased insecticide resistance was applied during the laboratory experiments after the greenhouse selection.

LC_{50}'s were determined using the vial bioassay of Staetz *et al.* (1992). A sample of 30-40 vigorous adults were aspirated from infested cotton leaves into a glass pipet and then gently blown into a glass vial. Each vial was coated with a specified concentration of bifenthrin dissolved in acetone and each dosage was replicated three times. Six different dosages, ranging from 0.003 to 125 µg/ vial, were tested against the susceptible and resistant colonies. Untreated vials were used as a control. After collecting the adults in the vial, they were held at 28 ± 5°C and 80 ± 5% RH in the laboratory. Dead adults were designated as those individuals that could not move, walk or fly. Mortality was assessed after four hours. No mortality was observed in the untreated controls. Dose-mortality regressions were estimated by the probit analysis of POLO (Russell *et al.* 1977). Regressions were also tested for sameness and parrallelism using POLO (Russell *et al.* 1977).

Both the resistant and susceptible colonies were held in isolation cages on clean cotton plants and extra care was taken to prevent any mixing of the colonies. The greenhouse-selected resistant strain was held without selection with bifenthrin treatments for 6 months to determine if resistance to bifenthrin would decrease with time. Bioassays for mortality responses to

bifenthrin were conducted monthly from March-September 1994 for both resistant and susceptible colonies to coincide with a single generation (Azab *et al.* 1971).

The fourth instar nymphs of resistant (R) and susceptible (S) whiteflies were cut from infested leaves of cotton plants and placed into gelatin capsules until adults emerged. Sexual determination of adults was made under the microscope (Gupta 1970). One female from one colony and one male from the other colony were placed in a gelatin capsule on a fully expanded leaf of a pre-square cotton plant and sealed with clay (Handi-Tak®, Super Glue Corp., Hollis, NY). In these tests the female diploid condition was indicated by RR or SS, for resistant and susceptible colonies, respectively, and the male haploid condition was indicated by R or S. A male from a RR♀ x S♂ cross was indicated as R_S. The resulting F_1 hybrid progenies were then backcrossed to their respective parental strains. At least 30 adults per vial (dose) from each cross and backcross were bioassayed for response to bifenthrin. Bioassays of males or females alone in the resistant and susceptible colonies were also conducted to determine if bifenthrin resistance differed between the sexes (or ploidy levels).

Stone' s (1968) formula was used to estimate the degree of domination (D) for the F_1 progeny. This formula results in a value of -1 if the resistance is fully recessive, a value of 0 if there is no dominance, and a value of +1 if the resistance is fully dominant (Keena and Granett 1990). The dose-mortality data for the backcrosses were further analyzed to determine whether the responses fit a model for single gene, Mendelian inheritance (1:1). In addition, inheritance models from 1:0.1 to 1:0.9 (RS) or 0.1:1 to 0.9:1 (SR) were tested for goodness of fit. The expected responses of the backcross populations for each concentration tested were obtained according to Georghiou's (1969) formula. The χ^2 goodness-fit values were calculated from the observed and expected responses to determine how well the observed results fit the model.

RESULTS AND DISCUSSION

The LC_{50} to bifenthrin beginning with a susceptible collection from a cotton field in July 1993 [0.014 (0.011-0.018), n=267, slope=1.92±0.22] was increased with six applications of bifenthrin (0.1 kg AI/473 l/ha applied once per whitefly generation) in the greenhouse prior to a final selection (Riley, unpublished data). Once the resistant colony was isolated in a cage, comparisons between dates over six consecutive months indicated no significant change in LC_{50} to bifenthrin in the offspring of the resistant strain (Table 1). Therefore, the resistance was stable in the population for six months (i.e., the slopes of the regression lines estimated for each generation were not significantly different). The resistance alleles were maintained in the absence of bifenthrin selection and no genetic drift was apparent once bifenthrin resistance in the insect was obtained. This genetic stability under isolated conditions could be related to parthenogenic production of males that conserve the genetics of a resistant female parent. One hypothesis for the observed data is that females from the R genotype might have two R alleles which exhibited strong penetrance. The male progenies produced by these females would carry the strong monoploid allele through facultative arrhenotoky. This assumes that the male progeny with the resistant allele mate with female progeny which are homozygous for the RR genotype, resulting in progeny with tightly coupled R alleles. Thus, each progeny in alternate generations would be at least 50% homozygous, thereby maintaining a stable resistant population. The LC_{50}'s of the S and R colonies were 0.041 and 24.9 µg/vial, respectively, resulting in 608 fold resistance ratio (Table 2). Quantification of the LC_{50} in the F_1 and F_2 generations of the reciprocal crosses corroborated the resistance stability of silverleaf whitefly. In contrast to the resistant strain, the responses to bifenthrin in susceptible populations were significantly different, suggesting that the frequency of the R allele might fluctuate in the susceptible populations.

TABLE 1. Stability of Bifenthrin Toxicity in Susceptible and Resistant Silverleaf Whitefly.

Colony	Date	n	LC_{50} (μg/vial 95%CI)	Slope ± SE	Factor[a]	χ^2
SS♀xS♂	03/17	173	0.088 (0.037-0.205)[b]	0.56 ± 0.08	0.85	1.9
"	04/14	239	0.009 (0.005-0.018)[b]	0.68 ± 0.08	0.88	2.6
"	04/29	134	0.010 (0.002-0.057)[b]	0.47 ± 0.29	0.50	1.5
"	06/03	154	0.009 (0.003-0.028)[b]	0.55 ± 0.09	0.15	0.5
"	07/13	140	0.038 (0.026-0.057)[b]	1.45 ± 0.29	0.18	0.5
"	08/03	119	0.041 (0.029-0.064)[b]	1.71 ± 0.36	0.54	1.6
"	09/09	290	0.010 (0.004-0.032)[b]	0.71 ± 0.07	1.34	5.4
RR♀xR♂	03/17	137	19.92 (12.99-33.38)[c]	2.49 ± 0.39	1.02	3.1
"	04/14	164	27.05 (20.09-40.65)[c]	1.90± 0.32	0.11	0.3
"	04/29	150	21.76 (17.05-28.30)[c]	2.30 ± 0.33	0.29	2.8
"	07/13	157	20.01 (9.11-42.51)[c]	2.41 ± 0.35	2.19	6.6
"	08/03	171	24.93 (11.76-40.58)[c]	2.01 ± 0.31	1.39	5.5
"	09/09	214	23.81 (19.13-29.58)[c]	2.26 ± 0.26	0.57	1.7

[a]Heterogeneity described by Russell *et al.* (1977).
[b]LC_{50} values within same strain were significantly different (P<0.05) using tests for sameness and parallelism in which hypothesis was rejected (POLO, Russell *et al.* 1977).
[c]LC_{50} values within same strain were not significantly different (P<0.05) using tests for sameness and parallelism in which hypothesis was accepted (POLO, Russell *et al.* 1977).

Bifenthrin resistance in *B. argentifolii* appeared to be inherited as an incompletely dominant trait. Overall, there were some differences in the dose-mortality response of males and females to bifenthrin dependant on whether the population tested was susceptible or resistant. The males of the susceptible parent population responded to bifenthrin the same as the susceptible females. However, the males differed significantly from females in the resistant population (Table 2). The dose-response lines for the parental SS (73% ♀) and RR (72% ♀) colonies and the reciprocal RS (50% ♀) and SR (68% ♀) crosses were all parallel (Fig. 1). The intercepts of lines for RR♀ x S♂ and SS♀ x R♂ were not equal and both differed from the RR or SS colonies (Table 2). In addition, the 95% confidence intervals of reciprocal crosses did not overlap. We suggest that differences in the percentage of females in the reciprocal cross populations and/or parental extranuclear effects (Hoyer and Plapp 1971) might have contributed to these results. The LC_{50}'s of the F_1 or F_2 generations in both the reciprocal crosses and sexes in the resistant strain were significantly different (Table 2). Estimates of dominance (D) for RR♀ x S♂ and SS♀ x R♂ were 0.91 and 0.51, respectively, indicating that bifenthrin resistance was inherited to a greater degree from a resistant (diploid) female than from a resistant (haploid) male.

TABLE 2. Responses to Bifenthrin and Dominance (D) of Bifenthrin Resistance in Susceptible, Resistant, and Crossed Populations of Silverleaf Whitefly.

Crosses	n	LC_{50} (95%CI)μg/vial	Slope ± SE	Ratio[a]	D[b]	Factor[c]	χ^2
SS x S	119	0.041 (0.029-0.064)	1.71 ± 0.36	1		0.54	1.6
RR x R	171	24.93 (11.8-40.6)	2.01 ± 0.31	608		1.39	5.5
F_1(RRxS)	140	18.64 (12.4-31.0)	1.33 ± 0.26	455	0.9	0.26	1.0
F_1(SSxR)	160	5.17 (3.28-7.74)	1.28 ± 0.25	126	0.5	0.44	1.8
F_2(RRxS)	157	15.85 (9.50-32.6)	1.94 ± 0.29	387		1.62	0.7
F_2(SSxR)	167	4.94 (3.65-6.65)	1.83 ± 0.25	120		0.85	2.3
BC(RSxR)	438	23.02 (15.9-36.3)	0.93 ± 0.10	561		0.77	5.4
BC(RRxR_s)	247	21.90 (12.1-54.3)	0.86 ± 0.13	534		0.20	1.4
BC(RSxS)	138	20.24 (9.72-51.6)	1.04 ± 0.21	494		0.57	1.7
BC(RRxS_R)	100	11.40 (3.09-106)	0.56 ± 0.13	278		0.78	2.3
BC(SRxR)	214	5.98 (0.95-447)	0.36 ± 0.13	146		0.32	0.9
BC(SRxS)	374	2.28 (1.44-4.24)	0.86 ± 0.10	56		0.92	6.5
BC(SSxS_R)	282	2.25 (1.21-5.76)	0.78 ± 0.12	55		0.66	4.6
BC(SSxR_S)	91	0.80 (0.23-2.62)	0.64 ± 0.12	2		0.26	0.8
SS♀	352	0.007 (0.001-0.12)[d]	0.45 ± 0.05			3.15	12.
S♂	313	0.009 (0.001-0.44)[d]	0.43 ± 0.05			4.14	16.
RR♀	324	32.28 (20.1-51.4)[e]	1.56 ± 0.20			1.20	3.6
R♂	311	21.81 (18.1-26.0)[e]	2.29 ± 0.25			0.24	0.7

[a]Determined by dividing the LC_{50} of the resistant strain by the LC_{50} of the susceptible.
[b]Degree of dominance (Stone 1968).
[c]Described by Russell et al (1977).
[d]LC_{50} values were not significant different (P=0.05) using tests for sameness and parallelism in which hypothesis was accepted (POLO, Russell. 1977).
[e]LC_{50} values were significant different (P=0.05) using tests for sameness and parallelism in which hypothesis was rejected (POLO, Russell *et al.* 1977).

Segregation was not apparent in the backcrosses of F_1 males with the opposite female parent, which was expected with male haploidy (Fig. 2). However, apparent segregation did occur when the F_1 males were backcrossed to their respective parent females (Fig. 3). This was not consistant with the haploid male possessing a full complement of either the resistant or susceptible factors. Females exhibited a greater degree of resistance with a greater range of responses (flatter slope value) than males (Table 2), so the haploid male probably did not possess all the resistant factors. Furthermore, males produced by a suspected homozygous parent may not be genetically identical. In a subsample from the resistant population, slight but significant differences occurred in resistant males from non-mated and mated females (LC_{50s} = 87 (62-125) and 51 (31-91), slopes = 2.3±0.4 and 3.4±0.6, n=89 and 99, respectively; SAME hypothesis rejected, PARALLEL hypothesis accepted, POLO). Also, the dose-mortality lines calculated in these tests combined male and female progeny, so variation in the progeny due to

ploidy differences was included in these estimates. This additional source of variation could affect LC_{50} measurements in progeny, particularly where progeny sex ratios differed from the 75% ♀ parent population (e.g., RR♀ x S_S♂ progeny was 50% ♀, Figure 1).

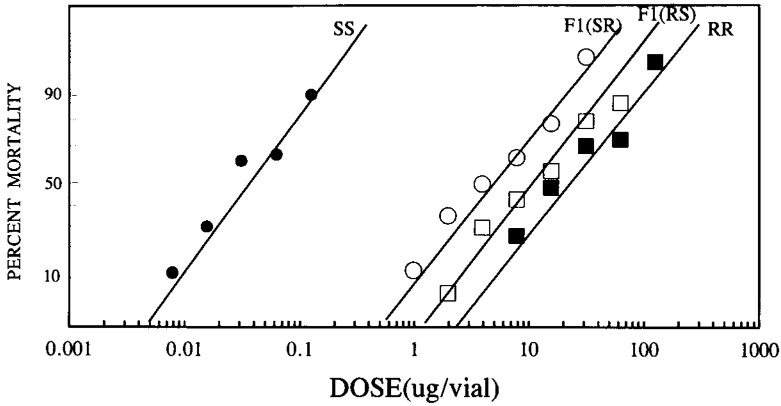


FIG. 1. Bifenthrin dose-mortality lines (Savin *et al.* 1977) for silverleaf whitefly parental populations and reciprocal crosses; the susceptible (dots: SS, 75% ♀), resistant (filled squares: RR, 73% ♀), and F_1 (squares: RR♀ x S_S♂, 50% ♀; circles: SS♀ x R_R♂, 73% ♀) populations.

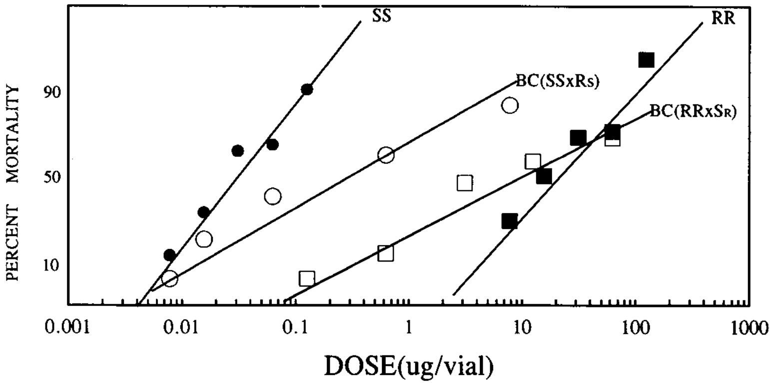


FIG. 2. Bifenthrin dose-mortality lines for silverleaf whitefly parental populations and backcrosses; specifically, the susceptible (dots: SS), resistant (filled squares: RR), and backcross (BC) populations (squares: RR♀ x S_R♂, circles: SS♀ x R_S♂).

Segregation was apparent in all four backcrosses with F_1 females because distinct plateaus were evident in the dose-response lines (Figs. 4, 5). Observed mortalities in the RS♀ x R♂, RR♀ x R_S♂ and RS♀ x S♂ crosses and the SR♀ x S♂ and SS♀ x S_R♂ crosses were similar (Table 2), in which the plateaus appeared at about 35-45% mortality rather than 50% (as

expected at least for the SR♀ x S♂, SR♀ x R♂, RS♀ x R♂ and RS♀ x S♂, crosses). Assuming a 1:1 segregation of males and females, the observed mortalities for SR♀ x S♂ and SS♀ x S_R♂ were significantly different from the expected mortalities (χ^2 > 15.507; df = 8; $P < 0.05$). In a non-parthenogenic situation for the Lepidoptera, Payne *et al.* (1985) suggested that there might be some lethal interaction in both crosses. Payne *et al.* (1985) explained that an unlinked gene from a parental background was lethal to another parental genotype, but was not lethal to the progeny genotype, resulting in a ratio different from the expected 1:1. This phenomenon is unknown for the silverleaf whitefly at present, but we offer this explanation as a possible contributing factor to our results. In our tests, ratio of χ^2 goodness-fit for SS/SR was 0.6: 1 (Table 3), suggesting that the backcross progeny might have received less than half of the lethal allele. This was consistent with the plateaus in the backcross lines at ca. 35-45% mortality (Figs. 3-5). Although data observed in the backcrosses RR♀ x R_S♂, RS♀ x R♂, RS♀ x S♂ and SR♀ x R♂ were not significantly different from the 1:1 model, a plateau at about 35-45% mortality also was observed in two of the other backcrosses (Figs. 3-5). Based on the LC_{50} values of reciprocal crosses, we suspect that the the most likely ratio was 1: 0.9 (RR/RS).

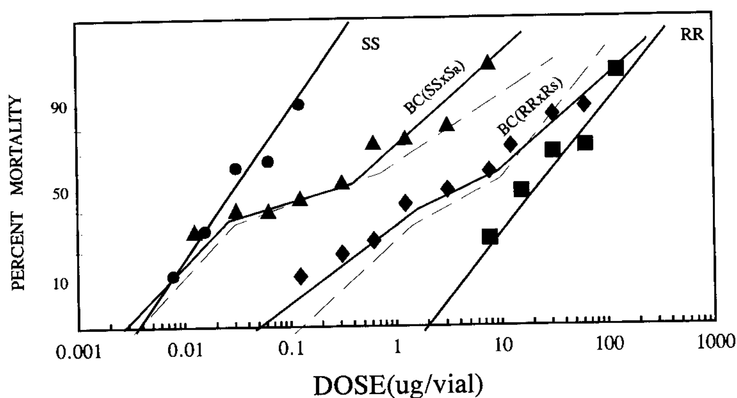


FIG. 3. Bifenthrin dose-mortality lines for silverleaf whitefly parental populations and backcrosses; specifically, the susceptible (dots: SS), resistant (filled squares: RR), and backcross populations (triangles: SS♀ x S_R♂, 64% ♀; diamond: RR♀ x R_S♂, 68% ♀). The dashed curve indicates expected mortalities for BC assuming 0.6: 1 (SS/SR) and 1: 0.9 (RR/RS) inheritance ratios using the observed dose-mortality responses of the susceptible (SS), resistant (RR) and F_1 (SR) genotypes.

These data also suggested that more than one factor participated in the inheritance of resistance to bifenthrin. These factors probably contributed to the variability of dose-response lines, the greater resistance of the SR genotype in the hybrid state, and the SR genotype in the backcross (Payne *et al.* 1985, Halliday and Georghiou 1985, Roush *et al.* 1986). The lower than expected mortality might be the result of one or more minor factors which play a significant subsidiary role in inheritance by the silverleaf whitefly. Specifically, the insecticide tolerance in the reciprocal crosses could have been increased due to one or more minor factors. Also, small plateaus appear in Figs. 3-5 at approximately 75-85% mortality, and observed and expected lines differed at some points along the probit lines. This suggested that there might

be at least two genes for the inheritance of bifenthrin resistance by the silverleaf whitefly. Since the genetics of *Bemisia* appears complex, it is not clear how these multiple genes control the inheritance of resistance to bifenthrin at the current time. Despite the minor factors or the complication of the 0.6: 1 (SS/SR) and 1: 0.9 (RR/RS) models, the distinct plateaus in four backcrosses were strong evidence for segregation of an incompletely dominant factor for resistance. To our knowledge, this is the first demonstration of segregation of a bifenthrin resistant gene in the silverleaf whitefly.

TABLE 3. χ^2 Tests for Different Ratios of Observed to Expected Values.

Ratio	$RRxR_S$	RSxR	RSxS	Ratio	$SSxS_R$	SRxS	SRxR
1:0.1	89.57	65.86	71.29	0.1:1	473.3	465.8	1271.0
1:0.2	61.99	45.79	48.45	0.2:1	217.2	222.8	627.4
1:0.3	47.87	38.34	38.16	0.3:1	95.4	110.4	360.3
1:0.4	36.83	31.97	29.78	0.4:1	57.2	44.5	220.2
1:0.5	28.33	26.76	23.21	0.5:1	27.6	25.7	134.6
1:0.6	21.70	22.68	18.56	0.6:1	12.3	10.5	80.2
1:0.7	16.33	19.39	14.38	0.7:1	6.1	4.9	47.6
1:0.8	13.06	16.87	11.38	0.8:1	6.5	4.9	24.4
1:0.9	7.37	14.69	9.09	0.9:1	11.5	10.1	11.8
1:1	7.55	12.40	7.05	1:1	20.1	18.9	6.8

[a]df=8; P(0.05) = 15.507.

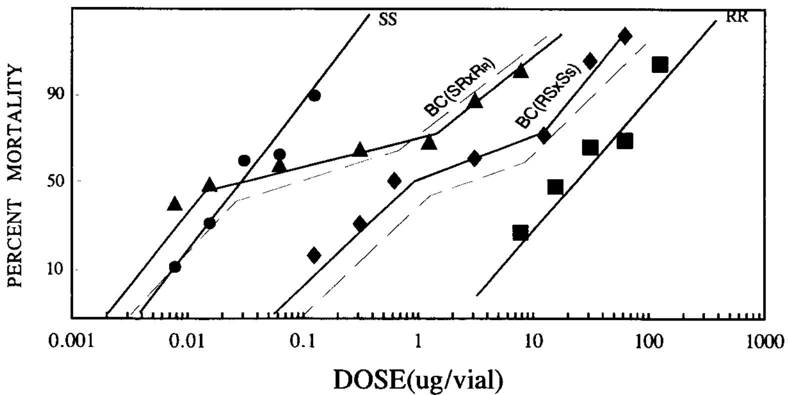


FIG. 4. Bifenthrin dose-mortality lines for silverleaf whitefly parental populations and backcrosses; specifically, the susceptible (dots: SS), resistant (filled squares: RR), and backcrosses (triangles: SR♀ x R♂, diamond: RS♀ x S♂). The dashed curve indicates expected mortalities for BC assuming 0.6: 1 (SS/SR) and 1: 0.9 (RR/RS) inheritance ratios using the observed dose-mortality responses of the susceptible (SS), resistant (RR) and F1 (SR) genotypes.

This study showed that bifenthrin resistance in the silverleaf whitefly was inherited as an incompletely dominant factor. The lack of information on the genetics and sexual determination of *Bemisia* made it difficult to interpret the results obtained in these tests; however, the resistance factors appear to be inherited to a greater extent through females than males. Also, the expression of resistance was greater in females than males. More specific data on differences between females and males than was obtained in these tests are needed to elucidate the inheritance and expression of resistance to bifenthrin in *Bemisia*.

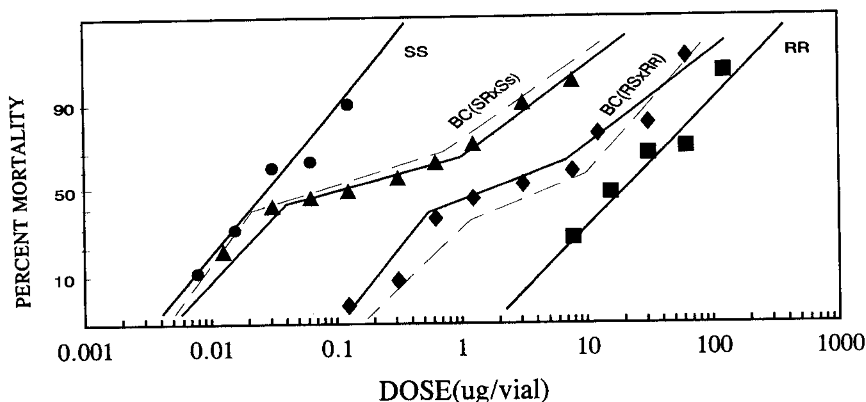


FIG. 5. Bifenthrin dose-mortality lines for silverleaf whitefly parental and backcrosses; specifically, the susceptible (dots: SS), resistant (filled squares: RR), and backcrosses (triangles: SR♀ x S♂, 60% ♀; diamond: RS♀ x R♂, 82% ♀). The dashed curve indicates expected mortalities for BC assuming 0.6: 1 (SS/SR) and 1: 0.9 (RR/RS) inheritance ratios using the observed dose/mortality responses of the susceptible (SS), resistant (RR) and F_1 (RS) genotypes.

In conclusion, the stability of resistance for the six-month period measured in this study could pose a serious problem for the management of insecticide resistance in the silverleaf whitefly. This could be particularly true under isolated conditions similar to the cages used in these tests. An enclosed greenhouse could sustain insecticide resistance in a selected whitefly population for at least 6 months according to these studies. However under field conditions, resistance to bifenthrin appears to be variable at best (Wolfenbarger and Riley 1994). Therefore, a better understanding of the bionomics of resistant whitefly populations is needed to understand the fate of resistance under field conditions.

ACKNOWLEDGMENTS

We gratefully acknowledge the technical support of M. M. Izeta, J. I. Martinez and C. T. Perez, and the critical reviews by N. Anthony, R. Constance, G. Mocelin and J. Ruberson. Financial support was provided by the USDA specific cooperative agreement 58-6204-3-025 (TX) and the Texas Agricultural Experiment Station. We thank the Institute of Plant Protection, Chinese Academy of Agricultural Science, Beijing, China for their support of this project.

LITERATURE CITED

Azab, A. K., M. M. Megahed, and D. H. Elmirsawi. 1971. On the biology of *Bemisia tabaci* (GENN.). Bull. Entomol. Soc. Egypte 55: 305-315.

Bellows, T. S. Jr., T. M. Perring, R. J. Gill, and D. H. Headrick. 1994. Description of a species of *Bemisia* (Homoptera: Aleyrodidae). Ann. Entomol. Soc. Am. 87: 195-206.

Byrne, F. J., I. Denholm, L. C. Birnie, A. L. Devonshire, and M. W. Rowland. 1992. Analysis of insecticide resistance in the Whitefly, *Bemisia tabaci,* pp.165-178. *In* Denholm, I., A. L. Devonshire and D. W. Hollomon [eds.], Resistance' 91: Achievements and developments in combating pesticide resistance. Elsevier, London.

Dittrich, V., S. O. Hassan, and G. H. Ernst. 1985. Sudanese cotton and the whitefly: a case study of the emergence of a new primary pest. Crop Protection 4: 161-176.

Dittrich, V., G. H. Ernst, O. Ruesch, and S. Uk. 1990. Resistance mechanisms in sweetpotato whitefly (Homoptera:Aleyrodidae) populations from Sudan, Turkey, Guatemala, and Nicaragua. J. Econ. Entomol. 83: 1665-1670.

Georghiou, G. P. 1969. Genetics of resistance to insecticides in houseflies and mosquitos. Exp.Parasitol. 26: 224-255.

Gupta, P. C. 1970. External morphology of *Bemisia gossypiperda* (M. and L.) a vector of plant virus diseases (Homoptera:Aleyrodidae). Zoologische Beiträge 18: 1-23.

Halliday, W. R., and G. P. Georghiou. 1985. Inheritance of resistance to permethrin and DDT in the southern house mosquito (Diptera: Culicidae). J. Econ. Entomol. 78: 762-767.

Hoyer, R. F., and F. W. Plapp, Jr. 1971. Insecticide resistance in the house fly: effect of a modifier gene in combination with major genes which confer resistance. J. Econ. Entomol. 64: 1051-1055.

Keena, M. A., and J. Granett. 1990. Genetic analysis of propargite resistance in pacific spider mites and twospotted spider mites (Acari:Tetranychidae). J. Econ. Entomol. 83: 655-661.

Kerr, W. E. 1962. Genetics of sex determination. Annu. Rev. Entomol. 7: 431-457.

Payne, G. T., B. J. Disney, and T. M. Brown. 1985. Field surveillance and laboratory selection for pyrethroid resistance in the tobacco budworm in South Carolina. J. Agric. Entomol. 2: 85-92.

Perring, T. M., A. D. Cooper, R. J. Rodriguez, C. A. Farrar, and T. S. Bellows, Jr. 1993. Identification of a whitefly species by genome and behavioral studies. Science 259: 74-77.

Perry, A. S. 1985. The relative susceptibility to several insecticides of adult whiteflies (*Bemisia tabaci*) from various cotton-growing areas in Israel. Phytoparasitica 13: 77-78.

Prabhaker, N., D. L.Coudriet, and D. E. Meyerdirk. 1985. Insecticide resistance in the sweetpotato whitefly, *Bemisia tabaci* (Homoptera:Aleyrodidae). J. Econ. Entomol. 78: 748-752.

Roush, R. T., R. L. Combs, T. C. Randolph, J. Macdonald, and J. A. Hawkins. 1986. Inheritance and effective dominance of pyrethroid resistance in the horn fly (Diptera:Muscidae). J. Econ. Entomol. 79: 1178-1182.

Russell, R. M., J. L. Robertson, and N. E. Savin. 1977. POLO: a new computer program for probit analysis. Rev. Entomol. Soc. Am. 23: 209-215.

Savin, N. E., J. L. Robertson, and R. M. Russell. 1977. A critical review of bioassay in insecticide research: likelihood ratio test of dose-mortality regression. Bull. Entomol. Soc. Am. 23: 257-266.

Staetz, C. A., K. A. Boyler, E. V. Gage, D. G. Riley, and D. A. Wolfenbarger. 1992. Vial bioassay for contact insecticides for adult whiteflies, *Bemisia tabaci,* pp 704-707. *In* D. J. Herber and D. A. Richter [eds.], Proceedings, Beltwide Cotton Production Conference, Memphis, TN.

Stone, B. F. 1968. A formula for determining degree of dominance in cases of monofactorial inheritance of resistance to Chemicals. Bull. W. H. O. 38: 325-326.

Wolfenbarger, D. A., and D. G. Riley. 1994. Toxicity of mixtures of insecticides and insecticides alone against b-strain sweetpotato whitefly, pp 1214-1216. *In* D. J. Herber and D. A. Richter [eds.], Proceedings, Beltwide Cotton Production Conference, San Antonio, TX.

OVIPOSITIONAL RESPONSE OF THE TOBACCO BUDWORM[1] AND BOLLWORM[2] TO CHICKPEA[3,4]

T. N. Shaver and J. D. Lopez, Jr.

Southern Crops Research Laboratory, USDA-ARS
Areawide Pest Management Research Unit
College Station, Texas 77845

ABSTRACT

Ovipositional response of tobacco budworm, *Heliothis virescens* F., to chickpea, *Cicer arietinum* L., was determined using fruiting terminals and methylene chloride extracts of fruiting terminals. Also, comparisons of ovipositional responses were made between chickpea and cotton, *Gossypium hirsutum* L., for the tobacco budworm and bollworm, *Helicoverpa zea* (Boddie). Of the total eggs oviposited by tobacco budworm, 86% were deposited on chickpea in a choice test between fruiting forms of chickpea and cotton. Mean numbers of eggs laid per female in a night were the same in comparisons of cotton versus chickpea and cotton versus cotton indicating preference for oviposition on chickpea. The presence of chickpea did not stimulate tobacco budworm moths to lay more eggs. Leaf texture of chickpea was not a factor in the ovipositional preference as determined by covering chickpea terminals with black broadcloth. There was no significant difference in the numbers of eggs oviposited by bollworm on chickpea or cotton. An ovipositional attractant was extracted with methylene chloride as evidenced by a significant preference of tobacco budworm moths to oviposit on broadcloth treated with a methylene chloride extract compared to a solvent blank.

INTRODUCTION

The chickpea or garbanzo bean, *Cicer arietinum L.*, is a legume of considerable economic importance in India, Pakistan, Ethiopia, and Iran where its most important insect pest is *Heliothis armigera* Hubner. Chickpea is a crop predominately of temperate climates; in the United States it is a favorable host of tobacco budworm, *Heliothis virescens* F., in small commercial plantings in Arizona where it may be utilized as an early season trap crop for this insect pest (Potter and Watson 1983). Large populations of the tobacco budworm also have been found in fields

---

[1] *Heliothis virescens* (F.) (Lepidoptera: Noctuidae)
[2] *Helicoverpa zea* (Bodie) (Lepidoptera:Noctuidae)
[3] *Cicer arietinum* L.
[4] Mention of a commercial product or vendor does not constitute an endorsement by the United States Department of Agriculture.

of chickpea in the Lower Rio Grande Valley of Texas and throughout northern Mexico (Potter and Watson 1983). In the United States, tobacco budworm is an important insect pest of cotton, a major agricultural commodity of the South.

Insects respond to a variety of chemical or physical stimuli. They are attracted to host plants and are stimulated to feed and/or oviposit by chemicals (kairomones) produced by the plant (Dethier et al. 1960). Saxena and Rembold (1984) demonstrated a larval attractant for *Heliothis armigera* Hubner using hexane extracts of chickpea seeds. Rembold et al. (1989a) identified 82 compounds from floured seed of chickpea; 16 were tested in an olfactometer using first-instar larvae of *H. armigera*. Significant positive response was obtained from delta-3-carene, myrcene, 1-pentanol, and alpha-pinene (Rembold et al. 1989b). Rembold et al. (1991) found that a mixture of these four chemicals was attractive to mated, egg-laying *H. armigera*, but had no effect on males or unmated females.

Knowledge of the chemical basis of insect behavioral responses to specific host plants can be important in developing strategies for alternate control procedures of target insect pests. The potential of developing an attractant for tobacco budworm from chickpea appears to be sound and has a reasonable probability of success. Incorporation of chemicals that elicit behavioral responses, such as attractants and stimulants, with other pest control measures would be beneficial to areawide management of insect pests such as the tobacco budworm.

As a preliminary step in developing this improved control technology, we evaluated the ovipositional response of the tobacco budworm to chickpea. The objectives of our study were to evaluate the relative ovipositional response of tobacco budworm to chickpea and cotton and to extract an ovipositional attractant/stimulant from chickpea. Response of the bollworm, *Helicoverpa zea* Boddie, to chickpea and cotton was also evaluated.

MATERIALS AND METHODS

To demonstrate an ovipositional preference of tobacco budworm to chickpea, terminals containing fruiting forms were collected from field grown chickpea (variety 'UC-5' or 'Surutoto') and from cotton ('Stoneville 213'). Three terminals were placed in separate 125-ml Erlenmeyer flasks containing approximately 80 ml of water to prevent desiccation and wilting of the plant material. The plant samples for both chickpea and cotton were positioned so that each had ca. 25 cm of leaves and fruiting forms above the top of the flask. One flask of each plant type was placed in a wire mesh cage, 60 cm on each side. Tobacco budworm female moths used in these tests were taken from a laboratory colony and confined upon emergence with an equal number of male moths to ensure that females were gravid at 3 days of age. Five mated, 3-day old female tobacco budworms were introduced into cages containing plant materials at 1600 hours. Moths remained in the cages for a 16-h ovipositional period with a dark cycle between 1900 and 0630 hours. Plant samples were removed from the cages at the end of the 16-h oviposition period, and each terminal was carefully examined for total numbers of eggs. Fresh plants and moths were used each night and the relative position of cotton and chickpea was rotated each night to minimize positional effects on oviposition. A total of six cages were set up each night and the test was conducted for 12

nights. Similar tests were conducted with laboratory-reared females of bollworm. Concurrently, a series of comparisons was conducted with tobacco budworm only in the same manner but comparing oviposition on two flasks of cotton terminals in the same cage to test the validity of results obtained by this procedure.

A second series of comparisons was made with plant terminals confined in black broadcloth pouches ca. 5.0 x 2.5 cm to determine if plant texture had an effect on ovipositional preference of the tobacco budworm to chickpea. Terminals and fruiting forms of chickpea or cotton were placed in pouches of black broadcloth (prepared by folding a piece of cloth over the plant material and stapling the edges). An untreated check was prepared by filling a pouch to similar bulkiness with cloth. One pouch containing cotton terminals and one pouch containing chickpea terminals were placed in a 500-ml cylindrical plastic container, 6.5 cm deep x 10 cm diameter. A 6-cm diameter hole was cut at the top of the container and was covered with hardware cloth to provide ventilation. Alternately, one pouch containing plant terminals and one blank pouch were placed in the 500-ml test chamber. One 3-day old mated female tobacco budworm moth was placed in each test chamber at 1600 h and removed 16-h later, at which time eggs deposited on each of the pouches were counted.

Solvent extracts were prepared from fresh chickpea terminals by macerating the plant material in a blender to provide 50 g plant material (fresh weight) in 250 ml solvent (water, methylene chloride, or hexane). The plant material plus solvent was allowed to sit for 1 h after which time the solvent was decanted and a second 250 ml aliquot of solvent added to the plant material. After an additional hour, the solvent was decanted and residual amounts of solvent were recovered from the plant material by filtration. Extracts were combined and diluted with solvent to give an extract equivalent to 0.1 g fresh plant material per 1 ml solvent. Preliminary bioassays indicated that water extracts were inactive and that methylene chloride extracts were more active than those prepared with hexane.

Methylene chloride extracts of chickpea were bioassayed by two methods. In the first method, 2.5 cm x 5.0 cm strips of black broadcloth were treated with a methylene chloride extract of fresh plant material by soaking the strips in extract. Blank strips of the same size were prepared by soaking them in solvent alone. Both treated and blank strips were allowed to air-dry for at least 10 min; one of each was placed in a 500 ml plastic container together with a mated 3-day old tobacco budworm female moth at about 1600 hours for 16 h (overnight) with an 11.5 h dark period. In the second method, an ovipositional chamber was constructed from ice cream cartons and was similar to the "T" tunnel olfactometer used by Deutsch (1968). Two 3.8-liter cylindrical ice cream cartons were taped together to give a 50 cm long x 24 cm diameter cylindrical chamber. A hole was cut in the center of the chamber to accommodate a 0.95-liter cylindrical carton to be used as the moth holding chamber and release site. Large sheets (30 cm X 30 cm) of black broadcloth were soaked to saturation in a methylene chloride extract of chickpea terminals (0.1 g plant material per 1 ml methylene chloride) and allowed to air-dry. For the control, a sheet of black broadcloth of the same dimensions was prepared by soaking in methylene chloride. The treated sheet was fitted over one end of the oviposition chamber and the control sheet was fitted over the other end.

Each was held in place with a rubber band. Ten mated 3 day-old tobacco budworm females were then released into the 0.95-liter container, and allowed to remain in the system for 16 h, after which time the treated and control broadcloth sheets were removed and checked for total number of oviposited eggs. Newly-treated broadcloth sheets and fresh insects were used each night; relative position of the treated and control sheets was adjusted by rotation each night to minimize positional effects. Two chambers were run each night for a total of six nights.

All data were analyzed by Student's *t* test with a=0.05.

RESULTS AND DISCUSSION

Tobacco budworm showed a definite ovipositional preference for chickpea compared to cotton (Table 1), with over 86% of eggs deposited on chickpea in a two-way comparison test using terminals containing fruiting forms of both plants in a large cage. Concurrently, a series of tests were run by the same

TABLE 1. Oviposition of Tobacco Budworm and Bollworm on Cotton and Chickpea Terminals Under Laboratory Conditions.

	Mean oviposition/female/night[ab]	
	Cotton	Chickpea
H. virescens	4.7 ± 1.2(1119)a	30.6 ± 5.1(7350)b
H. zea	16.6 ± 1.7(2660)a	12.5 ± 1.8(2007)a
	Cotton	Cotton
H. virescens	18.4 ± 3.6(1653)a	16.3 ± 2.9(1465)a

[a]Values in parentheses are total number of eggs laid
[b]Means in a row not followed by the same letter are significantly different, Student's *t* test, P=0.005.

technique but comparing oviposition on terminals from two different cotton plants in each cage to validate the experimental technique. In these tests, there was no significant difference in oviposition on the two cotton plants, indicating that differences between chickpea and cotton were real and not due to experimental technique. Also, the mean number of eggs laid per cage per female in a night was the same in the cotton versus chickpea comparison (35.3) and in cotton versus cotton comparison (32.7), indicating that the chickpea was not stimulating tobacco budworm moths to oviposit more eggs, but rather was influencing where eggs were deposited. When ovipositional preference of bollworm was determined on these plants in the same manner, no significant difference in oviposition on cotton or chickpea was observed, with means of 16.6 and 12.5 eggs laid per night per female, respectively, indicating that bollworm did not show an oviposition preference for chickpea.

Although a definite ovipositional response was observed in the preceding tests, the experimental design could not

differentiate between chemical or physical response resulting from factors such as surface texture of the plants. When terminals and fruiting forms of chickpea were encased in black broadcloth and tested against a cloth pouch of the same dimensions, containing no plant material, 86.1% of the eggs were laid on pouches containing the plant parts (Table 2).

TABLE 2. Oviposition of Tobacco Budworm on Black Broadcloth Treated with Methylene Chloride Extract of Chickpea Terminals and Fruiting Forms.

	Mean no. of eggs/female/night[ab]	
Assay method	Extract	Blank
500 ml chamber, plant[c]	35.8 ± 8.2(1074)a	5.8 ±2.7 (175)b
500 ml chamber, extract	37.4 ± 7.4(2394)a	8.8 ± 2.4(565)b
T-chamber	58.1 ± 7.4(3721)a	9.4 ± 4.1(601)b

[a]Values in parentheses are total number of eggs laid
[b]Means in a row not followed by the same letter are significantly different, Student's *t* test, P=0.005.
[c]Plant material was enclosed in 5.0 x 2.5 cm black broadcloth pouch and tested in 500 ml cylindrical plastic containers

This would indicate that there is a chemical factor in chickpea that causes an ovipositional preference because any physical preference was minimized or eliminated by the plant parts being covered by the broadcloth.

When methylene chloride extracts of chickpea were tested in 500 ml chambers, 81.0% of eggs were laid on broadcloth strips treated with extract, with a mean of 37.4 and 8.8 eggs oviposited per female per night on strips treated with extract or methylene chloride alone, respectively. Preliminary tests with extracts of chickpea prepared with methylene chloride, hexane, and water indicated that approximately equal numbers of eggs were oviposited on broadcloth strips treated with water extracts and blank strips treated with water. Deutsch (1968) reported no significant differences in oviposition on treated discs compared to control discs when water was used as a solvent for extracting tobacco leaves for potential ovipositional stimulants. However, slightly more eggs were deposited on broadcloth strips impregnated with hexane extract of chickpea (65%) compared with solvent blank in our preliminary tests. When methylene chloride extracts were tested in the "T" chamber, 86.1% of eggs were deposited on broadcloth treated with extract compared with broadcloth treated with solvent only.

Results of these tests demonstrate that chickpea contains a chemically-mediated oviposition stimulant/attractant that can be extracted with methylene chloride. Tests with plant material in large cages and with extract in 500-ml cages could not differentiate between an ovipositional stimulant or an attractant since insects could easily come in contact with the material.

Identification of the volatile plant compounds which influence oviposition could be useful in several different ways. Active compounds produced by chickpea could then be increased by plant breeding for maximum benefit (as trap plants) or decreased to reduce the attractiveness of a particular variety and thus reduce potential for insect damage. Also, a synthetic mixture of attractive chemicals could be used to lure adults to toxic baits or adult moths to traps. Thus, any one or a combination of these uses of the active chemicals could be beneficial as an integral part of areawide control programs where a combination of control methods may be preferable to one method and may reduce the use of broad spectrum insecticides.

LITERATURE CITED

Dethier, V. G., L. B Browne and C. N. Smith. 1960. The designation of chemicals in terms of responses they elicit from insects. J. Econ. Entomol. 53:134-136.

Deutsch, R. G. 1968. Ovipositional preferences of the tobacco budworm, *Heliothis virescens* (Fabricius). Ph.D Thesis, Texas A&M University, 35 pp.

Potter, M. F., and T. F. Watson. 1983. Garbanzo bean as a potential feeding stimulant for use with a nuclear polyhedrosis virus of the tobacco budworm (Lepidoptera:Noctuidae). J. Econ. Entomol. 79:449-451.

Remold, H., P. Woolner, S. Nit, H. Kollmannsberger, and F. Drawert. 1989a. Volatile components of chickpea (*Cicer arietinum* L.) seed. J. Agric. Food Chem. 37:659-662.

Remold, H., P. Woolner, and A. K. Singh. 1989b. Attractiveness of volatile chickpea (*Cicer arietinum*) seed components to *Heliothis armigera* larvae (Lep., Nocuidae). J. Appl. Ent. 107:65-70.

Remold, H., A. C. Kohne, and A. Schroth. 1991. Behavioral response of *Heliothis armigera* Hb. (Lep., Noctuidae) moths on a synthetic chickpea (*Cicer arietinum* L.) kairomone. J. Appl. Ent. 112:254-262.

Saxena,N. P., and H. Remold. 1984. Attraction of Heliothis armigera larvae by chickpea seed powder constituents. Z. angew. Intimal. 97:145-153.

EFFECTS OF IMIDACLOPRID ON COLONIZATION OF APHIDS[1] AND SILVERLEAF WHITEFLY[2] AND GROWTH, YIELD AND PHYTOTOXICITY IN CAULIFLOWER

E. T. Natwick, J. C. Palumbo[3], and C. E. Engle[4]

University of California Desert Research and Extension Center 1050 E. Holton Rd. Holtville, California 92250-9615

ABSTRACT

Imidacloprid transplant drench root plug treatments of cauliflower and imidacloprid treatments preplant in-furrow soil injections were evaluated for their effects on colonization of silverleaf whitefly, *Bemisia argentifolii* Bellows & Perring, green peach aphid, *Myzus persicae* (Sulzer), and cabbage aphid, *Brevicoryne brassicae* (Linnaeus). Whitefly treatments included imidacloprid root plug treatments of 180, 270, and 360 g AI/ha, an in-furrow treatment of 360 g AI/ha, and an untreated control. Aphid treatments included imidacloprid root plug treatments of 90, 180, and 270 g AI/ha, an in-furrow treatment of 360 g AI/ha, and an untreated control. The untreated control plants had significantly greater numbers of apterous aphids and whitefly nymphs than imidacloprid treatments. The imidacloprid in-furrow treatment had significantly fewer whitefly immatures than other treatments on several sampling dates. Phytotoxicity evaluations revealed some leaf tissue necrosis from root plug treatments of imidacloprid during the fall planting. Plant growth was retarded, harvest was delayed, and yield of kg of marketable heads was lower for the untreated control cauliflower plants due to higher whitefly infestation. Reduced yields due to aphids was not observed in the fall planting.

INTRODUCTION

The green peach aphid, *Myzus persicae* (Sulzer), and cabbage aphid, *Brevicoryne brassicae* (Linnaeus), are common pests of cauliflower, *Brassica oleracea* var. *botrytis* in the western United States. These polyphagous aphids cause economic damage to cauliflower through direct injury and contamination of heads (Blackman and Eastop 1984, Anonymous 1987). Most Western cauliflower is field packed and must be relatively free of aphid contamination (Anonymous 1987, Mayberry et al. 1994). Consequently, cauliflower is routinely treated with insecticides throughout the season to prevent aphid contamination.

Aphids in cauliflower are presently controlled with acephate, chlorpyrifos, dimethoate, methomyl, or methamidophos (Chaney et al. 1994). Green peach aphid and cabbage aphid populations utilize distinctly different portion of cauliflower plants. Green peach aphids prefer to feed on the underside of lower leaves and are protected from many foliar insecticide applications. Cabbage aphids prefer to colonize the youngest leaves and flowering parts and are

[1]Homoptera: Aphididae
[2]Homoptera: Aleyrodidae
[3]University of Arizona, Yuma Ag Center, Yuma, Arizona.
[4]Bayer Corporation, Fallbrook, California.

sheltered by the dense foliage surrounding the developing head making insecticidal control difficult (Anonymous 1987).

The control of green peach aphid is further complicated by its resistance to nearly all aphicides (Georghiou and Lagunes-Tejeda 1991). Once green peach aphid or cabbage aphid have successfully colonized a cauliflower plant it is difficult to achieve control (Anonymous 1987, Palumbo and Kerns 1994).

The sweetpotato whitefly, *Bemisia tabaci* (Gennaduis) strain B, later described as a new whitefly species, *B. argentifolii* Bellows & Perring, (Bellows et al. 1994) first became a pest of economic importance in cauliflower in the western United States in 1990 (Perring et al. 1991). This polyphagous whitefly causes economic damage to cauliflower through direct feeding causing stunting and delayed harvest and indirect injury from salivary toxins that bleach the floral stalks (Parrella et al. 1992). This pest prefers to colonize the underside of lower leaves allowing it to escape contact from many foliar applied insecticides (Chu et al. 1995). A systemic insecticide that is efficacious against all three of these pests would alleviate many of the coverage problems associated with control of aphids and whitefly in cauliflower. Imidacloprid, a new chloronicotyl insecticide, has systemic activity through soil application and controls aphids and whiteflies. Unlike most other systemic insecticides, imidacloprid is relatively immobile in the soil and requires precise placement where root uptake can occur (Mullins 1993). Performance of the compound varies depending on the crop and the method of application (Oetting and Anderson 1990). Imidacloprid has been used as a seed treatment to protect cereal crops from Russian wheat aphid, *Diuraphis noxia*, (Pike et al. 1993) and cotton from cotton aphid, *Aphis gossypii*, (Nauen and Elbert 1994). Imidacloprid in-furrow is efficacious for control of green peach aphid in lettuce (Palumbo and Kerns 1994). Imidacloprid has a US EPA registration for control of aphids and whitefly on cauliflower. However, no information is available concerning the effectiveness of imidacloprid as a treatment for cauliflower seedling plugs in trays prior to transplanting for protection from green peach aphid, cabbage aphid and the silverleaf whitefly.

Our objectives were to compare the effects of imidacloprid placement at 5 cm below the level of the cauliflower transplant plug in the seedbed, to the effects of imidacloprid treatments directly applied to transplant plugs in trays prior to transplanting on colonization of green peach aphid, cabbage aphid and the silverleaf whitefly, and on phytotoxicity of the imidacloprid treatments.

MATERIALS AND METHODS

Field studies were conducted in two trials during 1994 at the University of California Desert Research and Extension Center, Holtville, CA, and at the University of Arizona Yuma Agricultural Center, Yuma, AZ. In both trials, ' Snow Crown' cauliflower transplants were transplanted into single row beds on 1.02 m centers on 14 September at Holtville, and on 3 November at Yuma. Plots at both locations were arranged in a Latin square design with five replications. In-furrow preplant soil applications of imidacloprid (Admire® 2F, Bayer Corp., Kansas City, MO) were applied 5 cm sub surface, below the transplant line in 15.3 liter/ha final dilution. Transplant drench root plug imidacloprid treatments were uniformly sprayed over transplant flats in 2 liter final dilutions, followed by a 1 liter water can drench. Plants were allowed to set for 12 hours before transplanting. Plots at Holtville consisted of 6 beds, 15 m long with a buffer of two beds between plots. Treatments at Holtville consisted of the following: 1) transplant drench (imidacloprid, 180 g AI/ha); 2) transplant drench (imidacloprid, 270 g AI/ha); 3) transplant drench (imidacloprid, 360 g AI/ha); 4) preplant soil application, (imidacloprid, 360 g AI/ha) placed 5 cm sub surface; 5) untreated control. Plots at Yuma consisted of four beds, 20 m long with a buffer of two beds between plots. Treatments at Yuma consisted of the following: 1) transplant drench (imidacloprid, 90 g AI/ha); 2) transplant drench (imidacloprid,

180 g AI/ha); 3) transplant drench (imidacloprid, 270 g AI/ha); 4) preplant soil application, (imidacloprid, 360 g AI/ha) placed 5 cm sub surface; 5) untreated control.

Insect, plant growth, phytotoxicity, and marketability data were collected only from the inner two beds of each plot. Numbers of whitefly adults and nymphs were estimated weekly, at Holtville, from 27 September through 5 December. Whitefly adults were extracted into plastic vials from 10 plants per plot using a modified Dustbuster™ (Black & Decker® Inc., Shelton, CT) insect vacuum and counted in a laboratory using a 10X magnifying head set. Nymph densities were estimated from two disks (1.25 cm in diameter) per leaf from five leaves per plot. Five plants were randomly selected in each plot and a basal leaf extracted from each plant was placed in a dated, labeled bag for transport to the laboratory where nymphs were counted with the aid of a stereo microscope.

Plant growth was estimated by measuring the canopy height in cm from 10 randomly selected plants per plot at Holtville. Plant height in cm was estimated 31 October, 7, and 14 November. Yield was estimated by harvesting marketable cauliflower heads from 4 m on each of the two center rows in the middle of each plot at Holtville as the cauliflower heads matured. Harvest yield as numbers of heads and weight of heads was estimated on 19, 22, 28 December, and 3 and 8 January.

Numbers of aphids per leaf were estimated on 1, 20 December and 10, 20 and 31 January at Yuma. Ten plants per replicate were randomly selected in each plot and two leaves on the lower half of each plant were sampled using the methodology developed by Palumbo and Kerns (1994). The numbers and species of alate and apterous aphids were counted with the aid of a stereo microscope. Infestation levels were also estimated at harvest by randomly selecting ten plants within each replicate and recording the percentage of heads infested as (>1 aphid, but <4 aphids/ plant) and (> 5 aphids/plant).

Phytotoxic ratings were estimated from ten randomly selected plants per plot at 2, 4, and 8 wk after transplanting in Yuma and at 2 wk after transplanting in Holtville. The rating at Yuma was as follows: 0= no spotting and necrosis; 1= light spotting and necrosis; 2=moderate spotting and necrosis; 3= heavy spotting and necrosis. The rating at Holtville was as follows: 1= no necrosis; 2= light necrosis on 1 or 2 leaves; 3= necrosis on >2, but < 4 leaves; 4= necrosis on $\geq$ 4 leaves; 5= dead plant.

Aphid counts from collection cans, whitefly adult counts from suction samples, and whitefly nymph counts from leaf disk samples were transformed ($\log_{10}$+1) prior to analysis of variance to stabilize variances which were found to be heterogeneous. Counts of infested plants were converted to percentages with an arcsine transformation before further analyses. Treatment differences for aphid data sets were estimated with analysis of variance (ANOVA) and mean separation with a protected $LSD_{0.05}$ [PROC GLM, SAS Institute (1988)]. Whitefly data sets were analyzed by ANOVA, and mean separations with $LSD_{0.05}$ (MSTAT-C 1988).

RESULTS AND DISCUSSION

Holtville Studies. Silverleaf whitefly adults colonized and deposited eggs on cauliflower transplants in the slat house for 2 d prior to transplanting. After transplanting, silverleaf whitefly adults Immediately immigrated into plots colonizing all transplants. Cauliflower transplants with imidacloprid root plug treatments all had significantly fewer whitefly adults than the untreated control 13 d after transplanting (Table 1). The numbers of whitefly adults on in-furrow imidacloprid treated transplants were not different from the untreated transplants 13 d after planting. Numbers of whitefly adults for the root plug treatments were not different from the untreated transplants 19 d after planting, but the in-furrow imidacloprid treatment had significantly fewer adults than the untreated control.

TABLE 1. Effect of Imidacloprid Soil and Root Plug Treatments on *B. argentifolii* Adults in Cauliflower, Holtville, California, 1994.

		Mean[a] population per plant at sampling date										
Treatment	g AI/ha	27 Sept	3 Oct	10 Oct	17 Oct	24 Oct	31 Oct	7 Nov	14 Nov	21 Nov	28 Nov	5 Dec
Untreated	-----	94.3 a	103.6 a	152.1 a	66.1 a	86.3 a	53.9 a	12.4 a	15.0 a	1.1 a	2.3	2.5 a
Drench[b]	180	60.3 b	82.9 ab	91.0 b	23.9 b	37.2 bc	32.1 b	8.0 ab	11.0 ab	0.6 ab	1.4	0.8 b
Drench[b]	270	63.2 b	73.4 ab	59.7 b	28.0 b	39.2 bc	27.1 bc	6.6 b	10.5 ab	0.3 b	1.5	1.3 b
Drench[b]	360	66.7 b	81.0 ab	78.9 b	28.9 b	46.6 b	18.7 bc	7.5 ab	10.1 ab	0.5 b	0.9	0.9 b
Soil[c]	360	74.8 ab	53.3 b	65.1 b	12.7 c	15.9 c	12.8 c	3.6 b	3.9 b	0.3 b	0.3	0.3 b

[a]Mean separation within columns by $LSD_{0.05}$.
[b]Transplant drench root plug treatments were uniformly sprayed over each flat in 2 liter final dilutions on 13 September.
[c]In-furrow preplant soil application was placed 5 cm sub surface on 13 September.

TABLE 2. Effect of Imidacloprid Soil and Root Plug Treatments on *B. argentifolii* Nymphs in Cauliflower, Holtville, California, 1994.

		Mean[a] population per cm^2 at sampling date										
Treatment	g AI/ha	27 Sept	3 Oct	10 Oct	17 Oct	24 Oct	31 Oct	7 Nov	14 Nov	21 Nov	28 Nov	5 Dec
Control	-----	8.8 a	50.0 a	63.0 a	23.8 a	35.7 a	70.9 a	35.0 a	41.9 a	20.8 a	19.6 a	15.8 a
Drench[b]	180	2.7 b	13.8 b	21.6 b	11.7 ab	16.2 b	20.7 b	19.7 ab	14.1 c	11.2 ab	8.0 b	6.2 ab
Drench[b]	270	3.1 b	10.1 b	11.2 bc	1.8 b	12.2 b	10.5 bc	8.8 bc	24.6 b	12.8 ab	5.4 b	5.2 b
Drench[b]	360	2.8 b	14.3 b	12.0 bc	16.0 a	15.2 b	20.8 b	15.7 ab	10.7 c	7.6 b	4.3 bc	3.3 bc
Soil[c]	360	2.1 b	4.7 b	3.9 c	2.2 b	5.7 c	9.2 c	4.4 c	4.5 d	4.9 c	2.4 c	1.3 c

[a]Mean separation within columns by $LSD_{0.05}$.
[b]Transplant drench root plug treatments were uniformly sprayed over each flat in 2 liter final dilutions on 13 September.
[c]In-furrow preplant soil application was placed 5 cm sub surface on 13 September.

The in-furrow treatment had significantly fewer whitefly adults than the untreated control on seven consecutive weekly sampling dates from 3 October to 14 November (19 - 61 d after planting), and on 5 December (73 d after planting). The root plug treatments all had significantly fewer adults than the untreated control transplants on four consecutive weekly sampling dates from 10 October to 31 October (26 -43 d after planting) and on 5 December (73 d after planting). The imidacloprid in-furrow treatment had significantly fewer adults than all other imidacloprid treatments on 17 October, significantly fewer adults than the 270 g AI/ha rate of the imidacloprid root plug treatment on 24 October, and significantly fewer adults than the 360 g AI/ha rate of the imidacloprid root plug treatment on 31 October. There were no differences among the imidacloprid treatments and the untreated control on 21 and 28 November (Table 1).

The imidacloprid in-furrow treated plants had significantly fewer whitefly nymphs than the untreated control plants on all sampling dates from 27 September to 5 December, and significantly fewer nymphs than all of the root plug treatments on 24 October and 14 and 21 November (Table 2). All of the imidacloprid root plug treated plants had significantly fewer whitefly nymphs than the untreated control plants on 27 September to 10 October. In-furrow, imidacloprid treated plants had significantly fewer whitefly nymphs than all of the root plug treated plants on 24 October and 14 and 21 November.

The imidacloprid root plug treated transplants all had necrosis on leaf margins at the Holtville trial 2 wk after applications. The phytotoxic rating for all of the root plug treated transplants was significantly greater than the untreated control plants and the in-furrow imidacloprid treatment on 27 September (Table 3). There were no differences for phytotoxic ratings among the root plug treatments on 27 September.

TABLE 3. Phytotoxicity Evaluations[a] of Root Plug and In-Furrow Treatments Two Weeks Post Application of Imidacloprid to Cauliflower Transplants, Holtville, California, 1994-1995.

Treatment	g AI/ha	Phytotoxicity[b]
Control	-----	1.24 b
Drench[c]	180	2.04 a
Drench[c]	270	1.94 a
Drench[c]	360	2.06 a
Soil[d]	360	1.16 b

[a]1= no phytotoxicity, 2=slight necrosis on 1 or 2 leaf margins, 3= marginal necrosis on more than 2 leaves, 4= sever necrosis on most of the plant, 5= dead plant.
[b]Mean separation within columns by $LSD_{0.05}$.
[c]Transplant drench root plug treatments were uniformly sprayed over each flat in 2 liter final dilutions on 13 September.
[d]In-furrow preplant soil application was placed 5 cm sub surface on 13 September.

Untreated control plants were stunted by whitefly feeding (Table 4). The height in cm of the untreated plants was significantly less than the imidacloprid treated plants on all sampling dates (Table 5). In-furrow, imidacloprid treated plants were significantly taller than the 270 g AI/ha rate of the imidacloprid root plug treated plants at on 31 October and the 180 g AI/ha and 270 g AI/ha rates on 14 November, but no differences were observed among the imidacloprid treatments for plant height on 7 November.

TABLE 4. Plant Height and Number and Weight of Cauliflower Heads per 0.002 Acres Regressed of Numbers of Silverleaf Whitefly Nymphs per cm^2, Holtville, California, 1994.

Sample	n[a]	Range	a[a]	b[a]	s[a]	t[a]	p[a]	r^2
Height (cm)	15	8.2-20.5	90.16	-4.22	0.63	6.72	<0.001	0.776
Number of heads	5	19.8-26.2	117.14	-4.46	1.26	3.54	0.038	0.806
Weight (kg)	5	12.3-21.9	75.36	-1.52	0.14	10.68	0.002	0.974

[a]n = number of observations, a = y-intercept, b = slope, s = standard error of slope, t = computed to test the significance of the regression, p = probability of getting the t value by chance, r^2 = coefficient of determination. Linear regression equation is $y = a + bx$.

TABLE 5. Plant Height in cm Seven, Eight and Nine Weeks post Application of Imidacloprid to Cauliflower Transplants, Holtville California, 1994.

		Mean[a] plant height (cm) at sampling date		
Treatment	g AI/ha	31 October	7 November	14 November
Drench[b]	180	16.5 ab	18.0 a	18.9 b
Drench[b]	270	15.8 b	18.0 a	18.7 b
Drench[b]	360	16.2 ab	18.0 a	20.0 ab
Soil[c]	360	17.2 a	18.8 a	20.5 a
Control	-----	8.2 c	10.1 b	11.6 c

[a]Mean separation within columns by $LSD_{0.05}$.
[b]Transplant drench root plug treatments were uniformly sprayed over each flat in 2 liter final dilutions on 13 September.
[c]In-furrow preplant soil application was placed 5 cm sub surface on 13 September.

The untreated control had fewer marketable heads and harvest was delayed 14 d due to dense whitefly populations, compared to the imidacloprid treatments (Tables 4 and 6). No cauliflower was harvested from the untreated control on 14, 19 and 22 December, but there was no difference in the season total numbers of marketable heads among the treatments. There were no differences among the imidacloprid treatments in numbers of heads harvested on the five harvest dates from 22 December to 8 January, nor for the season total.

The season total kg/0.002 acre of cauliflower heads for the untreated control was significant less than the season totals for the imidacloprid treatments due to the dense whitefly populations on the untreated plants (Tables 4 and 7). There were no differences among the imidacloprid treatments for kg of heads from 28 December to 8 January, nor were there any differences among the imidacloprid treatments for season total kg of cauliflower heads.

Yuma Studies. Aphid species consisted predominately of green peach aphid and cabbage aphid. There were no aphids detected in any of the plots samples on 1 December (27 d after planting). Cabbage aphid was only present on 20 January (6%) and 31 January (23%). There were no differences among the treatment means for alate aphids on any of the sampling dates (Table 8). The untreated control plants had significantly more apterous aphids than any of the

TABLE 6. Number of Marketable Cauliflower Heads per 0.002 Acres for Imidacloprid treatments and the Untreated Control, Holtville, California, 1994-1995.

Treatment	g AI/ha	Mean[a] number heads at sampling date						Season Total
		14 Dec	19 Dec	22 Dec	28 Dec	3 Jan	8 Jan	
Drench[b]	180	1.8 a	6.2 b	5.0 a	8.0 a	0.8 b	0.0 a	21.8 a
Drench[b]	270	0.2 b	7.6 ab	4.0 a	10.6 a	1.6 b	0.0 a	24.0 a
Drench[b]	360	1.4 a	6.2 b	6.2 a	9.2 a	0.0 b	0.0 a	23.0 a
Soil[c]	360	0.6 ab	9.8 a	5.2 a	8.4 a	0.0 b	2.2 a	26.2 a
Control	-----	0.0 b	0.0 c	0.0 b	7.4 a	7.4 a	5.0 a	19.8 a

[a]Mean separation within columns by $LSD_{0.05}$.
[b]Transplant drench root plug treatments were uniformly sprayed over each flat in 2 liter final dilutions on 13 September.
[c]In-furrow preplant soil application was placed 5 cm sub surface on 13 September.

TABLE 7. Weight of Cauliflower Heads per 0.002 Acres for Imidacloprid Treatments and the Untreated Control, Holtville, California, 1994-1995.

Treatment	g AI/ha	Mean[a] weight (kg) of heads at sampling date						Season Total
		14 Dec	19 Dec	22 Dec	28 Dec	3 Jan	8 Jan	
Control	-----	0.00 c	0.00 c	0.00 c	4.99 b	4.57 a	2.76 a	12.34 b
Drench[b]	180	1.84 a	4.95 b	3.63 ab	6.78 ab	0.54 b	0.00 a	17.74 a
Drench[b]	270	0.15 c	6.82 ab	2.82 b	9.01 a	1.06 b	0.00 a	19.87 a
Drench[b]	360	1.32 ab	4.98 b	4.82 a	7.83 ab	0.00 b	0.00 a	18.94 a
Soil[c]	360	0.46 bc	7.96 a	3.89 ab	8.14 ab	0.00 b	1.42 a	21.86 a

[a]Mean separation within columns by $LSD_{0.05}$.
[b]Transplant drench root plug treatments were uniformly sprayed over each flat in 2 liter final dilutions on 13 September.
[c]In-furrow preplant soil application was placed 5 cm sub surface on 13 September.

imidacloprid treated plants on sampling dates of 20 December (46 d after planting) through 31 January (88 d after planting). There were no differences among imidacloprid treatment means for apterous aphids, except on 20 January the 270 g AI/ha imidacloprid root plug treated plants had significantly fewer apterous aphids than the 90 g AI/ha imidacloprid root plug treated plants (Table 8). At harvest the untreated control had a significantly greater percentage of heads infested with apterous aphids than imidacloprid treatments (Table 9). Alate aphids were found in 76 percent of all heads.

No phytotoxicity due to imidacloprid was detected on any of the sampling dates at Yuma. Light spotting observed on plants at Yuma, 4 and 8 wk after imidacloprid treatments, was not due to imidacloprid. Spotting and necrosis was positively identified as Downey mildew, *Peronospora parasitica*, (M. Matheron, personal communication).

TABLE 8. Application of Imidacloprid to Cauliflower Transplants Prior to Transplanting for Control of Aphids, Planted 2 November, 1994, Yuma, Arizona.

Date	g AI/ha	Treatment Method	Mean no. aphids per leaf[a]	
			Alate	Apterous
1 Dec	360	Soil[b]	0.0 a	0.0 a
	270	Drench[c]	0.0 a	0.0 a
	180	Drench	0.0 a	0.0 a
	90	Drench	0.0 a	0.0 a
	0	Untreated	0.0 a	0.0 a
20 Dec	360	Soil	0.0 a	0.0 b
	270	Drench	0.0 a	0.0 b
	180	Drench	0.0 a	0.0 b
	90	Drench	0.2 a	0.0 b
	0	Untreated	0.4 a	2.8 a
10 Jan	360	Soil	0.3 a	0.1 b
	270	Drench	0.0 a	0.1 b
	180	Drench	0.1 a	0.0 b
	90	Drench	0.1 a	0.1 b
	0	Untreated	0.3 a	2.3 a
20 Jan	360	Soil	1.4 a	1.7 bc
	270	Drench	0.7 a	0.3 c
	180	Drench	1.0 a	1.6 bc
	90	Drench	1.1 a	3.1 b
	0	Untreated	1.7 a	23.0 a
31 Jan	360	Soil	2.4 a	2.4 b
	270	Drench	2.1 a	1.4 b
	180	Drench	2.0 a	1.8 b
	90	Drench	2.3 a	3.2 b
	0	Untreated	2.6 a	42.3 a

[a]Aphid species consisted predominantly of green peach aphid and cabbage aphid. Cabbage aphid only present on 20 January (6%) and 31 January (23%). Mean separation within columns by protected $LSD_{0.05}$.
[b]In-furrow preplant soil application was placed 5 cm sub surface on 2 November.
[c]Transplant drench root plug treatments were uniformly sprayed over each flat in 2 liter final dilutions on 2 November and allowed to soak into the soil.

All of the imidacloprid treatments were efficacious for control of green peach aphid, cabbage aphid, and whitefly in cauliflower. However, the phytotoxicity observed following the preplant drench with imidacloprid to transplant trays in the Holtville study are of concern and worthy of further investigation. Why was there no phytotoxicity in the Yuma study? Some possibilities are differences in potting soil, possibly there was a whitefly imidacloprid interaction, but the most plausible explanation is the extreme differences in day and night time temperatures in September (40 C days and 25 C nights) versus November (25 C days and 10 C nights). The various rates of imidacloprid treatments to root plugs of transplants in trays were as efficacious

as the 360 g AI/ha imidacloprid in-furrow treatment for aphid control. The in-furrow imidacloprid 360 g AI/ha treatment was somewhat more efficacious for whitefly control compared to the various rates of imidacloprid applied to the root plugs of cauliflower transplants. However, all of the imidacloprid treatments produced equal yields of marketable cauliflower heads with the same harvest dates, 9 d earlier than the untreated plots and of greater weight than the untreated control. At Yuma there were no differences among the imidacloprid treatments for aphid contamination in the cauliflower heads. Both the Holtville and Yuma data suggest that there could be a reduction in the rate of imidacloprid using a transplant drench to achieve the same levels of aphid and whitefly control as the industry standard of in-furrow soil injection treatment to cauliflower.

TABLE 9. Effect of Imidacloprid Treatments to Cauliflower Transplants prior Transplanting on Population Densities of Apterous Aphids at Harvest, Yuma, Arizona, 1994-1995.

		% heads infested with apterous aphids[a]	
Rate g AI/ha	Treatment	Infested	>5 aphids
360	Soil[b]	2.0 b	1.0 b
270	Drench[c]	2.0 b	0.0 b
180	Drench	1.0 b	0.0 b
90	Drench	2.0 b	1.0 b
0	Untreated	46.0 a	18.0 a

[a]Infested heads = greater than 1 apterous aphid, but fewer than 5 aphids. >5 aphids = 5 or more aphids on head at harvest. (n= 100 heads/tmt). Alate aphids found in 76% of all heads. Mean separation within columns by protected $LSD_{0.05}$.
[b]In-furrow preplant soil application was placed 5 cm sub surface on 2 November.
[c]Transplant drench root plug treatments were uniformly sprayed over each flat in 2 liter final dilutions on 2 November and allowed to soak into the soil.

LITERATURE CITED

Anonymous 1987. Integrated pest management for cole crops and lettuce. Univ. Calif. Statewide IPM Proj., Publication 3307. 112 pp.

Blackman, R. L., and V. F. Eastop. 1984. Aphids on the World's Crops: An identification Guide. John Whiley & Sons, New York. 466 pp.

Bellows, T. S., Jr., T. M. Perring, R. J. Gill, and D. H. Headrick. 1994. Description of a species of *Bemisia* (Homoptera: Aleyrodidae). Ann. Entomol. Soc. Am. 87:195-206.

Chaney, W. E., N. Toscano, W. Bentley, E. T. Natwick, M. L. Flint, S. T. Koike, H. S. Agamalian, M. LeStrange, W. Lanini, C. Bell, D. W. Cudney, U. C. Kodira, B. B. Westerdahl. 1994. Cole crop pest management Guidelines. Univ. Calif. IPM Proj., UCPMG Publication 25. 56 pp.

Chu, C. C.,T. J. Henneberry, and A. C. Cohen. 1995. *Bemisia argentifolii* (Homoptera: Aleyrodidae): Host preference and factors affecting oviposition and feeding site preference. Environ. Entomol. 24: 354-360.

Georghiou, G. P., and A. Lagunes-Tejeda. 1991. The Occurrence of Resistance to Pesticides in Arthropods. Food and Agriculture Organization of the United Nations, Rome. 318 pp.

MSTAT-C. 1988. MSTAT-C, a microcomputer program for the design, management, and analysis of agronomic research experiments. Michigan State Univ., East Lansing, MI.

Mayberry, K. S., E. T. Natwick, R. A. Gonzalez, G. J. Holmes, C. E. Bell, and K. M. Bali. 1994. Guide Lines to Production Costs and Practices 1994-1995: Vegetable Crops. Univ. Calif., Imperial County, Coop. Ext. Circular 104-V. 96 pp.

Mullins, J. W. 1993. Imidacloprid: A new nitroguanidine insecticide, p. 184 -198. *In* Pest Control With Environmental Safety, Am. Chem. Soc. Sym. Series 524.

Nauen, R., and A. Elbert. 1994. Effect of imidacloprid on aphids after seed treatment of cotton in laboratory and greenhouse experiments. Pflanzenschutz Nachrichten Bayer (English edition). 47: 177-210.

Oetting, R. D., and A. L. Anderson. 1990. Imidacloprid for control of whiteflies on greenhouse grown poinsettias, pp. 367-372. *In* Proceedings, Brighton Crop Protection Conference, Pest and Disease, Brighton, England.

Palumbo, J. C., and D. L. Kerns. 1994. Effects of imidacloprid as a soil treatment on colonization of green peach aphid and marketability of lettuce. Southwestern Entomologist. 19: 339-346.

Parrella, M. P., T. S. Bellows, R. J. Gill, J. K. Brown, and K. M. Heinz. 1992. Sweetpotato whitefly: prospects for biological control. California Agriculture. 46: 25-26.

Perring, T. M., A. Cooper, D. J. Kazmer, C. Shields, and J. Shields. 1991. California Agriculture. 45:10-12.

Pike, K. S., G. L. Reed, G. T. Graf, and D. Allison. 1993. Compatibility of imidacloprid with fungicides as a seed-treatment control of Russian wheat aphid and effect on germination, growth and yield of wheat and barley. J. Econ. Entomol. 86: 586-593.

EFFECT OF CYHALOTHRIN ON FIELD POPULATIONS OF THE COTTON APHID, *APHIS GOSSYPII* GLOVER[1], IN THE TEXAS HIGH PLAINS

P. W. Kidd, D. R. Rummel[2], and H. G. Thorvilson[3]

Texas Agricultural Extension Service
209 South 5th Street, Brownfield, TX 79316

ABSTRACT

Cotton aphid, *Aphis gossypii* Glover, population densities in untreated cotton plots were compared with those in plots receiving one and two applications of cyhalothrin (Karate®) and one application of dicrotophos (Bidrin®). The cyhalothrin applications were timed to simulate typical treatment(s) for bollworm, *Helicoverpa zea* (Boddie), infestations and the dicrotophos application targeted beneficial arthropods. Rapid increases in aphid population densities occurred within 2 wk of cyhalothrin applications, resulting in significant yield reductions. Aphid numbers in dicrotophos treated plots were not significantly different from those in untreated plots on any sample date. Increases in aphid numbers did not appear to be due to reductions in predator populations, and predator populations appeared to increase only in response to increasing aphid populations.

INTRODUCTION

Before 1975, widespread cotton aphid (*Aphis gossypii* Glover) infestations were unknown in Texas High Plains cotton. However, aphid infestations on cotton have been common in the Texas High Plains since 1975 when heavy and uniform infestations first occurred throughout the area (Rummel 1975). Since 1975, the intensity of infestation has varied considerably among years. Population densities as a whole consistently increased throughout the 1980's, and economic infestations were common late in the decade. Difficulty in controlling aphids and indications of insecticide resistance were noted during the 1989 season (Allen et al. 1990). Head (1992) noted that aphids were estimated to have caused more insect related losses in cotton than any other pest in the United States in 1991. Over 10 million of the 13 million acres harvested were classified as infested by aphids, resulting in a loss of more than 360,000 bales. Losses in Texas alone accounted for more than 333,000 bales. All cotton acreage in Texas Agricultural Extension District Two was reported infested, and infestations in over 80% of these acres exceeded treatment thresholds (Head 1992).

Factors that influence excessive cotton aphid population development are not well defined. Although many beneficial arthropods prey on cotton aphids, predator populations typically average less than 1% of aphid populations (Leser et al. 1992). The impact of this low predator population density in preventing cotton aphid infestations is unknown. These arthropods often lack the reproductive capacity to control aphids when environmental conditions are favorable for aphid reproduction, resulting in occasional economic damage. Weathersbee and Hardee (1993) reported that predation and parasitism appeared important in slowing cotton aphid population growth early in the season and possibly instigating its reversal.

[1] Homoptera: Aphididae

[2] Texas A&M University Agricultural Research and Extension Center, Route 3, Box 219, Lubbock, TX 79401.

[3] Texas Tech University, Department of Plant and Soil Science, Mailstop 2122, Lubbock, TX 79409.

Increased aphid population density may sometimes be a reaction to insecticides rather than a result of decreased predator population densities (Dunnam and Clark 1941, Slosser et al. 1989, Kerns and Gaylor 1991). Insecticide applications targeting other pests, such as the bollworm, *Helicoverpa zea* (Boddie), cause reductions in aphid predator populations, but effects of these predator reductions on aphid population density are unclear.

Calcium arsenate was commonly used in the 1940's to control boll weevil, *Anthonomus grandis* Boheman, infestations. Dunnam and Clark (1941) found that cotton aphids caged on calcium arsenate treated plants multiplied more rapidly than those caged on untreated plants. They concluded that factors other than reduction of predator and parasite populations stimulated aphid reproduction following calcium arsenate treatments. Ewing (1943) attributed premature leaf drop of 50 to 75% and yield losses of 164 and 243 pounds of seed cotton per acre to aphid injury in plots dusted with calcium arsenate. Slosser et al. (1989) reported that in the Texas High and Rolling Plains, the elimination of natural enemies with pesticides did not appear to cause the initial population increase of cotton aphids. These authors suggested that leaf nitrogen and carbohydrate content, which can be altered by pesticides, may be highly influential on cotton aphid population density development. Kerns and Gaylor (1991) found that sulprophos treated plots developed greater aphid numbers which could not be attributed to reduced predator populations. Kerns and Gaylor (1992) found no evidence of direct reproductive stimulation (hormoligosis) by sulprophos or cypermethrin in a laboratory study using leaf discs. These authors suspected some type of indirect stimulation (trophobiosis) was involved.

Synthetic pyrethroid insecticides are commonly used in the Texas High Plains to control bollworm infestations. Although they did not target the cotton aphid, most commonly used pyrethroids were reported to promote increases in aphid population densities in 1991, 1992 and 1993 (Leser 1994). Aphid population densities tended to explode within two weeks of a pyrethroid application. The cause of these aphid population explosions is unclear and these outbreaks might be easily avoided if producers had a better understanding of the impact of pyrethroids on cotton aphid population development. Therefore, the objective of the present study was to investigate the effect of a pyrethroid insecticide on the development of cotton aphid populations in cotton.

MATERIALS AND METHODS

Tests to determine the impact of a pyrethroid insecticide on cotton aphid population development were conducted in 1993 and 1994 at the Texas A&M University Agricultural Research and Extension Center, Lubbock, TX. During the 1993 growing season, cotton plots treated with two applications of cyhalothrin (Karate®) at a rate of 0.025 lbs AI per acre were compared with plots receiving one application of cyhalothrin and untreated plots. This experiment was expanded in 1994 to include cotton plots treated once with dicrotophos (Bidrin®) at a rate of 0.1 lbs AI per acre. Cyhalothrin applications were timed to simulate producer's treatment(s) for bollworm infestations and the dicrotophos application targeted beneficial arthropods. Cyhalothrin was applied in the two application treatments on 22 and 30 July 1993 and on 11 and 20 July 1994. Single application treatment plots were sprayed with cyhalothrin on 30 July 1993 and on 20 July 1994. The dicrotophos treatment received a single application on 27 July 1994. All insecticides were applied with a CO_2-powered, two-row boom sprayer equipped with drops.

Experiments were arranged in randomized block designs consisting of six blocks in both years. Cotton cultivars used in these experiments were GSC 71+ and All-Tex Atlas, planted at the Texas Agricultural Experiment Station, Lubbock, TX, on 3 June 1993 and 25 May 1994, respectively. Stand densities were thinned to three plants per foot on 29 June 1993 and 15 June 1994. Plots were four rows wide by 20 feet long in 1993 and four rows by 25 feet long in 1994. Treatment plots were separated by a four row buffer. The total number of aphids in the terminal area and one full-sized upper and middle leaf of ten randomly selected plants in each plot was counted on a weekly basis. The terminal area was defined as the area above the smallest fully opened leaf in the terminal region of the plant. This sample area was redefined in 1994 to include only the smallest fully opened leaf in the terminal region of the plant, rather than the entire terminal area. Sample size was reduced to two leaves on five randomly selected plants per plot when aphid population density substantially increased.

Whole plant examinations were made and insect predator populations were monitored by counting the total numbers of selected predators on each of the plants sampled. Insect predators monitored included lady beetle adults and larvae, primarily *Hippodamia* spp.; lacewing larvae, primarily *Chrysoperla* spp.; minute pirate bugs, *Orius* spp.; and big-eyed bugs, *Geocoris* spp. in 1993. Syrphid fly larvae, *Syrphus* and other spp., were also monitored in 1994.

Plots were sampled on a weekly basis from 19 July through 23 August 1993 and from 29 June through 22 August 1994. Two center rows of each plot were machine harvested for yield analysis on 3 November 1993 and 13 December 1994. Data were subjected to analysis of variance and Duncan's new multiple range test (DNMRT) was used to seperate means (SAS Institute 1985). Fiber quality, including micronaire, length, uniformity, and strength, was determined by analysis of sub-samples of each treatment and results were evaluated on an observational basis.

RESULTS AND DISCUSSION

1993 Experiment. Cotton aphid population densities during the 1993 experiment are shown in Fig. 1. On 19 July, prior to insecticide applications, a mean of less than one aphid per leaf was detected in all plots. Cotton aphid population densities in plots receiving two applications of cyhalothrin began to increase rapidly following the first application on 22 July. Samples indicated aphid numbers in excess of 16 per leaf prior to the second cyhalothrin application on 30 July. Cotton aphid population density surpassed the current treatment threshold of 50 aphids per leaf prior to 4 August and continued to increase rapidly, peaking at more than 384 aphids per leaf on 17 August. Aphid numbers were greater than the treatment threshold for a period of more than 2 wk and were significantly greater than aphid numbers in untreated plots for four consecutive weeks ($P<0.05$, DNMRT).

A mean of less than three aphids per leaf was detected on 30 July in plots scheduled for a single application of cyhalothrin (Fig. 1). Following the cyhalothrin application on 30 July, aphid numbers increased to 23.2 aphids per leaf on 10 August and peaked at more than 187 aphids per leaf on 17 August. Although the rate of increase in these plots was slower than that of plots receiving two cyhalothrin applications, aphid numbers exceeded treatment thresholds in both treatments within 2 wk following a cyhalothrin application.

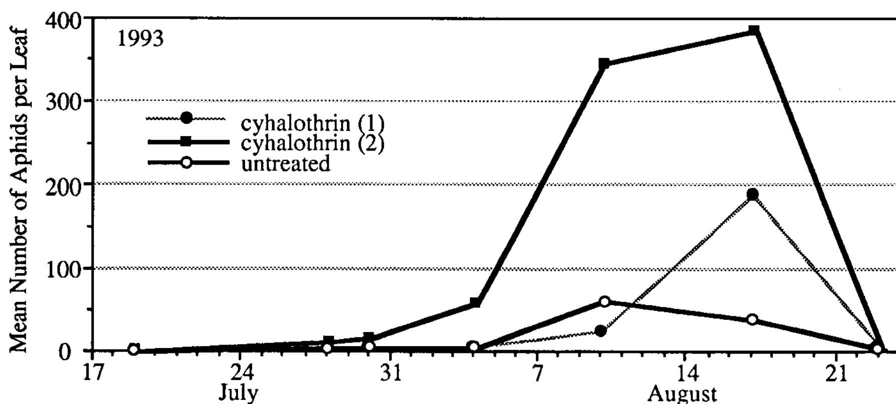


FIG. 1. Mean number of cotton aphids per leaf in untreated plots and in plots treated with one and two applications of cyhalothrin. Treatment dates: 1 application - 7/30; 2 applications - 7/22, 7/30. Lubbock, Texas, 1993.

In untreated plots (Fig. 1), aphid population density remained below a mean of six aphids per leaf until 10 August. Aphid numbers in these plots peaked at 63 aphids per leaf on 10 August and decreased to less than 19 aphids per leaf on 17 August. On 17 August and on a seasonal mean basis, cyhalothrin treated plots had significantly greater aphid numbers than untreated plots.

Cotton aphid populations declined quickly in all plots after 17 August. Inspections detected a mean of less than two aphids per leaf in each of the three treatments on 23 August. The cause of this drastic decline was attributed to the presence of high numbers of beneficial arthropods.

Aphid predator population densities, shown in Table 1, remained below one per plant in all treatment plots prior to 17 August. Although predator population densities were relatively low prior to this date, declines were noted following cyhalothrin applications. The lack of a substantial predator population for a period of approximately two weeks following the final cyhalothrin application on 30 July was attributed to residual activity of the insecticide. However, predator numbers in treated plots were never significantly smaller than those of untreated plots. Predator populations peaked in all plots on 17 August. Samples indicated mean predator population densities of 2.5 per plant in untreated plots, 4.1 per plant in cyhalothrin (1) plots, and 8.2 per plant in cyhalothrin (2) plots on 17 August. This increase in predator populations was believed to be in response to high aphid population densities.

Cotton aphid infestations significantly reduced yields in cyhalothrin treated plots (Table 2). The lowest yield of 463 lbs of lint per acre was from plots that received two applications. Plots that received one application produced 594 lbs of lint per acre and untreated plots produced 707 lbs of lint per acre. All of these yield differences were statistically significant ($P<0.05$, DNMRT) and were inversely proportional to cotton aphid population densities and duration of infestations (Fig. 2). No other cotton pests were detected at economically damaging levels throughout the duration of this experiment. Therefore, yield reductions were attributed solely to the effects of cotton aphid infestations. Though some differences in fiber quality were observed, these differences did not appear to be proportional to aphid population density (Table 3).

TABLE 1. Selected Beneficial Arthropods in Untreated Cotton Plots and in Plots Treated with One and Two Applications of Cyhalothrin. Lubbock, Texas, 1993.

Treatment [d]	Mean number of beneficials per plant [abc]							
	7/13	7/28	7/30	8/4	8/10	8/17	8/23	Seasonal mean [e]
Untreated	0.1 a	0.6a	0.5a	0.1a	0.3a	2.5a	1.7a	1.15a
Cyhalothrin (1)	--	--	0.7a	0.0a	0.1a	4.1 a b	1.5a	1.43a
Cyhalothrin (2)	0.1 a	0.1a	0.2a	0.1a	0.7a	8.2 b	1.1a	2.53 b

[a] Beneficial arthropods sampled: lady beetle adults and larvae, primarily *Hippodamia* spp.; lacewing larvae, *Chrysoperla* spp. and others; minute pirate bugs, *Orius* spp.; and big-eyed bugs, *Geocoris* spp.

[b] Dashes represent missing data. Cyhalothrin (1) plots were added on 7/30.

[c] Means followed by the same letter in a column are not significantly different ($P<0.05$, DNMRT)

[d] Treatment dates: cyhalothrin (1) - 7/30; cyhalothrin (2) - 7/22 and 7/30.

[e] Post-treatment seasonal mean of dates: 8/4, 8/10, 8/17 and 8/23.

TABLE 2. Cotton Lint Yield from Untreated Plots and from Plots Treated with One and Two Applications of Cyhalothrin. Lubbock, Texas, 1993.

Treatment [a]	Lbs. lint/acre [b]
Cyhalothrin (2)	463 a
Cyhalothrin (1)	594 b
Untreated	707 c

[a] Treatment dates: cyhalothrin (1) - 7/30; cyhalothrin (2) - 7/22 and 7/30.

[b] Means followed by the same letter are not significantly different ($P<0.05$, DNMRT)

1994 Experiment. Cotton aphid population densities in the 1994 experiment are shown in Fig. 3. Inspections showed that aphid numbers averaged less than one per leaf in all plots prior to the first cyhalothrin application on 11 July. Aphid populations began to increase in cyhalothrin (2) plots after this application. The second cyhalothrin application was made on 20 July, and samples indicated 11.7 aphids per leaf on 25 July. Aphid numbers continued to increase and were significantly greater than in all other treatments on 1 and 9 August ($P<0.05$, DNMRT). Aphid density peaked at 148.5 aphids per leaf on 9 August and leveled off at 145.1 aphids per leaf on 16 August. Cotton aphid numbers then decreased to less than three aphids per leaf on 22 August. Aphid population density in plots receiving two applications of cyhalothrin was greater than 50 aphids per leaf for a period of approximately two weeks. The seasonal mean of 38.5 aphids per leaf was significantly greater than that of all other treatments.

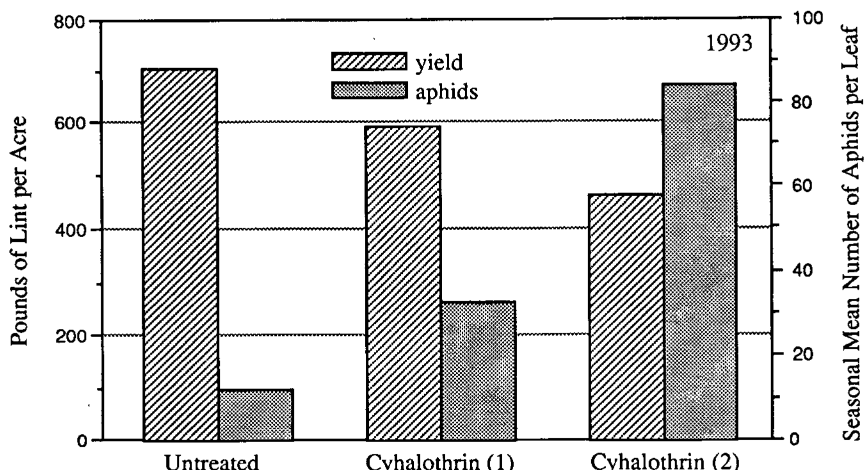


FIG. 2. Seasonal mean number of aphids per leaf and cotton lint yield from untreated plots, and plots treated with one and two applications of cyhalothrin. Lubbock, Texas, 1993.

TABLE 3. Fiber Quality Analysis Means from Untreated Plots, and Plots Treated with One and Two Applications of Cyhalothrin. Lubbock, Texas, 1993.

	Sample Mean [a]			
Treatment [b]	Length (in.)	Micronaire	Uniformity Ratio	Strength (g/tex)
Untreated	1.010	3.10	0.803	27.00
Cyhalothrin (1)	0.938	3.43	0.783	27.67
Cyhalothrin (2)	0.958	3.07	0.797	27.00

[a] Mean of three sub-samples.

[b] Treatment dates: cyhalothrin (1) - 7/30; cyhalothrin (2) - 7/22 and 7/30.

Cotton aphid population density in plots scheduled for a single application of cyhalothrin remained below one aphid per leaf prior to treatment on 20 July (Fig. 3). Aphid numbers increased immediately following this application to 7.5 aphids per leaf on 1 August. Aphid numbers continued to increase to 75.8 aphids per leaf on 9 August and peaked at 116.4 aphids per leaf on 16 August before declining to 1.4 per leaf the following week. Aphid numbers were significantly greater than those in untreated plots on 9 August and were significantly greater than those in untreated and dicrotophos treated plots on 16 August. Cotton aphid population density increased to more than 50 aphids per leaf within 17 days of the cyhalothrin application and remained at more than 50 aphids per leaf for approximately ten days.

Inspections of untreated check plots detected a gradual increase in cotton aphid population density to a peak of 31.0 aphids per leaf on 9 August (Fig. 3). Aphid numbers in these plots declined to 20.9 aphids per leaf on 16 August and 1.2 aphids per leaf on 22 August. Aphid population density in untreated plots was numerically less than all other treatments on all sample dates between 1 and 22 August, and was not significantly greater than any other treatment on any of the sample dates (P<0.05, DNMRT).

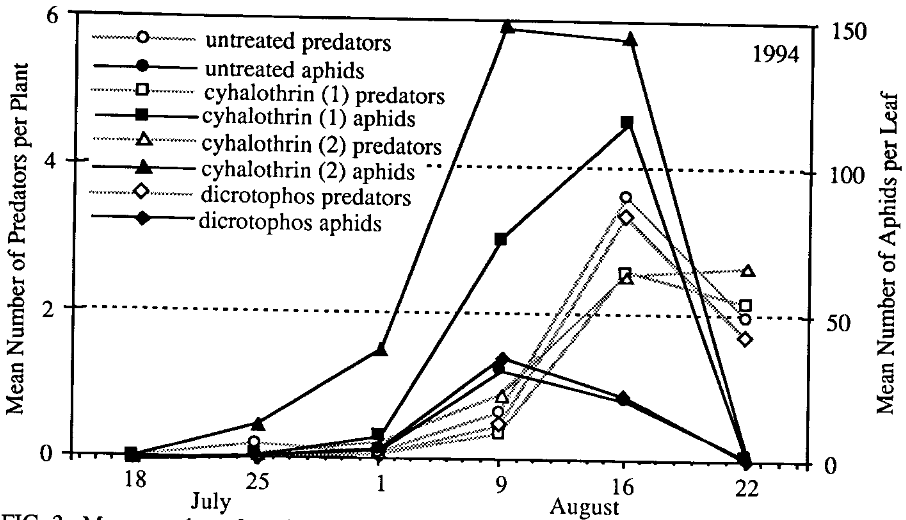


FIG. 3. Mean number of predators per plant and mean number of aphids per leaf in untreated plots, plots treated with dicrotophos, and plots treated with one and two applications of cyhalothrin. Lubbock, Texas, 1994. Treatment dates: cyhalothrin (1) - 7/20; cyhalothrin (2) - 7/11 and 7/20; dicrotophos - 7/27. Beneficial arthropods sampled: lady beetle adults and l;arvae, primarily *Hippodamia* spp.; lacewing larvae, *Chrysoperla* and other spp.; minute pirate bugs, *Orius* spp.; big-eyed bugs, *Geocoris* spp.; and syrphid fly larvae, *Syrphus* and other spp.

Dicrotophos treated plots averaged 1.1 aphids per leaf on 25 July, and received an application targeting beneficial arthropods on 27 July (Fig. 3). A reduction of beneficial arthropod numbers was not observed. This was attributed to low predator population density and predator movement among the small plots. Aphid populations did not respond to the dicrotophos treatment as they did to cyhalothrin. Cotton aphid population density increased gradually, peaked at 35.5 aphids per leaf on 9 August, and then declined to less than one aphid per leaf on 22 August. This seasonal pattern of aphid population development was very similar to that in untreated plots.

No significant differences were detected between selected aphid predator population densities of the four treatments, shown in Fig. 3, on any sample date (P<0.05, DNMRT). Considerably fewer predators were present in 1994 compared to 1993. The seasonal mean of untreated plots in 1993 was 1.2 predators per plant, compared to 0.7 per plant in 1994. Because of the small plot size, predators did not appear to be repelled from specific insecticide treated plots and moved freely among different treatments. Population densities remained below one predator per plant until 16 August, 19 days after the final insecticide application, when they ranged from 2.5 to 3.6 predators per plant among the four treatments. This increase in predator populations coincided with increases in aphid populations and was believed to be solely in response to high aphid population densities.

Differences in cotton lint yields, shown in Table 4, were not statistically significant (P<0.05; DNMRT). The reduced impact of aphids on yield was believed to be due to decreased duration and density of aphid infestations in 1994 compared to 1993, and to the change in cotton cultivar. Though yield differences were not statistically significant, numerical differences were noted, and the aphid infestation in plots receiving two applications of

cyhalothrin did have a negative economic impact (Fig. 4). These plots produced 129 lbs of lint per acre less than untreated plots, which is the equivalent of an $83.85 per acre loss at $0.65 per pound.

TABLE 4. Cotton Lint Yield from Untreated Plots, Plots Treated with Dicrotophos, and Plots Treated with One and Two Applications of Cyhalothrin. Lubbock, Texas, 1994.

Treatment [a]	Lbs. lint/acre [b]
Cyhalothrin (2)	888a
Dicrotophos	980a
Untreated	1017a
Cyhalothrin (1)	1030a

[a] Treatment dates: cyhalothrin (1) - 7/20; cyhalothrin (2) - 7/11 and 7/20; dicrotophos - 7/27.
[b] Means followed by the same letter are not significantly different ($P<0.05$, DNMRT).

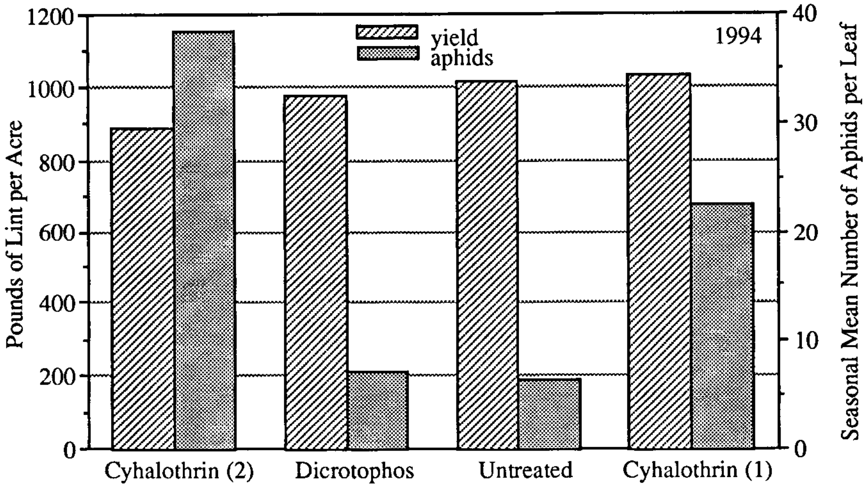


FIG. 4. Seasonal mean number of aphids per leaf and cotton lint yield from untreated plots, plots treated with dicrotophos, and plots treated with one and two applications of cyhalothrin. Lubbock, Texas, 1994.

Fiber quality analysis results are shown in Table 5. Aphid infestations appeared to have a negative impact on fiber strength. Plots receiving two applications of cyhalothrin, which had the highest aphid numbers, were the most negatively affected. These plots produced fiber with an average strength of 27.67 grams per tex, compared to 30.50 grams per tex in untreated plots, which had the lowest aphid numbers. Dicrotophos treated plots and plots receiving one and two applications of cyhalothrin produced fiber with an average strength of 29.67, 29.00 and 27.67 grams per tex, respectively. Length, micronaire, and uniformity did not appear to be affected by aphid infestations.

Rapid increases in the rate of aphid population development were observed in all cases following application of the pyrethroid insecticide cyhalothrin. These increases, which resulted in the development of significantly greater aphid population densities in cyhalothrin treated plots compared to untreated plots, did not appear to be due to decreased predator population densities. Increases in aphid population density appeared to be caused by some type of physiological change in either cotton aphids or cotton plants, which was promoted by cyhalothrin. Increasing predator numbers appeared to be a direct response to increasing aphid populations (Fig. 3). Though an increase in aphid numbers was noted following the dicrotophos application, this increase was very similar to that of untreated plots and did not appear to be related to the insecticide application.

TABLE 5. Fiber Quality Analysis Means from Untreated Plots, Plots Treated with Dicrotophos, and Plots Treated with One and Two Applications of Cyhalothrin. Lubbock, Texas, 1994.

Treatment [b]	Sample Mean [a]			
	Length (in.)	Micronaire	Uniformity Ratio	Strength (g/tex)
Untreated	1.039	3.95	0.813	30.50
Cyhalothrin (1)	1.063	3.93	0.815	29.00
Cyhalothrin (2)	1.042	4.07	0.820	27.67
Dicrotophos	1.063	3.83	0.820	29.67

[a] Mean of three sub-samples.

[b] Treatment dates: cyhalothrin (1) - 7/20; cyhalothrin (2) - 7/11 and 7/20; dicrotophos - 7/27.

Results of this study clearly show that cotton aphid infestations have a negative impact on cotton lint production. With exception of the cyhalothrin (1) treatment in the 1994 experiment, seasonal aphid population densities were inversely proportional to lint production (Figs. 2 and 4). Significant yield reductions appeared to occur when aphid numbers were greater than 50 per leaf for more than approximately ten days. Therefore, the current economic threshold of 50 aphids per leaf (Leser 1994) appears to be an acceptable level in the Texas High Plains. However, lint production may be influenced more by the duration than by the density of an aphid infestation. Producers should not take action when this threshold is met, but should monitor aphid populations more closely. An insecticide application may be justified if populations continue to increase. If population growth appears to be slowing or leveling off, it may be more advantageous to allow natural beneficial arthropod populations to control the infestation.

Successful cotton aphid management strategies in the Texas High Plains should include preservation and promotion of beneficial arthropod populations. Cotton aphid and predator populations should be closely monitored and should be included in cotton pest control decisions. Pyrethroid insecticides should be used only when other control methods will provide unacceptable results, and rapid increases in cotton aphid populations should be expected following applications of pyrethroid insecticides. Careful consideration of insecticide applications and promotion of predator populations should reduce the occurrence of economically damaging cotton aphid infestation developments in the Texas High Plains. These tactics and the consideration of area extension guidelines should provide reasonable management of the cotton aphid.

ACKNOWLEDGMENT

Funding and facilities for this study were provided by the Texas A&M University Agricultural Experiment Station, Lubbock, and Texas Tech University Department of Plant and Soil Science. The authors thank these agencies for their cooperation.

REFERENCES CITED

Allen, C. T., W. L. Multer, and V. Lucero. 1990. Seasonal changes in cotton aphid susceptibility to insecticides in west Texas cotton. pp. 287-290. *In* Proc. Beltwide Cotton Res. Conf., Nat. Cotton Council of Amer., Memphis, TN.

Dunnam, E. W., and J. C. Clark. 1941. Cotton aphid multiplication following treatment with calcium arsenate. J. Econ. Entomol. 34: 587-588.

Ewing, K. P. 1943. Cotton aphid damage and control in Texas. Jour. Econ. Entomol. 36: 598-601.

Head, R. B. 1992. Cotton insect losses 1991. pp. 621-625. *In* Proc. Beltwide Cotton Conf., Nat. Cotton Council of Amer., Memphis, TN.

Kerns, D. L., and M. J. Gaylor. 1991. Induction of cotton aphid outbreaks by the insecticide sulprofos. pp. 699-701. *In* Vol. II. Proc. Beltwide Cotton Conf., Nat. Cotton Council of Amer., Memphis, TN.

Kerns, D. L., and M. J. Gaylor. 1992. Sublethal effects of insecticides on cotton aphid reproduction and color morph development. Southwest. Entomol. 17: 245-250.

Leser, J. F., C. T. Allen, and T. W. Fuchs. 1992. Cotton aphid infestations in west Texas: A growing management problem. pp. 823-827. *In* Vol. II. Proc. Beltwide Cotton Conf., Nat. Cotton Council of Amer., Memphis, TN.

Leser, J. F. 1994. Management of cotton aphids: Texas style. pp. 137-141. *In* Proc. Beltwide Cotton Conf., Nat. Cotton Council of Amer., Memphis, TN.

Rummel, D. R. 1975. Focus on entomology. Texas Agri. Ext. Ser. Newsletter 14: 7 pp.

SAS Institute. 1985. SAS user's guide: statistics. SAS Institute, Cary, NC. 1290 pp.

Slosser, J. E., W. E. Pinchak, and D. R. Rummel. 1989. A review of known and potential factors affecting the population dynamics of the cotton aphid. Southwest. Entomol. 14: 302-313.

Weathersbee III, A. A., and D. D. Hardee. 1993. Indices of relative abundance for the cotton aphid, *Aphis gossypii* Glover, and associated parasitoids, pathogens and predators on six cotton cultivars. pp. 733-737. *In* Vol. II. Proc. Beltwide Cotton Conf., Nat. Cotton Council of Amer., Memphis, TN.

INFLUENCE OF GROUND COVER ON PHYTOPHAGOUS AND SAPROPHAGOUS ARTHROPODS IN PECAN TREES

Michael W. Smith, Don C. Arnold[1], Raymond D. Eikenbary[1], Natasha R. Rice, Asrat Shiferaw, Becky S. Cheary, and Becky L. Carroll

Department of Horticulture and Landscape Architecture, Oklahoma State University, Stillwater, OK 74078

ABSTRACT

Arthropods were collected, identified and counted on three dates to determine the effects of ground cover on the phytophagous and saprophagous arthropod community and density in pecan canopies. Ground covers were a grass sod [primarily bermudagrass, *Cynodon dactylon* (L.) Persoon], red clover (*Trifolium pratense* L.), or a mixture of crimson clover (*T. incarnatum* L.) and hairy vetch (*Vicia villosa* Roth). Arthropods collected included 11 orders of Insecta, plus Acarina and Isopoda. The order Coleoptera represented the largest number of families, followed by Lepidoptera, Diptera, Homoptera, Hemiptera, respectively. Ground cover affected density of some arthropods, but differences were usually small. The ground covers tested are unlikely to increase phytophagous arthropods in pecan.

INTRODUCTION

The use of legumes and mixtures of legumes and grasses as ground cover in pecan orchards offer certain advantages over perennial grass sod, such as supplying nitrogen and increasing densities of beneficial arthropods that may aid in the control of certain pecan pests (Bugg et al. 1991, Wood et al. 1983). Legumes were once commonly grown as cover or green manure crops in orchards until the arrival of inexpensive synthetic nitrogen fertilizers during the 1940's and 1950's (Tedders 1983). Interest in legumes as cover crops in orchards has increased recently because of high nitrogen costs, acquired pesticide resistance by certain insects and mites (Boethel 1981, Dutcher and Htay 1985), and outbreaks of secondary pests caused by elimination of natural enemies from pesticide applications (Mizell 1991).

Erdman (1967) estimated that nitrogen fixed by several legumes ranged from 57 to 207 kg/ha. Wood et al. (1983) reported that 'Amclo' arrowleaf clover (*Trifolium vesiculosum* Savi) adds about 112 kg/ha N to the soil of a pecan orchard. Rice (1994) found that a mixture of crimson clover (*T. incarnatum* L.) and hairy vetch (*Vicia villosa* Roth) supplied the equivalent of over 100 kg/ha N to the pecan orchard.

Three aphid species attacking pecan are yellow pecan aphid (*Monelliopsis pecanis* Bissell), blackmargined aphid (*Monellia caryella* (Fitch)), and black pecan aphid (*Melanocallis caryaefoliae* (Davis)) (Tedders et al. 1982). Feeding by the yellow pecan aphid and blackmargined aphid causes damage to pecan foliage by clogging phloem, inducing chlorosis, irreversibly suppressing photosynthesis, and indirectly by reducing nut size (Wood and Tedders

[1]Department of Entomology, Oklahoma State University, Stillwater, OK 74078.

1982, Wood et al. 1988). Black pecan aphid feeding causes necrosis in the surrounding mesophyll cells and can result in premature leaf abscission (Lakin 1972, Tedders et al. 1982). The pecan aphid complex is attacked by several species of predators and parasitoids (Edelson and Estes 1987, Liao et al. 1984). Certain legumes harbor large populations of aphids that attract predators and parasitoids (Smith et al. 1994). Aphids common on legumes in the southern U.S. are blue alfalfa aphid (*Acyrthosiphon kondoi* Shinji), pea aphid (*Acyrthosiphon pisum* (Harris)), cowpea aphid (*Aphis craccivora* Koch), and yellow clover aphid (*Therioaphis trifolii* (Monell)) (Bugg et al. 1990). Aphids found on understory legumes do not attack pecan trees. Predators and parasitoids that feed on these insects may attack aphids and other pests found in the canopy of pecan trees.

Bugg et al. (1991b) proposed a cover crop management system to enhance beneficial arthropods in pecan orchards. Several cool-season and warm-season cover crops were evaluated in Georgia, Oklahoma, and Massachusetts to increase aphidophagous insects and other entomophags to enhance biological control in vegetable and pecan agroecosystems (Wood et al. 1983, Bugg and Dutcher 1989, Bugg and Ellis 1990, Bugg et al. 1990, 1991a, Bugg and Dutcher 1993, Smith et al. 1994). Among several potential cool-season crops evaluated, certain legumes were promising candidates for increasing beneficial arthropods in pecan orchards.

Although several legumes have been evaluated for their effects on beneficial arthropod density and control of pecan aphids, extensive sampling has been limited to a few species. In this study we determined the effects of ground cover on phytophagous and saprophagous arthropods in the tree canopy. Beneficial arthropods collected in this study are reported elsewhere (Smith et al. 1996). Certain ground covers may increase arthropods potentially injurious to pecans, a scenario that has not been investigated. Not only is this useful in defining the effects of ground cover on arthropod density in pecan, it also provides new information on the diversity and density of phytophagous and saprophagous arthropods associated with pecan.

MATERIALS AND METHODS

We conducted the study in a commercially managed native pecan orchard about 15 km south of Sapulpa, Oklahoma. The soil was a Port loam (fine-silty, mixed, thermic; Cumulic Haplustoll; Mollisols), deep, well-drained, nearly level, and with high water holding capacity. This soil type is typical bottom land where pecan grows in Oklahoma. A stream bordered the orchard 400 m from the study area, and a 0.5 ha surface area pond was within 300 m of the site.

Insecticides applied during the study included chlorpyrifos on 21 April and 3 May, 1993, for phylloxera (*Phylloxera notabilis* Pergande, *P. russelae* Stoetzel, and *P. devastratrix* Pergande, Homoptera: Phylloxeridae) and on 17 June for pecan nut casebearer (*Acrobasis nuxvorella* Neunzig, Lepidoptera: Pyralidae), and carbaryl on 28 August and 15 September for pecan weevil (*Curculio caryae* (Horn), Coleoptera: Curculionidae) control. No other insecticides or fungicides were used in the orchard.

Red clover was planted during September 1990. Beginning in 1990, 'Dixie' crimson clover and hairy vetch were planted each year during late September. Stands were excellent each year. Crimson clover and hairy vetch senesced during June and acted as a mulch throughout the summer, resulting in few broadleaf or grassy weeds. The area with grass consisted mainly of closely-mowed bermudagrass. Each ground cover plot was about 10 ha in size.

We conducted the arthropod survey in 1993; three years after the ground covers were initially established. Three trees of about the same size within each ground cover type were selected for surveying arthropod fauna. Trees were 13.1±1.5 m (mean ± standard deviation) tall with a trunk area at breast height of 0.1±0.03 m^2, and the basal canopy area was 86±12 m^2. At dawn we covered the area below each tree with plastic, then applied esfenvalerate. After about two hours arthropods that had fallen to the ground were collected and placed in isopropyl alcohol

for later mounting, identification and counting. Samples were taken on nearly windless days with adequate personnel to allow collection of fallen arthropods within two hours. Trees were sampled on 24 May, 6 July and 1 September. Different trees were selected on each date. Data were analyzed by date using an analysis of variance. Significantly different means were separated by Duncan's multiple range test. If arthropod densities associated with the different ground cover treatments were not significantly different, data were pooled over the ground covers.

RESULTS AND DISCUSSION

Arthropods collected from pecan included eleven orders of Insecta plus Acarina and Isopoda (Table 1). Coleoptera contained the largest number of families (27) followed by Lepidoptera (16), Diptera (12), Homoptera (11), Hemiptera (11), Hymenoptera (5), Orthoptera (5), Thysanoptera (2), Isoptera (1), and Psocoptera (1). Unidentified material was counted as a single family, but in some cases several families may be represented in the unidentified material.

Several of the Coleoptera collected typically attack dead wood or weakened trees. Trees were damaged by cold temperatures during the fall of 1991, resulting in the death of several limbs up to 8 cm in diameter. Although trees were healthy and vigorous at the time of the study, some dead limbs were retained in the tree canopy, and probably contributed to the diversity and density of insects attacking dead or injured wood.

Twenty-seven species of Chrysomelidae were collected from pecan, mostly during May and July. This is a surprisingly large number of species to collect from a single host species during one year. Density of each species was low, but accumulated damage could negatively impact pecan production, especially when combined with other leaf feeding arthropods.

Several Curculionidae also were present; damage to pecan from most of these species is well documented. The Asiatic oak weevil, *Cyrtepistomus castaneus* (Roelofs), feeds on foliage as an adult; however, *C. castaneus* is not normally considered a pecan pest. In fact, Ferguson et al. (1991) found some *Carya* spp. to be unacceptable hosts for *C. castaneus*. Large densities of *C. castaneus* collected from pecan in July coupled with a lack of oaks or other potential hosts nearby caused us to suspect that pecan, *Carya illinoinensis*, was an acceptable host for *C. castaneus*, and that *C. castaneus* may be inflicting damage. Ferguson et al. (1991) concluded that *C. castaneus* contributed significantly to problems with oak forest regeneration in Missouri. Additional studies are needed to determine if pecan is an acceptable host for *C. castaneus*, and the extent of damage caused by this insect.

Twelve families of Diptera were collected; however, most of the species inhabited damp areas near the woods edge. It is unlikely that any of the species collected were contributing significantly to pecan damage.

Eleven families of Hemiptera were collected. Of these, the stink bugs (Pentatomidae) usually cause the greatest damage. Several of the stink bugs attack a wide range of host species (Arnold and Drew 1988). Some species have been observed in very high densities on certain legumes. Stink bugs cause damage to pecan by feeding on the fruit as it matures, causing black spots on the kernel and rendering it unmarketable. A major concern associated with use of legumes as a ground cover is increased damaged fruit from stink bug feeding. We collected nine species of stink bugs from pecan. Stink bug density in the pecan canopy was not affected by ground cover, and densities of each species were relatively low, especially in September when fruit damage is likely to occur.

Several species of leafhoppers, planthoppers and treehoppers were present. Densities of each species were low, except Cicadellidae was relatively high during July. Cicadellids were more abundant when crimson clover/hairy vetch was present as the ground cover. However, the density of cicadellids found in this study are unlikely to affect tree performance. Similarly, density of aphids on each sampling date was low.

Table 1. Influence of ground cover on arthropod densities in pecan canopies at selected times.

Arthropod	Ground cover	Arthropods/tree[a]					
		24 May		6 Jul		1 Sep	
	Uniramia: Insecta						
Coleoptera							
Alleculidae							
Lobopoda sp.	Pooled	0		0.9	(2.0)	0	
Undetermined sp.	Pooled	0.2	(0.4)	0		0	
Anobiidae							
Undetermined sp.	Pooled	0		1.0	(1.3)	0	
Anthicidae							
Notoxus sp.	Pooled	0		0		0.2	(0.4)
Bruchidae							
Bruchus brachialis F.	Pooled	0.1	(0.3)	0		0	
Buprestidae							
Agrilus sp.	Pooled	0.6	(0.7)	0		0	
Chrysobothris sp.	Pooled	0.2	(0.4)	--		0	
Lixus sp.	Pooled	0.1	(0.3)	0		0	
Pantomorus pallidus (Horn)	Pooled	0		0.1	(0.3)	0	
Undetermined spp.	Pooled	0.2	(0.4)	0.7	(1.1)	0	
Undetermined larvae	Pooled	0.3	(0.7)	0		0	
Elateridae							
Adelocera sp.	Pooled	0.1	(0.3)	0		0	
Conoderus lividus DeGeer	Pooled	0		22.3	(17.3)	2.4	(2.3)
C. vespertinus (F.)	Pooled	0		0.2	(0.4)	0	
C. sp.	Pooled	0.8	(1.2)	0.1	(0.3)	0.1	(0.3)
Glyphonyx sp.	Pooled	0.1	(0.3)	0.1	(0.3)	0.1	(0.3)
Limonius sp.	Pooled	0.3	(1.0)	0		0	
Melanotus sp.	Pooled	6.0	(8.4)	--		0	
	Grass	--		0. b		--	
	Clover/vetch	--		3.3 a	(0.6)	--	
	Red clover	--		1.3 b	(0.6)	--	
Endomychidae							
Lycoperdina ferruginea LeConte	Pooled	0		0.1	(0.3)	0	
Eucnemidae							
Undetermined sp.	Pooled	0		0.1	(0.3)	0	
Euglenidae							
Undetermined spp.	Pooled	0		0.4	(0.7)	0.1	(0.3)
Lagriidae							
Statira sp.	Pooled	0.3	(0.7)	0		0	
Languriidae							
Languria mozardi Latreille	Pooled	0		0.1	(0.3)	0.1	(0.3)
Lycidae							
Calopteron sp.	Pooled	0.2	(0.7)	0.2	(0.4)	0.1	(0.3)
Plateros sp.	Pooled	0		1.3	(1.3)	0	
Melandryidae							
Canifa sp.	Pooled	0.4	(0.5)	0.2	(0.4)	0	
Synchroa punctata Newman	Pooled	0		0.1	(0.3)	0	
Meloidae							
Epicauta funebris Horn	Pooled	0		0.1	(0.3)	0.3	(1.0)
Mordellidae							
Tomoxia carinata (Smith)	Pooled	0		0.4	(0.7)	0	
Undetermined sp.	Pooled	0		0		0.1	(0.3)
Rhynchitidae							
Eugnamptus sp.	Pooled	3.8	(8.9)	0		0	
Pterocolus ovatus (F.)	Pooled	0.1	(0.3)	0		0	

Table 1 cont.

Scarabaeidae							
Anomala innuba (F.)	Pooled	0		0.2	(0.4)	0	
A. marginata (F.)	Pooled	0		0.3	(0.7)	0.1	(0.3)
Cotinis nitida (L.)	Pooled	0		0.1	(0.3)	0	
Cyclocephala immaculata Olivier	Pooled	0		0.1	(0.3)	0	
Diplotaxis blanchardi Vaurie	Pooled	0		0.2	(0.4)	0	
Euphoria fulgida (F.)	Pooled	0.1	(0.3)	0.1	(0.3)	0	
Phyllophaga profunda (Blanchard)	Pooled	0		0.1	(0.3)	0	
Scolytidae							
Ips grandicollis (Eichhoff)	Pooled	0		0.2	(0.4)	0	
Scydmaenidae							
Undetermined sp.	Pooled	0		0.2	(0.4)	0	
Staphylinidae							
Platydracus cinnamopterus Gravenhorst	Pooled	0		0.1	(0.3)	0	
P. maculosus (Gravenhorst)	Pooled	0		0.1	(0.3)	0	
Undetermined spp.	Pooled	0.8	(1.2)	2.4	(2.4)	0.7	(1.1)
Tenebrionidae							
Undetermined sp.	Pooled	0.1	(0.3)	0		0	
	Grass	--		0. b[h]		--	
	Clover/vetch	--		1.3a	(0.6)	--	
	Red clover	--		0. b		--	
Dicerca sp.	Pooled	0.2	(0.4)	0.1	(0.3)	0	
Cerambycidae							
Acanthoderes quadrigibbus (Say)	Pooled	0		0.1	(0.3)	0	
Euderces reichei LeConte	Pooled	0.2	(0.4)	0.1	(0.3)	0	
Lepturges confluens (Haldeman)	Pooled	0		0.1	(0.3)	0	
Molorchus bimaculatus Say	Pooled	0.1	(0.3)	0		0	
Physocnemum brevilineum (Say)	Pooled	0		0.1	(0.3)	0	
Saperda discoidea F.	Pooled	0.1	(0.3)	0		0	
Stenosphenus notatus (Olivier)	Pooled	0.8	(1.4)	0		0	
Chrysomelidae							
Acalymma vittata (F.)	Pooled	0.3	(0.5)	0		0	
Anomoea rufifrons Lacordaire	Pooled	0		0.1	(0.3)	0	
Bassarius brunnipes (Olivier)	Pooled	0		0.3	(0.7)	0	
Colaspis brunnea (F.)	Pooled	0		0.7	(1.3)	0.1	(0.3)
Cryptocephalus fulguratus LeConte	Pooled	0		0.2	(0.7)	0	
C. guttulatus Olivier	Pooled	0		--		0	
	Grass	--		0. b		--	
	Clover/vetch	--		1.0 a	(0.)	--	
	Red clover	--		0.3 b	(0.6)	--	
C. leucomelas Suffrian	Pooled	0		0.2	(0.4)	0	
C. mutabilis Melsheimer	Pooled	0		0.1	(0.3)	0.3	(0.5)
C. nanus F.	Pooled	0		0.1	(0.3)	0	
C. sp.	Pooled	0.1	(0.3)	0		0	
Deloyala guttata (Olivier)	Pooled	0		0.2	(0.7)	0	
Diachus auratus (F.)	Pooled	0.2	(0.4)	0		0	
Glyptoscelis longior LeConte	Pooled	0.8	(1.4)	0		0	
Kuschelina gibbitarsa (Say)	Pooled	0.1	(0.3)	0		0	
Metachroma angustulum Crotch	Pooled	0		0.1	(0.3)	0	
Metriona bicolor (F.)	Pooled	0.1	(0.3)	0.1	(0.3)	0	
Nodonota tristis (Olivier)	Pooled	0		1.3	(1.6)	0	
Octotoma plicatula (F.)	Pooled	0.9	(2.3)	0.6	(1.0)	0	
Pachybrachis sp.	Pooled	0.1	(0.3)	0.2	(0.7)	0	
Paria quadriguttata LeConte	Pooled	0		0.3	(0.7)	0	
Plagiometriona clavata (F.)	Pooled	0		0		0.1	(0.3)
Rhabdopterus picipes (Olivier)	Pooled	0		3.1	(8.6)	0	
R. sp.	Pooled	1.4	(1.4)	0		0	
Zygogramma suturalis (F.)	Pooled	0.1	(0.3)	0		0	

Table 1 cont.

Taxon	Habitat	Mean	(SD)	Mean	(SD)	Mean	(SD)
Undetermined Alticinae	Pooled	0.8	(1.6)	2.6	(3.2)	0.4	(1.0)
Undetermined sp.	Pooled	0		0.1	(0.3)	0	
Cucujidae							
Ahasverus advena (Waltl)	Pooled	0		0.1	(0.3)	0.2	(0.7)
Telephanus velox Haldeman	Pooled	0		0.2	(0.7)	0	
Curculionidae							
Apion sp.	Pooled	0		0.2	(0.4)	0	
Conotrachelus elegans (Say)	Pooled	0		4.2	(2.5)	0.1	(0.3)
C. hicoriae Schoof	Pooled	0.2	(0.7)	2.3	(4.2)	0	
C. pecanae Buchanan	Pooled	0.2	(0.7)	0.2	(0.4)	0	
Curculio caryae (Horn)	Pooled	0		0		1.8	(2.6)
Cyrtepistomus castaneus (Roelofs)	Pooled	0		184.2	(96.7)	--	
	Grass	--		--		0. b	
	Clover/vetch	--		--		0.3 b	(0.6)
	Red clover	--		--		8.3 a	(2.1)
Hypera postica (Gyllenhal)	Pooled	0.8	(1.4)	0.1	(0.3)	0	
H. punctata (F.)	Pooled	0.2	(0.7)	0		0	
Listroderes costirostris Schoenherr	Pooled	0		0		0.1	(0.3)
Undetermined spp.	Pooled	0.7	(1.3)	3.3	(2.8)	0	
Undetermined larvae	Pooled	0		1.4	(1.9)	0.1	(0.3)
Collembola							
Entomobryidae							
Undetermined sp.	Pooled	0		0.1	(0.3)	0	
Sminthuridae							
Undetermined sp.	Pooled	0		0.1	(0.3)	0	
Undetermined sp.	Pooled	0.1	(0.3)	0		0	
Diptera							
Bibionidae							
Dilophis sp.	Pooled	11.6	(8.2)	0		0	
Penthetria sp.	Pooled	0.1	(0.3)	0		0	
Undetermined sp.	Pooled	0		0.4	(0.5)	0	
Ephydridae							
Ochthera sp.	Pooled	0		2.0	(2.0)	0	
Undetermined spp.	Pooled	0		--		0	
	Grass	--		0. b		--	
	Clover/vetch	--		0. b		--	
	Red clover	--		1.7 a	(1.2)	--	
Lauxaniidae							
Undetermined spp.	Pooled	0		1.4	(1.9)	2.1	(1.8)
Otitidae							
Delphinia picta F.	Pooled	0		0		0.4	(0.7)
Undetermined spp.	Pooled	0.7	(0.7)	0.1	(0.3)	0	
Phoridae							
Undetermined spp.	Pooled	0.8	(1.4)	0.6	(0.7)	0.3	(0.7)
Platypezidae							
Undetermined sp.	Pooled	0		0		0.2	(0.4)
Psilidae							
Undetermined spp.	Pooled	1.1	(2.1)	0		0	
Psychodidae							
Undetermined spp.	Pooled	0.2	(0.7)	0.1	(0.3)	0	
Sciaridae							
Undetermined spp.	Pooled	2.0	(1.9)	0.1	(0.3)	4.8	(12.9)
Tephritidae							
Undetermined sp.	Pooled	0		0.1	(0.3)	0.1	(0.3)
Tipulidae							
Undetermined spp.	Pooled	--		--		1.8	(2.4)
	Grass	2.7 c	(2.9)	0. b		--	
	Clover/vetch	7.0 b	(3.0)	14.0 a	(4.4)	--	
	Red clover	18.0 a	(8.9)	2.0 b	(1.0)	--	

Table 1 cont.

Taxon	Habitat						
Undetermined							
Undetermined spp.	Pooled	36.7	(33.3)	--		5.0	(10.3)
	Grass	--		7.0 b	(3.0)	--	
	Clover/vetch	--		29.7 a	(4.9)	--	
	Red clover	--		22.0 a	(14.0)	--	
Undetermined larvae	Pooled	0		0.3	(0.5)	0.3	(0.7)
Undetermined pupae	Pooled	0		0.2	(0.7)	0	
Hemiptera							
Alydidae							
Alydus eurinus (Say)	Pooled	0		0.3	(0.5)	0	
A. pilosulus Herrick-Schaeffer	Pooled	0		0.1	(0.3)	0	
Coreidae							
Acanthocephala terminalis (Dallas)	Pooled	0		0		0.2	(0.7)
Euthochtha galeator (F.)	Pooled	0		0.1	(0.3)	0	
Corimelaenidae							
Corimelaena lateralis (F.)	Pooled	0.1	(0.3)	0		0	
Galgupha atra Amyot & Serville	Pooled	0		0.1	(0.3)	0	
Cydnidae							
Pangaeus bilineatus (Say)	Pooled	0.2	(0.4)	0		0	
Sehirus cinctus (Palisot)	Pooled	0.1	(0.3)	0.6	(1.0)	0	
Lygaeidae							
Heraeus plebejus Stal	Pooled	0		0.2	(0.4)	0	
Kleidocerys resedae geminatus (Say)	Pooled	0.1	(0.3)	0		0	
Oedancala crassimana (F.)	Pooled	0		0.1	(0.3)	0	
O. dorsalis (Say)	Pooled	0.3	(0.5)	0		0	
Ozophora picturata Uhler	Pooled	0		0.2	(0.4)	0	
Xyonysius californicus (Stal)	Pooled	0.1	(0.3)	0		0	
Undetermined nymph	Pooled	0		0.1	(0.3)	0	
Miridae							
Agnocoris pulverulentus (Uhler)	Pooled	0		0.2	(0.4)	0	
Deraeocoris nebulosus (Uhler)	Pooled	0		1.4	(2.6)	0	
Diphleps unica Bergroth	Pooled	0		0.4	(0.7)	0	
Lopidea sp.	Pooled	1.8	(2.5)	0		0	
Lygocoris caryae (Knight)	Pooled	12.4	(15.0)	0.6	(1.1)	0	
L. caryae (Knight) nymphs	Pooled	15.4	(17.8)	0		0	
Lygus lineolaris (Palisot)	Pooled	--		0.4	(0.7)	0	
	Grass	1.3 b	(0.6)	--		--	
	Clover/vetch	13.7 a	(5.9)	--		--	
	Red clover	2.3 b	(1.5)	--		--	
L. lineolaris (Palisot) nymphs	Pooled	1.3	(3.0)	0		0	
Metriorrhynchomiris dislocatus (Say)	Pooled	0.7	(0.7)	0.3	(0.5)	0	
Myiomma cixiiforme (Uhler) nymph	Pooled	0.1	(0.3)	0		0	
Neurocolpus sp.	Pooled	0		0.1	(0.3)	0	
N. sp. nymphs	Pooled	--		0		0	
	Grass	2.0 a	(1.0)	--		--	
	Clover/vetch	0. b		--		--	
	Red clover	0.3 b	(0.6)	--		--	
Peritropis saldaeformis Uhler	Pooled	0		0.3	(0.5)	0	
Phytocoris spp.	Pooled	0.1	(0.3)	0.4	(1.0)	0	
Plagiognathus caryae Knight	Pooled	15.8	(34.1)	0.6	(1.3)	0	
P. caryae Knight nymphs	Pooled	11.4	(29.2)	0		0	
Prepops insitivus (Say)	Pooled	0.2	(0.7)	0		0	
Trigonotylus sp.	Pooled	0.1	(0.3)	0		0	
Undetermined spp.	Pooled	0		0.8	(1.1)	0	
Undetermined nymphs	Pooled	1.1	(3.3)	0.2	(0.7)	0	

Table 1 cont.

Taxon	Habitat						
Pentatomidae							
Acrosternum hilare (Say)	Pooled	0		0		0.2	(0.4)
Brochymena quadripustulata (F.)	Pooled	0		0		0.1	(0.3)
B. sp. nymphs	Pooled	0		3.4	(4.0)	0.1	(0.3)
Euschistus ictericus (L.)	Pooled	0		0.1	(0.3)	0	
E. servus (Say)	Pooled	0.1	(0.3)	0.4	(0.5)	0.1	(0.3)
E. tristigmus (Say)	Pooled	0		0.7	(2.0)	0	
Holcostethus limbolarius (Stal)	Pooled	0		0		0.1	(0.3)
Hymenarcys nervosa (Say)	Pooled	0.1	(0.3)	0		0	
Mormidea lugens (F.)	Pooled	0.2	(0.4)	0.1	(0.3)	0	
Piesmatidae							
Piesma cinereum (Say)	Pooled	0.3	(0.7)	0		0	
Rhopalidae							
Jadera haematoloma (Herrick-Schaeffer)	Pooled	0		0.1	(0.3)	0	
Tingidae							
Leptoypha costata Parshley	Pooled	0		0.1	(0.3)	0	
Undetermined							
Undetermined nymphs	Pooled	0		2.0	(4.6)	0	
Homoptera							
Acanaloniidae							
Acanalonia conica (Say)	Pooled	0		0.1	(0.3)	0	
Aphididae							
Longistigma caryae (Harris)	Pooled	0.8	(1.4)	0		0	
Monellia caryella (Fitch)	Pooled	13.0	(18.4)	2.3	(3.2)	0	
Undetermined spp.	Pooled	2.4	(5.5)	0		0	
Cercopidae							
Clastoptera achatina Germar	Pooled	0		1.2	(1.1)	0.6	(0.7)
C. achatina Germar nymphs	Pooled	0		--		0	
	Grass	--		3.3 b	(1.5)	--	
	Clover/vetch	--		2.0 c	(1.0)	--	
	Red clover	--		5.7 a	(0.6)	--	
Lepyronia quadrangularis (Say)	Pooled	0		0.1	(0.3)	0	
Cicadellidae							
Oncometopia nigricans Walker	Pooled	--		0		0	
	Grass	0. b		--		--	
	Clover/vetch	0. b		--		--	
	Red clover	7.0 a	(5.0)	--		--	
Undetermined spp.	Pooled	3.9	(3.8)	--		2.7	(2.7)
	Grass	--		15.7 b	(5.0)	--	
	Clover/vetch	--		41.0 a	(3.6)	--	
	Red clover	--		20.7 b	(11.0)	--	
Undetermined nymphs	Pooled	41.8	(39.1)	4.0	(3.6)	0.4	(0.5)
Cixiidae							
Oliarus aridus (Ball)	Pooled	0		1.2	(2.9)	0	
Delphacidae							
Delphacodes lutulenta (Van Duzee)	Pooled	0		0.1	(0.3)	0	
Liburniella ornata (Stal)	Pooled	0.1	(0.3)	0		0	
Pissonotus flabellatus (Ball)	Pooled	0		0		0.1	(0.3)
Derbidae							
Cedusa sp.	Pooled	0		0		0.1	(0.3)
Dictyopharidae							
Rhynchomitra microrhina (Walker)	Pooled	0		0		0.1	(0.3)
Scolops pungens (Germar)	Pooled	0		0.1	(0.3)	0	
Flatidae							
Anormenis septentrionalis (Spinola)	Pooled	0		3.7	(6.0)	0.3	(0.5)
Metcalfa pruinosa (Say)	Pooled	0		2.4	(5.5)	0.6	(0.5)

Table 1 cont.

Taxon	Treatment						
Membracidae							
Micrutalis calva (Say)	Pooled	0.1	(0.3)	0		0	
Platycotis vittata (F.)	Pooled	0		0.1	(0.3)	0	
Spissistilus borealis (Fairmaire)	Pooled	0		0.3	(0.5)	0	
Stictocephala taurina (Fitch)	Pooled	0		0.1	(0.3)	0.1	(0.3)
Telamona unicolor Fitch	Pooled	8.4	(7.4)	0.4	(1.0)	0	
Tortistilus inermis (F.)	Pooled	0.1	(0.3)	0.1	(0.3)	0	
Undetermined nymphs	Pooled	2.3	(3.5)	15.2	(26.3)	0	
Phylloxeridae							
Undetermined sp.	Pooled	0.3	(0.7)	0		0	
Hymenoptera							
Anthophoridae							
Undetermined sp.	Pooled	0.1	(0.3)	0		0	
Formicidae							
Camponotus pennsylvanicus (DeGeer)	Pooled	0.6	(1.0)	--		0.1	(0.3)
	Grass	--		12.3 a	(9.6)	--	
	Clover/vetch	--		0.3 b	(0.6)	--	
	Red clover	--		0. b		--	
C. pennsylvanicus (DeGeer) pupae	Pooled	0		1.9	(5.3)	0	
C. sp.	Pooled	0.4	(0.7)	0.2	(0.4)	0	
Crematogaster sp.	Pooled	0.4	(0.7)	0.4	(1.0)	0	
Formica pallidefulva Latreille	Pooled	0.2	(0.7)	0		0	
Prenolepis imparis (Say)	Pooled	0.2	(0.4)	0		0	
Tapinoma sessile (Say)	Pooled	0.1	(0.3)	0.1	(0.3)	0.1	(0.3)
Undetermined spp.	Pooled	0.6	(0.7)	3.0	(2.2)	2.4	(3.0)
Pergidae							
Acordulecera sp.	Pooled	0.3	(0.5)	0		0	
A. sp. larvae	Pooled	4.1	(2.5)	0		0	
Tenthredinidae							
Eupareophora parca (Cresson) larvae	Pooled	0.3	(1.0)	0		0	
Periclista marginicollis (Norton) larvae	Pooled	8.7	(6.1)	0		0	
Xiphydriidae							
Xiphydria tibialis Say	Pooled	0.1	(0.3)	0		0	
Isoptera							
Rhinotermitidae							
Reticulotermes sp.	Pooled	--		0		0	
	Grass	3.7 a	(1.2)	--		--	
	Clover/vetch	0.3 b	(0.6)	--		--	
	Red clover	0. b		--		--	
Lepidoptera							
Apaturidae							
Asterocampa celtis (Bois. & LeConte)	Pooled	0		0.1	(0.3)	0.1	(0.3)
Arctiidae							
Cisthene packardii (Grote)	Pooled	0.1	(0.3)	0		0	
Halisidota tesselaris (J.E. Smith)	Pooled	0		3.9	(3.5)	0.1	(0.3)
Undetermined larvae	Pooled	0.7	(1.9)	0.2	(0.7)	1.6	(3.2)
Gelechiidae							
Undetermined larvae	Pooled	--		0		0	
	Grass	24.7 a	(5.8)	--		--	
	Clover/vetch	5.0 b	(3.0)	--		--	
	Red clover	0.7 b	(1.2)	--		--	
Geometridae							
Undetermined larvae	Pooled	9.1	(6.4)	0.7	(0.7)	0.3	(0.7)
Hesperiidae							
Undetermined spp.	Pooled	0		0.2	(0.4)	0.3	(0.7)
Lasiocampidae							
Tolype velleda (Stoll) larvae	Pooled	0.4	(0.7)	0.1	(0.3)	0	

Table 1 cont.

Limacodidae							
Euclea sp. larvae	Pooled	0		1.2	(1.5)	0.6	(1.1)
Phobetron pithecium (J.E. Smith) larva	Pooled	0		0.1	(0.3)	0	
Undetermined larvae	Pooled	0		0.4	(1.0)	0	
Lycaenidae							
Undetermined larvae	Pooled	0.4	(0.7)	0		0	
Noctuidae							
Bleptina caradrinalis Guenee	Pooled	0		0.1	(0.3)	0	
Catocala sp. larvae	Pooled	2.2	(3.5)	0.4	(0.7)	0	
Orthosia hibisci (Guenee)larvae	Pooled	28.3	(27.0)	0		0	
Peridroma saucia (Hubner) larvae	Pooled	0.2	(0.7)	0		0	
Plathypena scabra (F.)	Pooled	0.3	(0.7)	0.1	(0.3)	0.1	(0.3)
Undetermined moths	Pooled	0.3	(0.7)	0.1	(0.3)	0	
Undetermined larvae	Pooled	5.5	(7.1)	26.7	(10.8)	--	
	Grass	--		--		1.7 b	(2.1)
	Clover/vetch	--		--		8.7 a	(4.0)
	Red clover	--		--		1.7 b	(1.5)
Notodontidae							
Heterocampa guttivitta Walker larvae	Pooled	2.4	(3.2)	2.9	(3.2)	--	
	Grass	--		--		1.7 b	(2.1)
	Clover/vetch	--		--		4.3 a	(0.6)
	Red clover	--		--		0.7 b	(0.6)
Schizura leptinoides (Grote)	Pooled	0		0		0.1	(0.3)
S. leptinoides (Grote) larvae	Pooled	0		1.9	(1.5)	0.6	(0.7)
Pyralidae							
Acrobasis nuxvorella Neunzig	Pooled	0		0.1	(0.3)	0	
Eustixia pupula Hubner	Pooled	0.1	(0.3)	0		0	
Undetermined sp.	Pooled	0		0		0.2	(0.7)
Saturniidae							
Automeris io (F.) larvae	Pooled	0		0.3	(0.7)	0.3	(0.7)
Undetermined larvae	Pooled	0.3	(1.0)	0		0	
Sphingidae							
Laothoe juglandis (J.E. Smith) larvae	Pooled	0		--		--	
	Grass	--		3.3 b	(1.5)	1.0 b	(1.7)
	Clover/vetch	--		0.3 c	(0.6)	8.7 a	(1.2)
	Red clover	--		7.7 a	(2.1)	6.0 a	(3.5)
Undetermined larvae	Pooled	0		0		0.7	(0.9)
Tortricidae							
Cydia caryana Fitch	Pooled	0.3	(0.5)	0.7	(1.7)	0.9	(1.5)
Undetermined larvae	Pooled	--		0		0	
	Grass	20.3 a	(10.3)	--		--	
	Clover/vetch	5.0 b	(4.0)	--		--	
	Red clover	1.3 b	(0.6)	--		--	
Zygaenidae							
Harrisina americana (Guerin)	Pooled	0.1	(0.3)	0		0	
Undetermined							
Undetermined spp.	Pooled	2.9	(4.0)	11.6	(8.3)	3.7	(4.0)
Undetermined larvae	Pooled	4.3	(4.2)	3.4	(3.5)	0.7	(1.1)
Orthoptera							
Acrididae							
Chortophaga viridifasciata (DeGeer)	Pooled	0.1	(0.3)	0		0.4	(1.3)
Dichromorpha viridis (Scudder)	Pooled	0		0		0.1	(0.3)
Melanoplus femurrubrum (DeGeer)	Pooled	0		0		0.2	(0.7)
M. sp. nymphs	Pooled	0		0.4	(0.9)	0.2	(0.4)

Table 1 cont.

Taxon	Ground cover	Mean	(SD)	Mean	(SD)	Mean	(SD)
Undetermined nymphs	Pooled	0.2	(0.7)	1.1	(1.3)	--	
	Grass	--		--		1.7 a	(0.6)
	Clover/vetch	--		--		0. b	
	Red clover	--		--		0.3 b	(0.6)
Blattellidae							
Parcoblatta sp.	Pooled	0		0.2	(0.4)	0	
P. sp. nymphs	Pooled	0		0.7	(1.4)	0.1	(0.3)
Undetermined ootheca	Pooled	0		0.1	(0.3)	0	
Gryllidae							
Allonemobius fasciatus (DeGeer)	Pooled	0		3.0	(6.2)	1.6	(1.8)
A. fasciatus (DeGeer) nymphs	Pooled	0		4.9	(6.2)	1.3	(1.1)
Anaxipha exigua (Say)	Pooled	0		0.1	(0.3)	0	
Cycloptilum sp.	Pooled	0		0		0.1	(0.3)
C. sp. nymph	Pooled	0		0.1	(0.3)	0	
Gryllus sp.	Pooled	0.2	(0.7)	0		1.2	(1.9)
G. sp. nymphs	Pooled	0		0		0.4	(0.5)
Hapithus agitator Uhler	Pooled	0		0		0.3	(0.7)
H. agitator Uhler nymphs	Pooled	0		0		0.1	(0.3)
Neoxabea bipunctata (DeGeer)	Pooled	0		0.6	(0.9)	0	
Oecanthus niveus (DeGeer)	Pooled	0		1.4	(3.3)	0.6	(0.7)
O. niveus (DeGeer) nymphs	Pooled	0		1.6	(1.7)	0.3	(0.7)
Undetermined nymphs	Pooled	0.2	(0.7)	0.3	(0.5)	0	
Tetrigidae							
Tetrix arenosa angusta Hancock	Pooled	0		0		0.1	(0.3)
Tettigidea lateralis (Say)	Pooled	0		0.7	(1.3)	0.2	(0.4)
Undetermined nymphs	Pooled	0.1	(0.3)	0.3	(1.0)	0	
Tettigoniidae							
Conocephalus faciatus (DeGeer)	Pooled	0		0.1	(0.3)	0.2	(0.7)
C. strictus (Scudder)	Pooled	0		0		0.7	(2.0)
Microcentrum rhombifolium Saussure	Pooled	0		0		0.2	(0.4)
Neoconocephalus sp. nymph	Pooled	0		0		0.1	(0.3)
Orchelimum nigripes Scudder	Pooled	0		0		0.1	(0.3)
O. silvaticum McNeill	Pooled	0		0		0.1	(0.3)
O. sp. nymphs	Pooled	0		2.2	(2.2)	0	
Scudderia texensis Saussure & Pictet	Pooled	0		0.4	(0.5)	0	
Undetermined nymphs	Pooled	2.7	(3.0)	0.4	(0.9)	0	
Psocoptera							
Undetermined spp.	Pooled	1.3	(1.5)	1.2	(1.1)	0	
Thysanoptera							
Phlaeothripidae							
Undetermined spp.	Pooled	0.6	(0.7)	2.7	(2.6)	0	
Thripidae							
Undetermined spp.	Pooled	240.3	(188.8)	9.9	(10.4)	0.1	(0.3)
	Chelicerata: Arachnida						
Acarina							
Undetermined spp.	Pooled	30.1	(42.6)	55.8	(89.5)	26.2	(36.1)
	Crustacea: Malacostraca						
Isopoda							
Undetermined spp.	Pooled	0.1	(0.3)	0.4	(0.5)	1.1	(2.3)

[a]Mean of 3 (ground covers significantly different) or 9 (ground covers were pooled) trees. Standard deviation is shown in parenthesis.

[b]Means followed by the same letter within a species and date are not significantly different by Duncan's multiple range test, 5% level. Data were pooled over ground covers if Fisher's F-test indicated that treatments were not different.

Five families of Hymenoptera were collected from pecan. Some of these such as Pergidae and Tenthredinidae, occasionally inflict significant damage to pecan. However, densities collected in this study were low and not affected by ground cover type.

Termites were more abundant during May in trees with grass than legume ground cover, but this was probably associated with the presence of a nearby termite colony rather than the ground cover.

Several Lepidoptera families, most of which feed on foliage, were collected. Densities of one family, Gelechiidae, were quite high during May when grass was present as the ground cover. *Orthosia hibisci* (Guenee) (Noctuidae) were abundant during May, but none were collected during July or September. Many of the Lepidoptera foliage feeders were collected during May, indicating that their combined damage may be a source of leaf loss or damage during the early spring. Several Lepidoptera foliage feeders were collected during July and September suggesting that they contribute to leaf loss throughout the growing season.

Few families of Orthoptera were collected from pecan. Acridids are rarely a problem on pecan, except when trees are small. Densities of other Orthoptera collected were low and are generally not considered a problem on pecan.

Barklice typically feed on molds, fungi, pollen, and dead insect fragments. We collected a few specimen during May and July.

Numerous Thysanoptera were collected during May, followed by a sharp decline in density during July, and even fewer in September. Thrips densities were similar regardless of ground cover type.

Large densities of Acarina were collected during each sampling date. These were not separated into predatory and phytophagous mites, and it is likely that both were present. Phytophagous mites pose a serious problem in some areas where pecans are grown, but rarely build to damaging levels in Oklahoma.

Isopoda have not been reported to cause economic damage to pecan. We collected a few specimen during each sampling period.

In conclusion, arthropods can cause substantial leaf loss in pecan (Ring et al. 1985). Results indicate that the ground covers tested in this study are unlikely to cause an increase in phytophagous arthropods that would be injurious to pecan. The legume ground covers used in this study attract beneficial arthropods (Smith et al. 1994), that may suppress certain pest species in pecan.

ACKNOWLEDGEMENTS

Approved for publication by the Dean of the Oklahoma Agricultural Experiment Station. This project was supported by U.S. Department of Agriculture Low Input Sustainable Agriculture Southern Region grant number LS91-36-63 and the Oklahoma Agriculture Experiment Station.

LITERATURE CITED

Arnold, D. C., and W. A. Drew. 1988. The Pentatomidea (Hemiptera) of Oklahoma. Okla. Agri. Expt. Sta. Bull. T-166.

Boethel, D.J. 1981. Resistance in the pecan leaf scorch mite, *Eotetranychus hicoriae* (McGregor) (Acari: Tetranychidae): implications in pecan pest management. Misc. Pub. Entomol. Soc. Am. 12:31-44.

Bugg, R. L., and J. D. Dutcher. 1989. Warm-season cover crops for pecan orchards: horticultural and entomological implications. Biol. Agri. and Hort. 6:123-148.

Bugg, R. L., and J. D. Dutcher. 1993. *Sesbania exaltata* (Rafinesque-Schmaltz) cory (Fabaceae) as a warm-season cover crop in pecan orchards: effects of aphidophagous coccinellidae and pecan aphids. Biol. Agri. Hort. 9:215-229.

Bugg, R. L., and R. T. Ellis. 1990. Insects associated with cover crops in Massachusetts. Biol. Agri. Hort. 7:47-68.

Bugg, R. L., J. D. Dutcher, and P. J. McNiell. 1991a. Cool-season cover crops in the pecan orchard understory: effects on Coccinellidae (Coleoptera) and pecan aphids (Homoptera: Aphididae). Biol. Cont. 1:8-15.

Bugg, R. L., M. Sarrantonio, J. D. Dutcher, and S. C. Phatak. 1991b. Understory cover crops in pecan orchards: possible management systems. Am. J. Alt. Agri. 6:50-62.

Bugg, R. L., S. C. Phatak, and J. D. Dutcher. 1990. Insects associated with cool-season cover crops in southern Georgia: implications for pest control in truck-farm and pecan agroecosystems. Biol. Agri. Hort. 7:17-45.

Dutcher, J.D., and U.T. Htay. 1985. Resurgence and insecticide resistance problems in pecan aphid management. pp. 17-29. *In* W.W. Neel, W.L. Tedders, and J.D. Dutcher [eds.], Aphids and phylloxeras of pecan. Univ. Georgia Expt. Sta., Spec. Publi. 38.

Edelson, J. V., and P. M. Estes. 1987. Seasonal abundance and distribution of predators and parasites associated with *Monelliopsis pecanis* Bissell and *Monellia caryella* (Fitch) (Homoptera: Aphidae). J. Entomol. Sci. 22:336-347.

Erdman, L.W. 1967. Legume inoculation: what it is, what it does. Agricultural Research Service, U.S.D.A., Farmers Bull. No. 2003.

Ferguson, C. S., M. J. Linit, and G. Krause. 1991. Host-plant preference of the Asiatic oak weevil (Coleoptera: Curculionidae). Environ. Entomol. 20:1427-1432.

Lakin, K. R. 1972. An investigation of the damage associated with *Tinocallis caryaefoliae* (Davis) feeding on pecan leaves. M.S. Thesis, Dept. Entomol., Auburn Univ. 47p.

Liao, H-t., M. K. Harris, F. E. Gilstrap, D. A. Dean, C. W. Agnew, C. J. Michels, and F. Mansour. 1984. Natural enemies and other factors affecting seasonal abundance of the blackmargined aphid on pecan. Southwest. Entomol. 9: 404-420.

Mizell lll, R.F. 1991. Pesticides and beneficial insects: application of current knowledge and future needs. p 47-54. *In* B.W. Wood and J.A. Payne [eds.], Pecan Husbandry: challenges and opportunities. First National Pecan Workshop Proceedings. A.R.S., U.S.D.A., ARS-96.

Rice, N. R. 1994. Assessment of legume and non-legume ground covers and augmentation of aphid predators for low-input pecan management. M.S. Thesis, Dept. Hort. L.A., Oklahoma State Univ. 132p.

Ring, D. R., M. K. Harris, and R. Olszak. 1985. Life tables for pecan leaves in Texas. J. Econ. Entomol. 78:888-894.

Smith, M. W., D. C. Arnold, R. D. Eikenbary, N. R. Rice, A. Shiferaw, B. S. Cheary, and B. L. Carroll. 1996. Influence of ground cover on beneficial arthropods in pecan. Biol. Control 6:164-176.

Smith, M. W., R. D. Eikenbary, D. C. Arnold, B. S. Landgraf, G. G. Taylor, G. E. Barlow, B. L. Carroll, B. S. Cheary, N. R. Rice, and R. Knight. 1994. Screening cool-season legume cover crops for pecan orchards. Am. J. Alt. Agri. 9:127-135.

Tedders, W. L. 1983. Insect management in deciduous orchard ecosystems: habitat manipulation. Environ. Mgmt. 7:29-34.

Tedders, W. L., B. W. Wood, and J. W. Snow. 1982. Effects of feeding by *Monelliopsis nigropunctata, Monellia caryella,* and *Melanocallis caryaefoliae* on growth of pecan seedlings in the greenhouse. J. Econ. Entomol. 75:287-291.

Wood, B. W., W. White, and W. L. Tedders. 1983. Use of legumes in pecan orchards. Ann. Rept. Northern Nut Growers' Assn. 74:147-150.

Wood, B. W., and W. L. Tedders. 1982. Effects of an infestation of blackmargined aphid on carbohydrates in mature 'Stuart' pecan. HortScience 17:236-238.

Wood, B. W., W. L. Tedders, and C. C. Reilly. 1988. Sooty mold fungus on pecan foliage suppresses light penetration and net photosynthesis. HortScience 23:851-853.

FLIGHT PERIODS IN TEXAS OF THREE PARASITES (DIPTERA: PYRGOTIDAE) OF ADULT *PHYLLOPHAGA* SPP. (COLEOPTERA: SCARABAEIDAE), AND EGG PRODUCTION BY *PYRGOTA UNDATA*[1]

R. L. Crocker, L. A. Rodriguez-del-Bosque[2] W. T. Nailon, Jr., and X. Wei

Texas Agricultural Experiment Station,
Texas A&M University Research & Extension Center,
17360 Coit Road; Dallas, Texas 75252-6599

ABSTRACT

Flights by *Pyrgota undata* Wiedemann and *Sphecomyiella valida* (Harris) (Diptera: Pyrgotidae), parasites of adult *Phyllophaga* spp. (Coleoptera: Scarabaeidae), were monitored three and a partial fourth years at Dallas, Texas, by means of UV light traps. Pyrgotid flight periods in other Texas counties were estimated from museum specimens dating back to 1926. *Pyrgota undata* were trapped in Dallas County from 5 Apr–15 May; museum specimens for other Texas counties span 24 Mar (Brazos and Williamson Counties) through 7 Jun (Gonzales County). Only two *S. valida* were taken in Dallas County, one on 21 Apr and one on 10 May; records for other Texas counties cover 9 May (Brazos County) through 25 Jul (Williamson County). A third pyrgotid, *Boreothrinax maculipennis* (Macquart), was not collected in Dallas County; its records elsewhere in Texas range from 26 Mar (Anderson County) to 5 May (Cherokee County). Late season *Phyllophaga* flights escape exposure to pyrgotids. *Pyrgota undata*, *S. valida*, and *B. maculipennis* records include 7, 15, and 7 Texas counties, respectively; their overall sex ratios were 40:10, 22:28, and 17:7 females:males, respectively. *Pyrgota undata* females contained 0–161 (mean = 84.5, median = 91.0) eggs. No seasonal trend was evident in the numbers of eggs per *Py. undata* female.

INTRODUCTION

The larvae (white grubs) of certain *Phyllophaga* spp. (Coleoptera: Scarabaeidae: Melolonthinae) are highly destructive pests of many cultivated plants including turfgrasses, forage grasses, corn, small grains, strawberries, potato tubers, sugar cane, and young nursery trees. In Texas and northeastern Mexico, *Phyllophaga crinita* Burmeister is the key pest in the group, although *Ph. congrua* (LeConte) and other species at times also cause significant damage. Even though many natural enemies of *Phyllophaga* are known, present white grub management practices are based almost exclusively on the use of chemical pesticides.

Parasitic flies (Diptera: Pyrgotidae) are among the diverse natural enemies of *Phyllophaga* spp. and other scarabaeids. Pyrgotids include about 330 species worldwide,

[1] For clarity, '*Phyllophaga*' is abbreviated '*Ph*'. and '*Pyrgota*' is abbreviated '*Py.*' throughout the paper.

[2] Campo Experimental Río Bravo, CIRNE-INIFAP-SARH, A.P.172, Río Bravo, Tamps., 88900, México.

eight of which occur in five genera in America north of Mexico (Steyskal 1978). A female pyrgotid parasitizes an adult scarabaeid by assaulting a selected host at night while the latter is in flight or feeding. In effecting oviposition, the fly thrusts its sharp sclerotized ovipositor into the soft dorsal surface of the host's abdomen and inserts an oblong white egg into the beetle's haemocoel (Burks 1952, Davis 1919, Forbes 1907, Steyskal 1978).

Pyrgota undata Wiedemann and *Sphecomyiella valida* (Harris) (Diptera: Pyrgotidae) are widely occurring North American parasites of adult *Phyllophaga* spp. (Davis 1913, 1916, 1918, 1922, 1929). Until now little has been reported beyond their geographic range, some of their hosts in the northern United States, and fragmentary information on their dates of occurrence in a few locations. Davis (1919) recorded the parasites' known geographic ranges and hosts. Of the 19 *Phyllophaga* spp. known to be hosts of one or both parasites (Davis 1919), 14 inhabit Texas and four others are found in states adjoining Texas (Luginbill and Painter 1953).

Pyrgota undata and *S. valida* occur as far north as Manitoba and Ontario, Canada, respectively; both species range in the United States from Arizona to North Dakota to Texas and eastward (Davis 1919, Lago 1981, Steyskal 1965, 1978). Petch and Hammond (1926) and Poprawski (1994) reared *Py. valida* from *Phyllophaga anxia* (LeConte) in Quebec, Canada. *Boreothrinax maculipennis* (Macquart) (Diptera: Pyrgotidae) is known from Kentucky to Maryland, south to Texas and Florida (Steyskal 1965), and west to Iowa and Arizona (Steyskal 1978).

Burks (1952) speculated on the synchronization of beetle and fly generations. Davis (1919) stated that female beetles are utilized more frequently as hosts, and that small species of beetles parasitized by *Py. undata* may produce a higher proportion of male flies than do larger beetles. Davis noted that parasitized beetles live only 10–14 days, and that *Py. undata's* developmental time from oviposition to pupation is about 3 weeks. He estimated that the parasite's egg stage lasts 5–6 days, with the entire life cycle normally taking one or rarely two years. Only one larva develops in each host (Davis 1919, Steyskal 1987).

Due to pyrgotids' nocturnal habits, they are difficult to collect although both sexes are attracted to light (Davis 1919) and occasionally moderate numbers are collected in light traps (Forbes 1907, Gates and Peters 1962, Lago 1981, Harris and White 1984). Petch and Hammond (1926) recovered as many as 20 *Py. undata* from light traps on peak nights. Other than at light, the most commonly cited means of collection has been from parasitized hosts. We know of no previous studies of the reproductive flights or reproductive potential of pyrgotids.

Petch and Hammond (1926) wrote that because less than 0.1 percent of *Ph. anxia* adults in flight were parasitized by *Py. undata*, the fly probably was not an effective check. Clausen (1940) concluded that pyrgotids appear to be of very little value in the control of scarabaeid pests; in contrast, Steyskal (1978, 1987) and Lago (1981) stated that pyrgotids can be responsible for marked decreases in the populations of white grubs. The paucity of information on pyrgotids precludes definitive acceptance of either conclusion. Our objective was to further bioecological research on pyrgotids by elucidating what species are present in Texas, their seasonal occurrence, which local *Phyllophaga* spp. are known or probable hosts, and the oviposition potential of *Py. undata*.

MATERIALS AND METHODS

Pyrgotid and *Phyllophaga* spp. adults were trapped at the Texas A&M University (TAMU) Research and Extension Center in northern Dallas, TX, in 1992, 1994, and 1995 for the entire flight period of *Phyllophaga* spp. (Apr–Sep). In 1993, pyrgotids were

sampled 1–15 May. Daily collections were made using four general purpose UV light insect traps (similar to model 110103-2, Ellisco, Inc., Philadelphia, PA) equipped with reflective baffles and a 15 w fluorescent bulb (G.E. F15T8-BL). The traps (ca. 100 m apart) were visually separated by a large building, so that they were independently sampling approximately the same surrounding habitat (mixed crop land, forage grasses, and turfgrasses). Pyrgotid flies and adult scarabs were removed daily from each trap, identified, and preserved in 75% isopropanol. Fourteen female *Py. undata* collected across their flight season were dissected, and the numbers and development of eggs per ovary were recorded. Ranges of dates for *Phyllophaga* spp. flights in Dallas County during pyrgotid flight periods are based on 1979–1994 season-long daily UV light trapping (RLC unpublished data).

The pyrgotids in the TAMU Department of Entomology Insect Collection in College Station, TX, were catalogued as to species, sex, date, site of collection, and method of collection. These flies included representatives of three species (*B. maculipennis*, *Py. undata*, and *S. valida*) taken in various years back through 1926 in Brazos County and elsewhere in Texas. It is not know how long trapping was conducted at these sites; however, our experience in Dallas County suggests that the 1931 Brazos County data may be complete. Ranges of dates for *Phyllophaga* spp. flights in Brazos County during pyrgotid flight periods are based on 1982 and 1983 season-long daily UV light trapping in Bryan (RLC unpublished data).

Analysis of variance and regression analyses were preformed using SuperAnova® version 1.11 (Gagnon et al. 1989). Descriptive statistics were computed using StatView® version 4.01 (Haycock et al. 1993).

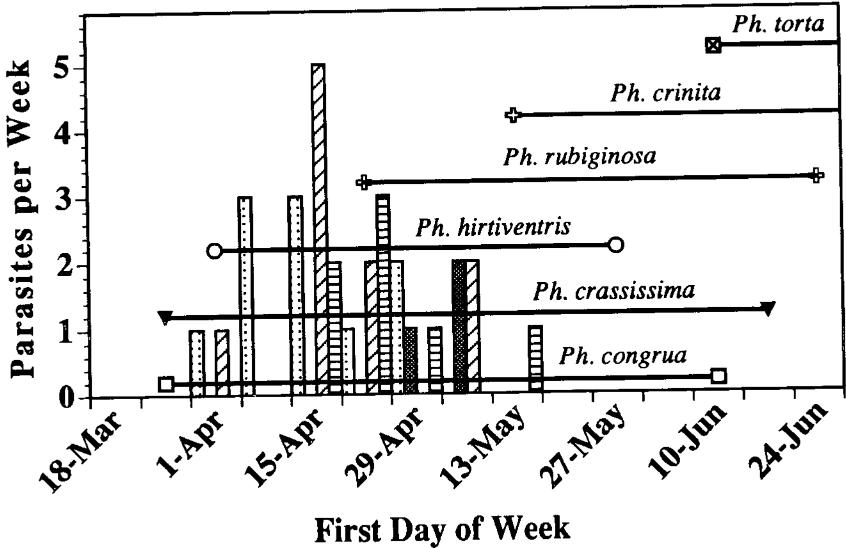


FIG. 1. Total weekly numbers of *Pyrgota undata* collected in Dallas, Texas, in UV light traps in 1992 (light grey), 1993 (dark grey; partial year's data), 1994 (diagonal stripes), and 1995 (horizontal stripes). Horizontal lines (at arbitrary heights) depict local historical date ranges for flight activity of *Phyllophaga* spp. contemporaneous with the parasites.

RESULTS AND DISCUSSION

Pyrgota undata were captured in Dallas 6 Apr–2 May 1992, 3 May–8 May 1993 (trapping in 1993 was only 1–15 May) , 5 Apr–10 May 1994, and 19 Apr–15 May 1995 (Fig. 1). The earliest and latest seasonal collection dates on museum specimens of *Py. undata* from Brazos County are 24 Mar (1930) and 4 May (1931), with all but three individuals being captured 8–23 Apr (Fig. 2a). Miscellaneous museum specimens ('f' and 'm' denote 'female' and 'male', respectively) are from the following Texas counties: Erath (1f, 26 Apr 1982; 1f, 28 Apr 1982), Gonzales (1f, 7 Jun 1969), Montgomery (1f, 27 Apr–3 May 1987), San Augustine (1f, 24 Apr 1989), and Williamson (1f, 1 Jun 1961; 1f 24 Mar 1963; 1f, 25 Mar 1963; 1m, 28 Mar 1963). It appears that *Py. undata* fly somewhat earlier in Texas than in Illinois (where *Py. undata* are most common 20–24 May), but that they probably resemble *Py. undata* in Illinois in being univoltine (Davis 1919). Petch and Hammond (1926) captured *Py. undata* even later (3– 24 Jun) in Quebec, Canada.

Although *Py. undata* have been collected more frequently in Texas than have either of the other two pyrgotids, our four UV light traps in Dallas captured a total of only 30 specimens over three and a fraction years, and the TAMU Insect Collection contains but 31 Texas specimens collected since 1930. Lago (1981) first reported pyrgotids in Mississippi; he catalogued 19 *Py. undata* collected 10 Apr–8 Jul (1916–1980). These low numbers of *Py. undata* for Texas and Mississippi contrast with the "very conspicuous numbers" (Davis 1919) or "abundant" (Forbes 1907) specimens reported for Illinois.

Sphecomyiella valida also appears to be uncommon in Texas. The TAMU Insect Collection (with dates as early as 1922) includes only 30 of the flies from Brazos County (Fig. 2) plus 19 specimens from elsewhere in Texas. Seasonally the Brazos County collection dates are 9 Mar (1926) to 26 Jun (1979), with one individual (excluded from Fig. 2) collected between 23 Oct and 6 Nov (1988); all except four specimens were collected by 19 Apr (1931). Our four Dallas traps took only one male and one female of *S. valida*, collected 21 Apr 1995 and 10 May 1993, respectively. Miscellaneous museum specimens represent the following other Texas counties: Anderson (2f, 10 Apr 1966; 1f, 14–21 Jul 1974), Aransas (1m, 22 Apr 1962), Brewster (1f, 24 Jul 1968), Cameron (1f, 19–20 Jun 1981), Cherokee (1f, 6 Apr 1962), Dimmitt (1m, 5 Apr 1935; 1f, 1 May 1936), Erath (1f, 9 Jun 1982; 1f, 11 Jun 1982), Hidalgo (1f, Jun 1981), Mill (1m, 21 Apr 1922), Sutton (1f, Apr 1931), Tom Green (1m, 7 May 1932), Walker (1f, 6 May 1971), and Williamson (1f, 7 Apr 1965; 1f, 25 Jul 1968). Lago (1981) detailed 21 specimens collected 7 Apr–28 Aug (1905–1980) in Mississippi.

Boreothrinax maculipennis was the least commonly encountered of the pyrgotids. It was not found in Dallas traps, and specimens (1930–1982) in the TAMU Insect Collection included only 17 individuals from Brazos County and seven of the flies from other Texas Counties. TAMU Insect Collection specimens from Brazos County range seasonally only from about 26 Mar (1982) through 18 Apr (1930) as follows: 1f, 26 Mar–2 Apr 1982; 1f, 27 Mar 1967; 1m, 29 Mar 1967; 1m, 1 Apr 1949; 1f, 2 Apr 1967; 1f, 4 Apr 1940; 1m + 1f, 5 Apr 1967; 1f, 8 Apr 1979; 3f, 14 Apr 1931; 1f, 14 Apr 1957; 3f, 15 Apr 1931; 1f, 18 Apr 1930. Other specimens in the TAMU Insect Collection were taken 26 Mar (1986) through 5 May (1952) in the following Texas counties: Anderson (3m, 10 Apr 1966), Cherokee (1f, 5 May 1952), Hidalgo (1f, 26 Mar 1986),Liberty (1f, 4 May 1934), and Williamson (1m, 13 Apr 1965). This range of dates is much narrower than are those of the other two pyrgotids. Lago (1981) noted that he had captured *B. maculipennis* only once in Mississippi and detailed 24 Mississippi records dating 1970–1979.

Davis (1919) described *S. valida* as being less common than *Py. undata* in Illinois. Texas data show considerable variability among sites as to apparent relative abundances of

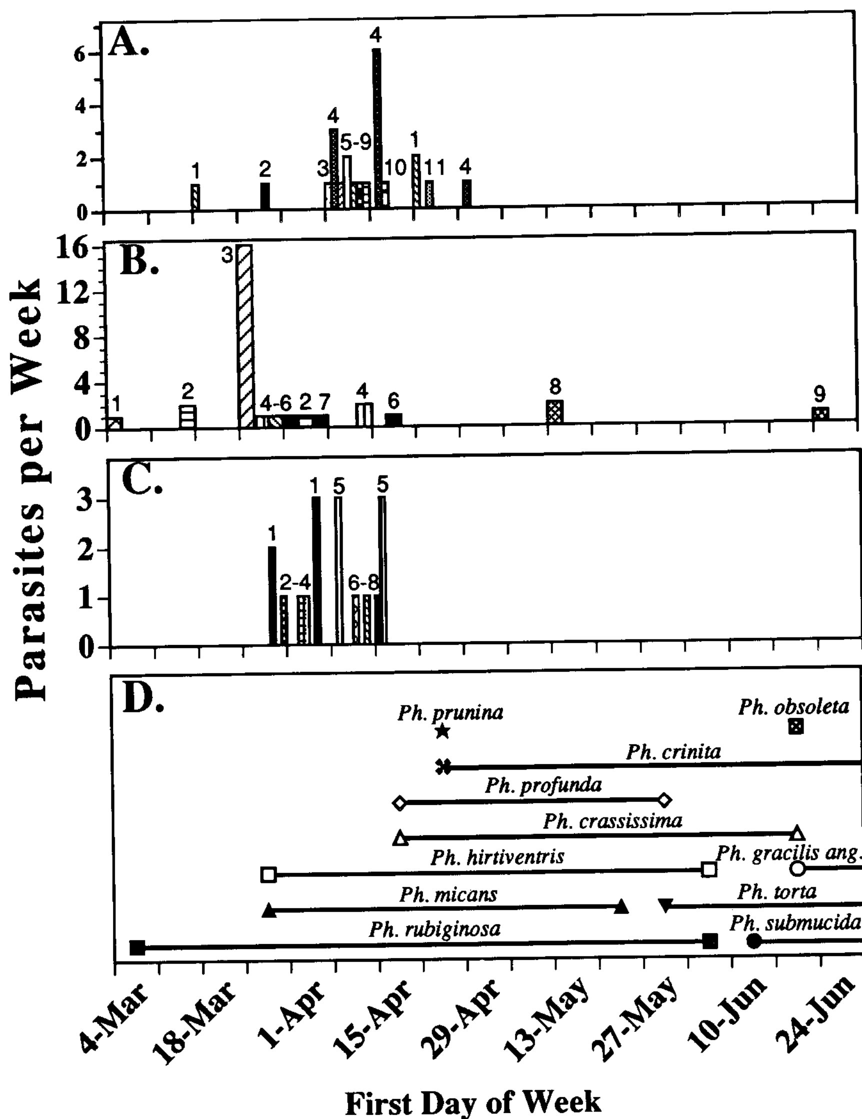


FIG 2. Total weekly numbers of adult pyrgotids in miscellaneous collections from Brazos County, Texas, versus potential host species of *Phyllophaga*. A. *Pyrgota undata* taken 1930 (#1), 1967 (#2), 1946 (#3), 1931 (#4), 1949 (#5), 1965 (#6), 1969 (#7), 1978 (#8), 1979 (#9), 1943 (#10), and 1937 (#11). B. *Sphecomyiella valida* collected 1926 (#1), 1949 (#2), 1929 (#3), 1964 (#4), 1982 (#5), 1931 (#6), 1978 (#7), 1957 (May, week unknown) (#8), and 1979 (#9). C. *Boreothrinax maculipennis* for 1930 (#8), 1931 (#5), 1940 (#3), 1949 (#4), 1957 (#7), 1967 (#1), 1979 (#7), and 1982 (#2). D. Horizontal lines (at arbitrary heights) depict local historical date ranges for flight activity of *Phyllophaga* spp. contemporaneous with the parasites.

species. These data suggest that *Py. undata* [represented by 62 specimens (11 males plus 46 females plus 5 unsexed) from 7 counties] occurs widely in eastern Texas with females trapped about 4x more frequently than are males. Petch and Hammond (1926) noted that 68.7% of 73 *Py. undata* dissected from light–trapped *Ph. anxia* adults were female. *Sphecomyiella valida* [with 51 specimens (29 males plus 22 females) collected in 16 counties] appears to be encountered at about a 3:2 m:f sex ratio, with populations from East Texas to at least as far west as the Big Bend area. *Boreothrinax maculipennis* [with 24 specimens (7 males plus 17 females) collected in 6 counties] may be the least abundant of the Texas pyrgotids, with females of this species about 2x more commonly captured than are males. These estimated sex ratios may be an artifact of collection techniques; also, the high concentration of entomologists at TAMU in Brazos County appears to bias the geographic distribution of data.

Pyrgota undata flights in Dallas County (Fig. 1) are well timed for parasitization of its known host, *Ph. hirtiventris*, as well as for possible hosts *Ph. crassissima* [a host of *S. valida* (Davis 1919)] and *Ph. congrua*. It also may utilize the early portion of flights by *Ph. rubiginosa* (LeConte) and the very earliest flight activity of *Ph. crinita*, but *Ph. torta* (LeConte), *Ph. glabricula* (LeConte), and *Ph. affabilis* occur too late in the year to be potential hosts. Further south in Brazos County, flights of *Py. undata* apparently begin before any potential hosts other than *Ph. rubiginosa* are available (Fig. 2a, d). However, *Ph. hirtiventris* and *Ph. micans* are present during most of the parasite's reproductive season, and the earliest flying *Ph. crassissima*, *Ph. profunda*, *Ph. prunina*, and *Ph. crinita* are concomitant with the trailing edge of *Py. undata*'s flight period. Brazos County *Py. undata* have ceased to fly, however, by the time *Ph. torta*, *Ph. gracilis angulata* Glascow, *Ph. obsoleta* (Blanchard), and *Ph. submucida* (LeConte) are present. It may be that the termination of *Py. undata* flights in Dallas and Brazos Counties, while potential and known hosts still are available, is due to limited adaptation to environmental stresses in the southern extreme of the parasite's range. At the northern extreme of the parasite's range, Petch and Hammond (1926) observed that *Py. undata* in Quebec, Canada, were not seen until over 3 weeks into the flight period of *Ph. anxia*.

Flights of *S. valida* in Brazos County begin too early to take advantage of its known host, *Ph. crassissima*. Although most of the records for *S. valida* are concentrated early in the flight seasons of *Ph. rubiginosa*, *Ph. micans*, and *Ph. hirtiventris*, this parasite's reproductive period overlaps those of at least eight other *Phyllophaga* spp. (Fig. 2b, d). The flights of *S. valida* in Dallas County agree with those of *Ph. crassissima*, *Ph. congrua*, *Ph. hirtiventris*, and *Ph. rubiginosa*; however, little can be drawn from the collection of two parasites (21 Apr and 10 May). No hosts of *B. maculipennis* are known, but the data suggest *Ph. rubiginosa*, *Ph. micans*, and *Ph. hirtiventris* as likely hosts in Brazos County (Fig. 2c, d).

In Indiana, Davis (1919) found 54 and 98 eggs in two female *Py. undata*. Our *Py. undata* contained 0–161 ($n = 14$, mean = 84.5, S.D. = 55.5, skewness = -0.167, kurtosis = -1.240, median = 91.0) eggs per female. The negative kurtosis value indicates that the distribution of egg counts is platykurtic (i.e., more dispersed than in a 'normal' distribution). The negative skewness of the distribution and the median being greater than the mean indicate that most of the extreme counts were less than the mean. Egg counts for the right ovary and left ovary of a given female were highly correlated ($F = 95.1676$, $P < 0.0001$, $R^2 = .896$). Neither regression analysis (df 1, 12; F 0.20; $P = 0.66$) nor plotting of the data indicated that the number of eggs per female changes as the season progresses.

Although pyrgotids are widely distributed over Texas, more work is needed to elucidate their geographic ranges, host preferences, and impact as natural enemies of *Phyllophaga*. Both of the reported host species in Dallas County, *Ph. hirtiventris* and *Ph.*

crassissima, tend to be much less abundant than the dominant spring species, *Ph. congrua* (R.L.C., unpublished data). Field data are needed to determine whether parasitism is a factor in this. Although *Py. undata* and *S. valida* only are known to parasitize *Phyllophaga* spp., the total literature on these pyrgotids and on *B. maculipennis* is much too limited to exclude the possibility that they may also parasitize scarabaeids in other genera. Most of the *Py. undata* collected in UV light traps in Dallas were females; it is unknown whether they were attracted to trapped potential hosts rather than simply to the light. Conversely, captured male parasites may have been responding in some cases to female parasites in or around the trap. It would be desirable to test traps baited with hosts or with parasites.

ACKNOWLEDGMENT

We thank Michael Klein (USDA, ARS Horticultural Insects Research Laboratory, OARDC, Wooster, Ohio), Rosa Maria Crocker, Allen E. Knutson, James A. Reinert, and Edgar A. Rojas (Texas A&M University REC, Dallas, Texas) for critical review of this manuscript.

LITERATURE CITED

Burks, B. D. 1952. Insects, enemies of insects, p. 373–380. *In* A. Stefferud [ed.], Insects: the yearbook of agriculture, 82nd Congress, 2nd Session, House Document No. 413. US Government Printing Office. 780 p.

Clausen, C. P. 1940. Entomophagous insects. McGraw–Hill Book Co., Inc. NY. 688 p.

Cole, F. R. 1969. The flies of Western North America. Univ. CA Press, Berkeley. 693 p.

Davis, J. J. 1913. Common white grubs. USDA Farmers' Bull. 543: 1–20.

Davis, J. J. 1916. A progress report on white grub investigations. J. Econ. Entomol. 9: 261–281.

Davis, J. J. 1918. Common white grubs. USDA Farmers' Bull. No. 940: 1–28.

Davis, J. J. 1919. Contributions to a knowledge of the natural enemies of *Phyllophaga*. Ill. Nat. Hist. Surv. Bull. 13: 53–138 + 15 pl.

Davis, J. J. 1922. Common white grubs. USDA Farmers' Bull. No. 940. 30 p.

Davis, J. J. 1929. Common white grubs. USDA Farmers' Bull. No. 940: 1–27.

Forbes, S. A. 1907. On the life history, habits, and economic relations of the white grubs and May-beetles. Univ. Ill. Agric. Exp. Sta. Bull. 116: 447–480.

Gagnon, J., J. M. Roth, M. Carroll, R. Hofmann, K. A. Haycock, J. Plamondon, D. S. Feldman, Jr., and J. Simpson. 1989. SuperANOVA: accessible general linear modeling. Abacus Concepts, Inc., Berkeley, CA. 316 p.

Gates, D. E., and L. L. Peters. 1962. Insects in Kansas. Ext. Div., Kansas State Univ., Manhattan (2nd ed.). 307 p.

Harris, K. M., and I. M. White. 1984. Diptera. International course on applied taxonomy of insects and mites of agricultural importance. Commonw. Inst. Entomol., British Museum (Natural History), London.

Haycock, K. A., J. Roth, J. Gagnon, W. F. Finzer, and C. Soper. 1993. Statview: the ultimate integrated data analysis & presentation system. Abacus Concepts, Inc., Berkeley, CA. 466 p.

Lago, P. K. 1981. Records of Pyrgotidae from Mississippi, with additional notes on the distribution of *Sphecomyiella valida* (Harris) and *Pyrgota undata* Wiedemann (Diptera). Entomol. News 92: 115–118.

Luginbill, P., Sr., and H. R. Painter. 1953. May beetles of the United States and Canada. USDA Tech. Bull. 1060, Washington, D.C. 102 p.

Petch, C. E., and G. H. Hammond. 1926. Parasites of white grubs in southern Quebec: a progress report. Entomol. Soc. Ontario Ann. Rep. 56:85–91.

Poprawski, T. J. Insect parasites and predators of *Phyllophaga anxia* (LeConte) (Col., Scarabaeidae) in Quebec, Canada. J. Appl. Entomol. 117: 1–9.

Steyskal, G. C. 1965. Family Pyrgotidae, p. 657–658. *In* A. Stone [ed.], A catalog of the Diptera of America north of Mexico. USDA, ARS Agric. Handbook 276.

Steyskal, G. C. 1978. Synopsis of the North American Pyrgotidae (Diptera). Proc. Entomol. Soc. Wash. 80: 149–155.

Steyskal, G. C. 1987. Pyrgotidae, p. 813–816 *In* J. F. McAlpine, B. V. Peterson, G. E. Shewell, H. J. Teskey, J. R. Vockeroth, and D. M. Wood, [eds.], Manual of Nearctic Diptera, Vol. 2, Research Branch Agric. Canada Monogr. 28, Biosystematics Res. Inst., Ottawa, Canada.

COMPORTAMIENTO DE UNA NUEVA TRAMPA PARA ESCARABAJOS MELOLONTIDOS[1], DESTRUCTORES DE RAICES

M. Camino-Lavín, A. Jiménez-Pérez, V. Castrejón-Gómez, F. Castrejón-Ayala y R. Figueroa-Brito

Centro De Desarrollo De Productos Bióticos-IPN. Km. 8.5 Carr. Yautepec-Jojutla. Apartado Postal 24, Yautepec, Morelos, México.

ABSTRACT

A new trap for melolonthid scarabs, named CSAT-m, is described and studied in order to understand how it works. Eight chemicals from pineapple were evaluated as attractants with this trap, while ripe pineapple was used as control. Temperature of air inside and outside the CSAT-m trap was recorded during the study. The occurrence of a full solar eclipse in the area provided an oportunity to study these temperature differencials, observing a 5°C difference in a short time between inside and outside the trap. Methyl acetate and ethyl acetate were the most volatile of the tested compounds; their use resulted in the most insects captured, specially the melonthids *Cotinis mutabilis*, *Cyclocephalla guttata* and *Phyllophaga* spp. These beetles are important pests because the adults feed on economic fruit and their larvae damage the roots of crops. Highest numbers of insects were attracted and captured when these chemicals were used during sunny days, when more chemicals were dispersed. Trap efficiency should be considered in terms of level of lure dispersion, not just in terms of time of operation.

RESUMEN

Se estudió el comportamiento de una trampa para escarabajos melolóntidos denominada CSAT-m. Se probaron 8 substancias químicas de la piña madura en 8 trampas CSAT-m, teniendo como testigo una trampa CSAT cebada con piña madura. Se registró la temperatura dentro y fuera de las trampas CSAT-m, así como durante un eclipse total de sol. Los acetatos de etilo y de metilo fueron las substancias más volátiles y atrajeron el mayor número de especies, destacándose los melolóntidos mayates destructores de raíces: *Cotinis mutabilis*, *Cyclocephalla guttata* y *Phyllophaga* spp. Estos fueron capturados en días soleados cuando ocurre en mayor grado la dispersión de los cebos. Durante el eclipse, la temperatura descendió 5°C. Se propone que la eficiencia de una trampa de este tipo sea medida en función del tiempo en que existe intercambio de aire dentro de la trampa y no del tiempo en uso.

INTRODUCCION

El uso de trampas para capturar insectos se emplea con diferentes propósitos que quedan englobados en dos objetivos: Monitoreo y supresión de poblaciones. Es importante conocer el funcionamiento de una trampa, sobre todo cuando un cebo está involucrado, para optimizar la captura de insectos. Entre los principales factores que se deben de conocer están: a) Las condiciones ambientales que favorecen el buen funcionamiento de la trampa (día, noche, temperatura, etc.); b) el diseño propio de la trampa; c) las propiedades químicas de los cebos utilizados (punto de ebullición, peso molecular, etc.), ya que de estas dependerá la volatilización de las substancias químicas; y d) el funcionamiento, es

---

[1] Coleoptera: Scarabeidae: Melolonthinae

decir, de como se liberan los cebos de las trampas y son dispersados por el viento. Cupperus et al. (1990) mencionan que cuando se usan trampas es importante elegir las condiciones ambientales ideales para colocar las trampas, ya que hay un "efecto de sitio" o preferencia de los insectos hacia algunas trampas que se encuentran colocadas en situaciones óptimas para la especie a colectarse (temperatura, humedad, viento, luminosidad, etc.). Camino (1975) diseñó una trampa para capturar el picudo de la palma de coco, *Rhynchophorus palmarum* (Linneo), usando como atrayente o cebo frutas en fermentación. La trampa fue hecha de una cubeta de plástico (24 cm de ancho y 35 cm de alto), con una cubierta a manera de techo y un frasco de vidrio de 3 l en la parte inferior, en donde se coloca el cebo (2 kg de fruta, más un litro de agua), la trampa se cuelga a 1.2 m sobre la superficie del suelo. La trampa se denominó "CSAT", el mejor cebo fue la piña madura.

Camino et al. (1992) probaron en condiciones de laboratorio algunas substancias aisladas de la piña (Ping Wu et al. 1991) para atraer a *R. palmarum* y reportaron que la atracción hacia algunos compuestos se presentó solo cuando había una alta humedad y durante la noche. En el presente trabajo se evalúa el comportamiento de una nueva trampa basada en una modificación de la trampa CSAT, para la captura de escarabajos (mayates) destructores de raíces (Scarabeidae: Melolonthinae), considera las condiciones ambientales y sus efectos sobre el funcionamiento de la trampa y la evaporación de los cebos químicos usados.

MATERIALES Y METODOS

Trampa CSAT Modificada.- Se desarrolló una trampa la cual es una modificación de la trampa "CSAT" (Fig. 1) reportada por Camino (1975). La nueva trampa denominada CSAT-m se diferencia de la anterior por tener un frasquito de 8 ml en lugar de un frasco de 3 l, donde se coloca el atrayente, una cubierta plana sobre la boca de la cubeta, y en su parte media, un embudo de plástico de 6 cm de diámetro por donde penetran los insectos (Fig. 1).

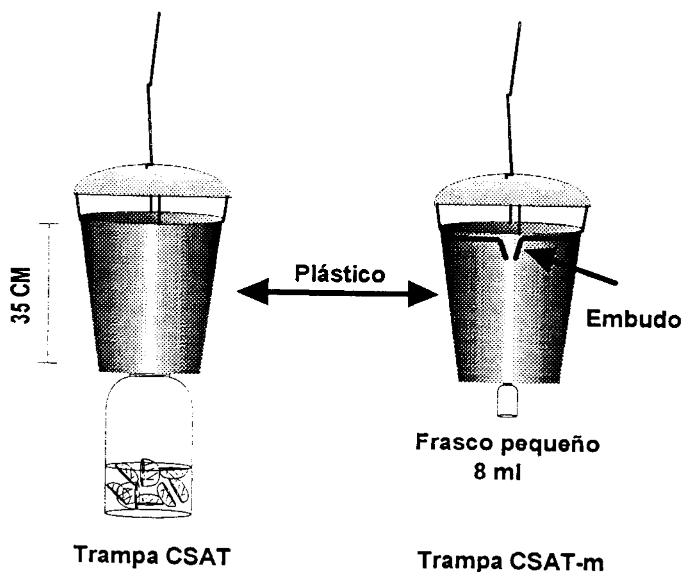


Fig. 1 Trampa CSAT y CSAT modificada

El trabajo se realizó en el campo experimental del Centro de Desarrollo de Productos Bióticos (CEPROBI) del Instituto Politécnico Nacional, localizado a 5 km al sur de la ciudad de Yautepec, Morelos, México. En la estación experimental se encuentran algunos cultivos como maíz (*Zea mays*), papaya (*Carica papaya*), *Bromelia hemisphaerica* y *Leucoprenma mexicana*. La vegetación natural está compuesta por *Ipomea* spp, *Prosopis juliflora*, *Caesalpinia* spp, *Leucaena glauca* y otros. Existe una estacionalidad bien marcada, siendo la época seca de diciembre a mayo y la húmeda de junio a diciembre.

Nueve trampas amarillas (este color fue usado para coleópteros en la trampa CSAT (Camino 1975)) fueron colocadas en sitios diferentes en el campo experimental y fueron colgadas de árboles frutales a 1.2 m de altura. Las diferentes substancias químicas aisladas de piña madura se colocaron en los frasquitos de vidrio de la trampa CSAT-m (Tabla 1). La trampa 9 (CSAT) sirvió como testigo, ya que contenía piña madura. Las substancias químicas identificadas de la piña fueron obtenidas de Aldrich Chem. Co. (Ciudad de México, D.F. México).

TABLA 1. *S*ubstancias *P*robadas en las *T*rampas *M*odificadas

TRAMPA No.	NOMBRE QUÍMICO	% PUREZA	PUNTO DE EBULLICIÓN (°C)	PESO MOLECULAR
1	LACTATO DE ETILO	98%	154	118.13
2	ISOVALERATO DE ETILO	98%	131-133	130.19
3	ACETATO DE ETILO	99%	77	86.13
4	METIL VALERATO	99%	128	116.16
5	BUTIRATO DE ETILO	99%	120	116.16
6	ACETATO DE METILO	99%	57.5	74.08
7	BUTIRATO DE METILO	99%	102-103	102.13
8	4-ALIL-1,2-DIMETOXIBENCENO	99%	254-255	178.28
9	PIÑA MADURA		TESTIGO (2 kg en 1 l de agua)	

Durante junio de 1991, se tomó la temperatura dentro y fuera de las trampas CSAT-m, usando un termómetro digital Modelo N-08528-60 (Cole-Parmer) con dos terminales o microconectores (exactitud de ± 0.05% de lectura). Después de que las substancias químicas fueron colocadas dentro de las trampas, se registró la temperatura interna y externa de las mismas. Las temperaturas se tomaron en tiempos diferentes en días en que se presentaron condiciones climáticas diferentes; se tomaron a las 8:00, 9:00, 15:00 y 16:00 hr en los días y condiciones climáticas de: junio 24, nublado; junio 25, soleado; junio 26, lluvioso; junio 27, lluvioso y nublado; y agosto 3, lluvioso y nublado. Se midió la cantidad de las 8 substancias que se evaporó diariamente bajo prueba y cuando se evaporaron los 5 ml colocados inicialmente, se llenaron de nuevo los frasquitos.

Los insectos capturados fueron colocados en frasquitos con alcohol al 70% y enviados al Colegio de Postgraduados en Ciencias Agrícolas, (Montecillos, Estado de México, México), para su identificación taxonómica (realizada por MC Socorro Anaya). Se realizó un análisis estadístico de los datos de las temperaturas, calculando el coeficiente de correlación entre las temperaturas dentro y fuera de las trampas.

*E*clipse *S*olar.- El día 11 de julio de 1991, se presentó un eclipse total de sol en el área de estudio y esto se tomó como modelo de "noche corta" y fue utilizado para obtener información sobre el comportamiento de la trampa CSAT-m, bajo estas circunstancias, ya que ocurrieron cambios climáticos importantes, sobre todo de temperatura

durante el eclipse total de sol. Durante el eclipse, se escogieron al azar tres trampas CSAT-m de las nueve colocadas en el campo experimental. En estas tres trampas se registró la temperatura del aire dentro y fuera de las mismas, cada 5 minutos durante el tiempo que duró el eclipse total de sol. Se tomaron temperaturas desde las 12:25 a 13:20 primera fase: obscuridad parcial; y de las 13:28 a 14:20 (tercera fase: obscuridad parcial). Durante la segunda fase u obscuridad total de las 13:21 a las 13:27 se registró la temperatura cada minuto. Durante el 15 de julio de 1991, se tomaron temperaturas dentro y fuera de las mismas trampas a las mismas horas y tiempos en que el día del eclipse se había presentado (11 de julio). El análisis estadístico de los datos durante y después del eclipse se realizó igual que durante la primera parte.

RESULTADOS Y DISCUSIÓN

El acetato de metilo (6) y el acetato de etilo (3) fueron los compuestos más volátiles (Fig. 2), por lo cual requirieron mayor cantidad; se agregaron 5 ml cada dos días para el primero y cada tres para el segundo. El 4-alil-1,2-dimetoxibenceno (8) fue el menos volátil, y la evaporación de los 5 ml tomó 10 días. Esto concuerda con el peso molecular y el punto de ebullición de las substancias probadas (Tabla 1).

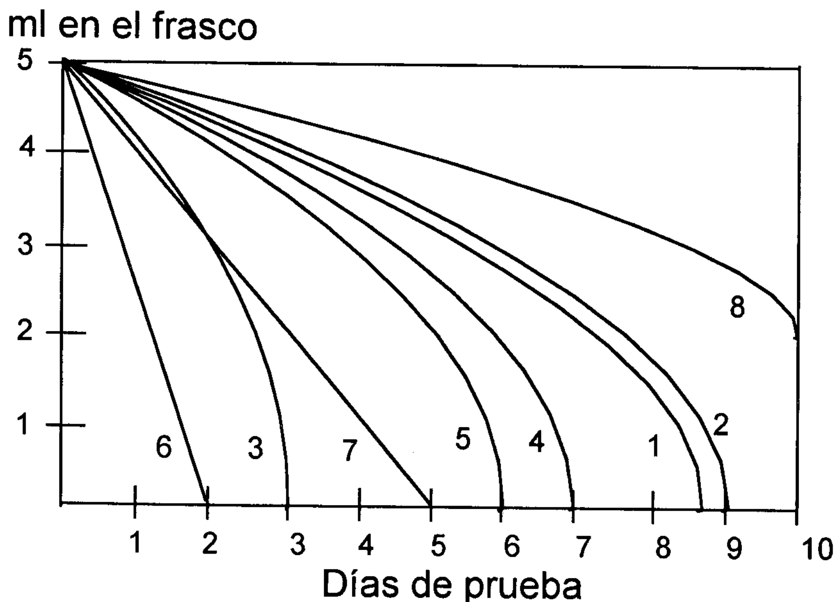


Fig. 2. Evaporación de las substancias químicas probadas (ver Tabla 1 para los compuestos).

El coeficiente de correlación (r) de las temperaturas dentro y fuera de las trampas CSAT-m fue bajo en días lluviosos o nublados (Fig. 3), mientras que en condiciones soleadas la correlación fue alta (más de 0.8). Esto puede explicarse por la diferencia en temperaturas dentro y fuera de la trampa, resultando en un intercambio de aire debido a supuestas corrientes de aire convectivas, las cuales acarrearían con si los vapores de los atrayentes.

La trampa testigo cebada con piña madura capturó el mayor número de especies de insectos, pertenecientes a 5 órdenes: Coleoptera (Scarabeidae: Melolonthinae, Cerambycidae, Carabidae, Cucujidae, Elateridae, Chrysomelidae), Lepidoptera (Nymphalidae, Noctuidae, Geometridae, Arctiidae, Lycaenidae), Diptera (Sarcophagidae, Tachinidae, Muscidae, Pyrgotidae, Drosophilidae), Hemiptera (Alydae, Reduviidae) e Hymenoptera (Vespidae, Sphecidae). Se destacaron los escarabajos melolóntidos *Cotinis mutabilis*, *Cotinis* spp, *Cyclocephalla guttata* y *Phyllophaga* spp; en total, 22 familias y 23 especies. La trampa CSAT-m cebada con acetato de etilo capturó insectos en 5 órdenes: Coleoptera (Scarabeidae:Melolonthinae), Lepidoptera (Noctuidae, Geometridae, Arctiidae, Lycaenidae), Diptera (Drosophilidae), Hemiptera (Reduviidae), Hymenoptera , (Vespidae, Sphecidae); en total, 9 familias y 10 especies, sobresaliendo *Cotinis* sp. y *Cyclocephalla guttata*. Siguió en orden de captura, el butirato de etilo con 4 órdenes: Coleoptera (Scarabeidae: Melolonthinae), Lepidoptera (Noctuidae, Lycaenidae), Diptera (Calliphoridae), y Hemiptera (Reduviidae); en total, 5 familias y 7 especies, sobresaliendo *C. guttata*, *Cyclocephalla* sp y *Phyllophaga* sp. Siguió el acetato de metilo con 4 familias y 6 especies, no encontrándose ningún melolóntido, pero si califóridos (Diptera) tales como *Cochliomyia* sp. La trampa cebada con 4-alil-1,2-dimetoxibenceno capturó insectos de 2 órdenes y 3 familias, pero ningún melolóntido (Coleoptera). El lactato de etilo e isovalerato de etilo capturaron tres especies de insectos en dos familias; entre ellas *C. guttata* y *C. mutabilis*, y por último, el metil valerato y butirato de metilo capturaron solo una especie cada uno.

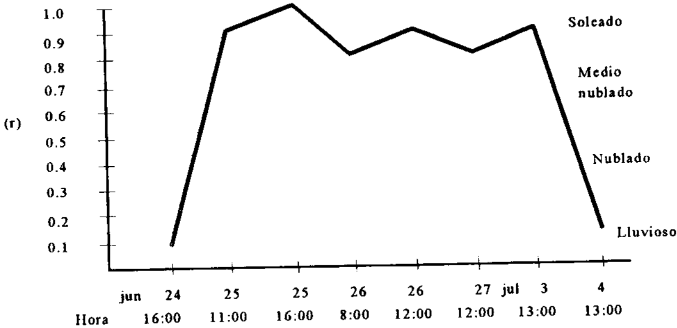


FIG. 3. Coeficiente de correlación (r) entre las temperaturas dentro y fuera de las trampas CSAT-M

Las especies de melolóntidos antes mencionadas son de importancia agrícola, ya que los adultos ocasionan daños a frutos con cáscara blanda facilitando el ataque por otros insectos y propiciando la presencia de hongos que causan pudriciones (Morón y Terón 1988). Además, las larvas de *Phyllophaga* destruyen el sistema radicular de las plantas, produciendo una disminución del número de frutos y semillas y ocasionado su muerte. Las capturas se realizaron en días soleados o medianamente nublados, desde el amanecer hasta el atardecer (junio 25-27 y julio 3).

Durante la obscuridad total causada por el eclipse, la temperatura ambiental bajó 5°C, después fue constante durante 6 minutos y luego se elevó de nuevo 5°C. Las inversiones de temperatura se presentaron después de la segunda fase del eclipse, por lo que se asume que el mismo fenómeno ocurre que el explicado por la figura 3.

Hubo diferencia significativa (P=0.05) entre las temperaturas externas e internas en los días de julio 10 y julio 15, y hubo diferencia altamente significativa (P=0.01) el día del eclipse (julio 11). Esto puede explicarse por la falta de inversiones de temperatura durante la obscuridad total del eclipse, mientras que si hubo inversiones de temperatura en el día 15 a esta misma hora (13:24 a 13:30 hr). De aquí podemos pensar que la mayor parte de las inversiones de temperatura ocurren durante el amanecer y que varias inversiones ocurren durante el día, sobre todo en días soleados y se capturarán mas especies en estas condiciones.

TABLA 2. Coeficiente de correlación (r) de temperaturas dentro y fuera de trampas CSAT-m en tres días diferentes [a]

Día	Temperatura	r	Significancia [b]
Antes del eclipse 10 Julio	Afuera Dentro	0.2362	S
Día del eclipse 11 Julio	Afuera Dentro	0.8768	AS
Después del eclipse 15 Julio	Afuera Dentro	0.3288	S

[a] Las temperaturas se midieron al mismo tiempo de 13:22 a 13:36 cada minuto (n=5)

[b] S= Significativo (P=0.05%)
AS= Altamente significativo (P= 0.01%)

La trampa CSAT-m funciona mejor que la CSAT y durante el presente estudio se conoció cuando ocurren las inversiones de temperatura y como ocurren, por lo que se recomienda usar estas trampas en días soleados o medianamente nublados. Esta información propone que la eficiencia de una trampa de este tipo debe ser medida en función del tiempo en que existe un intercambio de aire y no del tiempo cronológico.

AGRADECIMIENTO

Esta contribución es parte del proyecto DEPI 945307 del Instituto Politécnico Nacional. Los autores son becarios COFAA-IPN.

LITERATURA CITADA

Camino-Lavín, M. 1975. Capture of *Rhynchophorus palmarum* (L.), a coconut palm pest in colored traps in three plant communities in Sánchez Magallanes and Paraíso, Tabasco, México. Folia Entomol. Mex. 33:63-64.

Camino-Lavín, M., A. Jiménez-Pérez y F. Castrejón-Ayala 1992. Pruebas de atrayentes químicos para la captura de *Rhynchophorus palmarum* (L.). Rev. Latinoamer. Quim. 22/23: 11-13.

Cupperus, G. W, W. S. Fargo, P.W. Flinn, and D. W. Hagstrum. 1990. Variables affecting capture of stored-grain insects in probe traps. J. Kans. Entomol. Soc. 63:486-489.

Morrón, M. A. y R. A. Terrón. 1988. Entomología Práctica. Instituto de Ecología A. C., México. 504 pp.

Ping-Wu, May-Chien Kuo, G. H. Thomas, R. T. Rosen and Chi-Tang Ho. 1991. Free and glycosidically bound aroma compounds in pineapple (*Ananas comosus* L. Merr). J. Agric. Food. Chem. 39: 170-172.

VARIACION TEMPORAL DE LA ARANEOFAUNA EN FRUTALES DE LA REGION DEL CABO, BAJA CALIFORNIA SUR MEXICO.

M. L. Jiménez y A. Tejas.

División de Biología Terrestre, Centro de Investigaciones Biológicas del Noroeste, Apdo. Postal 128, La Paz, B.C.S. 23000 México

ABSTRACT

A study was conducted from March, 1992 to February, 1993 of the spider fauna and its seasonal variation in a mango and citrus grove in an ejido in Baja California Sur, Mexico. Spider fauna was sampled 11 times/month along a 50 m transect. A total of 1,699 spiders in 41 species were obtained, of which 55% were wandering spiders and 45% were aerial web spinners. Most species collected belong to the families Theridiidae (19%), Araneidae (12%) and Salticidae (12%). The most abundant species were: *Lyssomanes pescadero* (28%), *Wulfila immacullela* (9%), *Leucauge venusta* (9%) and *Uloborus glomosus* (9%). The relative abundance of spiders was higher from early fall to spring, and lowest during summer. Because of its abundance an its hunting behavior, *L. pescadero* should be considered as a potential biological control of the Mexican fruit fly, *Anastrepha ludens*.

RESUMEN

Se realizó un estudio de la composición de especies de arañas en un huerto de mangos (*Mangifera indica L.*) y cítricos (*Citrus* spp.) en el Ejido "El Pescadero", Baja California Sur, México de marzo de 1992 a febrero de 1993. Se realizaron colectas diurnas mensuales a lo largo de un transecto de 50 m. Se obtuvo un total de 1,699 arañas de 41 especies, de las cuales el 55.2% fueron cazadoras errantes y 44.7% cazadoras de red. Las familias más ricas específicamente fueron Theridiidae (19.5%), Araneidae (12.1%), Salticidae (12.2%). Las especies más abundantes fueron *Lyssomanes pescadero* (28.3%), *Wulfila immaculella* (8.7%), *Leucauge venusta* (9.5%) y *Uloborus glomosus* (8.6%). La mayor abundancia relativa de las arañas se observó en otoño de 1992 a primavera de 1993. Por su abundancia y estrategias de captura, *L. pescadero* puede ser agente de control para la mosca mexicana de la fruta, *Anastrepha ludens*.

INTRODUCCION.

En los últimos 20 años se han realizado estudios que destacan la importancia de las arañas como controladores de plagas agrícolas, tanto en cultivos temporales como en huertos de cítricos (Muma 1975), y manzanos (Dondale *et al.* 1979). En estos agroecosistemas, las arañas constituyen una gran parte de los artrópodos depredadores de ácaros, áfidos y polillas

(Mansour *et al.* 1983). Su acción es muy eficaz en el control de plagas de insectos (Ibarra-Núñez 1990), lo cual permite una reducción en el uso de insecticidas y en los costos. No obstante, aún se desconoce su potencialidad y limitaciones como controladores biológicos (Nyffeler *et al.* 1988). Por lo que el conocimiento de su papel ecológico en las zonas agrícolas es preponderante, debido a su abundancia en ese tipo de ecosistemas (Dean *et al.* 1987, Breene *et al.* 1988).

Especies tales como *Argiope aurantia* Lucas y *Phiddipus audax* (Hentz) son depredadoras eficaces de la pulga saltona del algodón (*Pseudatomoscelis seriatus* (Reuter)) en Texas , así como de huevos y larvas del gusano elotero (*Heliothis armigera* (F.)) (Dean *et al.* 1987).

En Baja California Sur, los agroecosistemas están rodeados de extensas zonas cubiertas por vegetación xerófila, las cuales son contrastantes por su microclima, ya que los huertos presentan mayor humedad y menor variación diurna de temperaturas, lo cual puede favorecer la presencia de una gran diversidad de artópodos que encuentran ahí refugio y alimento. Por lo anterior, este trabajo tuvo como objetivos determinar la composición de las especies de arañas y su fluctuación temporal en un huerto de cítricos y mangos.

MATERIALES Y METODOS

El estudio se realizó de marzo de 1992 a febrero de 1993, en un huerto de 1.5 ha, sembrado con mangos (*Mangifera indica* L.), cítricos (*Citrus aurantius* L., *C. limetta* Risso (Christm.) y *Citrus* spp.) y otros, en un rancho del Ejido "El Pescadero" (23° 17' N y 110° 09' W), Baja California Sur. La proporción de árboles de mangos fue de 89.2% y la de cítricos 10.7%. El quelite (*Amaranthus palmeri* S. Wats), el tacote (*Viguiera tomentosa* A. Gray), la malva (*Melochia tomentosa* L.), el pasto buffer (*Cenchrus ciliaris* L.), y el huizapol (*Cenchrus echinatus* L.) dominaron el sustrato bajo del huerto. Se realizaron muestreos diurnos mensuales, durante once meses (en julio no se colectó) en un area de 50 x 30 m. En el estrato herbáceo como en el arbóreo, y a una altura de 2 m máximo, se utilizaron redes de golpeo de 50 cm de diámetro y de fondo plano 50 x 50 x 10. Los ejemplares se conservaron en alcohol etílico al 70% para su posterior identificación y cuantificación.

Datos de temperatura y precipitación fueron obtenidos de la estación ubicada a 1.5 km. del sitio de estudio y fueron proporcionados por la Secretaría de Agricultura y Recursos Hidráulicos de La Paz, B.C.S.

RESULTADOS Y DISCUSION

Se obtuvo un total de 1,699 arañas pertenecientes a 41 especies, 34 géneros y 16 familias. Las familias más ricas específicamente fueron: Theridiidae (19.5%), Araneidae (12.2%) y Salticidae (12.2%) (Tabla 1). Las especies más abundantes fueron *Lyssomanes pescadero* Jiménez y Tejas (28.36%), *Wulfila immacullela* (Gertsch) (8.71%), *Leucauge venusta* (Walckenaer) (9.53%) y *Uloborus glomosus* (Walckenaer)(8.64%). Las arañas cazadoras de red representaron el 44.7%, siendo la familia Theridiidae (ocho especies) la más diversa. Las cazadoras errantes fueron en un 55.2% dominando las de la familia Salticidae (cinco especies).

Los juveniles fueron los más abundantes (80%), seguidos de las hembras (13%) y los machos (7.0%). La abundancia relativa de arañas fue alta a principios de la primavera y paulatinamente fue descendiendo, hasta llegar al mínimo a mediados del verano. Desde finales de esta estación empezó a incrementarse considerablemente, tendiendo máximos en otoño e invierno (Tabla 2). Evidentemente, las especies que marcaron estos cambios fueron *Lyssomanes pescadero*, *Wulfila immaculella*, *Leucauge venusta* y *U. glomosus*. Se hizo un análisis de correlación entre las variables ambientales (temperatura media y precipitación mensuales) y la abundancia de las arañas y no se encontró ninguna correlación entre estos parámetros y las abundancias relativas ($r=-0.4$, ns, $p>0.05$), pero se observó que durante el verano (Tabla 2) esta sufrió un descenso notable debido al aumento de temperatura. Sin embargo, la presencia de arañas se mantuvo casi constante durante el resto del año, debido a las condiciones microclimáticas del huerto, propiciadas por el riego.

El huerto estudiado comparte once especies con comunidades de arañas registradas por Mansour, et al., (1982) y Young y Edwards (1990) en cítricos, alfalfa y maíz en Estados Unidos. De estas, *Leucauge venusta*, *Acacesia amata* (Hentz) y *Uloborus glomosus* son más frecuentes en caña de azúcar, maíz, algodón, alfalfa, soya y cítricos.

Durante el estudio, el huerto fue fumigado por los agricultores para combatir a la mosca mexicana de la fruta, *Anastrepha ludens*, y también fué objeto de otras prácticas agrícolas, como la quema de frutos, aunque no fue desyerbado. De acuerdo con Young y Edwards (1990), las prácticas agrícolas y la aplicación de insecticidas dañan más a las arañas tejedoras que a las errantes. En este estudio, las arañas fueron abundantes durante los meses de mayor humedad, debido al riego. Esto quizá pudo ser ocasionado por la protección que las malezas brindan a las arañas, especialmente a las tejedoras, ya que como indican Riechert y Lockley (1984), las plantas les proveen de un excelente refugio.

Las cazadoras de redes como *Dictyna mulegensis* Chamberlin, *Theridion crispulum* Simon y *Anelosimus studiosus* (Hentz) fueron más frecuentemente capturadas en árboles de mango y cítricos a 1.5 m de altura aproximadamente, en tanto que *Gasteracantha cancriformis* (L.) fué observada en árboles de mango a más de 4 m de altura. *Lyssomanes pescadero* fue la araña más numerosa en la vegetación arbórea, en el envés de las hojas. Su abundancia está correlacionada con la abundancia de la mosca mexicana de la fruta (Jiménez *et al.* 1990). Ambas poblaciones presentaron su abundancia máxima a principios del otoño. Sadana y Kumari (1991) demostraron que la araña *Lyssomanes sikkimensis* Tikader es un depredador excelente de la cigarra del mango ("mango hopper") *(Idioscopus clypealis* (Lethierry)), por lo que es de considerar que *L. pescadero* pudiera ser un agente de control biológico para la mosca mexicana de la fruta. Hasta hace 15 años, *Lyssomanes* spp. habían sido reportadas sólo para los trópicos húmedos (Galiano 1980). Jiménez y Tejas (1993) encontraron frecuentemente a dos especies de este género (*L. burrera* Jiménez y Tejas y *Lyssomanes* sp) en la vegetación riparia de la Sierra de La Laguna, B.C.S., en plena zona templada. Consideramos que en El Pescadero, la humedad debida al riego agrícola, y la vegetación arbórea, crearon un microhabitat que ha favorecido el establecimiento de las tres

TABLA 1. Composición y abundancia relativa (%) de arañas cazadoras de redes y cazadoras errantes de un huerto de mangos y cítricos del Ejido "El Pescadero", B.C.S., México, obtenida de colectas realizadas de marzo de 1992 a febrero de 1993. J= Juveniles; H= hembras; M=machos.

FAMILIA Especie	Abundancia (No.) J	 H	 M	 Total	 %
TEJEDORAS DE REDES					
ARANEIDAE					
Araneus pegnia (Walckenaer)	20	3	2	25	1.50
Eustala sp.	39	0	0	39	2.29
Larinia sp.	3	0	0	3	0.17
Gasteracantha cancriformis (L.)	5	1	2	8	0.47
Acacesia hamata (Hentz)	7	0	0	7	0.41
DICTYNIDAE					
Dictyna mulegensis Chamberlin	70	21	33	124	7.29
Dictyna tucsona Chamberlin	1	0	0	1	0.05
Dictyna varyna Chamberlin y Gertsch	3	1	2	6	0.35
Dictyna dauna Chamberlin y Gertsch	0	1	0	1	0.05
THERIDIIDAE					
Theridion crispulum Simon	57	34	37	128	8.00
Theridion possitivum Chamberlin	10	2	10	22	1.29
Theridion sp.	3	0	5	8	0.47
Euryopis taczanowskii Keyserling	5	0	4	9	0.52
Tidarren fordum (Keyserling)	2	0	2	4	0.23
Anelosimus studiosus (Hentz)	27	5	10	42	2.47
Argyrodes projiciens (O.P. Cambridge)	6	1	4	11	0.64
Argyrodes subdolus (O.P. Cambridge	10	0	2	12	0.70
TETRAGNATHIDAE					
Leucauge venusta (Walckenaer)	157	1	4	162	9.53
ULOBORIDAE					
Uloborus glomosus (Walckenaer)	90	16	28	134	8.00
Philoponella oweni (Chamberlin)	9	4	0	13	1.00
PHOLCIDAE	1	0	0	1	0.05
Subtotal	525	90	145	760	45.48
CAZADORAS ERRANTES					
ANYPHAENIDAE					
Aysha cambridgei Bryant	102	1	1	104	6.12
Wulfila immaculella (Gertsch)	142	1	5	148	8.71
CLUBIONIDAE					
Castanieira sp.	7	0	0	7	0.41
Cheiracanthium sp.	71	0	0	71	4.17
Trachelas speciosus Banks	3	1	1	5	0.29
GNAPHOSIDAE					
Cessonia sp.	3	0	0	3	0.17
HETEROPODIDAE					
Olios septicus Chamberlin	8	0	3	11	0.64
MIMETIDAE					
Mimetus hesperus Chamberlin	13	1	1	15	0.88
OXYOPIDAE					
Hamataliwa grisea Keyserling	14	0	0	14	0.82
Hamataliwa helia (Chamberlin)	1	1	0	2	0.11
SELENOPIDAE					
Selenops sp.	2	0	0	2	0.11
PISAURIDAE					
Tinus sp.	4	0	0	4	0.23
SALTICIDAE					
Lyssomanes pescadero Jiménez y Tejas	440	8	34	482	28.36
Sitticus peninsulanus (Banks)	7	8	15	30	0.17
Metaphiddipus sp.	2	1	2	5	0.29
Dendriphantes melanomerus Chamberlin	0	2	0	2	0.11
Thiodina sylvana Hentz	16	2	2	20	1.17
THOMISIDAE					
Misumenops dubius (Walckenaer)	4	1	3	8	0.47
Misumenoides sp.	2	1	0	3	0.17
Isaloides sp.	3	0	0	3	0.17
Subtotal	844	282	67	939	54.52
TOTALES	1369	118	212	1699	100.0

TABLA 2. Temperatura, Precipitación y Abundancia Relativa de Arañas de un Huerto Frutal de Baja California Sur, México, durante 1992-1993.

Estación	Temperatura X (° C)	Precipitación X (mm)	Abundancia relativa X(%)
Primavera	20.2	0.06	30.31
Verano	25.5	0.76	6.53
Otoño	25.7	0.56	32.37
Invierno	20.4	1.03	30.78

especies.

Leucauge venusta es una araña comunmente asociada a los sembrados de gramíneas, leguminosas, cítricos y algodón (Young y Edwards 1990, Mansour *et al.* 1982). Se establece en la vegetación baja (Alvarez del Toro 1992), o en bajos estratos de vegetación más alta y con gran humedad. *U. glomosus* comparte los mismos sembradíos con *L. venusta* excepto los cítricos (Young y Edwards 1990). Sin embargo, en este estudio, la relativa frecuencia de sus individuos de todas las edades, sugiere que puede tener varias generaciones al año, manteniendo una población casi constante.

Uloborus glomosus es dominante en los algodonales, donde los áfidos constituyen entre el 35 y 90% de su alimento (Nyffeler *et al.* 1989). Esto sugiere que la especie pudiera ser utilizada como un controlador biológico, aunque la disposición horizontal de su telaraña sólo le permite atrapar insectos corredores que se precipitan, permitiendo el escape de los voladores (Nyffeler y Dean 1989).

AGRADECIMIENTO

Agradecemos al Biol. Jorge Llinas, al Dr. G. Ibarra-Núñez y a los revisores anónimos por su comentarios y sugerencias a este manuscrito. Este estudio fué realizado por el apoyo económico del Centro de Investigaciones del Noroeste, S.C., la Secretaría de Educación Pública (SEP) y el Consejo Nacional de Ciencias y Tecnología (CONACyT).

LITERATURA CITADA

Alvarez del Toro, M. 1992. Arañas de Chiapas. Univ. Aut. de Chis. México. 297 pp.

Breene, R.G., W.L. Sterling, and D.A. Dean. 1988. Spider and ant predators of the cotton fleahopper on wolly croton. Southwest. Entomol. 3:177-183.

Dean, D.A., W.L. Sterling, M. Nyffeler, and R.G. Breene. 1987. Foranging by selected spiders predators on the cotton fleahopper and other prey. Southwest. Entomol. 12:263-270.

Dondale, C.D., B. Parent, and D. Pitre. 1979. A 6 year study of spiders (Araneae) in a Quebec apple orchard. Can. Entomol.111:377-380.

Galiano, M.E. 1980. Revisión del género *Lyssomanes* Hentz, 1845 (Araneae, Salticidae). Op. Lilloana 30:1-104.

Ibarra-Nuñez, G. 1990. Los artrópodos asociados a cafetos en un cafetal mixto del Soconusco. Chiapas I. Variedad y Abundancia. Fol. Entomol. Mex. 79:207-231.

Jiménez, M.L., A. Tejas, Y R. Servin. 1990. La mosca de la fruta del género *Anastrepha*. En: Uso y Manejo de los

Recursos Naturales en la Sierra de La Laguna, B.C.S., A. Ortega (Ed.) CIB. 233-249 pp.
Jiménez, M.L., and A. Tejas. 1993. Two new species of the genus *Lyssomanes* (Hentz) from the Cape Region, B.C.S. México. J. Arachnol. 21:205-208.
Mansour F., J.W. Ross, G.B. Edwards, W.H. Whitcomb and D.B. Richman. 1982. Spiders of Florida Citrus Groves. The Fla. Entomol. 65:514-522.
Mansour, F., D.B. Richman, and W.H. Whitcomb, 1983. Spider Management in Agroecosystems: Habitat Manipulation. Enviro. Manag. 7:43-49.
Muma, M.H. 1975. Spiders in Florida citrus groves. Fla.Entomol. 58:83-90.
Nyffeler, M., D.A. Dean, and W.L. Sterling. 1989. Prey selection and predatory importance of orb-weaving spiders (Araneae) (Araneidae, Uloboridae) in Texas cotton. Environ. Entomol. 18:373-380.
Riechert, S.E. and T. Lockley, 1984. Spiders as biological Control Agents. Ann. Rev. Entomol. 29:299-320.
Sadana, G.L. and Kumari, M. 1991. Predatory potential of the *Lyssomanes sikkimensis* Tikader on the mango hopper, Idioscopus clypealis (Lethierry). Entomol. 16:283-285.
Young, O. and G.B. Edwards. 1990. Spiders of United States field crops and their potential effects on crop pest. J. Arachnol.18:1-27.

VOL. 21 NO. 3 SOUTHWESTERN ENTOMOLOGIST SEPT. 1996
SCIENTIFIC NOTE

SCANNING ELECTRON MICROSCOPY OF THE OVIPOSITOR OF THE HORN FLY

D. E. Bay[1], S.M. Meola[2], and S. L. White[3]

Behavioral studies have demonstrated the antennae of the horn fly, *Haematobia irritans* (L.), to be the primary receptor organs responsible for attraction of the female to bovine manure pats for the purpose of oviposition (Bay, unpublished data). A survey of the sensilla located thereon has been published by White and Bay (1980). In addition to chemoreceptive sensilla located on the antennae and palps, the ovipositors of several muscoid fly species [e.g., *Musca autumnalis* De Geer, *Phormia regina* (Meigen), and *Lucilia cuprina* (Wied.)] have also been demonstrated to possess chemoreceptors permitting oriented movements of that organ as well as inciting oviposition itself (Hooper et al. 1972, Rice 1976, Merritt and Rice 1984, Merritt 1987). No information, however, exists concerning the elicitation of horn fly oviposition or the nature of the sensilla involved. The purpose of the present study, therefore, was to examine the types and distributions of sensilla on the horn fly ovipositor using scanning electron microscopy (SEM).

Ovipositors were excised and prepared for study by fixation for 3 h in a 3% solution of glutaraldehyde buffered to 7.4 pH with 0.2 Millonig's phosphate buffer (Millonig 1962) at 4°C. Following three rinses in a phosphate buffer rinse over 30 min, ovipositors were postfixed for 1 h in a 1% solution of phosphate-buffered osmium tetroxide and rinsed with phosphate buffer for an additional 30 min. Fixed samples were then dehydrated for 10 min each through an ascending series of ethanol (50, 75, 85, 95, and 100%, respectively) and critical point dried, mounted on aluminum studs, coated in vacuo with gold-palladium, and examined by scanning electron microscopy.

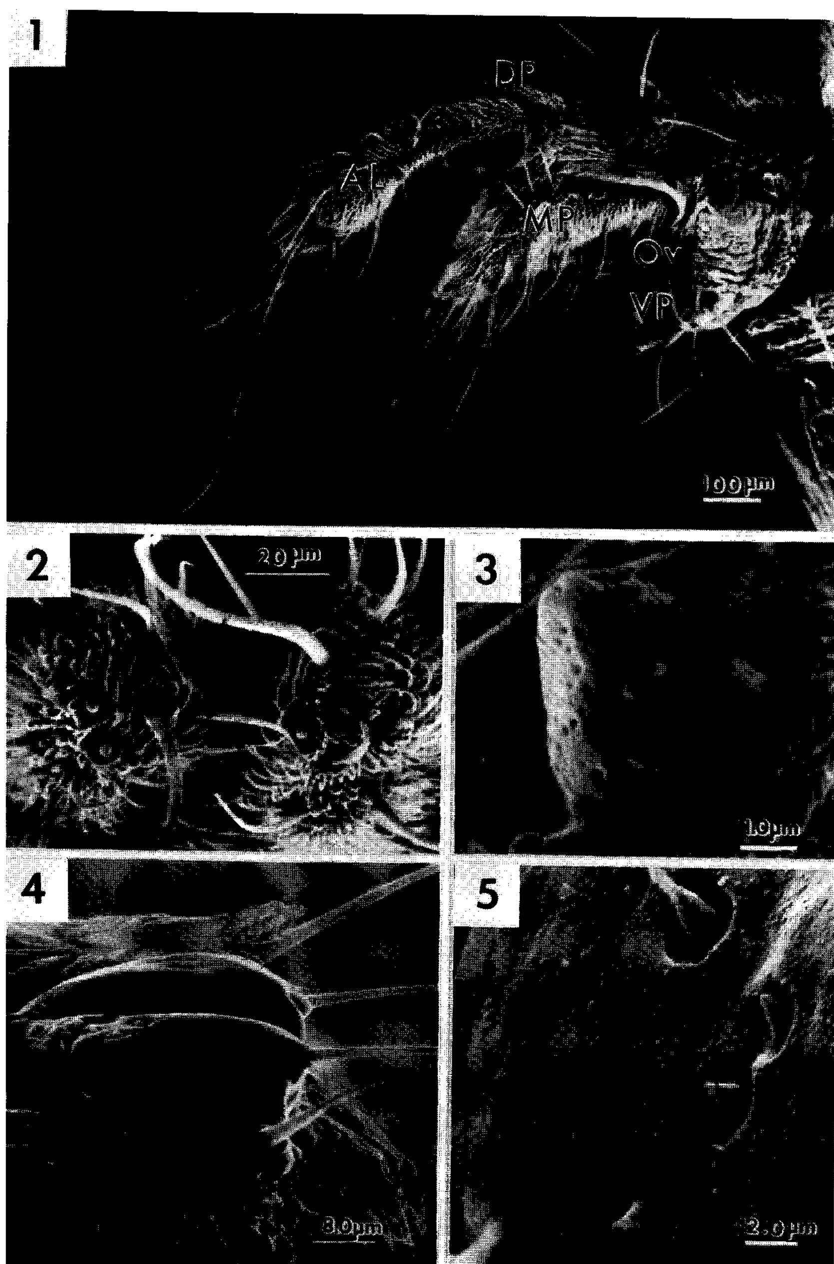
Morphologically, the ovipositor of the horn fly is similar to that of other muscoid flies (Wallis 1962, Hooper et al. 1972) in that it consists essentially of a telescoping tube formed by the modified 6th-9th abdominal segments retracted within the anterior portion of the abdomen. Hydrostatic pressure and intrinsic musculature appear responsible for extension and positioning movements. Rings of 8-10 articulated hairs averaging 110, 120, and 90 μ in length encircle the distal margins of the 6th, 7th, and 8th segments, respectively. All sensillia on the 9th abdominal segment are located on the following six plates: dorsal plate, paired anal leaflets, median plate, and paired ventral plates.

Dorsal Plate. The dorsal plate (Fig. 1) is a long, transversely curved structure, 175 μm long and 125 μm wide, situated anteriordorsal to the paired anal leaflets. Two pairs of posteriorly directed, tactile hairs are located distally on the plate; each inner and outer hair measures approximately 75 and 100 μm, respectively, in length. The remainder of the dorsal plate is densely beset with numerous microtrichiae.

Anal Leaflets. The anal leaflets (Fig. 1) are paired, bulbous, stalked structures situated on either side of the anal opening at the ovipositor terminus. Each leaflet is approximately 325 μm in length and 75 μm in width. The greatest accumulation of sensilla on the horn fly ovipositor occurs on these leaflets. As noted by Rice (1976) for the black blow fly, this accumulation of sensilla is not surprising since the anal leaflets form the leading edge of the egg-laying apparatus, and sensory feedback from this region is crucial in determining the suitability of ovipositional substrates.

Each anal leaflet has 27 long tactile hairs, ranging from approximately 25 μm to 600 μm in length. Most of these hairs are directed posteriorly and are admirably situated to monitor guidance of the ovipositor and physical placement of eggs within the manure pat ovipostional substrate. In addition, the terminal end of each leaflet also bears a pair of

[1] Department of Entomology, Texas A&M University, College Station, Texas 77843.
[2] USDA-ARS, Food Animal Protection Research Laboratory, College Station, Texas 77845.
[3] Lilly Research Laboratory, Greenfield, Indiana 46140.



FIGS. 1-5: Scanning electron micrograph of the horn fly ovipositor. 1. Lateroventral view of extended terminus showing the dorsal plate (DP), anal leaflets (AL), median plate (ML), ventral plates (VP), and oviduct opening (Ov). 2. Terminus of paired anal leaflets showing paired, multiporous sensory pegs. 3. Multiporous sensory peg on anal leaflet 4. Paired ventral plates. 5. Sunken cone-shaped receptors on ventral plates.

sensory pegs, ca. 3 μm in diameter by 4.5 μm in length (Fig. 2), each of which is situated on an elevated circular base ca. 12 μm in diamter. The surfaces of each peg are irregularly covered by numerous pores (Fig. 3), the presence of which are indicative of an olfactory function (Slifer et al 1957). As with the tactile hairs, these pegs are ideally located to receive chemical information of an olfactory nature from the ovipositional substrate.

Median Plate. Situated anterioventral to the anal opening and the paired anal leaflets, the flat, club-shaped medial plate (Fig. 1) averages approximately 185 μm in width and 275 μm in length. As with the dorsal plate, the median plate bears only tactile hairs. Fourteen pairs of these hairs, ranging in length from 20 μm basally to 50 μm distally are bilaterally distributed over the entire ventral surface. Two similar unpaired hairs, ca. 25 μm in length, are located basally and distally, respectively, along the midline. An additional 12 pairs of tactile hairs are located laterally on the posterior two-thirds of the plate. Although most of these latter hairs average approximately 100 μm in length, two of the more apical pairs exceed 300 μm. As with the dorsal plate, the remainder of the ventral plate is covered with numerous microtrichiae.

The external opening of the oviduct is situated ventrally, at the anterior margin of the medial plate, between segments 8 and 9 (Fig. 1). During the act of oviposition, eggs must pass beneath the medial plate as they exit this opening. Deflection of these articulated hairs would provide sensory feedback to the female, permitting monitoring of egg passage and its subsequent placement. Return of the deflected hairs to their normal position would indicate to the female that the egg had been placed in the ovipositional substrate so that another egg could be extruded.

Ventral Plates. The paired ventral plates (Figs. 1, 4), ca. 50 μm in width and length, occupy the area lateroventral to the oviduct opening. Each plate is characterized by a medial and lateral tactile hair measuring ca. 75 and 100 μm, respectively, in length. The posterior surface of each ventral plate also bears 4-5 sunken cone-shaped receptors (Figs. 4, 5), ca. 2 μm in length, which appear similar morphologically to the sensory pits described by Hooper et al. (1972) on the ventral plates of the ovipositor of the face fly. Unlike the other plates, microtrichiae are absent from these ventral plates.

In summary, this SEM study provides morphological evidence that the horn fly ovipositor bears both tactile and olfactory sensory organs. Tactile hairs are present on all the plates comprising the ovipositor, but are especially numerous on the median plate and the paired anal leaflets. Olfactory sense organs consist of paired pegs with pores situated on the terminus of each anal leaflet. We are unable to ascribe a role for the sensory structures located on the ventral plates.

LITERATURE CITED

Hooper, Ruth L., C. W Pitts, and Jane A. Westfall. 1972. Sense organs on the ovipositor of the face fly, *Musca autumnalis*. Ann. Entomol. Soc. Amer. 65:577-586.

Merritt, D. J. 1987. The cercal sensilla of the blow fly, *Lucila cuprina*. I. Structure of the sockets and distal dendritic regions. Tissue Cell. 19:287-300.

Merritt, D. J., and M. J. Rice. 1984. Innervation of the cercal sensilla on the ovipositor of the Australian sheep blow fly, *Lucilia cuprina* Physiol. Entomol. 9:37-48.

Millonig, G. 1962. Further observations on a phosphate buffer for osmium solutions in fixation. Proc. 5th Int. Congr. Electron Microsc. 2:8.

Rice, M. J. 1976. Contact chemoreceptors on the ovipositor of *Lucilia cuprina* (Wied.), the Australian sheep blow fly. Aust. J. Zool. 24:353-360.

Slifer, E.H., J.J. Prestage, and H.W. Beams. The fine structure of the long basiconic sensory pegs of the grasshopper (Orthoperta, Acrididae) with special reference to those on the antennae. J. Morphol. 101:359-397.

Wallis, D. I. 1962. The sense organs on the ovipositor of the blowfly, *Phormia regina* Meigen. J. Insect Physiol. 8:453-467.

White, Sandy L., and D. E. Bay. 1980. Antennal olfactory sensilla of the horn fly, *Haematobia irritans irritans* (L.) (Diptera:Muscidae). J. Kans. Entomol. Soc. 53:641-652.

VOL. 21 NO. 3 SOUTHWESTERN ENTOMOLOGIST SEPT. 1996
SCIENTIFIC NOTE

MAGNITUDE OF SURVIVAL OF OVERWINTERING SORGHUM MIDGE[1]

Dale A. Mott, George L. Teetes, and Michael I. Biggerstaff[2]

Department of Entomology
Texas A&M University
College Station, TX 77843-2475

No information exists on overwintering survival of sorghum midge, *Stenodiplosis sorghicola* (Coquillett), a key insect pest of sorghum, *Sorghum bicolor* (L.) Moench (Young and Teetes 1977). Sorghum midges overwinter as larvae within johnsongrass, *Sorghum halepense* (L.) Pers., and sorghum spikelets that fall to the ground and become covered with litter or are disked into the soil during crop residue destruction (Baxendale and Teetes 1983ab). Sorghum midges emerge in the spring to lay eggs of the first generation in flowering johnsongrass spikelets (Baxendale and Teetes 1983b). Adult female sorghum midges disperse from johnsongrass to the first flowering sorghum and can cause economic damage to later flowering sorghum after only a single additional generation (Baxendale et al. 1984b). Baxendale (1983) found that overwintered sorghum midges at College Station emerged between 26 April and the end of June but did not quantify the number of sorghum midges that entered diapause during the summer/fall of the preceding year. Knowledge of the magnitude of survival of overwintering sorghum midges would be beneficial to attempts to computer simulate sorghum midge population dynamics.

Field experiments were conducted at the Texas A&M University Research Farm, 13 km southwest of College Station. Mean number of diapausing sorghum midge larvae per square meter of field on 23 August 1994 was estimated based on 20.5 panicles per each of 20, 1-m^2 areas in the field, 36 rachis branches per each of 30 panicles, and number of diapausing sorghum midges per each of 45 rachis branches taken from each of the top, middle, and bottom thirds of 140 field-collected panicles. Diapausing sorghum midge larvae were detected by squeezing rachis branches in a squeeze device (Montoya 1965). Silken cocoons dissected from 10 spikelets that produced orange-colored droplets when squeezed verified the presence of diapausing larvae. The 140 panicles and 60 additional panicles randomly collected from the field on 23 August were kept dry at room temperature in a laboratory.

Overwintering survival was assessed by exposing diapausing sorghum midges to three sets of conditions. In January 1995, 48 pyramid traps, 12 in each of four replications, were set up in a field where sorghum residue had been shredded and disked 20 September 1994. Replications were randomly arranged in the field, as were the 12 pyramid traps per replication. Each 1-m^2 pyramid trap was constructed of a wooden frame covered by Lumite[R] screening. A canning jar, coated on the

[1]Diptera: Cecidomyiidae
[2]Department of Meteorology, Texas A&M University, College Station, TX 77843-3150

inside with vegetable oil, was screwed into an inverted lid attached to the top of the trap. Sorghum midges emerging after overwintering within sorghum spikelets in the soil flew into the jar and stuck in the oil. Used jars were replaced three times each week from 1 March - 30 June 1995. Sorghum midges in used jars were removed in a laboratory and placed in 70% ethanol. Samples of possible sorghum midges were sent for identification to Dr. Raymond J. Gagne, Research Entomologist, USDA Systematic Entomology Laboratory, Communications and Taxonomic Service Unit, Beltsville, MD.

Half of the 200 field-collected panicles were placed on 10 February 1995 in 10 flats in a greenhouse and covered with ≤10 cm of field-collected soil. Two flats were covered by a pyramid trap (previously described). Soil and panicles in flats were sufficiently moistened every 2 wk to wet all panicles. The remaining 100 panicles were on 24 February 1995 buried 2.54-10 cm deep in soil beneath five pyramid traps in an area of the field where no sorghum had been grown the previous 2 yr but near where the 200 panicles were collected and field emergence of sorghum midges was monitored.

A mean of 1.8 diapausing sorghum midges per rachis branch was found by using the squeeze device. This number corresponded to a mean of 1,325 sorghum midges per square meter that entered diapause in sorghum panicles shredded and disked in 1994.

Sorghum midges emerged for 69 d, from 11 April - 19 June, from sorghum residue in the field (Fig 1.). A total of 511 (10.6 per m^2) sorghum midges were captured in the 48 pyramid traps. Peak emergence was on 3 May, when a total of 42 (0.88 per m^2) sorghum midges emerged. Baxendale (1983) observed peak emergence approximately 10 d later. Estimated survival in the field, 0.8%, was based on numbers of sorghum midges entering diapause in 1994 and emerging in spring 1995 (Table 1.).

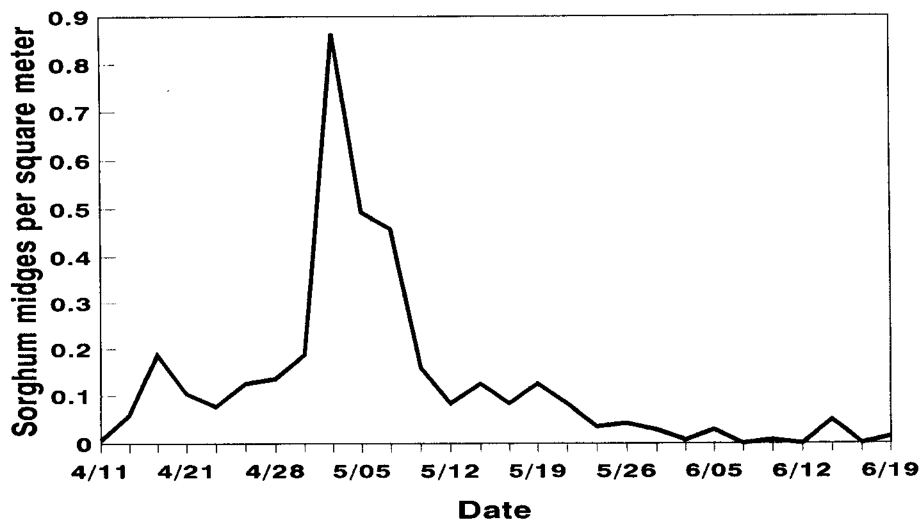


FIG. 1. Number of overwintered sorghum midges emerging per square meter in a field planted to sorghum the previous year, College Station, Texas, 1995.

TABLE 1. Number of Overwintering Sorghum Midges Per Square Meter Surviving to Emerge from Sorghum Spikelets, College Station, TX, 1995.

Treatment	Diapaused sorghum midges emerging per square meter[a]	Percent survival
Field - all winter	10.6a	0.8
Field - spring only	80.0b	6.0
Greenhouse	1161.0c	87.7

[a]Within a column, means followed by a different letter are significantly different, $P < 0.05$, LSD.

Significantly more sorghum midges emerged from field-collected sorghum panicles stored in a laboratory and then placed in the field (80 per m^2) or greenhouse (1161 per m^2) than from spikelets remaining in the field the entire winter. Six and 88% of sorghum midges diapausing in field-collected panicles brought into the laboratory for part of the winter survived to emerge when the panicles were placed during the spring in the field or greenhouse, respectively. Only 0.8% survived the winter in the field. The number of sorghum midges that emerged from panicles in the greenhouse was not very different than that estimated to have entered diapause in 1994.

Information on overwintering survival will be included in computer simulation models to improve prediction of sorghum midge population dynamics (Baxendale 1984ab, Jost 1993, Pendleton et al. 1994). Information in this paper will provide starting time and abundance of the first nondiapausing sorghum midge generation of the season.

ACKNOWLEDGMENT

This research was supported by grants 010366-145 from the Advanced Technology Program sponsored by the Texas Higher Education Coordinating Board and A.I.D./DAN-1254-G-00-0021-00 from the Agency for International Development through the Sorghum/Millet Collaborative Research/Support Program (INTSORMIL).

LITERATURE CITED

Baxendale, F. P. 1983. Modeling seasonal development of the sorghum midge, *Contarinia sorghicola* (Coquillett). Ph.D. dissertation, Texas A&M University, College Station.

Baxendale, F. P., and G. L. Teetes. 1983a. Factors influencing adult emergence from diapausing sorghum midge, *Contarinia sorghicola* (Diptera: Cecidomyiidae). Environ. Entomol. 12: 1064-1067.

1983b. Thermal requirements for emergence of overwintered sorghum midge (Diptera: Cecidomyiidae). Environ. Entomol. 12: 1078-1082.

Baxendale, F. P., G. L. Teetes, and P. J. H. Sharpe. 1984a. Temperature-dependent model for sorghum midge (Diptera: Cecidomyiidae) spring emergence. Environ. Entomol. 13: 1566-1571.

Baxendale, F. P., G. L. Teetes, P. J. H. Sharpe, and H. Wu. 1984b. Temperature-dependent model for development of non-diapausing sorghum midge (Diptera: Cecidomyiidae). Environ. Entomol. 13: 1572-1576.

Jost, D. J. 1993. Refinement and validation of the sorghum midge (Diptera: Cecidomyiidae) component of the grain sorghum crop growth model -- SORKAM. M.S. thesis, Texas A&M University, College Station.

Montoya, E. L. 1965. A squeeze device for detection of larvae of the sorghum midge, *Contarinia sorghicola* (Coquillett). J. Econ. Entomol. 58: 938-940.

Pendleton, B. B., G. L. Teetes, and M. E. Makela. 1994. Predicting sorghum midge (Diptera: Cecidomyiidae) generations and abundance. J. Econ. Entomol. 87: 993-998.

Young, W. R., and G. L. Teetes. 1977. Sorghum entomology. Ann. Rev. Entomol. 22: 193-218.

VOL. 21 NO. 3 SOUTHWESTERN ENTOMOLOGIST SEPT. 1996
SCIENTIFIC NOTE

EFFECT OF SORGHUM RESIDUE DESTRUCTION ON ABUNDANCE OF SORGHUM MIDGES[1] EMERGING FROM DIAPAUSE

Dale A. Mott, George L. Teetes, Bonnie B. Pendleton, and Roger M. Anderson

Department of Entomology
Texas A&M University
College Station, TX 77843-2475

The sorghum midge, *Stenodiplosis sorghicola* (Coquillett), is a key insect pest of sorghum, *Sorghum bicolor* (L.) Moench (Young and Teetes 1977). A proportion of larvae in each generation diapause within johnsongrass, *Sorghum halepense* (L.) Pers. or sorghum spikelets that fall to the ground and become covered with litter or are disked into the soil during crop residue destruction (Baxendale and Teetes 1983ab). Sorghum midges emerge in the spring from diapaused larvae to lay eggs of the first generation in johnsongrass (Baxendale and Teetes 1983b). The small, orange-colored flies disperse from johnsongrass to the first flowering sorghum and can cause economic damage after only a single additional generation (Baxendale et al. 1984). Larval feeding on developing kernels causes direct yield loss.

Little research has been conducted on effects of different crop residue destruction procedures on the number of overwintering sorghum midges that emerge in the spring, and only Mott (1995) has assessed the proportion of sorghum midges that survive overwintering. Roth and Pitre (1975), in Mississippi, assessed adult sorghum midge emergence from fields where sorghum crop residue from the previous year had not been disturbed, been plowed in mid-March, been disked in the fall and wheat planted in the field, or been disked and plowed in the fall and wheat planted in the field. They reported that tillage practices alone did not appreciably affect the number of adult sorghum midges emerging in the spring.

Field experiments were conducted at the Texas A&M University Research Farm, 13 km southwest of College Station. Mean number of diapausing sorghum midge larvae per square meter of field on 23 August 1994 was estimated to be 1,325. This estimate was based on 20.5 panicles per each of 20, 1-m^2 areas in the field, 36 rachis branches per each of 30 panicles, and 1.8 diapausing sorghum midges per each of 140 rachis branches from 140 field-collected panicles. Diapausing sorghum midge larvae were detected by squeezing rachis branches in a squeeze device (Montoya 1965). A silken cocoon dissected from each of 10 spikelets that produced an orange-colored droplet when squeezed verified the presence of a diapausing larva.

Crop residue in different areas of the field was destroyed on 20 September 1994 by shredding only, shredding and disking, or shedding, disking, and deep plowing. The three treatments were randomly arranged and replicated four times.

[1]Diptera: Cecidomyiidae

In January 1995, a set of four pyramid traps was placed in each replication of the three residue destruction treatment areas (a total of 48 traps). The 1-m^2 pyramid traps were constructed of a wooden frame covered by Lumite[R] screening. A canning jar, coated on the inside with vegetable oil, was screwed into an inverted lid attached to the top of the trap. Sorghum midges emerging from the soil after overwintering flew into the jar and stuck in the oil. Used jars were replaced three times each week from 1 March - 30 June 1995. Sorghum midges in used jars were removed in a laboratory and placed in 70% ethanol. Samples of possible sorghum midges were sent for identification to Dr. Raymond J. Gagne, Research Entomologist, USDA Systematic Entomology Laboratory, Communications and Taxonomic Service Unit, Beltsville, MD.

A total of 386 sorghum midges emerged between 18 April and 19 June 1995 into the 48 pyramid traps. Sorghum midges emerged for 49, 62, and 50 d from areas where sorghum residue had been shredded only, shredded and disked, and shredded, disked, and deep plowed, respectively (Fig. 1). Peak emergence occurred between 1 and 7 May. Approximately 40 sorghum midges emerged from each crop residue destruction treatment on the day of peak emergence. More than 3.5 times as many sorghum midges had emerged by 3 May from shredded and disked residue (72) as from the other two treatments combined (20).

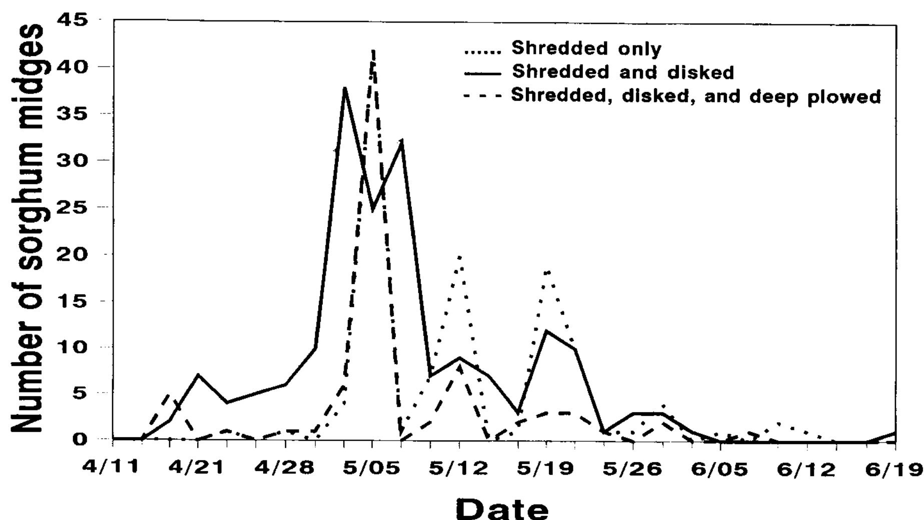


FIG. 1. Number of overwintered sorghum midges to emerge from shredded only, shredded and disked, and shredded, disked, and deep-plowed sorghum residue, College Station, Texas, 1995.

Significantly fewer (F = 3.59, P = 0.03) sorghum midges emerged from shredded, disked, and deep-plowed residue (82, 51,244 per hectare) than from shredded and disked residue (185, 115,614 per hectare) (Table 1). One hundred nineteen (74,366 per hectare) sorghum midges emerged from shredded only sorghum residue.

TABLE 1. Number of Overwintering Sorghum Midges Emerging from Shredded Only, Shredded and Disked, or Shredded, Disked, and Deep-Plowed Sorghum Residue, College Station, TX, 1995.

Treatment	Total number of sorghum midges[ab]	Number of sorghum midges per hectare
Shredded only	119ab	74,366
Shredded and disked	185a	115,614
Shredded, disked, and deep plowed	82b	51,244

[a]Within a column, means followed by the same letter are not significantly different at the 5% probability level, by LSD.
[b]Total number of sorghum midges to emerge into 16 pyramid traps per treatment.

These results differ from those of Roth and Pitre (1975). Shredding, disking, and deep plowing sorghum residue led to highest mortality and lowest emergence of overwintering sorghum midges. Forty-four and 64% fewer sorghum midges emerged from shredded, disked, and deep-plowed residue as from shredded only and shredded and disked residue, respectively. By reducing the number of sorghum midges that emerge from overwintering diapause, this insect pest would be less abundant, and damaging infestations would be delayed.

ACKNOWLEDGMENT

This research was supported by grants 010366-145 from the Advanced Technology Program sponsored by the Texas Higher Education Coordinating Board and A.I.D./DAN-1254-G-00-0021-00 from the Agency for International Development through the Sorghum/Millet Collaborative Research/Support Program (INTSORMIL). The assistance of Drs. Michael I. Biggerstaff and John A. Jackman is acknowledged.

LITERATURE CITED

Baxendale, F. P., and G. L. Teetes. 1983a. Factors influencing adult emergence from diapausing sorghum midge, *Contarinia sorghicola* (Diptera: Cecidomyiidae). Environ. Entomol. 12: 1064-1067.

1983b. Thermal requirements for emergence of overwintered sorghum midge (Diptera: Cecidomyiidae). Environ. Entomol. 12: 1078-1082.

Baxendale, F. P., G. L. Teetes, P. J. H. Sharpe, and H. Wu. 1984. Temperature-dependent model for development of non-diapausing sorghum midge (Diptera: Cecidomyiidae). Environ. Entomol. 13: 1572-1576.

Montoya, E. L. 1965. A squeeze device for detection of larvae of the sorghum midge, *Contarinia sorghicola* (Coquillett). J. Econ. Entomol. 58: 938-940.

Mott, D. A. 1995. Overwintering survival and role of moisture in diapause termination and subsequent emergence of sorghum midge (Diptera: Cecidomyiidae). M.S. thesis, Texas A&M University, College Station.

Roth, J. P., and H. N. Pitre. 1975. The sorghum midge in Mississippi. Mississippi Agric. Forest. Exp. Stn. Bull. 836.

Young, W. R., and G. L. Teetes. 1977. Sorghum entomology. Ann. Rev. Entomol. 22: 193-218.

SCIENTIFIC NOTE

NATIVE PARASITOIDS OF CITRUS LEAFMINER *PHYLLOCNISTIS CITRELLA*[1] STAINTON IN COLIMA, MEXICO

Miguel Agustín Perales-Gutiérrez, Hugo César Arredondo-Bernal, Enrique Garza-González and Luís Alberto Aguirre-Uribe.

Centro Nacional de Referencia de Control Biológico, DGSV-SAGAR
Apdo. Postal 133, Tecomán, Colima, México. 28130.

Citrus Leafminer (CLM), *Phyllocnistis citrella* Stainton, a phytophagous pest of citrus species and other Rutaceae, is native to Asia (Knapp 1995), was initially reported in Florida (Hepnner 1993), from which it spread to other areas of the southwestern United States. In Mexico, CLM was reported in the State of Tamaulipas in September 1994; all citrus growing areas in Yucatán, Veracruz, Tabasco, San Luis Potosí, Nuevo León and Colima are currently infested. CLM attacks leaves of young flushes, eventually young stems and rarely fruits (Sponagel and Díaz 1994). Neonate larvae bore through the leaf epidermis and begin ingesting cell sap. The damaged area becomes dried and curled, reducing the leaf surface responsible for photosynthesis; secondary damage includes disease transmission and premature defoliation (Sponagel and Díaz 1994).

As part of an exploration program of natural enemies of CLM in Mexico, the complex of native parasitoids of CLM were studied in the citrus growing areas of northern Colima State, from November 1994 to May 1995. Collections of flushes on lemon plants attacked by CLM were transported to the Quarantine Unit in Centro Nacional de Referencia de Control Biológico in Tecomán, Mexico, where damaged leaves were examined. Leaves with parasitized hosts were individually placed in Petri dishes at 27 °C, and held under daily observation to adult emergence.

A total of 1899 larvae, 172 prepupae, and 1822 pupae of CLM resulted from these collections and parasitoids recovered were *Cirrospilus quadristriatus* (Subba Rao and Ramamani) *Cirrospilus* spp., *Closterocerus* spp., *Horismenus* sp., and *Zagrammosoma multilineatum* (Ashmead) (Hymenoptera: Eulophide); *Elasmus* sp. (Hymenoptera: Elasmide); and an unidentified genus of Encyrtidae. Table 1 lists the parasitoids detected as well as the development stage of the host where the parasites larvae were observed. All of the above are ectoparasitoids, except *Closterocerus* spp. and probability *Horismenus* sp. Cumulative percentage parasitism averaged 6.4% on second-instar larvae, 66% on third-instar larvae, 17% on prepupae, and 10.6 % on pupae.

This is the first step in assessing the value of each parasitoid on the regulation of pest population. A knowledge of natural enemies of CLM in Mexico is also useful in decision making for classical biological control. The characteristics of exotic entomophagous can be chosen to complement the effect of native parasitoids.

---

[1] Lepidoptera: Phyllocnistidae.

TABLE 1. Citrus Leafminer Parasitoids in Colima.

Family	Taxon [a]	Stage parasitized	Biology
Eulophidae	*Cirrospilus quadristriatus*	Larva 3 Pupa	Primary ectoparasitoid and secondary ectoparasitoid of pupae
	Cirrospilus spp.	Larva 2, 3 Prepupa Pupa	Primary ectoparasitoid and secondary ectoparasitoid of larvae
	Closterocerus spp.	Larva 2, 3	Primary endoparasitoid
	Horismenus sp.	Prepupa or pupa	Unknown
	Zagrammosoma multilineatum	Larva 3 Prepupa Pupa	Primary ectoparasitoid and secondary ectoparasitoid of larvae
Elasmidae	*Elasmus* sp.	Pupa	Primary ectoparasitoid and secondary ectoparasitoid of larvae
Encyrtidae	unidentified genus	Prepupa or pupa	Unknown

[a] Identified by M. Schauff. U.S. National Museum. Washington, U.S.A.

This research was supported by Consejo Nacional de Ciencia y Tecnología (CONACYT) through the project No. 95-01-005 "Manejo Integrado del Minador de la Hoja (*Phyllocnistis citrella* Stainton) en Limón Mexicano".

LITERATURE CITED

Heppner, J.B. 1993. Citrus leafminer, *Phyllocnistis citrella* Stainton (Lepidoptera: Gracillariidae: Phyllocnistinae). Fla. Dept. Agric. & Consumer Services, Division of Plant Industry, Gainesville. Entomology Circular No. 359. 2 p.

Knapp, J.L. 1995. Citrus Leafminer Workshop. Florida Cooperative Extension Service, Institute of Food and Agricultural Sciences. University of Florida, Gainesville. February 8-9, 1995, Orlando, Florida. 25 p.

Sponagel, K.W. and F. J. Díaz. 1994. El minador de la hoja de los cítricos *Phyllocnistis citrella*: un insecto-plaga de importancia económica en la citricultura de Honduras. Fundación Hondureña de Investigación Agrícola, La Lima, Cortés, Honduras. 25 p.

PINK BOLLWORM ADULT AND LARVAL SUSCEPTIBILITY TO STEINERNEMATID NEMATODES AND NEMATODE PERSISTENCE IN THE SOIL IN LABORATORY AND FIELD TESTS IN ARIZONA

T. J. Henneberry, L. Forlow Jech and R. A. Burke
USDA, ARS, PWA, Western Cotton Research Laboratory,
4135 E. Broadway, Phoenix, AZ 85040-8830

ABSTRACT

Percentages of infected pink bollworm, *Pectinophora gossypiella* (Saunders), (PBW) adults emerging from pupae placed on soil treated with *Steinernema carpocapsae* (Weiser) or *S. riobravis* Cabanillas, Poinar and Raulston were 16.7-34.0% and 20.7-26.7%, respectively. Some (0-13%) pupae that did not produce adults were also infected. Under laboratory conditions in moist soil, PBW larval mortalities were 91.9% on the day of treatment and 5.1% on day 35 following treatment for larvae released on soil surfaces treated with *S. riobravis* and 50.0 and 7.0%, respectively, for larvae buried 1.7 cm in the soil. Mortalities for larvae released on soil surfaces treated with *S. carpocapsae* were 90.5% on the day of treatment and 38.5% on day 35 following treatment. Mortality of larvae buried 1.7 cm in the soil was 31.0% on the day of treatment and 3.0% on day 35 following treatment. When soil treated with *S. riobravis* was allowed to dry between PBW larval releases on soil surfaces or burial in soil, but wetted on the day of larval exposure, percentage mortalities ranged from 37.3-97.8% for larvae exposed on the soil surface and 62-84% for buried larvae over a 35-day test period. Also, under laboratory conditions *S. riobravis* parasitized higher percentages of PBW larvae buried in the soil than *S. carpocapsae* but *S. carpocapsae* parasitized higher numbers of larvae released on the soil surface than did *S. riobravis*.

Under field conditions, with irrigations about every 14-21 days, PBW larval mortalities after exposure to soil samples from plots treated with *S. riobravis* at the rate of 5 nematodes per cm^2 of soil surface were 50% on the day of treatment and 2.5% on day 90 following treatment. Larval mortality percentages after exposure to soil samples from plots treated with *S. carpocapsae* at the rate of 5 per cm^2 of soil surface were 32.5, 15.3, 5.3 and 2.5, respectively, for the day of treatment and day 1, 7, and 15 following treatment. No further mortality occurred in bioassays conducted up to 90 days following treatment. With plots treated with 25 nematodes per cm^2 of soil surface, PBW larval mortalities ranged from 100% on the day of treatment to 7.5% on day 63 following treatment with *S. riobravis* and 92.5% on the day of treatment to 5% on day 7 following treatment with *S. carpocapsae*. Percentages of larval mortality after exposure to soil samples from plots treated with *S. riobravis* increased after each irrigation, but did not increase after exposure to soil samples from plots treated with *S. carpocapsae*.

INTRODUCTION

Pink bollworm (PBW), *Pectinophora gossypiella* (Saunders), last stage larvae have been reported highly susceptible to the Kapow strain of *Steinernema carpocapsae*

(Weiser) (Lindegren et al. 1992, 1993c) and *S. riobravis* (Henneberry et al. 1996). The results of studies with six species of entomopathogenic nematodes and using greater wax moth, *Galleria mellonella* (L.) as the insect host substitute for PBW, indicated that there was extensive variation between the six species with respect to their ability to penetrate a 5-cm sand barrier and infect larvae (Lindegren et al. 1993b). Of those species evaluated, the newly described *S. riobravis* Cabanillas, Poinar and Raulston (Cabanillas et al. 1994) was rated best in relation to host searching efficiency. *S. riobravis*, indigenous to the Rio Grande Valley of Texas, was discovered parasitizing corn earworm, *Helicoverpa zea* (Boddie) and fall armyworm, *Spodoptera frugiperda* (J. E. Smith) in the soil (Raulston et al. 1992). PBW larvae are very vulnerable to attack in their soil habitat during fall, winter and spring diapause (Henneberry 1986). Also, during the growing season, PBW larvae that develop in squares or bolls tunnel out of the fruiting forms in the last larval stage to pupate in or on the soil (Butler and Henneberry 1976a). This behavioral characteristic may offer an opportunity to consider biological control with nematodes during the growing season. Although PBW pupae are not susceptible to *S. carpocapsae* or *S. riobravis* infection (Lindegren et al. 1993c, Henneberry et al. 1995), no information exists regarding the susceptibility of PBW adults. Lingren (1983) reported that most PBW moth emergence occurs between 0800 and 1100 h. Eclosion of the adults involves a series of behavioral characteristics involving pupal movement, pupal case splitting and wing expansion, extension and relaxation. Thereafter, little movement of adults occurred for 2-3 h. Consideration of these behavioral descriptions suggested the possibility that newly emerged adults exposed for 2-3 h on nematode treated soil might also be susceptible to infection. As many as five generations of PBW may occur during the Arizona cotton growing season (Slosser and Watson 1972) which may extend from mid-March to mid-October. Thus, persistence of a natural enemy that inhabits the soil would be an important factor in determining the potential biological control of PBW. Heavy reliance on insecticides for control of PBW has resulted in resistance, adverse effects on non-target organisms, secondary pest resurgence, and concern for the environment. The urgent need for biological components of integrated management systems for PBW prompted us to determine the susceptibility of PBW adults to nematode infection and to compare the persistence of *S. carpocapsae* and *S. riobravis* in treated soil in the laboratory and field as a factor in determining PBW biological control potential. We also compared *S. riobravis* and *S. carpocapsae* parasitization of PBW larvae buried in the soil or released on the soil surface.

MATERIALS AND METHODS

PBW larvae and pupae used in the studies were obtained from the laboratory culture at the USDA-ARS, Western Cotton Research Laboratory, Phoenix, Arizona. Rearing methods were as described by Bartlett and Wolf (1985). *S. carpocapsae* and *S. riobravis* nematodes were reared using the *in vivo* method of Lindegren et al. (1993a).

Adult Susceptibility - Laboratory. Susceptibility to nematode infection of newly emerged PBW adults was determined in 300 ml waxed paper containers, 9.5 cm diameter and 6 cm high, each holding 70 g of soil. Ten darkly pigmented pupae (24h-eclosion), per container, were selected for the experiments (Butler and Henneberry 1976b). In Experiment 1, soil in the containers was treated with *S. carpocapsae* in effective juvenile (IJ) nematodes at rates of 150 or 500 per container and in Experiment 2 with 39, 117 or 392 per container. In both tests *S. carpocapsae* and *S. riobravis* IJs were applied to the soil surface in 23 ml of water. Soil in control containers were treated with 23 ml of water

alone. Each experiment was replicated 15 times. Emerged moths and remaining pupae were dissected and examined for the presence of nematodes after 72 h.

Nematode Persistence in Soil - Laboratory. Persistence of the two nematode species in the soil under laboratory conditions was determined in cylindrical cardboard containers that were 15 cm in diameter and 13 cm high. For Experiment 3, about 700 g of soil was placed in each of 210 containers and watered with 130 ml of distilled water which thoroughly soaked the soil. Seventy of the containers were untreated controls. The remaining 140 were treated, one half in each case, with 150 IJ *S. riobravis* or *S. carpocapsae* per container.

Immediately after treatment (Day 0) and every 4-5 days thereafter for 35 days, 10 PBW larvae were either buried in the soil or released onto the soil surface in five untreated, five *S. riobravis*-treated or five *S. carpocapsae*-treated containers. Larvae were buried (1.7 cm) individually in each of ten plastic biopsy cassettes (Tissue Path IV®, Curtin Matheson Scientific, Inc.). All soil from the original untreated or nematode-treated containers was used to cover the larvae in the cassettes. Ten ml of water was added to each container following the introduction of larvae and also to all remaining containers to maintain moist soil conditions. The average laboratory temperature during the experiment was 26.6° C. PBW larval mortality in all cases was determined and larval dissections performed to determine the presence of nematodes 72 h after exposure to untreated or nematode treated soil. The experiment was conducted two times in randomized block designs with five replications each.

Effects of soil drying on *S. riobravis* infection of PBW larvae were determined in Experiment 4 in the containers described. One half of 140 containers with wetted soil (130 ml of distilled water) were treated at the rate of 150 *S. riobravis* IJs per container. The remaining containers were untreated controls. PBW larvae in biopsy cassettes were buried as described or released free on the soil surface in each of five control or five nematode treated containers on the day of treatment (Day 0) and thereafter every 4-7 days for 35 days. No additional water was added to the containers on day 0. However, 5, 20, 35, 35, 50 and 50 ml of water was added to each control container and each container treated with nematodes when PBW larvae were introduced on days 5, 11, 17, 21, 28 and 35, respectively, after nematode treatment. The amount of water added at each date following treatment was determined by weight loss of the soil in containers due to evaporation. Thus, soil with *S. riobravis* nematodes was dried for 0-35 days before additional water was added and PBW larvae introduced to the treated soil. PBW mortality was determined 72 h following burial or surface release on treated or untreated soil. The Experiment was conducted in a randomized block design of 5 replications.

Comparison of S. riobravis and S. carpocapsae larval parasitization - laboratory. Two Experiments (5 and 6) were conducted to verify the different larval mortalities observed between *S. carpocapsae* and *S. riobravis* for larvae released on the soil surface or buried in the soil. For Experiment 5, 275 containers with soil were prepared as described for Experiment 3. Seventy-five containers, in each case, were untreated, *S. riobravis*-treated or *S. carpocapsae*-treated (150/container); 25, in each case, of the untreated, *S. riobravis*, and *S. carpocapsae*-treated containers received no additional water, 25 were watered weekly and 25 were watered only at the time larvae were introduced on the soil surface in the containers. PBW larvae were introduced onto the soil surface into untreated *S. riobravis* and *S. carpocapsae* containers that were not watered, watered weekly or watered at the time of larval introduction on days 1, 8, 15, 22 and 29 days, respectively, following nematode treatment. The experimental design was a randomized complete block with five replications. Larvae were recovered 72 h following release in the containers and dissected to determine the presence of nematodes.

For Experiment 6, there were 60 containers (15 cm diameter x 5 cm high). Containers with bottoms removed were placed on inverted container lids. Ten biopsy cassettes, each containing one PBW larvae, were placed in the bottoms of 15 container lids. Approximately 2.5 cm of soil was placed in each of the 60 containers. Of the 15 containers with buried larvae, five containers were untreated and five, in each case, *S. riobravis* or *S. carpocapsae*-treated. In a second group of five untreated, five *S. riobravis* and five *S. carpocapsae*-treated containers ten free PBW larvae were released onto the soil surfaces. On day 3 following nematode treatments, 30 remaining containers were gently removed from the inverted container lids. Ten biopsy containers, each containing one PBW larvae, were placed in the bottoms of five untreated, five *S. riobravis*-treated and five *S. carpocapsae*-treated container lids. Containers were replaced on the inverted container lids. Ten free PBW larvae were released on the surface of the second group of five untreated, five *S. riobravis* and five containers with soil treated with *S. carpocapsae*. Larvae were recovered from the soil surface or from buried cassettes after 24 h exposure and dissected to determine the presence of nematodes.

Nematode Persistence in Soil - Field. Experiments 7 and 8 were conducted to determine the persistence of *S. riobravis* and *S. carpocapsae* nematodes under field conditions at Phoenix, Arizona. The experiments were conducted in 27.4 m^2 plots arranged in randomized block designs with four replications. In Experiment 7, treatments were *S. riobravis* and *S. carpocapsae* at the rate of five nematodes per cm^2 of soil surface. In Experiment 8, treatments were with *S. riobravis* or *S. carpocapsae* at the rate of 25 nematodes per cm^2 of soil surface. In both experiments, all plots were furrow irrigated 1 day prior to treatment; nematodes were applied to the plots in 8 liters of water with a sprinkling can 1 day later. Control plots were treated with 8 liters of water alone. To simulate a typical Arizona cotton irrigation schedule, all plots were irrigated every 22-23 days for March through May in Experiment 7 and every 14 days in all other cases after nematode treatment. There were six irrigations in Experiment 7 and five irrigations in Experiment 8. Morning (0730 h) and midafternoon (1430 h.) soil temperatures at 2.5 -5.0 cm depths were taken with pocket dial thermometers (VWR, Phoenix, Arizona) 5 days a week for the duration of each experiment. Soil samples from each plot, in each experiment, were taken on the day of treatment (Day 0), the day following treatment (Day 1) and at approximate 7-day intervals thereafter for 90 days in Experiment 7 and 63 days for Experiment 8. Soil was collected in 5-cm deep circular aluminum cylinders with a volume of 90.5 cm^3. All soil in each sample from each plot was spread evenly in individual 25 x 150 mm petri dishes and moistened with 3 ml of water. Ten last instar PBW larvae were released onto the soil surfaces in each petri dish. Petri dishes with soil and larvae were held at 26.7° C in constant temperature boxes for 72 h, when dead and living larvae were recorded in each dish.

All data were analyzed using analysis of variance (ANOVA) and means separated, contingent upon a significant F test, using the method of least significant differences. All percentages were transformed to arcsins before ANOVA procedures.

RESULTS

Adult Susceptibility-Laboratory. Percentages of moth emergence from PBW pupae placed on soil treated with *S. carpocapsae* treated soil averaged 87.3% for the 500 IJ rate and 95.3% for the 150 IJ rate compared to 98.7% for the untreated control soil (Table 1, Experiment 1). Moth emerged from pupae on soil treated with *S. carpocapsae* nematodes at the rate of 150 or 500 IJs were 16.7% and 33.3% infected with nematodes,respectively. Also, the pupae remaining were 3.0% and 13.0% infected following the two treatments,

respectively. For Experiment 2, there were no significant differences between PBW emergence of moths from pupae exposed on soil treated with *S. riobravis*, *S. carpocapsae* or to untreated soil (Table 1, Experiment 2). Percentages of infected moths ranged from 20.7-34.0% for *S carpocapsae* and 20.7-26 for *S. riobravis*, and < 3.0% of the remaining pupae treated with either nematode species were infected.

TABLE 1. Mean[a] (± SE) Percentages of Pink Bollworm Moth Emergence, and Nematode Infected Moths and Pupae Following Pupal Exposure to *S. riobravis* or *S. Carposcapsae* Treated and Untreated Soil. Experiments 1 and 2.

Nematodes/ container	% Moth emergence	Percentages of Emerged moths infected	Percentages of Remaining pupae infected	Total infected
		Experiment 1		
Untreated	98.7 ± 0.9 a	0.0 ± 0.0 c	0.0 ± 0.0 b	0.0 ± 0.0 c
S. carposcapsae				
150	95.3 ± 1.7 a	16.7 ± 3.2 b	3.0 ± 1.5 b	19.7 ± 4.0 b
500	87.3 ± 3.2 b	33.3 ± 3.5 a	13.0 ± 4.7 a	46.3 ± 6.3 a
		Experiment 2		
Untreated	86.7 ± 5.9 a	0.0 ± 0.0 c	0.0 ± 0.0 c	0.0 ± 0.0
S. carposcapsae				
39	80.7 ± 6.8 a	20.7 ± 6.2 b	0.7 ± 0.7 b	21.4 ± 6.3 b
117	83.3 ± 5.7 a	24.0 ± 5.8 ab	0.0 ± 0.0 ab	24.0 ± 5.8 ab
392	79.3 ± 5.7 a	34.0 ± 8.9 a	2.7 ± 1.2 a	36.6 ± 8.9 a
S. *riobravis*				
39	83.3 ± 4.4 a	20.7 ± 6.7 b	1.3 ± 0.9 b	22.0 ± 6.6 b
117	80.7 ± 5.8 a	25.3 ± 8.0 ab	2.0 ± 1.5 ab	27.3 ± 8.0 ab
392	82.7 ± 4.1 a	26.7 ± 7.2 ab	1.3 ± 0.9 ab	28.0 ± 7.3 ab

[a] Means of 15 replications. Means in a column within an experiment not followed by the same letter are significantly different $P \leq 0.05$. Method of least significant differences.

Nematode Persistence in Soil - Laboratory. Under laboratory conditions, in continually moist soil, there were no significant differences between percentage mortalities of untreated PBW larvae buried in the soil in biopsy containers compared with those released on the soil surface (Table 2, Experiment 3). Average percentage mortalities for all days showed that both nematode species infected more larvae released on the surface soil as compared to buried larvae. Higher larval mortalities following release on the soil surface occurred with *S. carpocapsae* than with *S. riobravis*. Highest larval mortalities occurred on the day of treatment and in general, for each species, mortalities decreased thereafter through day 35 of the experiment. For Experiment 4, with *S. riobravis* nematodes, after wetting the soil with 130 ml of water at the time of nematode treatment, the soil with nematodes was allowed to dry out between time intervals for each subsequent PBW larval introduction (Table 3). Increasing amounts of water ranging from 5-50 ml were required to wet the soil as the drying time increased. Under these conditions, overall percentages of larval mortality for untreated buried larvae and larvae released on the soil surface were

not significantly different. However, variability was high and mortality of 12.6 and 18.9% on days 11 and 28 occurred for larvae released on the untreated soil surface. Larvae were not nematode infected and were probably injured dur-ing handling. Average PBW larval mortality ranged from 51.4-85.1% for *S. riobravis*-treated containers over the 35 days of the experiment.

TABLE 2. Mean[a] (± SE) Percentage Mortalities of Pink Bollworm Larvae When Buried or Released On the Surfaces of Untreated or *S. riobravis* or *S. carpocapsae*[b] Treated Soils. Experiment 3.

Days after nematode treatment	Larvae		Main Effect Mean for days
	On soil surface	Buried	
Untreated			
0	0.0 ± 0.0 l	1.0 ± 1.0 kl	0.5 ± 0.5 F
5	1.4 ± 1.4 kl	2.0 ± 1.3 kl	1.7 ± 0.9 F
11	0.0 ± 0.0 l	0.0 ± 0.0 l	0.0 ± 0.0 F
17	1.0 ± 0.0 kl	0.0 ± 0.0 l	0.5 ± 0.5 F
21	1.1 ± 1.1 kl	2.0 ± 1.3 kl	1.6 ± 0.9 F
28	4.4 ± 3.4 jl	1.0 ± 1.6 kl	2.7 ± 1.8 F
35	1.0 ± 1.0 kl	0.0 ± 0.0 l	0.5 ± 0.5 F
Mean	1.3 ± 0.6 D	0.9 ± 0.3 D	1.1 ± 0.6 X
S. riobravis			
0	91.9 ± 3.3 a	50.0 ± 9.8 cd	71.0 ± 7.0 A
5	77.0 ± 6.1 ab	45.0 ± 12.8 d-f	61.0 ± 7.8 AB
11	81.3 ± 4.6 ab	48.0 ± 13.6 d-f	64.7 ± 8.0 AB
17	39.5 ± 6.9 c-e	30.0 ± 6.5 ef	34.8 ± 4.8 CD
21	33.2 ± 6.2 d-f	8.0 ± 2.5 hi	20.6 ± 4.4 E
28	22.2 ± 5.5 fg	7.1 ± 3.4 h-k	14.7 ± 3.6 E
35	5.1 ± 2.7 g-l	7.0 ± 1.0 kl	3.1 ± 1.5 F
Mean	50.0 ± 4.2 B	27.0 ± 3.9 C	38.5 ± 3.0 Y
S. carpocapsae			
0	90.5 ± 3.7 a	31.0 ± 7.1 d-f	60.7 ± 7.8 AB
5	96.9 ± 2.2 a	30.0 ± 8.3 ef	63.5 ± 8.7 AB
11	95.9 ± 1.7 a	35.0 ± 6.0 d-f	65.5 ± 7.6 A
17	78.7 ± 5.2 ab	18.3 ± 4.4 fg	48.5 ± 7.7 BC
21	86.9 ± 4.2 ab	10.0 ± 4.0 hi	48.5 ± 9.3 CD
28	60.0 ± 9.1 bc	13.0 ± 4.7 gh	36.5 ± 7.3 D
35	38.5 ± 8.0 c-e	3.0 ± 1.5 j-l	20.7 ± 5.7 E
Mean	78.2 ± 3.1 A	20.0 ± 2.4 C	49.1 ± 3.2 Z
Main Effect			
Surface/buried	43.2 ± 2.8 A	16.0 ± 1.7 B	--- ---

[a] Means of 10 replications. Larvae treatment means or overall means in a column or row not followed by the same lower case letter are significantly different $P \leq 0.05$. Overall means for days after treatment in a column not followed by the same capital letter are significantly different $P \leq 0.05$. Method of least significant differences.
[b] 150 infective juvenile nematodes/container.

TABLE 3. Mean[a] (± SE) Percentage Mortalities of Pink Bollworm Larvae Following Release on Surface or Buried in *S. riobravis*[b] Treated or Untreated Soils[c]. Experiment 4.

Days after nematode treatment	Water[d] added (ml)	Larvae		Main Effect mean for days
		On soil surface	Buried	
Untreated				
0	0	0.0 ± 0.0 i	0.0 ± 0.0 i	0.0 ± 0.0 e
5	5	0.0 ± 0.0 i	0.0 ± 0.0 i	0.0 ± 0.0 e
11	20	12.6 ± 7.9 gh	6.0 ± 2.5 g-i	9.3 ± 4.1 d
17	35	0.0 ± 0.0 i	0.0 ± 0.0 i	0.0 ± 0.0 e
21	35	0.0 ± 0.0 i	0.0 ± 0.0 i	0.0 ± 0.0 e
28	50	18.9 ± 7.6 fg	0.0 ± 0.0 i	9.4 ± 4.8 d
35	50	5.8 ± 3.6 g-1	2.0 ± 2.0 hi	3.9 ± 2.1 de
Mean Control/Surface/Buried				
		5.3 ± 1.9 B	1.1 ± 0.6 B	--- ---
S. riobravis				
0	0	97.8 ± 4.4 a	62.0 ± 8.6 e	79.9 ± 7.3 a
5	5	80.7 ± 3.6 b-e	74.0 ± 6.0 b-e	77.3 ± 4.2 ab
11	20	37.3 ± 1.7 f	65.6 ± 8.6 de	51.4 ± 8.8 c
17	35	69.2 ± 3.1 c-e	76.0 ± 6.8 b-e	72.6 ± 4.7 b
21	35	64.5 ± 2.9 e	84.0 ± 6.8 bc	74.2 ± 6.0 ab
28	50	74.0 ± 3.3 b-e	74.0 ± 5.1 b-e	74.0 ± 6.4 ab
35	50	86.8 ± 3.9 ab	83.0 ± 5.6 b-d	85.1 ± 4.0 a
Mean Treated Surface/Buried				
		79.9 ± 4.3 A	74.1 ± 2.7 A	--- ---

[a] Means of 5 replications not followed by the same lower case letter or overall means not followed by the same capital letter are significantly different $P \leq 0.05$. Method of least significant differences.
[b] 150 Infective juvenile nematodes/container.
[c] Soil treated with 130 ml of water on the day of treatment.
[d] Amount of water added on the day after nematode treatment that PBW larvae were buried or released on the soil surface.

Comparison of S. riobravis and S. carpocapsae larval parasitization - laboratory. In Experiment 5, there were no significant differences in larval mortality for unwatered, watered weekly or watered at the time of larval introduction into untreated soil. Table 4 shows the combined data for days following nematode treatment and the affects of *S. riobravis* and *S. carpocapsae* on percentages of PBW larval mortality following release on soil surfaces. Results verified that higher percentages of PBW larvae were infected when released on the surface of *S. carpocapsae*-treated soil compared with percentages infected after release on the surface of *S. riobravis*-treated soil. Results of Experiment 6 (Table 5) further emphasized this difference and also showed that *S. riobravis* on the day of and 3 days following treatment parasitized significantly higher percentages of PBW larvae buried 2.5 cm in the soil compared with *S. carpocapsae*.

Nematode Persistence in Soil - Field. During 28 March to 27 June, average morn-

TABLE 4. Mean[a] (± SE) Percentage Mortalities of Pink Bollworm Larvae Following Releases on Soil Surfaces of *S. riobravis* or *S. carpocapsae*. Experiment 5.

Days after nematode treatment	Treatment		
	Untreated	*S. riobravis*	*S. carpocapsae*
1	0.0 ± 0.0 g	72.3 ± 5.0 c	96.0 ± 1.6 a
8	0.0 ± 0.0 g	36.4 ± 6.6 de	94.5 ± 1.4 a
15	0.0 ± 0.0 g	25.9 ± 3.7 e	82.9 ± 3.1 b
22	0.7 ± 0.7 g	27.4 ± 6.9 e	86.2 ± 2.8 b
29	0.0 ± 0.0 g	7.2 ± 2.3 f	45.1 ± 4.3 d
Overall Mean	0.1 ± 0.1 C	33.8 ± 3.4 B	80.9 ± 2.5 A

[a] Means of 15 observations per treatment. Means in the same column or row not Followed by the same letter are significantly different. Overall means in a row not followed by the same capital letter are significantly different. Method of least significant differences $P \leq 0.5$.

TABLE 5. Mean (± SE)[a] Percentage Mortalities of Pink Bollworm Larvae Buried or Released on the Surfaces of Soil Treated with *S. riobravis* or *S. carpocapsae* or Untreated Soils. Experiment 6.

Treatment	Day of treatment	Day 3 following treatment
Untreated		
Buried	0.0 ± 0.0 c	0.8 ± 0.5 e
Surface	0.5 ± 0.5 c	0.0 ± 0.0 e
S. riobravis		
Buried	18.0 ± 2.3 b	25.2 ± 1.5 c
Surface	57.3 ± 3.7 a	44.1 ± 0.7 b
S. carpocapsae		
Buried	0.4 ± 0.4 c	4.0 ± 1.1 d
Surface	64.0 ± 3.4 a	86.2 ± 0.2 a
Means for nematodes		
Untreated	0.3 ± 0.3 C	0.4 ± 0.3 C
S. riobravis	37.7 ± 6.9 A	34.7 ± 3.3 B
S. carpocapsae	32.2 ± 10.7 B	45.1 ± 13.8 A
Means buried - surface		
Buried	6.1 ± 2.4 B	10.0 ± 3.0 B
Surface	40.6 ± 7.8 A	43.5 ± 9.5 A

[a] Means of 5 replications, 5 observations per replication. Means in a column not followed by the same letter are significantly different. Method of least significant differences $P \leq 0.05$.

ing soil temperatures ranged from 62-81 °F and midday temperatures from 84-109° F (Table 6). PBW larval mortality after exposure to soil from untreated control plots over the 90 days of the experiment averaged 0.2 ± 0.2%. Larval mortality after exposure to soil samples treated with *S. riobravis* nematodes (5 per cm^2 of soil surface) ranged from 50-60% for the first 7 days following treatment. Percentage mortalities decreased to 28 and 10% on days 15 and 22 following treatment, respectively, and increased on day 28 following nematode treatment and 7 days after irrigation. Similar increases in PBW larval mortality occurred after exposure to soil samples 12, 5, and 4 days after irrigation's on 10 and 24 May and 7 June. In contrast, PBW larval mortality after exposure to soil samples from plots treated with *S. carpocapsae* nematodes (five per cm^2 of soil surface) was 33% on the day of treatment and decreased to 2.5% on day 15 following treatment. No further mortality occurred with larvae exposed to soil samples on days 22-90 following treatment.

From 25 April to 27 June, average morning and midday soil temperatures ranged from 60-84° F and 75-112° F, respectively, (Table 6). Average PBW larvae mortality after exposure to soil from untreated plots was 0.5%. Mortality of PBW larvae exposed to soil samples from plots treated with 25 *S. riobravis* nematodes per cm^2 of soil surface ranged from 70-100% for the first 7 days following treatment. Mortality increased followed irrigation's. Larval mortalities of PBW larvae exposed to soil from plots treated with 25 *S. carpocapsae*/cm^2 of soil surface were 92.5, 80.0, 5.0, and 0.0%, respectively, for soil samples taken on the day of treatment and on 1, 7 and 15 days following treatment. No larval mortality occurred when larvae were exposed to soil samples taken on day 22 through 63 following treatment, irrespective of irrigation scheduling.

DISCUSSION

PBW pupae are not susceptible to *S. carpocapsae* or *S. riobravis* infection unless injured or the pupal integument is undeveloped to allow a point of entry (Lindegren et al. 1993c, Henneberry et al. 1995). There have been no attempts to determine PBW adult susceptibility until the current studies. Emerged moths from pupae placed on the soil surfaces treated with nematodes were infected at levels ranging from 17-34% indicating a low but potentially useful, effect of soil inoculation for infecting PBW adults. The fact that some of the pupae (0-13%) that did not produce adults also were infected suggests that at least some of the nematodes probably entered the pupal case to infect adults during the eclosion process. Additional studies will have to be done to fully define the exact time of nematode entrance into and infection of PBW adults.

Location of the host is an important factor affecting entomopathogenic nematode efficacy (Kaya 1990), in part because of the different host search strategies exhibited by different nematode species (Kaya et al. 1993). For example, Alatorre-Rosas and Kaya (1990) reported that when *S. carpocapsae* and *Heterorhabditis bacteriophora* Poinar were placed in the same containers with hosts, *S. carpocapsae* infected more hosts near the soil surface, but *H. bacteriophora* infected more hosts deeper in the soil. Kaya et al. (1993) applied combinations of *S. carpocapsae* with "sit and wait" predatory behavior and *H. bacteriophora* with "foraging" behavior for control of black cutworm, *Agrotis epsilon* (Hufnagel) .(feeding near or at soil surface). and black vine weevil, .*Otiorhynchus sulcatus* (F.) (root feeder) in the same soil habitat. *S. carpocapsae* was effective against black cutworm and *H.bacteriophora* effective against black vine weevil demonstrating the potential of combinations of entomopathogenic nematodes for control of two pest species in the same habitat, but with different ecological niches. Results also illustrated the importance of knowing and exploiting the behavioral characteristics of entomopathogenic nematodes in biological control implementation. In general, in our studies, *S. riobravis*

TABLE 6. Mean (± SE)[a] Percentages of Pink Bollworm Larval Mortality After Exposure to Soil Samples from Irrigated[b] Untreated Field Plots or Field Plots Treated with *S. riobravis* or *S. carpocapsae* at the Rate of 5 per cm² of Soil Surface for Experiment 7 and 25 per cm² for Experiment 8.

Days After		Average Weekly Temps		Treatment		
Nematode treatment	Last irrigation	0730 h	1430 h	Untreated	*S. Riobravis*	*S. Carpocapsae*
			Experiment 7 (March 28-June 27)			
0	1	66	84	0.0 ± 0.0 i	50.0 ± 12.3 a-c	32.5 ± 4.8 b-d
1	2	66	84	0.0 ± 0.0 i	60.0 ± 7.1 a	15.3 ± 6.4 d-h
7	8	68	90	0.0 ± 0.0 i	55.3 ± 20.5 a	5.3 ± 3.1 g-i
15	16	71	96	2.5 ± 2.5 hi	27.5 ± 6.3 c-e	2.5 ± 2.5 h-i
22	1	68	93	0.0 ± 0.0 i	10.0 ± 4.1 e-i	0.0 ± 0.0 i
28	7	62	85	0.0 ± 0.0 i	57.5 ± 13.2 ab	0.0 ± 0.0 i
35	14	72	103	0.0 ± 0.0 i	25.0 ± 12.6 d-f	0.0 ± 0.0 i
43	1	66	87	0.0 ± 0.0 i	12.8 ± 9.4 f-i	0.0 ± 0.0 i
48	5	73	97	0.0 ± 0.0 i	5.0 ± 5.0 g-i	0.0 ± 0.0 i
56	12	74	85	0.0 ± 0.0 i	20.0 ± 13.5 d-g	0.0 ± 0.0 i
63	5	81	107	0.0 ± 0.0 i	30.3 ± 23.4 cd	0.0 ± 0.0 i
69	11	71	100	0.0 ± 0.0 i	0.0 ± 0.0 i	0.0 ± 0.0 i
76	4	80	109	0.0 ± 0.0 i	20.0 ± 14.1 hi	0.0 ± 0.0 i
83	11	78	96	0.0 ± 0.0 i	2.5 ± 2.5 i	0.0 ± 0.0 i
90	4	---	----	0.0 ± 0.0 i	2.5 ± 2.5 i	0.0 ± 0.0 I
Seasonal Means:						
		---	----	0.2 ± 0.2 C	25.2 ± 39 A	3.7 ± 1.4 B
			Experiment 8 (April 25-June 7)			
0	1	60	75	0.0 ± 0.0 e	100.0 ± 0.0 a	92.5 ± 4.8 ab
1	2	60	75	2.5 ± 2.5 de	100.0 ± 0.0 a	80.0 ± 7.1 bc
7	8	71	103	0.0 ± 0.0 e	70.0 ± 2.4 c	5.0 ± 5.0 de
15	2	66	85	0.0 ± 0.0 e	10.0 ± 5.8 de	0.0 ± 0.0 e
21	8	75	103	0.0 ± 0.0 e	67.5 ± 17.9 c	0.0 ± 0.0 e
28	1	72	82	0.0 ± 0.0 e	82.2 ± 7.4 bc	0.0 ± 0.0 e
36	8	84	112	2.5 ± 2.5 de	20.0 ± 14.1 d	0.0 ± 0.0 e
42	1	71	98	0.0 ± 0.0 e	20.0 ± 16.8 d	0.0 ± 0.0 e
49	8	80	107	0.0 ± 0.0 e	7.5 ± 4.8 de	0.0 ± 0.0 e
56	1	78	93	0.0 ± 0.0 e	17.5 ± 6.3 d	0.0 ± 0.0 e
63	8	---	----	0.0 ± 0.0 e	7.5 ± 2.5 de	0.0 ± 0.0 e
Seasonal Means:						
		---	----	0.5 ± 0.3 C	45.7 ± 6.4 A	16.1 ± 5.1 B

[a] Means of 4 replications. Means in a row or column not followed by the same letter are significantly different. Seasonal means in a row not followed by the same capital letter are significantly different. In each $P \leq 0.05$, method of least significant differences.

[b] Irrigation's of 0.3 m of water/0.4 ha on 3/28, 4/19, 5/10, 5/24, 6/7, and 6/21 for Experiment 7 and 0.3 m of water/0.4 ha on 4/25, 5/9, 5/23, 616, and 6/20 for Experiment 8.

infected higher numbers of PBW larvae buried in the soil compared with *S. carpocapsae*, but the reverse occurred for PBW larvae released on the soil surface. Results indicated that *S. carpocapsae* may be a useful biological control agent during the cotton growing season when PBW larvae tunnel out of fruiting forms and fall to the soil surface. In the field, percentages of buried PBW larvae infected by *S. riobravis* were higher than those infected by *S. carpocapsae*. Lindegren et al. (1993b) found that *S. riobravis* penetrated 5-cm sand barriers to infect greater percentages of wax moth larvae, *Galleria mellonella* (L.) compared to *S. carpocapsae*. Differences between the two species may have occurred because of the difference in nematode penetration ability in sand and soil or the fact that high nematode mortality occurred over the increasing time intervals between introduction of host larvae when soil moisture levels were maintained at high levels. Nematodes were expected to remain active, but PBW larval infection percentages for both species decreased. The role of moisture in entomopathogenic nematode activity is well known (Poinar 1979), and the rate of nematode desiccation appears to play an important role in survival. Simons and Poinar (1973) showed that prolonged drying, as may occur under soil conditions, resulted in immobile, collapsed or twisted appearance, but *S. carpocapsae* readily revived when immersed in water. This appears to have occurred in our studies when soil with *S. riobravis* was allowed to dry for periods of 5 to as long as 35 days between PBW larval introduction. Under these conditions, an average of 74-80% larval mortality occurred over the 35 days of the laboratory experiment. The phenomena of an exceptional *S. riobravis* survival mechanism in the soil was further substantiated from results of the field tests where some PBW larval infection occurred for as long as 63-90 days compared to 7-15 days for *S. carpocapsae*. Further, increasing levels of PBW larval infection in relation to irrigations indicated that gradual drying out of the soil environment between irrigations induced the gradual nematode desiccation and survival mechanisms discussed by Poinar (1979).

Both steinernematid nematode species used in our studies have a place in PBW integrated pest management programs. Additional field tests are being conducted to determine the most effective time, application rates and most efficacious nematode species in relation to PBW and cotton plant growth phenology. Gouge et al. (1996) reported significant reductions in PBW boll infestations and 19% increased cotton yield in cotton fields treated with *S. riobravis* compared with untreated fields. Thus, implementation of *S. riobravis* as a biocontrol agent for PBW control in cotton appears feasible.

LITERATURE CITED

Alatorre-Rosas, R., and H. K. Kaya. 1990. Interspecific competition between entomopathogenic nematodes in the genera *Heterorhabditis* and *Steinernema* for an insect host in sand. J. Invertebr. Pathol. 55: 179-188.

Bartlett, A. C., and W. W. Wolf. 1985. *Pectinophora gossypiella*, pp. 415-430. *In* Sengh-Pond, and R. F. More, [eds.] Handbook of Insect Rearing, Elsevier, Amsterdam.

Butler, G. D., Jr., and T. J. Henneberry. 1976a. Biology, behavior and effects of larvae of pink bollworm in cotton flowers. Environ. Entomol. 5: 970-972.

Butler, G. D., Jr., and T. J. Henneberry. 1976b. Temperature dependent development rate tables for insects associated with cotton in the Southwest. USDA-ARS, W-38, 36 pp.

Cabanillas, H. E., G. E. Poinar, Jr., and J. R. Raulston. 1994. *Steinernema riobravis* n. sp. (Rhabditida: Steinernematidae) from Texas Fundam. Appl. Nematol. 17: 123-131.

Gouge, D. H. L. L. Reaves, M. M. Stoltman, J. R. VanBerkum, R. A. Burke, L. J. Forlow Jech, and T. J. Henneberry. 1996. Control of pink bollworm *Pectinophora gossypiella* (Saunders) (Lepidoptera: Gelechiidae) larvae using the entomopathogenic nematodes *Steinernema riobravis* (Cabanillas, Poinar, and Raulston) (Rhabditides: Steinernematidae), pp. 1078-1082. *In* P. Dugger, and D. Richter, [eds.] Proc. Beltwide Cotton Prod Res. Conf., Natl. Cotton Council of Amer., Memphis, TN.

Henneberry, T. J. 1986. Pink bollworm management in cotton in the Southwestern United States. USDA-ARS, ARS-51. National Tech. Inf. Serv., Springfield, VA 45 pp.

Henneberry, T. J., J. E. Lindegren, L. Forlow Jech, and R. A. Burke. 1995. Pink bollworm (Lepidoptera: Gelechiidae), cabbage looper and beet armyworm (Lepidoptera: Noctuidae) pupal susceptibility to steinernematid nematodes (Rhabdita: Steinernematidae). J. Econ. Entomol. 88: 835-839.

Henneberry, T. J., L. F. Jech, R. A. Burke, and J. E. Lindegren. 1996. Temperature effects on infection and mortality of *Pectinophora gossypiella* (Lepidoptera: Gelechiidae) larvae by entomopathogenic nematode species. Environ. Entomol. 25: 179-183.

Kaya, H. K. 1990. Soil ecology, pp. 93-115. *In* R. Gaugler, and H. K. Kaya, [eds.] Entomopathogenic nematodes in biological control. CRC Press, Boca Raton, Fla.

Kaya, H. K., T. M. Burlando, and G. S. Thurston. 1993. Two entomopathogenic nematode species with different search strategies for insect suppression. Environ. Entomol. 22: 859-864.

Lindegren, J. E., K. A. Valero, and B. E. Mackey. 1993a. Simple *in vivo* production and storage methods for *Steinernema carpocapsae* infective juveniles. J. Nematol. 25: 193-197.

Lindegren, J. E., K. A. Valero, and L. J. Forlow Jech. 1993b. Host searching response of various entomopathogenic nematodes: sand barrier bioassay, pp. 1044-1045. *In* D. J Herber, and D. A. Richter, [eds.] Proc. Beltwide Cotton Prod. Res. Conf., Natl. Cotton council of Amer., Memphis, TN.

Lindegren, J. E., K. F. Meyer, T. J. Henneberry, P. V. Vail, L. J. Forlow Jech, and K. A. Valero. 1993c. Susceptibility of pink bollworm (Lepidoptera: Gelechiidae) soil associated stages to the entomopathogenic nematode *Steinernema carpocapsae* (Rhabditida: Steinernematidae). Southwestern Entomol. 18: 113-120.

Lindegren, J. E., T. J. Henneberry, and L. Forlow Jech. 1992. Mortality response of pink bollworm to the entomopathogenic nematode *Steinernema carpocapsae*, pp. 930-931. *In* D. J. Herber, and D. A. Richter, [eds.] Proc. Beltwide Cotton Prod. Res. Conf., Natl. Cotton Council of Amer., Memphis, TN.

Lingren, P. D. 1983. Behavior of pink bollworm (Lepidoptera: Gelechiidae) adults during eclosion to departure from site of emergence. Ann. Entomol. Soc. Am. 76: 657-660.

Poinar, G. O., Jr. 1979. Nematodes for biological control of insects, pp. 128-159. CRC Press, Inc., Boca Raton, Florida.

Raulston, J. R., S. D. Pair, J. Loera, and H. E. Cabanillas. 1992. Prepupal and pupal parasitism of *Helicoverpa zea* and *Spodoptera frugiperda* (Lepidoptera: Noctuidae) by *Steinernema* sp. in corn fields in the lower Rio Grande Valley. J. Econ. Entomol. 85: 1666-1670.

Simons, W. R., and G. O. Poinar, Jr. 1973. The ability of *Neoaplectana carpocapsae* (Steinernematide: Nematoidea) to survive extended periods of desiccation. J. Invertebr. Pathol. 22: 228-230.

Slosser, J. E., and T. F. Watson. 1972. Population growth of the pink bollworm. Arizona Agric. Expt. Stn. Bull. 195, 32 pp.

ABAXIAL SURFACE AND EMULSIFIED LEAF pH OF COTTON, *GOSSYPIUM* SPP.

W.J. Smith, C. Wayne Smith, and Robert L. Meagher, Jr.

Graduate Research Assistant and Professor, Department of Soil & Crop Sciences, Heep Center, Texas A&M University, College Station, TX 77843-2474; Research Associate Entomologist, USDA-ARS, CVAVE, 1700 S.W. 23rd Dr., Gainesville, FL.

ABSTRACT

The abaxial surface pH of the uppermost main stem leaf measuring at least 3 cm and the oldest fully expanded main stem leaf from the lower canopy of seventeen genotypes were determined when plants, grown under greenhouse conditions, were typically at the first square stage of growth and four weeks later. Emulsified leaf pH was determined on the crude extract after grinding leaf tissue in deionized water. Only minor differences were found in abaxial leaf surface or emulsified leaf pH relative to plant age or leaf position. Abaxial leaf surface pH ranged from 8.24 to 9.63 and emulsified leaf pH ranged from 5.75 to 6.56 among the seventeen genotypes evaluated.

INTRODUCTION

Insects caused an estimated loss of over a million bales of cotton annually in the U.S. during 1989-1994 (Carter 1996). Losses in 1995 more than doubled to 2.3 million bales. Unrealized income plus cost of control in 1995 were valued at 889 million dollars. The major insect pests of cotton include the boll weevil, *Anthonomus grandis grandis* (Boheman), the bollworm/tobacco budworm complex, *Helicoverpa zea* (Boddie)/*Heliothis virescens* (F.), the plant bug complex, *Lygus* spp., *Neurocolpus nubilus* (Say), and *Pseudatomoscelis seriatus* (Reuter), aphids, *Aphis gossypii* (Glover), pink bollworm, *Pectinophora gossypiella* (Saunders), the whitefly complex, *Bemisia argentifolii* (Bellows and Perring) and *B. Tabaci* (Gennadius), and the most recent addition to the major pest list, beet armyworm, *Spodoptera exigua* (Hubner) (Metcalf and Metcalf 1993).

Insecticides have been the mainstay of insect control since the USDA recommended calcium arsenate for the control of boll weevil about 1920. Synthetic chlorinated hydrocarbon insecticides were introduced during the 1940's, followed by the organophosphates in the 1950's. Boll weevil resistance to the chlorinated hydrocarbon compounds was evident by the 1950's (Brazzel 1961, Roussel and Clower 1955), and by 1965 resistant bollworm and budworm populations were evident (Adkisson 1964, Adkisson and Nemec 1966, Brazzel 1963, 1964). Carter (1996) recently reported that the cotton producer does not have the insecticide chemistry to deal with even moderately resistant populations of tobacco budworm. Similar scenarios can be documented for many of the insect pests of cotton relative to the development of resistance to classes of insecticides.

A number of alternative control strategies have been reported. Integrated pest management has been effective as a concept for reducing dependency on chemical insecticides (Carruth and Moore 1973, Collins et al. 1979, Frisbie et al.1976, Larson et al.1975, Sterling and Haney 1973). Biological agents and insecticides based on naturally produced metabolites may provide effective control of some insect pests in the immediate future (Dimock 1996, Thompson 1996). Transgenic Bt cotton cultivars were available commercially for the first time in 1996. These unique cotton cultivars genetically produce the same protein, CryIA(c), as produced by the bacteria, *Bacillus thuringiensis*, that is toxic to some lepidopterous insect pests of cotton. This biological insecticide has been sold in the U.S. for over 35 years (Navon 1993). Resistance potential in *Heliothis virescens* to this method of insecticide delivery has been documented already (Gould et al. 1995).

Several plant morphological or allelochemical traits have been studied for their host plant resistance properties. Frego bracts (Jones et al. 1987, Pieters and Bird 1976), varying levels of plant pubescence (Butler et al. 1991, Butler and Henneberry 1984, Meredith and Schuster 1979, Stephens and Lee 1961, Walker and Niles 1973, Wannamaker 1957), absence of leaf, bract, and/or floral nectaries (Benedict and Leigh 1976, Meredith 1976, Schuster and Maxwell 1974, Wilson 1982), plant pigmentation (Bailey 1981, Jones et al. 1987), and gossypol content (Lukefahr and Martin 1966, Singh and Weaver 1971, Zummo et al. 1984) have been implicated as possible host plant resistant traits to one or more insect pests of cotton. Others include okra leaf shaped leaves, earliness of maturity, other allelochemicals, and genetic background (Butler et al. 1988, Butter et al. 1992, Ozgur and Sekeroglu 1986, Sippell et al. 1987, Smith 1992). One other possible source of resistance that has received little attention is leaf pH (Berlinger et al. 1983, Berlinger 1986).

Cotton has an unusual and little understood characteristic of having a very high leaf surface pH, averaging about 10, compared with most plant species, that average around 7 (Harr et al. 1980). This value reportedly varies by leaf age and by species. This high surface pH is due to epidermal glands similar to hydathodes that secrete cations, mostly Ca, Mg, and K onto the leaf surface (Elleman and Entwhistle 1982, Harr et al. 1980). Harr et al. (1980) reported that this high surface pH can be detrimental to pesticide longevity and fungal pathogens.

Many insects may prefer plant tissue of a specific age or during specific portions of the growing season. Some of this preference may be explained by the fact that the pH of cell sap changes with leaf age (Harr et al. 1980, Husain et al. 1936). Berlinger et al. (1983) determined that *B. tabaci* could distinguish pH of artificial diets in increments as low as 0.25 units, with a preference for diets buffered to pH's from 6.0 to 7.25.

The objective of this study was to determine the genetic variability for abaxial leaf surface and emulsified leaf pH among seventeen cotton genotypes.

MATERIALS AND METHODS

Four replications, consisting of one plant each, of seventeen diverse cotton genotypes were grown under greenhouse culture in 1992 and 1993. The selection of the seventeen genotypes evaluated for pH in this study was based on several criteria, including leaf pubescence, foliage color, leaf shape, species, earliness of maturity, and yield potential (Table 1). Several of these genotypes had been observed by the junior authors to be especially susceptible or apparently resistant to *B. argentifolii* in 1991 in the Lower Rio Grande Valley of Texas. Deltapine 50 is considered one of the most field resistant current cultivars available to producers while Stoneville 453 is considered one of the most whitefly susceptible current cultivars. Pima S6 was included because it represents a different species, *Gossypium barbadense*, than the other sixteen that are *G. hirsutum*, the species normally grown in Texas.

Strain 86L^{2}14 L has okra leaf shaped leaves as does strain MACAOS that also has red foliar pigmentation. Tamcot CAB-CS and MACAOS are from the Texas Agricultural Experiment Station, Stoneville 453 and Deltapine 50 are current cultivars from the those companies, Lone Star is an obsolete cultivar released for production in Texas in the early 1900's, and the numbered strains were developed by the corresponding author at Texas A&M. As noted earlier, these seventeen genotypes represented a range in leaf trichome density, from the obsolete cultivar Lone Star with three trichomes cm^{-2} of leaf surface to strain 89E51 and Stoneville 453 that averaged 119 trichomes cm^{-2} (Smith 1994).

TABLE 1. Phenotypic Characteristics of Cotton Genotypes Evaluated for Abaxial Leaf Surface and Emulsified Leaf pH.

Genotype	Leaf shape	Visible leaf pubescence	Foliage color
Deltapine 50[a]	normal	smooth	green
Stoneville 453[a]	normal	very hairy	green
Tamcot CAB-CS[a]	normal	smooth	green
Lone Star[b]	normal	smooth	green
Pima S6[a,c]	normal	very hairy	green
86E20	normal	smooth	green
86L^{2}9	normal	moderate	green
86L^{2}14L	okra	smooth	green
88G104	normal	smooth	green
89E51	normal	very hairy	green
89E62	normal	smooth	green
89F46H	normal	hairy	green
89F46S	normal	moderate	green
90C19H	normal	moderate	green
90C19S	normal	smooth	green
90J57	normal	smooth	green
MACAOS	okra	moderate	red

[a] Current commercial cultivar.
[b] Obsolete commercial cultivar.
[c] *Gossypium barbadense*; all other genotypes are *G. hirsutum*.

Seeds were placed into moistened peat pellets for germination on 17 September and 8 December 1992. Seedlings were transplanted about ten days later to 7.6 liter pots filled with a commercial potting mixture. Plants were fertilized once with 16 g/pot of a commercial fertilizer with a formulation of 21-7-11 (N-P-K). Soil tests of the potting mixture used indicated that all other plant nutrients were sufficient. Plants were placed in a large, walk-in, organdy cage to exclude insects. Plants were not treated with insecticides to avoid chemically influencing leaf surface pH but were periodically hand sprayed with deionized water to dislodge and kill insect pests (*Trialeurodes* sp. and aphids). Efforts were made to avoid any of these immatures when recording leaf surface pH but some insects were present on some leaves when emulsified.

The pH of the abaxial leaf surfaces and emulsified leaf pH were determined for the youngest main stem leaf measuring at least 3 cm in diameter, and on the oldest, normal shaped main stem leaf from the lower canopy. Measurements were taken when plants across the seventeen genotypes typically had reached first square, 27 and 28 October 1992 for the first planting and 22 to 26 February 1993 for the second planting. A second measurement was taken

4-weeks post first square stage. Plants were typically blooming at 4-weeks post first square for the first planting but not for the second planting. This was probably caused by reduced incoming radiant energy and reduced temperatures encountered during the winter months.

Abaxial pH was measured with an Orion flat surface pH electrode. One droplet, 55 μl, of deionized water was placed between major veins on the undersurface of the leaf. The electrode was placed on the water droplet and pH recorded from an Orion pH meter after 90 sec. Emulsified leaf pH was determined as outlined by Berlinger et al. (1983). One gram of upper, expanding main stem leaf tissue from each plant and three grams of lower, fully expanded main stem leaf tissue were collected from each plant. Eight ml of deionized water were added to the 1 g sample and 24 ml were added to the older plant leaf tissue. The mixtures were ground until smooth, approximately 45 sec., with a tissue homogenizer. The pH of the crude extract was taken with an Orion pH probe and meter. Three readings of each sample were taken after stirring and averaged to provide a final pH value.

Pots were arranged in the greenhouse in a randomized complete block design. Data were analyzed as a split plot with sampling dates split to genotypes and genotypes split to leaf sampled. Means were separated by the Waller-Duncan LSD at k=100 which approximates the 5% probability level.

RESULTS AND DISCUSSION

Plant age, first square and 4-weeks post first square, genotype, and leaf position significantly affected abaxial leaf surface pH during 1992 and 1993, while emulsified leaf pH was affected by genotype and leaf sampled in both years (Table 2). Genotype x plant age interaction was significant during 1992 for abaxial leaf surface pH while leaf sampled x plant age interactions were significant for leaf surface pH during 1993 and for emulsified leaf pH during both years.

TABLE 2. Mean Squares for Abaxial Leaf Surface and Emulsified Leaf pH of Upper and Lower Main Stem Leaves of Seventeen Cotton Genotypes Greenhouse Grown at College Station, Texas during 1992 and 1993.

		Abaxial surface		Emulsified leaf	
Source	Df	1992	1993	1992	1993
PA	1	5.42[a]	36.01[a]	0.83	0.20
Error a	3	0.30	1.71	0.00	0.08
G	16	1.82[b]	1.11[b]	0.23[b]	0.11[a]
G x PA	16	0.86[b]	0.18	0.04	0.02
Error b	93	0.27	0.36	0.07	0.01
L	1	23.92[b]	1.77[b]	0.46[b]	0.52[b]
L x PA	1	0.17	8.95[b]	3.81[b]	0.29[b]
L x G	16	0.18	0.29	0.07	0.01
L x PA x G	16	0.25	0.17	0.06	0.01
Error c	93	0.23	0.25	0.06	0.01

PA=Plant age (first square and first square plus 4 weeks); G=genotype; L=leaf sampled (top most expanding leaf and oldest fully expanded leaf).

[a, b] Significant at P=0.05 and 0.01, respectively.

Abaxial leaf surface pH averaged 9.06 at the first square stage of growth during 1992 and increased to 9.37 four weeks later when the plants were beginning to flower. Average pH

during 1993 at first square was 9.31, higher than in the first planting, but dropped 0.75 units to 8.56 four weeks later. Emulsified leaf pH of the seventeen genotypes averaged 6.16 units at first square during 1992, lower than 1993 when the average pH was 6.38. There was not a difference in the emulsified leaf pH between sampling dates during 1993 with values of 5.98 and 5.91 for first square and first square plus four weeks, respectively.

Abaxial leaf surface and emulsified leaf pH varied by location of the leaf sampled in both years (Table 2). The significant first order interactions of leaf position with plant age were caused by differences in direction of response across plant ages. In all cases, pH varied by less than 0.5 units (data not shown). Across these seventeen genotypes, the surface pH of the upper-most main stem leaf measuring 3 cm in diameter averaged 8.91 during 1992 and 8.85 during 1993, significantly less than that of the lower main stem leaves that averaged 9.53 and 9.03 respectively for 1992 and 1993. Emulsified upper main stem leaf pH of the seventeen genotypes averaged 6.18 at first square during 1992, while lower leaves averaged 6.44 units. The opposite trend was observed during 1993 with the pH of emulsified upper leaves having a higher pH at 5.99 than lower main stem leaves at 5.90.

Leaf surface pH ranged from 8.41 for Pima S6 during 1992 to 9.63 for Stoneville 453 (Table 3). Pima S6 and 90J57 were among the lowest in abaxial leaf surface pH while Stoneville 453, Deltapine 50, 89F46H, 86E20 and several others were significantly higher during both years. Somewhat of the same trend was observed for emulsified leaf pH with Pima S6 and 90J57 having the lowest or near the lowest pH during both 1992 and 1993. Even so, the ranges in pH observed were not greatly encouraging relative to genetic modification of leaf pH in cotton.

TABLE 3. Genotypic Means[a] of Abaxial Leaf Surface and Emulsified Leaf pH for Seventeen Cotton Genotypes.

	Abaxial surface		Emulsified leaf	
Genotypes	1992	1993	1992	1993
Stoneville 453	9.63 a	8.98 a-c	6.56 a	6.02 bc
Deltapine 50	9.59 ab	9.07 a-c	6.37 a-e	6.01 bc
89F46H	9.50 ab	9.02 a-c	6.50 a-c	5.94 c
86E20	9.44 a-c	9.21 ab	6.46 a-d	6.06 ab
89E51	9.39 a-c	8.77 cd	6.51 a-c	6.00 bc
Lone Star	9.39 a-c	8.77 cd	6.32 a-e	5.86 ef
$86L^2 14L$	9.39 a-c	8.85 bc	6.54 ab	5.95 cd
89E62	9.37 a-c	9.20 ab	6.43 a-d	6.11 a
90C19S	9.34 a-d	9.26 a	6.12 ef	5.94 c-e
89F46S	9.33 a-d	9.07 a-c	6.28 b-e	5.97 c
MACAOS	9.31 a-d	9.00 a-c	6.26 c-e	5.82 fg
$86L^2 9$	9.27 b-d	8.76 cd	6.22 d-f	6.01 bc
Tam. CAB-CS	9.13 c-e	9.00 a-c	6.23 d-f	5.87 d-f
90C19H	9.05 de	9.00 a-c	6.23 d-f	5.86 ef
88G104	8.94 e	9.26 a	6.34 a-e	5.98 bc
90J57	8.46 f	8.49 de	6.12 ef	5.87 d-f
Pima S6	8.41 f	8.24 e	5.97 f	5.75 g
Test mean	9.23	8.94	6.31	5.95
CV (%)	5.2	5.6	3.8	1.7

[a] Means followed by the same letter within columns are not different according to Waller-Duncan LSD at k=100.

While the objectives of this research did not include direct correlation of pH with any specific insect activity, observations of *B. argentifolii* colonization of these 17 genotypes under greenhouse and field conditions provided no clear distinction in susceptibility between those genotypes with the highest and lowest pH (Smith 1994). Deltapine 50 is one of the most resistant cultivars to *B. argentifolii* under field conditions in the Lower Rio Grande Valley of Texas while Stoneville 453 is one of the most susceptible. These two cultivars were near identical in abaxial surface and emulsified leaf pH in this study, suggesting that these characteristics are not useful selection criteria for *B. argentifolii* resistance. On the other hand, the number of genotypes evaluated in this study might be too small to conclude that pH could not be manipulated as a host plant resistance trait in cotton for *B. argentifolii* or other cotton insect pests.

LITERATURE CITED

Adkisson, P.L. 1964. Comparative effectiveness of several insecticides for controlling bollworms and tobacco budworms. Texas Agri. Exp. Sta. MP-709.

Adkisson, P.L., and S.J. Nemec. 1966. Comparative effectiveness of certain insecticides for killing bollworms and tobacco budworms. Texas Agri. Exp. Sta. Bull. 1048.

Bailey, J.C. 1981. Growth comparison of *Heliothis virescens* (F.) larvae fed white, yellow and orange cotton pollen. Proc. Beltwide Cotton Prod. Res. Conf. 1981:79.

Benedict, J.H., and T.F. Leigh. 1976. Host plant resistance in cotton to lygus bugs and other insect pests. Proc. Western Cotton Prod. Conf. 1976:80-83.

Berlinger, M.J., Z. Magal, and A. Benzioni. 1983. The importance of pH in food selection by the tobacco whitefly *Bemisia tabaci*. Phytoparasitica 11:151-160.

Berlinger, M.J. 1986. Host plant resistance to *Bemisia tabaci*. Agric. Ecosyst. Environ. 17:69-82.

Brazzel, J.R. 1961. Boll weevil resistance to insecticides in Texas in 1960. Texas Agri. Exp. Sta. PR-2171.

Brazzel, J.R. 1963. Resistance to DDT in *Heliothis virescens*. J. Econ. Entomol. 56:561.

Brazzel, J.R. 1964. DDT resistance in *Heliothis zea*. J. Econ. Entomol. 57:455.

Butler, G.D., Jr., and T.J. Henneberry. 1984. *Bemisia tabaci*: The effect of cotton leaf pubescence on abundance. Southwest. Entomol. 9:91-94.

Butler, G.D., Jr., D. Rimon, and T.J. Henneberry. 1988. *Bemisia tabaci* (Homoptera: Aleyrodidae): populations on different cotton varieties and cotton stickiness in Israel. Crop Prot. 7:43-47.

Butler, G.D., Jr., F.D. Wilson, and G. Fisher. 1991. Cotton leaf trichomes and populations of *Empoasca lybica* and *Bemisia tabaci*. Crop Prot. 10:461-464.

Butter, N.S., B.K. Vir, G. Kaur, T.H. Singh, and R.K. Raheja. 1992. Biochemical basis of resistance to whitefly *Bemisia tabaci* Genn. (Aleyrodidae: Homoptera) in cotton. Trop. Agric. 69:119-122.

Carruth, L.A., and L. Moore. 1973. Cotton scouting and pesticide use in East Arizona. J. Econ.Entomol. 66:187-190.

Carter, F.L. 1996. The 1995 insect year. Proc. Beltwide Cotton Prod. Conf. 1996:6.

Collins, G.S., R.D. Lacewell, and J. Norman. 1979. Economic comparison of alternative cotton production practices: Texas Lower Rio Grande Valley. So. J. Agri. Econ. 11:79-82.

Dimock, M.B. 1996. Spod-X LC: Biological insecticide for beet armyworm. Proc. Beltwide Cotton Prod. Conf. 1996:53.

Elleman, C.J., and P.F. Entwhistle. 1982. A study of glands on cotton responsible for the high pH and cation concentration of the leaf surface. Ann. Appl. Biol. 100:553-558.

Frisbie, R.E., J.M. Sprott, R.D. Lacewell, R.D. Parker, W.E. Buxkemper, W.E. Baghley, and J.W. Norman. 1976. A practical method of economically evaluating an operational cotton pest management program in Texas. J. Econ. Entomol. 69:211-214.

Gould, F., A. Anderson, A. Reynolds, L. Bumgarner, and W. Moar. 1995. Selection and genetic analysis of a *Heliothis virescens* (Lepidoptera: Noctuidae) strain with high levels of resistance to *Bacillus thuringiensis* toxins. J. Econ. Entomol. 88:1545-1559.

Harr, J., R. Guggenheim, T. Boller, and J.J. Oertli. 1980. High pH values on the leaf surface of commercial cotton varieties. Cot. Fib. Trop. 35:379-383.

Husain, M.A., A.N. Puri, and K.N. Trehan. 1936. Cell sap acidity and the incidence of whitefly (*Bemisia gossypiperda*) on cottons. Current Sci. 4:486-487.

Jones, L.E., J.I. Dickson, and J.P. Beasley. 1987. Preference and nonpreference of boll weevils to selected cotton. Proc. Beltwide Cotton Prod. Res. Conf. 1987:98-101.

Larson, J.L., R.D. Lacewell, J.D. Casey, L.N. Namken, M.D. Heilman, and R.D. Parker. 1975. Impact of short season cotton production in Texas Lower Rio Grande Valley on producer returns, insecticide use and energy consumption. Texas Agri. Exp. Sta. MP-1204.

Lukefahr, M.J., and D.F. Martin. 1966. Cotton-plant pigments as a source of resistance to the bollworm and tobacco budworm. J. Econ. Entomol. 59:176-179.

Meredith, W.R. 1976. Nectariless cottons. Proc. Beltwide Cotton Prod. Res. Conf. 1976:34-37.

Meredith, W.R., Jr., and M.F. Schuster. 1979. Tolerance of glabrous and pubescent cottons to tarnished plant bugs. Crop Sci. 19:484-488.

Metcalf, R.L., and R.A. Metcalf. 1993. Destructive and Useful Insects. McGraw-Hill, Inc., New York.

Navon, A. 1993. Control of lepidopteran pests with *Bacillus thuringiensis*, pp. 125-176. *In* P.F. Entwistle, J.S. Cory, M.J. Bailey, and S. Higgs [eds.] *Bacillus thuringiensis*, An Environmental Biopesticide: Theory and Practice. J. Wiley, New York.

Ozgur, A.F., and E. Sekeroglu. 1986. Population development of *Bemisia tabaci* (Homoptera: Aleyrodidae) on various cotton cultivars in Cukurova, Turkey. Agric. Ecosyst. Environ. 17:83-88.

Pieters, E.P., and L.S. Bird. 1976. Field studies of boll weevil resistant cotton lines possessing the okra leaf-frego bract characters. Crop Sci. 17:431-433.

Roussel, J.S., and D.F. Clower. 1955. Resistance to the chlorinated hydrocarbon insecticides in the boll weevil (*Anthonomus grandis*, Boh.). Louisiana Agri. Exp. Sta. C. 41.

Schuster, M.F., and F.G. Maxwell. 1974. The impact of nectariless cotton on plant bugs, bollworm, and beneficial insects. Proc. Beltwide Cotton Prod. Res. Conf. 1974:26-27.

Sippell, D.W., O.S. Bindra, and H. Khalifa. 1987. Resistance to whitefly (*Bemisia tabaci*) in cotton (*Gossypium hirsutum*) in the Sudan. Crop Prot. 6:171-178.

Singh, I.D.,and J.B. Weaver, Jr. 1971. Growth and infestation of boll weevils on normal-glanded, glandless, and high gossypol strains of cotton. J. Econ. Entomol. 65:821-824.

Smith, C. Wayne. 1992. History and status of host plant resistance in cotton to insects in the United States. Adv. Agro. 48:251-296.

Smith, W.J. 1994. Resistant characteristics of cotton to sweet potato whitefly (*Bemisia tabaci* Gennadius). M.S. Thesis. Texas A&M University, College Station.

Stephens, S.G., and H.S. Lee. 1961. Further studies on the feeding and oviposition preferences of the boll weevil (*Anthonomus grandis*). J. Econ. Entomol. 54:1085-1090.

Sterling, W., and R.L. Haney. 1973. Cotton yield climbs, costs drop through pest management systems. Texas Agri. Prog. 19:4-7.

Thompson, G.D. 1996. Naturalyte insect control. Proc. Beltwide Cotton Prod. Conf. 1996:51.

Walker, J.K., and G.A. Niles. 1973. Studies of the effect of bollworms and cotton fleahoppers on yield reduction in different cotton genotypes. Proc. Beltwide Cotton Prod. Res. Conf. 1973:100-102.
Wannamaker, W.K. 1957. The effect of plant hairiness of cotton strains on boll weevil attack. J. Econ. Entomol. 50:418-423.
Wilson, F.D. 1982. Present state of the art and science of cotton breeding for insect resistance in the west. Proc. Beltwide Cotton Prod. Res. Conf. 1982:111-117.
Zummo, G.R., J.C. Segers, and J. R. Benedict. 1984. Seasonal phenology of allelochemicals in cotton and resistance to bollworm (Lepidoptera: Noctuidae). Environ. Entomol. 13:1287-1290.

COTTON LEAF SURFACE FEATURES SERVE AS BEHAVIORAL CUES TO SILVERLEAF WHITEFLIES[1]

Allen Carson Cohen[2], Chang-chi Chu[2], Thomas J. Henneberry[2], Thomas Freeman[3], James Buckner,[4] and Dennis Nelson[4]

ABSTRACT

We examined cotton leaves for correlations between surface structures and veins, using light and electron microscopy. Using *Bemisia argentifolii* Bellows and Perring egg placement, nymphal positions and crawler (first-instar nymph) behavior, we evaluated the responses of whitefly nymphs to leaf surface features. Two kinds of epidermal cells predominated the leaf abaxial surface: those underlying vascular bundles and those underlying the areoles (regions between veins). Lamina trichomes (simple and complex) originated from elongated epidermal cells overlaying the veins, including even the most minute (single-stranded) veins. All 2,000 lamina trichomes (non-glandular) that we examined originated from vascular bundle-associated epidermal cells. Areoles of fully expanded leaves had perimeters of 2.463 mm (± 0.1113 S.E., N= 10) and a mean area of 0.382 mm^2 (± 0.0374). Epidermal cells underlying areoles were isodiametric while those underlying veins were elongated. Eggs were generally deposited on the elongated epidermal cells associated with bundles or on cells within ca. 30 µm of those vascular bundle-associated epidermal cells. Crawlers walked about 2300 µm per minute until they settled upon feeding sites that were immediately under the minor veins, never more than about 60-80 µm from the edge of the abaxial bundle-associated epidermal cells. Crawlers spent at least 80% of their time in contact with bundle-associated epidermal cells, apparently making contact with these cells either with legs or antennae.

INTRODUCTION

The silverleaf whitefly, *Bemisia argentifolii* Bellows and Perring, is a devastating pest whose feeding range includes a large variety of host plants (Byrne and Bellows 1991, Bellows et al. 1994). The capacity of *B. argentifolii* to utilize its hosts to build large populations depends upon the ability of individuals to find minor veins in host leaves (Cohen et al. 1996). The highest mortality in the life cycle of *B. tabaci* (Gennadius) was shown to occur between egg hatch and beginning of the second instar (Horowitz et al.1984, Butler et al. 1984). Cohen et al. (1996) reported that all successfully growing

---

[1] Mention of a proprietary product does not constitute and endorsement by the U. S. Department of Agriculture.

[2] USDA, ARS, Western Cotton Research Laboratory, 4135 E. Broadway, Phoenix, AZ 85040-8830 USA.

[3] Electron Miscroscope Facility, North Dakota State University, Fargo, ND 58105.

[4] Biosciences Research Laboratory, USDA, ARS, P.O. Box 5674, Fargo, ND 58105.

immatures were connected via salivary sheaths to minor veins in cantaloupe, cotton, lantana, hibiscus and lettuce leaves. Earlier studies by Pollard (1955) and by Cohen et al. (1993) indicated that some nymphs of *B tabaci* and *B. argentifolii* were not connected via salivary sheaths to veins. These studies, undertaken with leaf sectioning techniques, proved to be less reliable than intact leaf staining and clearing methods (Cohen et al. 1996). It is now evident that successful development by *B. argentifolii* demands the ability of newly emerged first-instar nymphs, known as crawlers, to find minor veins which occupy less than 10% of the volume of the leaf (Chu et al. 1995, Cohen et al. 1996). There is also a time window of about 3 to 5 hours, after which crawlers will die if they do not succeed in reaching a feeding site (Cohen unpublished data). Furthermore, factors of host plant structure, including depth, frequency and size of minor veins impose stringent constraints upon the ability of crawlers to locate appropriate feeding sites (Cohen et al. 1996).

One surface feature that has received extensive attention relative to insect preferentia is the presence or absence of hairs or non-glandular trichomes. Several reports indicate preferences that many homopterous insects show for hirsute (hairy) leaves (Wilson et al. 1993; Butler and Wilson 1984; Weathersbee et al. 1995). Generally, these reports explain the basis of the preference as either added protection from desiccation or complication of searching by entomophages on hairy surfaces. Why trichomes are associated with vascular bundles is unknown.

We report here the results of studies on the relationship between surface features of cotton (*Gossypium hirsutum* L.) leaves and minor veins. Within this context, we further report on whitefly behavioral observations that appear to be correlated with location of veins, including oviposition, nymphal settling sites and locomotory activities of crawlers.

MATERIALS AND METHODS

We used cotton leaves from the cultivars Delta Pine 90 (DPL 90) and DPL 115. We prepared intact leaf disks (1.0 cm dia.) for light microscopy by staining them with McBride's acid fuschin (1% in 90:10 ethanol glacial acetic acid) for 4 h, destaining in 95% ethanol for 2 min, then clearing by autoclaving in lactic acid, glycerol, and water (1:1:1) at 121° C, 15 psi for 25 min. Disks were mounted under cover slips in fresh clearing agent. Stained, cleared disks were examined with a dissecting microscope equipped with a video imaging system and with a compound microscope equipped with a camera. This examination included measurements of the positioning of eggs and nymphs in relation to the distribution of minor veins, trichomes and cell surface features, including surface morphology of epidermal cells.

Leaves, for electron microscopy examination, were fixed in glutaraldehyde, dehydrated in EtOH and critical point dried using CO_2 as a transitional fluid or fixed and dehydrated in acidified 2,2-dimethoxypropane prior to critical point drying. Specimens were viewed with a JEOL JSM 6300 scanning electron microscope. The two selected photographs were taken at 15 kV. Electron micrographs were used to make measurements of the epidermal cells and for assessment of epidermal surface contour.

To study whitefly behavior, we exposed five DPL 115 (hairy-leafed isoline) to heavy infestations of whiteflies in a greenhouse for 2 days. Within ten-20 mm^2 randomly chosen abaxial surface sections, we counted the number of eggs within and outside of a 600 μm diameter around lamina trichomes. We chose this diameter as an extremely conservative distance that was less than the labium-ovipositor length of female *B. argentifolii*. Twenty newly-hatched first-instar nymphs were observed on fresh leaves and tracked with a

Boecleler VIA-170 video imaging system as they moved over the surface of leaves. All measurements were made in the laboratory at 25° C under fiber optic light illumination. Distances traveled over a 2-min interval and pattern of movement were recorded in relation to position of surface cells associated with vascular bundles. Positions of eggs were recorded in relation to vascular bundle-related surface cells, stomata, and trichomes. Measurements were made to determine the percentage of total leaf surface area that was within a 600 μm diameter of trichomes (the approximate distance between the labium and ovipositor). We conservatively estimated that, if a female inserted an egg within this area, she could have been in contact with a lamina trichome. Distances were measured between egg pedicels and trichomes using the video imaging system calibrated with a stage micrometer. Difference among these measurements were evaluated with χ^2 tests and ANOVA (SAS Institute 1988).

RESULTS

Non-glandular leaf trichomes all originated from vein-associated epidermal cells (Fig. 1), as evidenced by examination with light and electron microscopy. Epidermal cells in the areoles were about 35 x 35 μm each, while those associated with veins were about 80 x 22 μm each. Elongated epidermal cells (Figs. 1 and 2) were evident wherever veins were present, including even the finest, single-stranded veins. The hairy cotton isoline DPL 115 contained 48 (+/- 2.5) trichomes/ cm^2. All of the 2000 lamina trichomes examined originated from epidermal cells associated with vascular bundles. Eggs were usually deposited on these elongated epidermal cells associated with bundles or on cells within 30 μm of those bundle-associated epidermal cells.

Stomata on the adaxial surface were about half as numerous as those on the abaxial side of leaves (156.8 ±10.28/ mm^2 versus 321.6 ± 13.51/ mm^2, mean ± S.E., respectively; N=15). The width of bundles of minor vein-associated epidermal cells was 35.6 μm (± 0.86 S.E.; N=20), and the mean distance across areoles or between closest minor veins was 531 μm ± 28.3 μm, S.E.; N=100). Observation of 300 stained feeding sheaths in cleared leaves revealed no evidence of stylet penetration through stomatal openings.

FIG. 1. Abaxial surfaces of cotton leaves, showing the elongated cells associated with veins.

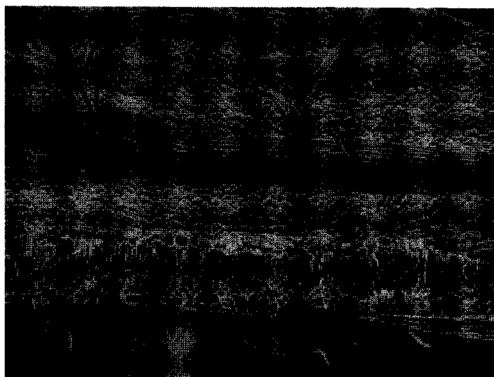


FIG. 2. A fracture section of a cotton leaf showing various surface features such as trichomes, major (raised) and minor veins (with their characteristic elongated surface cells). Internal features are also evident, including minor veins, upper and lower epidermis, spongy parenchyma and palisade layer. Lamina trichomes and a whitefly egg are visible on the undersurface.

Figure 3 is a feeding model based on a diagram of a potential feeding site superimposed on a cotton leaf surface with a single areole outlined. The perimeter of this structure was about 2,000 µm and the area within about 450,000 μm^2. Since a crawler's stylet tip cannot reach beyond 70-80 µm from the site of labial contact with the plant's surface, this feeding model was conservatively based on an 80 µm boundary around minor veins (Cohen et al. 1996). An area whose perimeter is about 80 µm from the minor vein

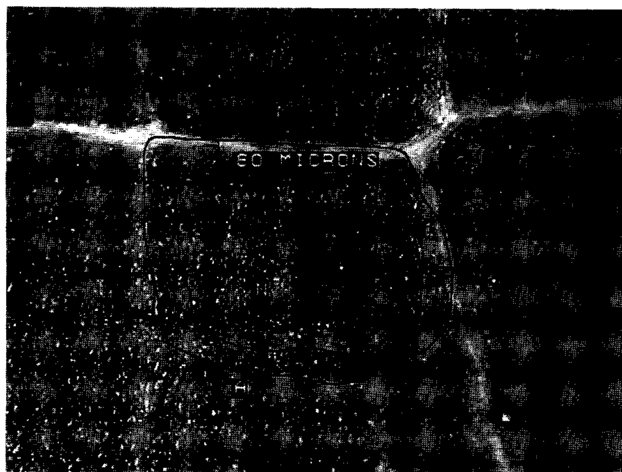


FIG. 3. Abaxial surface from a cotton leaf showing the minor veins, an areole labeled "A" and an area referred to as a non-target zone "N" (where probes by crawlers cannot reach veins). The non-target zone is about 57% of the entire areole.

surrounding the areole is demarked with another line. The area within this smaller region represented the total surface from which vascular bundles would be out of reach to a probing crawler (Fig. 3, "N"). Therefore, defined area of about 250,000 μm^2 is the area within the areole that was unavailable as a potentially usable feeding site. In this case, roughly 55% of the areole surface and surrounding minor veins were unavailable to a crawler searching for a vascular bundle to use as a feeding target. Areole perimeters measured 2.463 mm (± 0.111 S.E.; n= 10), and areoles had a mean area of 0.3821 mm (± 0.0374 S.E.; n= 10). Fig. 4 is a crawler on a leaf surface, showing the relative span of the legs in relationship with the size of the epidermal cells.

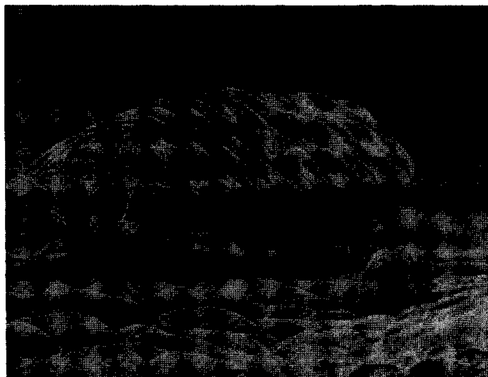


FIG. 4. A crawler on the abaxial surface of a cotton leaf, showing the relative size of surface features with the appendage span and setae of the searching whitefly. Individual epidermal cells and their irregular contour are visible.

In the ten, 20 mm^2 surfaces examined, there was a total of 81 eggs, 57 within the 600 µm diameter of the trichomes and 24 eggs outside of this diameter. The surface of the leaf within 600 µm of trichomes is 10.1 % of the leaf area (excluding major veins which *B. argentifolii* avoid as oviposition sites). Assuming that egg deposition is totally random, we would expect about 10% of the eggs (=8) would be placed within the 600 µm diameter around trichomes, and 90 % or 73 eggs would be placed outside of the 600 µm diameter. Therefore, the 57 eggs found within the diameter around trichomes is highly significant ($P < 0.005$; $\chi^2 = 301.2$; d.f. = 1).

Walking speed varied from a very fast pace with infrequent stops soon after hatch to a very slow pace with frequent stops prior to settling. During fast-paced movements, individuals walked an average of 2,300 µm per minute (± 280 µm, S.E.; n= 30) until they settled upon feeding sites that were within about 60 µm from the center of the abaxial bundle-associated epidermal cells. About 80% of searching time (= 96 s, ± 28 S.E. of 120 s; N = 30) was spent in contact with the elongated vein-associated epidermal cells, apparently making contact with these cells (possibly with legs or antennae). Crawlers, frequently back-tracked, turning around at a given site. Then they would either settle or commence walking again. None of the 30 crawlers that we observed walked onto the petiole nor made any other apparent effort to abandon the leaf upon which they were

situated. All of the crawlers observed here remained within an area of about 100 mm of the initial point where they were first detected. Although leaves were observed with the abaxial surface oriented upwards, there were no efforts by crawlers to go to the positional underside of the leaves. This is in contrast with adult behavior observed by Chu et al. 1995.

DISCUSSION

The selection of a host plant to be used by the developmental stages of aleyrodids is pre-determined by the mother's ovipositional choices (van Lenteren, and Noldus 1990, Byrne and Bellows 1991). However, the crawler selects a specific feeding site on the leaf, apparently according to surface features that provide cues that target plant tissue is within reach of the stylets (Cohen et al. 1996). For insects such as potato aphids that feed on larger veins, the prominence of leaf veins is a likely cue for commencement of feeding activity (Gibson 1972). While the location of feeding sites has been the subject of extensive investigation in aphids (*e.g.*, Pollard 1973, Klingauf 1987), there has been relatively little attention to the specific site-selection stimuli in aleyrodids (Cohen et al. 1996).

The importance of *B. argentifolii*'s host finding cues is underscored by these facts: 1) these insects are obligate phloem feeders (Cohen et al. 1996); 2) the reach of the stylets of first- instar nymphs of this species is limited to less than 70-80 μm from the point of labial contact to the vascular bundle; 3) crawlers can live for only a few hours before they either starve or desiccate if they do not reach a food source (Cohen, unpublished data); and 4) the principal mortality in *Bemisia* species occurs between egg hatch and the onset of the second-instar nymphal stage (Horowitz et al. 1984). The geometric model of feeding by *B. argentifolii* (Cohen et al. 1996) described the constraints of the feeding relationship. This includes the fact that whitefly nymphs must start their feeding probes on the vascular bundle-associated epidermal cells or within about 70-80 μm from these elongated epidermal cells. These constraints are apparent in Fig. 3 which shows that in a typical areole with an area of 450,000 μm^2, there is an area of about 250,000 μm^2 where initiation of a feeding probe could not possibly terminate in successful contact with the minor vein.

Using this model which includes the vascular bundle-associated epidermis and an area of about 80 μm around these bundles, and a non-target zone that includes the remaining epidermis where a probe will never result in contact with a minor vein, we have a clear picture of the crawler's potential choices. Based upon these measurements, if we assumed that the crawler made random probes on the abaxial surface, it would have a less than a 50% chance of reaching a target vein. Under these assumptions, if it missed the vein in the first probe, its chances of missing again in the second would also be a little more than 50% in subsequent probes. Therefore, we would expect that about 50% of all crawlers would miss once, 25% would miss twice, 12.5% would miss three times. Without knowing the limits of how many misses are possible before the crawler dies, it is impossible to predict how much accuracy is required of a crawler. However, considering the time and material investment involved in finding a suitable minor vein, it is probable that a random search would be highly non-adaptive. The fact that a crawler can move at a rate of 2,300 μm/min suggests a potential that a crawler can cover the full width or length of a cotton leaf in 2 hours. Although our observations reveal that crawlers spend considerable time turning and back-tracking, we have also noted that they search areas that amount to at least 1/4 of the surface area of a leaf before they settle.

If the search for suitable feeding sites is a non-random process, then the cues regarding the location of veins must be surface features. For insects such as potato aphids

that feed on large veins (Gibson 1972), location of the veins is simply the recognition of raised sites corresponding to major veins. However, the minor veins are far less conspicuous and not characterized by raised surface features.

We found that the minor veins in cotton leaves are associated with elongated epidermal cells. Vascular bundle-associated epidermal cells were approximately 80 x 22 µm, while the other epidermal cells were about 35 x 35 µm. Although the minor veins themselves were not readily visible in fresh leaves, these veins were evident under 200x magnification where they were seen to be darker green than areole regions. Under SEM, the vascular bundle-associated epidermal cells were apparent because of the elongated appearance of these cells compared to the isodiametric appearance of the other epidermal cells. Although it was not apparent from cross sections, SEM observations reveal slight depressions or ruts in the surface epidermal cells that are associated with minor bundles. While it is possible that this is an artifact resulting from differential drying, it may represent a natural contour of the surface of cotton leaves. Another characteristic feature that differentiates vascular bundle-associated epidermal cells from non-vascular associated epidermis is that trichomes were present only in the former. One other difference between the epidermal cells is that stomata only occurred in non-vascular-associated epidermal cells. There were about twice as many stomata on the abaxial surface than on the adaxial side of the leaf. However, out of several hundred stylet penetrations observed *B. argentifolii* feeding, none involved stomata; therefore, stomatal openings appear to be of little or no importance in this species.

Whatever the surface features were that allowed recognition of vascular bundle sites (visual and/or tactile), the crawlers were observed to spend 80% of their search time in contact with the vascular bundle-associated epidermal cells. Since these cells constitute less than 25% of the leaf surface, it must be concluded that search is not random, but rather is in response to some feature of the elongated epidermal cells. Since the surface comprised of elongated cells and the proximate epidermal cells within a border of 80 µm comprises nearly 50% of the area of a typical areole, we reasoned that if search is random, nymphs should spend about half their time in this zone and the other half in the non-target zone. The fact that nymphs spent 80% of their time within the target zone suggests that there is a contact stimulus to remain near bundles, and this pre-disposes nymphs to begin their probes close to sites where successful feeding is most likely.

This study has shown that in hairy cotton leaves such as DPL 115 (hairy isoline), there is an obvious preference for oviposition sites near the lamina trichomes. While it is not apparent from this study what the advantages are in placing eggs close to trichomes, it is possible that the proximity of the trichomes to vascular bundles might favor water uptake by the eggs, which have been shown to derive much of their water from plant cells (Byrne et al. 1990). The details and mechanisms of ovipositional site selection by *B. argentifolii* warrents further attention, especially in relationship to trichomes and other surface features.

We feel that it is important to understand this surface/ feeding relationship in order to develop alternative strategies to pesticide treatments to control whiteflies. Several studies have shown that whiteflies have definite preferences for certain kinds of host plants, in part, in relation to certain plant physical features (Chu et al. 1995; Cohen et al. 1996). They also show definite preferences for different varieties of host plants within a given species (Natwick et al. 1995, Rao et al. 1990, Puri et al. 1993, and Wilson et al. 1993). While the reality of these differences in feeding choice are well-documented, the basis is still poorly understood. If plant breeders or genetic engineers knew which surface features might confuse the normal stimulus-response pattern of crawlers, they could use

this as a first line of defense in developing a management system aimed at thwarting the impressive efficiency of colonization and host plant utilization of this important pest.

ACKNOWLEDGEMENT

We wish to thank Don Brummett and Jackie Cohen for their technical assistance, Drs. A. A. Weathersbee, III and W. R. Meredith, Jr., for their kind gift of several isolines of DPL 115 seed and Drs. Eric T. Natwick, Dan Gerling, Joe Leggett and Alvin Simmons for their helpful comments on an earlier version of this manuscript.

LITERATURE CITED

Byrne, D. N., A. C. Cohen, and E. A. Draeger. 1990. Water uptake from plant tissue by the egg pedicel of the greenhouse whitefly, *Trialurodes vaporariorum* (Westwood) (Homoptera: Aleyrodidae). Can. J. Zool. 68: 1193-1195.

Byrne, D. N., and T. S. Bellows. 1991. Whitefly biology. Ann. Rev. Entomol. 36: 431-457.

Bellows, T. S., T. M. Perring R. J. Gill, and D. H. Headrick. 1994. Description of a species of *Bemisia* (Homoptera: Aleyrodidae). Ann. Entomol. Soc. Amer. 87: 195-206.

Butler, G. D., and F. D. Wilson. 1984. Activity of adult whiteflies (Homoptera: Aleyrodidae) within plantings of different cotton strains and cultivars as determined by sticky-trap catches. J. Econ. Entomol. 77: 1137-1140.

Chu, C. C., T. J. Henneberry, and A. C. Cohen. 1995. Silverleaf whitefly (Homoptera: Aleyrodidae): Host preference and factors affecting oviposition and feeding site preference. Environ. Entomol. 24: 354-360.

Cohen, A. C., C. M. Newman, and D.L. Hendrix, 1993. Histochemical studies of feeding sites of *Bemisia tabaci* on cotton leaves, p. 959. *In*: D. J. Herber and D. A. Richter [eds.] Proceedings, Beltwide Cotton Conferences, 9-16 February, National Cotton Council of America, Memphis, TN.

Cohen, A. C., T. J. Henneberry, and C. C. Chu. 1996 Geometric relationships between whitefly feeding behavior and vascular bundle arrangements. Entomol. Exp. App. 78: 135-142.

Gibson, R.W. 1972, The distribution of aphids on potato leaves in relation to vein size. Entomol. Exp. App. 15: 213-223.

Horowitz, A. R., H. Podoler, and D. Gerling. 1984. Life table analysis for the tobacco whitefly *Bemisia tabaci* (Gennadius) in cotton fields in Israel. Ecol. Applic. 5: 221-233.

Inayatullah, C. M. A. Ghani, and A. Ghaffer, 1985. Cotton whitefly, *Bemisia tabaci* (Genn.) and its control.. Lahore, Punjab, Directorate of Agricultural Information. 90 pp.

Klingauf, F. A. 1987. Host plant finding and acceptance, pp. 209-223. *In* Aphids Their Biology, Natural Enemies and Control. Vol. A. A. K. Minks, and P. Harrewijn, [eds.]. Elsevier, Amsterdam.

Lenteren, J. C. van, and L. P. J .J. Noldus. 1990. Whitefly-plant relationships: behavioral and ecological aspects, pp. 47-89. *In* D. Gerling [ed.] Whiteflies: their Bionomics, Pest Status and Management. Intercept, Andover, UK.

Mauseth, J. D. 1988. Plant Anatomy. The Benjamin/Cummings Publishing Company, Inc. Menlo Park, CA. 560 pp.

Natwick, E. T., C. C. Chu, H. H. Perkins, T. J. Henneberry, and A. C. Cohen. 1995. Pima and upland cotton susceptibility to *Bemisia argentifolii* under desert conditions. Southwest. Entomol.. 20: 429-438.

Pollard, D. G. 1955. Feeding habits of the cotton whitefly *Bemisia tabaci* Genn. (Homoptera: Aleyrodidae). Ann. Appl. Biol. 43: 664-671.

Pollard, D. G. 1973. Plant penetration by feeding aphids (Hemiptera, Aphidoidea): a review. Bull. Entomol. Res. 62: 631-714.

Puri, S. N. A. S. Ansingkar, V. N. Ajankar, R. C. Lavekar, G. D. Butler, Jr., and T. J. Henneberry, 1993. Effect of cotton leaf morphology on incidence of *Bemisia tabaci* Genn. on cotton. J. Appl. Zool. Res. 4: 41-44.

Rao, N. Venugopal, A. S. Reddy, R. Ankaiah, Y. N. Rao, and S. M. Khasim. 1990. Incidence of whitefly (*Bemisia tabaci*) in relation to leaf characters of upland cotton (*Gossypium hirsutum*). Indian J. Agric. Res. 60: 619-624.

SAS Institute. 1988. SAS user's guide, basics. SAS Institute, Cary, NC.

Weathersbee, A. A., III, D. D. Hardee, and W. R. Meredith, Jr. 1995. Differences in yield responses to cotton aphids (Homoptera: Aphididae) between smooth-leaf and hairy-leaf isogene cotton lines. Econ. Entomol. 88: 749-754.

Wilson, F. D., H. M. Flint, B. R. Stapp, and N. J. Parks, 1993. Evaluation of cultivars, germplasm lines, and species of *Gossypium* for resistance to Biotype "B" of sweetpotato whitefly (Homoptera: Aleyrodidae). J. Environ. Entomol. 86: 1857-1862.

AN UNUSUAL CASE OF POLYGYNY IN *SOLENOPSIS INVICTA* BUREN[1]

S. Bradleigh Vinson and Sherry Ellison
Texas A&M University, Department of Entomology
College Station, TX 77843-2475

ABSTRACT

The occurrence of a polygyne colony of imported fire ants containing several physogastric queens is reported. Previous studies of polygyne colonies revealed that the queens are not physogastric, and individuals produce fewer eggs than monogyne queens. The physogastric polygyne condition, reported here, remained stable over the four months of study. Separation of the queens from the main colony and from each other resulted in their execution by workers. Whether the occurrence of multiple physogastric queens is a rare condition or represents a new form of fire ant remains to be determined. If polygyne colonies of physogastric queens occur with any frequency their existence would prove difficult to explain in regard to current ideas concerning queen number. For this reason the regulation of queen number is discussed.

INTRODUCTION

The red imported fire ant, *Solenopsis invicta* Buren was accidentally introduced into Mobile, Alabama, from South America between 1920-1930 (Vinson and Greenberg 1986). Since its introduction, this ant has gradually expanded its range to infest over 275 acres within all or part of nine southern states and Puerto Rico with a few infested counties occurring in Virginia, Tennessee, and Oklahoma (Callcott and Collins 1995). Fire ant populations within the United States are predominantly monogyne with the exception of Texas and several smaller areas scattered throughout the infested range (Porter et al. 1991, Porter 1992). The monogyne condition for this species was considered the norm until 1973 when Glancey et al. (1973) reported on the existence of two colonies north of Jackson, Mississippi estimated to be 6-8 months old with 20 inseminated females taken from one colony and two from another. Two more colonies with two workers producing queens were found near Gulfport, Mississippi (Glancey et al. 1973). Hung et al. (1974) reported the existence of many colonies of multiple queens in Texas which also contained numerous sterile males. Over the intervening years these polygyne populations have been found at more and more sites throughout the southeastern United States (Fletcher 1983; Porter et al. 1991, 1992; Porter 1992); however, their ability to outcompete the monogyne form and rapidly spread appears limited (Greenberg et al. 1992, Macom and Porter 1996).

[1]Hymenoptera: Formicidae

Polygyne colonies have been characterized by the presence of numerous inseminated and worker producing queens (Hung et al. 1974, Vargo and Fletcher 1986), by the presence of workers that are 33% smaller on the average (Greenberg et al. 1985), by workers that are less aggressive towards non-nestmate conspecifics (Mirenda and Vinson 1982), by higher mound density per hectare by a factor of two to three (Greenberg et al. 1992, Malcomb and Porter 1995, Porter 1992), by the presence of sterile males (Hung et al. 1974, Ross and Fletcher 1985), by uninseminated male producing gynes (Vargo and Ross 1989), and by queens that produce fewer eggs per day per queen (Fletcher et al. 1980, Ross 1988, Vargo and Fletcher 1989, see also Tschinkel and Howard 1978). This lower reproductive output of associated queens is not uniform, and the queens in a polygyne colony can differ substantially in the number of eggs each produces (Vargo and Fletcher 1989) and in the viability of their eggs (Vargo and Ross 1989). The numbers of queens in a polygyne colony may range from three to several hundred (Vargo and Fletcher 1989). However, the lower reproductive output of individual queens and the lack of physogastric queens in polygyne colonies is essential to current theories regarding the regulation of queen number and the evolution of polygyny (Fletcher and Blum 1981, 1983a,b).

The queens of well developed monogyne colonies are physogastric (i.e., the abdomen is swollen and these queens may oviposit upwards of 65 eggs/hr [Fletcher et al. 1980]). These queens, at least in east Texas (Ellison and Vinson, per obs.), are often covered with mites of the genus *Mesostigmata* (Hunter and Costa 1971, Hermann et al. 1970). In contrast, neither physogastric or heavily mited queens have been reported in polygyne colonies. Herein we report the occurrence of a multiple physogastric queen colony which was stable over the four months of study. These queens were also covered with mites.

MATERIALS AND METHODS

The colony, along with five others, was excavated from Brazos County from a known polygynous area and brought to the laboratory in a 5-gal bucket coated with talc to prevent the ants escape. After three days, the colony was separated from the soil by the "drip-method" (Jouvenaz et al. 1977). When the workers and queens were collected from the flooded bucket, two queens were observed that appeared to be physogastric, and they were placed, along with several others, in a rearing container with several thousand workers. The rearing container consisted of a shoe box, the sides of which were coated with Fluon® (ADI Fluon, ICI Americas Inc., Bayonne, NJ), and contained a 15-cm, plastic Petri dish containing a thin castone bottom to hold moisture. Four 3-cm holes were melted in the top of the Petri dish to allow the workers to enter or leave. The colony was provided an artificial diet described by Bhatkar and Whitcomb (1970), 2-3 ml of a 50% honey solution, and a couple of meal worms or crickets on a 2-day schedule routinely employed to maintain all of the laboratory colonies. Water was available ad libitum.

After one and one half months, the queens were located and three physogastric and mite-infested queens were found. These were also the only queens found. These were weighed, their egg production determined, and they were returned to the colony. Their egg production was determined by confining each queen under a 5-cm, inverted opaque plastic bottle cap for 5 hr on moist filter paper. After 5 hr the queens were returned to the colony and the eggs counted.

This colony was maintained for an additional two and a half months with the queens located and their visible condition recorded monthly. At four months the queens were removed and re-weighed. The queens were returned to the colony and six days later they

were again removed to determine if one was more dominate in attracting workers. This was done because in other polygyne colonies we have examined, one queen produces most of the eggs and attracts most of the workers (Chen 1996). A dominance test (Chen and Vinson, unpublished data) was used which consists of placing each queen under a screen cap (5 cm in diameter and 8 mm high) through which the workers can enter and leave but which confines the queen. Each screen cap with a queen was placed in a 9-cm, plastic Petri dish with a castone bottom to retain moisture. The Petri dish had several holes through which workers could pass. Each queen and her nest were placed an equivalent distance from the original nest and the workers were allowed to chose which queen to congregate around. This was repeated a second time, but with the approximately 10,000 workers removed to a clean 15-cm, castone bottom plastic nest as described above. The number of workers around each queen and the behavior of workers towards each queen was recorded 24 hr later.

RESULTS

Of the six collected colonies, all were polygyne with several inseminated queens. However, only the one colony contained physogastric or mited queens. A month and a half after collection, this one colony was found to contain three physogastric queens (Fig. 1A-C), as evidenced by an enlarged abdomen with distinct intersegmental membranes (Fig. 1D). Each queen was heavily mited, typical of queens in monogyne colonies (Fig. 2). A sample of worker head capsule measurements (N=31, $\bar{x}$ 0.701 mm SD=0.163 mm) indicated the colony was polygyne (Greenberg et al. 1985, 1992). The three queens weighed 20.6, 20.8, and 21.3 mg which is typical of physogastric monogyne queens (Vargo and Fletcher 1989). Weights of polygyne queens rarely exceed 14.2 mg (Vargo 1992). They produced 342, 368, and 402 eggs, respectively, in 5 hr for an average of 74 eggs/hr, slightly higher than that reported by Fletcher et al. (1980) for monogyne physogastric queens.

During the next two and one half months, the colony tripled in size with a high proportion of brood. During this period the three queens were generally clustered in the colony and remained physogastric and heavily mited.

After four months we decided to examine these queens again and determine if one was dominate. They were re-weighed and each lost a little weight, weighing 20.6, 15.6, and 18.4 mg, but were still physogastric. The queens remained heavily mited. The first dominance test showed no difference in recruitment of workers in 4.2 hr to each of the three queens, but the recruitment was lower than expected (<50 workers per queen) as the majority of workers remained with the large brood mass of the colony, and very little brood was transported to the queen locations.

The test was repeated with approximately 10,000 workers. After 24 hr the three queen containers and their associated ants were removed and each was placed in a clean shoe box, the ants counted, and the queens examined. We found that all three queens had been severely attacked. Queen 1 and 3 had recruited 3,177 and 4,557 workers, respectively, and both had their abdomen perforated. Both queens died within two hours. Queen 2, which earlier weighed the least, had 1,886 workers and several abdominal sclerites were chewed, but she remained intact. The workers continued to attack until she was returned to the original colony where she survived and remained physogastric.

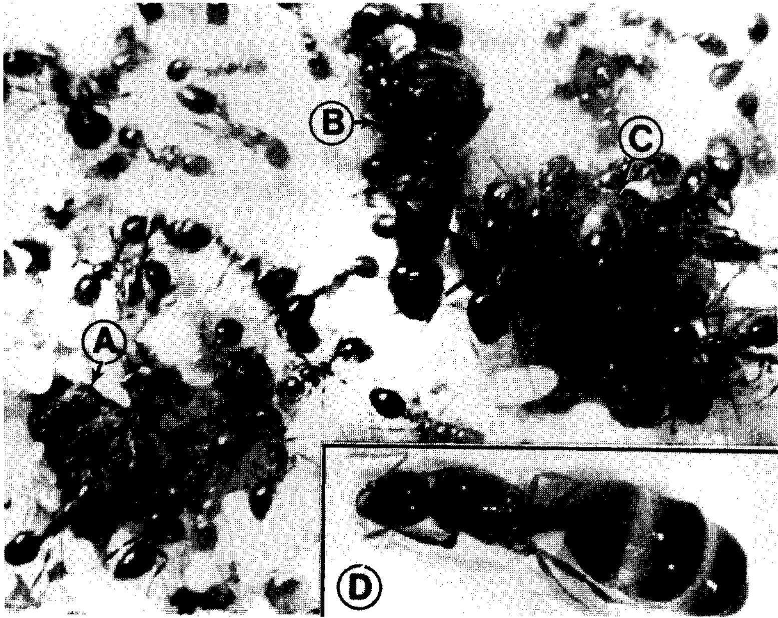


FIG. 1. Three physogastric queens can be seen in an imported fire ant colony (arrows). One of the three queens, which was similar to the other two, is seen in the insert.

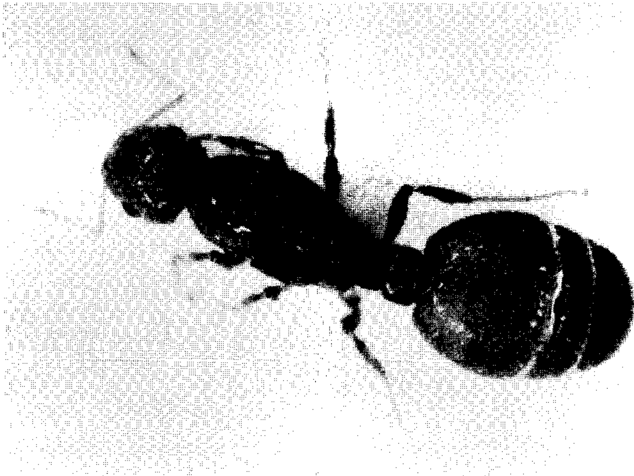


FIG. 2. A monogyne queen showing the heavy infestation of mites.

DISCUSSION

This report documents the occurrence of three highly reproductively active queens coexisting in the same colony. The other five colonies contained 5-12 inseminated queens which were not mited and none of which were physogastric. This suggests that this colony was not typical of the area. The maintenance of the highly reproductive physogastric condition in one colony was accompanied by the large amount of brood produced over the four months suggests the condition was stable. The high hourly egg production by each queen confirms that all three were contributing eggs to the colony and that the physogastric condition was not due to a disease or parasitism. Based on worker size the colony was consistent with the multiple queen form rather than an unusual form of the monogyne condition. Clearly egg production, in this example, was not suppressed. Yet the presence of three physogastric queens in the same colony is in contrast to the lower fecundity which is considered a general feature of queens in polygyne social insect colonies (Michener 1964).

Fletcher and Blum (1981, 1983b) suggested that pheromones from the queen influences reproductive development, whereas Vargo and Fletcher (1986) suggest the queen pheromones may effect larvae. Queen pheromones may also regulate queen number (Fletcher and Blum 1983a). As discussed by Fletcher and Blum (1983a), workers recognize a queen by a pheromone blend characteristic of each species. The pheromone varies in different queens so that each has a characteristic odor which workers can detect. A queen pheromone is also released (Fletcher and Blum 1983a) with an optimal level that influences worker behavior. Workers only tolerate a certain level of this pheromone. When a queen is lost, the pheromone level drops allowing workers to accept another queen. If there are many queens, some are executed bringing the pheromone level back within the optimal range.

In polygyne colonies the lower fecundity of queens is indicative of a lower pheromone production (Fletcher and Blum 1983a). Thus, several queens may coexist in a colony before the tolerance threshold of the workers is exceeded which is assumed to be maintained at some level approximating a monogyne colony with all the queens suppressed in pheromone and egg production (Vargo 1992). Although Fletcher and Blum (1983a) suggest that the workers of polygyne colonies may tolerate a higher total pheromone titer, it is unclear whether the pheromone titer of each queen must, nevertheless, be suppressed. An attempt to determine if one queen was dominate (Chen and Vinson, unpublished data) and, thus, presumed to have more pheromone, was not successful and indicated that the workers preferred to remain with the massive brood and the high levels of pheromone that presumably remained in the colony. When these workers were removed to a neutral environment and given access to these three queens, the attempted execution of the three queens, two being successful, is in contrast to the results of similar tests with multiple queen colonies where workers generally prefer one queen over the others and do not, in the time frame used here, execute the other queens. The results also suggest that these three queens derived some advantage by remaining in close contact within the brood chamber.

More importantly, this report documents for the first time, the occurrence of physogastric queens in multiple queen colonies. Whether this is a rare occurrence or represents a new form of multiple queen colonies remains to be determined.

REFERENCES

Bhatkar, A., and W. H. Whitcomb. 1970. Artificial diet for rearing various species of ants. Florida Entomol. 53: 229-232.

Callcott, A. M., and H. Collins. 1995. Invasion and range expansion of imported fire ants in North America from 1918 to 1993, pp. 50-61. *In:* Proc. International Pest Symposia and 1995 Imported Fire Ant Research Conference, San Antonio, TX.
Fletcher, D. J. C. 1983. Three newly-discovered polygynous populations of the fire ant, *Solenopsis invicta*, and their significance. J. Georgia Entomol. Soc. 18: 538-543.
Fletcher, D. J. C., and M. S. Blum. 1981. Pheromonal control of dealation and oogenesis in virgin queen fire ants. Science 212: 73-75.
Fletcher, D. J. C., and M. S. Blum. 1983a. Regulation of queen number by workers in colonies of social insects. Science 219: 213-314.
Fletcher, D. J. C., and M. S. Blum. 1983b. The inhibitory pheromone of queen fire ants: effects of disinhibition on dealation and oviposition by virgin queens. J. Comp. Physiol. A153: 467-475.
Fletcher, D. J. C., M. S. Blum, Y. N. Whitty, and T. V. Temple. 1980. Monogyny and polygyny in the fire ant, *Solenopsis invicta*. Ann. Entomol. Soc. Amer. 73: 658-661.
Glancey, B. M., C. H. Craig, C. E. Stringer, and P. M. Bishop. 1973. Multiple fertile queens in colonies of the imported fire ant, *Solenopsis invicta*. J. Ga. Entomol. Soc. 8: 237-238.
Greenberg, L., D. J. C. Fletcher and S. B. Vinson. 1985. Differences in worker size and mound distribution in monogynous and polygynous colonies of the fire ant *Solenopsis invicta* Buren. J. Kansas Entomol. Soc. 58:9-18.
Greenberg, L., S. B. Vinson, and S. Ellison. 1992. Nine-year study of a field containing both monogyne and polygyne red imported fire ants (Hymenoptera: Formicidae). Ann. Entomol. Soc. Am. 85: 686-695.
Hermann, H. R., M. S. Blum, and A. N. Hunt. 1970. Myrmecophilous arthropods associated with imported fire ant, *Solenopsis saevissima* (Hymenoptera: Formicidae). Proc. La. Acad. Sci. 33: 13-18.
Hung, A. C. F., S. B. Vinson and J. W. Summerlin. 1974. Male sterility in the red imported fire ant, *Solenopsis invicta* Buren. Ann. Entomol. Soc. Amer. 67:909-912.
Hunter, P. E., and M. Costa. 1971. Description of *Cymnolaelaps shealsi* N. sp. (Acarina: Mesostigmata) associated with the imported fire ant. J. Ga. Entomol. Soc. 6: 51-53.
Jouvenaz, D. P., G. E. Allen, W. A. Banks, and D. P. Wojcik. 1977. A survey of pathogens of fire ants, *Solenopsis* spp. in the Southeastern United States. Florida Entomol. 60: 275-279.
Macom, T. E., and S. D. Porter. 1996. Comparison of polygyne and monogyne fire ant (Hymenoptera: Formicidae) population densities. Ann. Entomol. Soc. Amer. 89: 535-543.
Michener, C. D. 1964. Reproductive efficiency in relation to colony size in hymenopterous societies. Insect Sociaux 11: 317-341.
Mirenda, J. T., and S. B. Vinson. 1982. Single queen and multiple queen colonies of imported fire ants in Texas. Southwest. Entomol. 7:135-141.
Porter, S. D. 1992. Frequency and distribution of polygyne fire ants (Hymenoptera: Formicidae) in Florida. Fla. Entomol. 75: 248-257.
Porter, S. D., A. Bhatkar, R. Mulder, S. B. Vinson, and D. J. Clair. 1991. Distribution and density of polygyne fire ants (Hymenoptera: Formicidae) in Texas. J. Econ. Entomol. 84: 866-874.
Porter, S. D., H. G. Fowler, and W. P. MacKay. 1992. Fire ant mound densities in the United States and Brazil (Hymenoptera: Formicidae). J. Econ. Entomol. 85: 1154-1161.

Ross, K. G. 1988. Differential reproduction in multiple-queen colonies of the fire ant *Solenopsis invicta* (Hymenoptera: Formicidae). Behav. Ecol. Sociobiol. 23: 341-355.

Ross, K. G., and D. J. C. Fletcher. 1985. Genetic origin of male diploidy in the fire ant, *Solenopsis invicta* (Hymenoptera: Formicidae) and its evolutionary significance. Evolution 39: 888-903.

Tschinkel, W. R., and D. F. Howard. 1978. Queen replacement in orphaned colonies of the fire ant, *Solenopsis invicta*. Behav. Ecol. Sociobiol. 3: 297-310.

Vargo, E. L. 1992. Mutual pheromonal inhibition among queens in polygyne colonies of the fire ant, *Solenopsis invicta*. Behav. Ecol. Sociobiol. 31: 205-210.

Vargo, E. L., and D. J. C. Fletcher. 1986. Queen number and the production of sexuals in the fire ant *Solenopsis invicta* (Hymenoptera: Formicidae). Behavioral Ecol. Sociobiol. 19: 41-47.

Vargo, E. L., and D. J. C. Fletcher. 1989. On the relationship between queen number and fecundity in polygyne colonies of the fire ant, *Solenopsis invicta*. Physiol. Entomol. 14: 223-232.

Vargo, E. L., and K. G. Ross. 1989. Differential viability of eggs laid by queens in polygyne colonies of the fire ant, *Solenopsis invicta*. J. Insect Physiol. 35: 587-593.

Vinson, S. B., and L. Greenberg. 1986. The biology, physiology, and ecology of imported fire ant, pp. 193-226. *In* S. B. Vinson [ed] Economic Impact and Control of Social Insects. Praeger Press. New York.

FREE-CHOICE OLFACTOMETER BIOASSAY SYSTEM FOR EVALUATING THE ATTRACTIVENESS OF PLANT VOLATILES TO ADULT *HELICOVERPA ZEA*[1]

K. R. Beerwinkle, T. N. Shaver, P. D. Lingren, and J. R. Raulston[2]
Areawide Pest Management Research Unit, USDA-ARS, 2771 F & B Road
College Station, TX 77845

ABSTRACT

A bioassay system to quantitatively evaluate adult *Helicoverpa zea* (Boddie) behavioral responses to plant volatile attractants is described. The system is comprised of two- and six-choice olfactometers, a purified air source, air flow controls, and an environmental room. The olfactometers are designed to provide for the testing and evaluation of volatiles from a variety of natural and synthetic sources. Results from selected bioassays of volatiles from natural plant sources and chemically-formulated plant mimics as attractants for adult *H. zea* are presented to illustrate operational characteristics of the system.

INTRODUCTION

Based on nocturnal observations of emergence and early post emergence behavior indicating food seeking is a high priority of newly-emerged adult *Helicoverpa zea* (Boddie), Lingren et al. (1987, 1988) proposed the use of feeding attractant baits containing toxicants (attracticides) as an adult control technique. The viability of this technique was demonstrated by Lingren et al. (1990) who reported major mortality among newly emerged *H. zea* adults that fed on attracticide baits banded around corn stubble in an emergence habitat. Beerwinkle et al. (1993) reported observations of intense feeding activity of *H. zea* adults $\leq$ 1 d of age on dallisgrass ergot honeydew.

Identification of chemical volatiles that effectively mimic natural plant food-source attractants is a prerequisite to the development of successful attracticide formulations. Adult *H. zea* and many other noctuid moths are polyphagous, and they feed on a wide range of cultivated and non-cultivated host plants (Fitt 1991). Several plant species that are attractive food sources for *H. zea* have been identified by direct field observations and by identification of pollen on collected insects (Bryant et al. 1991, Lingren et al. 1993). Bioassay techniques and apparatus were required for quantifying the behavioral responses of moths exposed to volatiles emanating from various natural plant materials and synthetic chemical formulations to assist in the isolation and identification of active attractant chemical compounds.

---

[1]Lepidoptera: Noctuidae
[2]Subtropical Cotton Insects Research Unit, USDA-ARS2413 East Hwy 83, Weslaco, TX 78596. In cooperation with the Texas Agricultural Experiment Station, Texas A&M University, College Station, TX. Mention of a trade name or a proprietary product is for specific information only and does not constitute a guarantee or warranty of the product by the U. S. Dept. of Agriculture or the Texas Agricultural Experiment Station and does not imply endorsement of the product over other products not mentioned.

As defined by Shorey (1970), a bioassay is an assay to determine the effects of various treatments on living organisms. Thus, a bioassay is the basic method for assessing the biological activity of plant volatiles as feeding attractants on the insects of interest. The two main types of bioassays are based on either electrophysiological or behavioral responses (Finch 1980). Bioassay apparatus used to observe and measure insect responses to odors are most commonly called olfactometers as proposed by Dethier (1947).

Olfactometers for evaluating the behavioral responses of insects to volatiles have taken many forms since each apparatus must invariably be tailored to suit a particular test insect (Finch 1980). Most laboratory olfactometer systems are basically one of two types. One type provides insects with one or several Y-junction choices. Volatiles under test issue through one arm of the Y and clean air through the other so that the insects have a choice (McIndoo 1926). Olfactometers of various configurations based on the Y-junction principle have been used extensively for evaluating olfactory responses of insects of various species (Nettles 1979, Martin et al. 1990, Trematerra and Capizzi 1991, Budenberg et al. 1993).

The second type of laboratory olfactometer that has been used extensively includes many variations of low-speed wind tunnels or flight chambers. Wind tunnel apparatus usually includes provisions for light, temperature, humidity, and airflow control to simulate critical field conditions for the insects under study. Many investigators have used wind tunnels to evaluate the olfactory responses of different insect species to various volatiles (Miller and Roelofs 1978; Cardé and Hagaman 1979; Hedin et al. 1986, Birch and White 1988; Tingle et al. 1989, 1990; Tingle and Mitchell 1992; Charlton and Cardé 1990; Sanders 1990; Haynes et al. 1991; Landolt et al. 1991; Mitchell et al. 1991; Heath et al. 1992).

Both free-choice Y-junction type and wind tunnel olfactometer apparatus are being used in our research to bioassay *H. zea* adult responses to plant feeding attractants. However, the purpose of this paper is to describe the free-choice olfactometer bioassay system developed for this research and to report some typical results obtained with its use.

MATERIALS AND METHODS

The free-choice olfactometer bioassay system consists of a series of two- and six-choice Y-junction type olfactometers, an air supply and conditioning system, and a laboratory environmental room.

Olfactometers. The basic two-choice olfactometer unit (Fig. 1) is constructed of clear acrylic and consists of a large rectangular moth-holding chamber (23 x 23 x 60 cm) (C, Fig. 1) with a pair of connected subchambers (A and B, Fig. 1), each of which serves the dual functions of holders for volatile samples and traps for attracted moths. The two subchambers are mounted in the floor of chamber C in positions such that their centers lie on a line parallel to the vertical plane of the front wall and their peripheries are about equidistant from the interior surface of the front and side walls, respectively. Each of the subchamber cylinders (8.3 cm ID by 33.0 cm height) is divided into two subcompartments of about equal volume by a screen partition. The bottom cap-plugs on the subchambers were constructed by machining the threads off 7.6-cm polyvinyl chloride (PVC) pipe plugs so they could be inserted inside the ends of the 8.3-cm ID subchamber tubing with close tolerances. A large neoprene o-ring was mounted on each plug so that it compressed against the lower edge of the circular subchamber wall to seal the opening when the plug was pinned in place.

In operation, pairs of test volatile sources, which may be bouquets of plant parts or chemical volatile dispensers of various types, are placed in the lower subcompartments of the subchambers (Fig. 2). Metered, prepurified air under positive pressure is delivered into each subchamber through an inlet in the bottom cap at a volume flow rate of about 12 liters/min. The air passes up through the subchambers carrying test-sample volatiles into the moth-holding

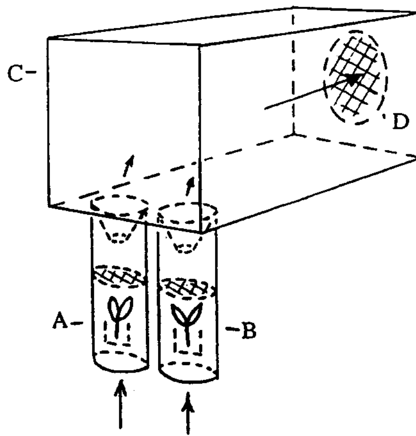


FIG. 1. Schematic of two-choice olfactometer apparatus. (A,B) Bait/trap subchambers. (C) Moth exposure chamber. (D) Exhaust port filter pad.

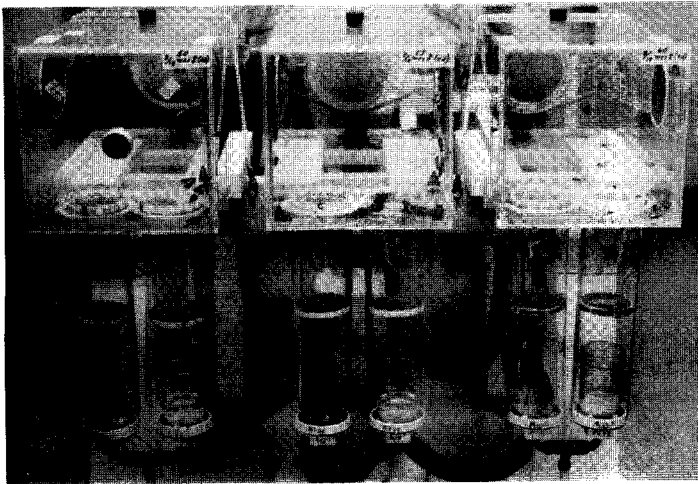


FIG. 2. End view of three, two-choice olfactometers illustrating the configuration of the apparatus and bait placement.

chamber. The two air-volatile mixtures enter the chamber in separate plumes whose integrities are briefly maintained before they intermix as the composite mixture flows horizontally to an exhaust port in the rear of the chamber. Moths are exposed to the volatiles in the holding chamber, and those that seek the source of the preferred attractant enter the upper subcompartment of the appropriate chamber (A or B, Fig. 1) through a screen funnel and become trapped. The screen partition in the subchamber prevents the moths from coming into contact with the volatile source.

Results obtained from typical bioassay experiments include counts of the numbers of moths responding to the respective baits, and time-of-response data obtained from video recordings made with a time-lapse video recorder (Panasonic model no. AG-6720) and a conventional video camera equipped with a night vision lens system (Dark Invader, B. E. Meyers & Co., Inc., Redmond, WA).

The two-choice olfactometer apparatus is designed for easy assembly and disassembly for servicing and cleaning. The two end caps of the moth-holding chamber are held in place with elastic straps made of surgical rubber tubing. The paired subchamber cylinders (A and B, Fig. 1) are connected to chamber C by inserting them about 1.5 cm through collars on close-fitting holes in the chamber floor and attaching them to the collars with removable pins. The cap-plugs on the bottom of the subchamber cylinders are also held in place with removable pins through pin holes in the subchamber walls.

The exhaust port (D, Fig. 1) of the moth-holding chamber is covered with a synthetic fiber pad that filters moth scales from the exhausting air. The exhaust ports of the several individual olfactometer units in the system are connected to a common exhaust manifold with appropriate lengths of 3.2-cm ID flexible polyvinyl tubing. The manifold is connected to a common exhaust duct which is constructed of 5.1-cm ID rigid PVC pipe. A squirrel-cage blower (Model 4C442, W. W. Granger, Lincolnshire, IL) mounted in series with the common exhaust duct pulls the air from the connected chambers and delivers it to the outside of the laboratory. The capacity of the fan is regulated such that the internal pressures of each moth-holding chamber in the system is maintained at a slight negative pressure ($\approx$ 0.4-mm water column) relative to the ambient external chamber pressure in the laboratory. Thus, each olfactometer unit is effectively a closed system and no test volatiles escape to the surrounding room atmosphere. Chamber internal pressures are monitored with a wall-mounted manometer (Mark II Series model MM-80, Dwyer Instruments, Inc., Michigan City, IN).

Six-choice olfactometer units (Fig. 3) were developed which operate on the same principles as described for the two-choice units. In the six-choice units, three pairs of subchambers are attached to the floor of the moth-holding chamber. The moth-holding chamber of the six-choice unit is constructed with a section of acrylic tubing (45.7 cm ID by 33.0 cm height) capped on the top and bottom with 6.4-mm thick acrylic plates. Air flows vertically from inlets in the bottom of the six subchambers through the subchambers and the main chamber to an exhaust port centrally located in the top of the unit. A screen partition in the main chamber is positioned about 5 cm from the top to keep resting moths more directly in the flowing air. The relative attractiveness of up to six separate volatile sources can be evaluated simultaneously in the 6-choice units. The volatile sources under test are placed in the lower compartments of the subchambers as described for the 2-choice chambers.

Air Supply System. Oil-free compressed air for the olfactometers is supplied from a continuously running compressor (Model 3040-P118B, Gast Manufacturing Corp., Benton Harbor, MI) that is remotely located from bioassay laboratory to minimize the effects of the inherent noise. The compressed air is regulated at a pressure of about 70 kPa (10 psi) with a pressure relief valve mounted on the compressor outlet. Before delivery to the bioassay laboratory the temperature of the hot, freshly-compressed air is cooled to about 26°C by flowing it through a cooling coil submersed in a controlled-temperature water bath.

The cooled air is then piped through 3.2-cm ID PVC pipe to the bioassay room where it is passed through a cylindrical filter chamber (15.2-cm ID by 61.0-cm height) filled with activated charcoal granules to remove any entrained volatiles or other contaminants. From the filter, the air passes into an output supply manifold where it is metered to individual olfactometer units through sight flow indicating meters (Model RMA-22-SSV, Dwyer Instruments, Inc., Michigan City, IN). One flowmeter is used for each pair of bait/trap subchambers of the olfactometers. Thus, one flowmeter is used for each 2-choice olfactometer and three flowmeters are used for each 6-choice unit.

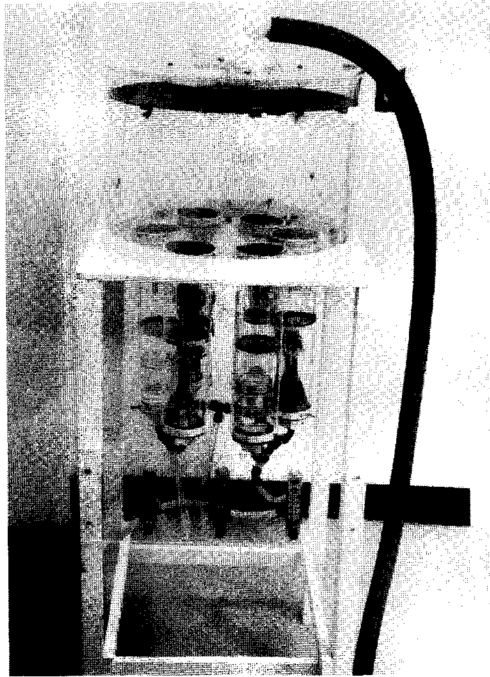


FIG. 3. Side view of a six-choice olfactometer.

From each flowmeter the air passes through an enclosed cylindrical humidifying chamber (10.2-cm ID by 45.7-cm height) constructed with a section of 10.2-cm ID PVC pipe with caps and associated PVC pipe fittings. The chamber is filled about two-thirds full with deionized water and the air is passed from the top of the chamber to the bottom through a section of 1.3-cm ID rigid PVC tubing that is terminated with a fine-mesh cloth sock. The air passes through the sock, bubbles up through the water, and exits the humidifier in a near saturated state. From the humidifiers, the conditioned air is delivered through appropriate lengths of clear 1.3-cm ID plastic tubing to the respective pairs of bait/trap subchambers of the individual olfactometer units.

Environmental Bioassay Room. The current bioassay system is comprised of seven, two-choice and two, six-choice olfactometer units set up in an insulated room (3.4 x 5.6 x 2.4 m) equipped with a dedicated air conditioning system, a duct mounted electrostatic filter, and auxiliary humidifiers. The walls and ceiling of the room are painted with washable semigloss white latex. Room lighting consists of two 80 W florescent ceiling mounted fixtures, four reflector-mounted 150 W incandescent flood lamps positioned to provide indirect illumination to the olfactometer chambers during simulated daylight periods, and six reflector-mounted, red-filtered 8 W incandescent lamps to furnish low levels of indirect red illumination during scotophase periods. The florescent lamps are controlled by a manual on/off switch, the flood lamps are controlled by an automatic dusk/dawn simulating controller (modified after Beerwinkle and Berry 1975), and the red-filtered lamps are controlled by a manual electronic dimmer switch. Daylight simulated illumination levels on the olfactometer units are about 700 lux and scotophase simulated levels are about 0.01 lux.

Typical Bioassay Procedures. Test baits for assaying moth attractance to natural plant volatiles are prepared by placing bouquets of flowering plants with cut stems in a beaker containing a chemical solution (Chrysal, Pokon & Chrysal - Naarden - Holland, Bussum, The Netherlands) used by florists to keep cut flowers fresh. Plant volatile blanks are beakers containing only the Chrysal solution. Test baits of chemically formulated mimics of natural plant volatiles are prepared by placing the chemical compounds on absorbent cotton dental rolls which are fitted into glass sleeves to retard evaporation. Chemical volatile blanks are prepared in the same manner except that the plant volatile mimicking chemicals are excluded.

Both feral *H. zea* adult males and laboratory-reared males and females are routinely used in the bioassay experiments. The feral males are collected from wire-mesh cone traps (Hartstack et al. 1979) baited with four-component pheromone lures (Hercon Environmental, Inc., Emigsville, PA) and located in an agricultural cropping area of the Brazos River Valley of Burleson County near College Station, TX. The laboratory moths are obtained as pupae from a colony maintained by the Subtropical Cotton Insects Research Unit, USDA-ARS-SARL, at Weslaco, TX. The larvae are reared and the pupae maintained on a 14:10 h light-dark cycle.

Bioassay experiments are conducted in a time-frame that corresponds to the natural nocturnal feeding behavior of the moths. Moths are placed in the holding chambers of the respective olfactometer units (usually 75 in the two-choice and 125 in the six-choice chambers) during the daylight hours. At that time, the chambers are fully configured with air supply and exhaust systems functioning and the room fully lighted to a level of about 700 lux. The moths are held in the chambers, undisturbed until the time of dusk simulation. A petri dish containing moistened cotton pads is placed in each holding chamber to provide a source of water to the moths. During the daylight period, no volatile sources are present in the bait chamber, and screen disks are positioned over the entrance funnels to the trap subchambers to prevent random trapping of moths before baits are placed.

At a time approximating natural sundown in the area, the fluorescent lights are manually turned off to begin dusk simulation in the laboratory and the automatic light dimming controller begins the dimming cycle for the incandescent flood lamps. The dimming cycle is completed in about 30 min when the floodlamps are fully extinguished. The red-filtered lamps, dimmed to provide a very low illumination level of about 0.01 lux, remain on throughout the scotophase period.

The volatile bait sources to be tested are placed in the bait chambers of the respective olfactometer units and the trap-entrance-blocking screen disks are removed during the dusk simulation period. After the night-vision video system is set up and activated, the olfactometer apparatus is left unattended until the following morning when the automatic light controller causes the incandescent flood lamps to start turning on at about 0700 h to begin a dawn simulation period. The moths trapped in the various trap subchambers are counted and other data from the night's test are collected beginning at about 0730 h. After the data are collected, the baits and trapped moths are removed from the subchambers, the subchambers are disassembled, washed, dried, and reassembled in preparation for the next night's test.

When sufficient moths are available, test moths are used only once in the olfactometer experiments. However, since a rather large number of moths is required for each night's test (about 775), both responding and non-responding moths that remain visually healthy are frequently reused in two or more successive experiments. Feral moths are preferred over laboratory-reared moths, and they are used when available.

RESULTS AND DISCUSSION

Olfactometer Bioassay Response Characteristics. Responses of *H. zea* moths to plant volatile sources of different concentrations in the two-choice chambers compared to that of blanks

have typically been qualitative, indicating whether or not the plant volatiles were attractive, but failing to indicate a well defined dose/response relationship. For example, in a series of assay tests in which moth responses to *Gaura suffulta* (Engelm.) bouquets of various sizes (1 to 48 stems with 1 to 3 blooms per stem) were compared to that of blanks, all assays demonstrated moth attractance to the plants, but there were only slight differences in the comparative moth responses to the plant samples and the respective blanks for the different plant-sample sizes (Fig. 4). However, moth responses to variable concentrations of volatiles provided by different sized

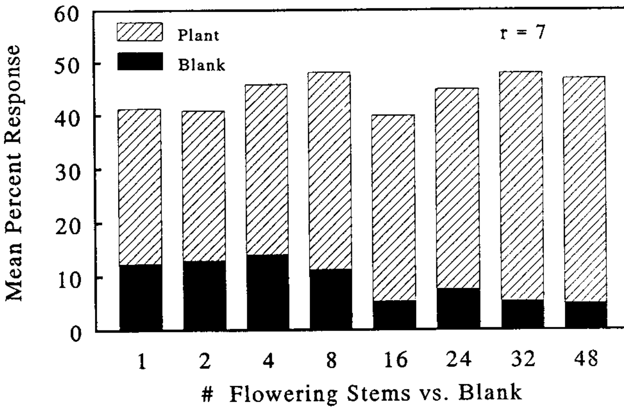


FIG. 4. Responses of feral male *H. zea* moths to volatiles of various concentrations from *G. suffulta* bouquets of different sizes compared to blanks, respectively, in two-choice olfactometers. Moth responses were significantly higher (ANOVA, $\alpha = 0.05$) to plant volatiles than to blanks in all comparisons, but the correlation of increased response with increased concentrations was poor ($r = 0.39$).

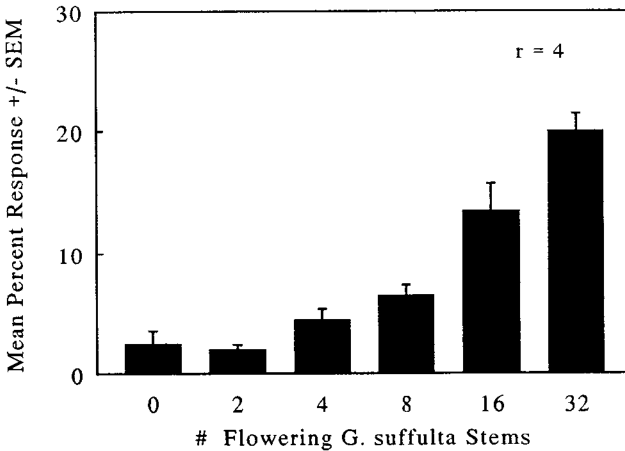


FIG. 5. Comparative responses of feral male *H. zea* moths to volatiles of various concentrations from *G. suffulta* bouquets of different sizes in six-choice olfactometers. The increase in moth response to increased volatile concentration (# of flowering stems) was linear over the range tested ($P < 0.05$, $r^2 = 0.874$).

bouquets of the same plant in the six-choice olfactometer units have tended to be proportional to the volatile concentrations (Fig. 5). Linear regression analysis of the data with number of flowering *G. suffulta* stems as the dependent variable and percentage moth response as the independent variable had an r-squared value of 0.87 indicating a linear dose/response relationship between the variables under the conditions they were tested. Thus, the six-choice chambers have been useful for assaying the relative attractiveness of up to six volatile sources in single tests. Typically, only 30-50 % of total numbers of moths exposed in either the two-choice or six-choice chambers responded to attractant baits during single overnight tests with these systems.

The olfactometer system was used in a series of bioassay experiments in an elimination process to isolate four major moth-attractant chemicals from among the group of 14 compounds originally identified in *Gaura drummondii* flower volatiles by Teranishi et al. (1991). Results from this series of bioassays with simultaneous evaluations of synthetic attractant compounds and natural plant bouquets, used as attractant standards, indicated apparent seasonal variations in *H.*

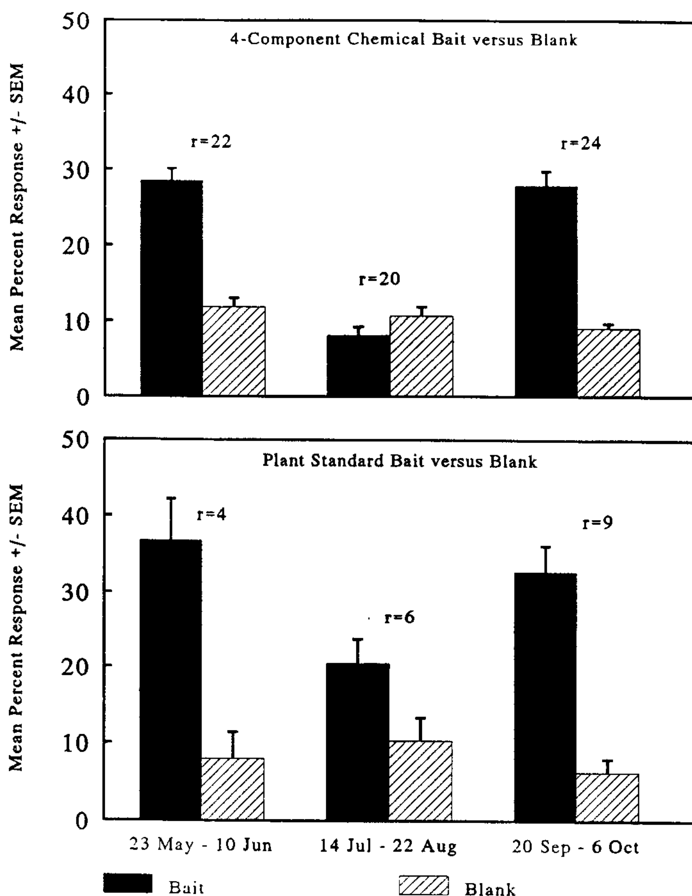


FIG. 6. Seasonal responses of feral male *H. zea* moths to a four-component chemical mixture that was formulated to mimic the natural volatiles of *Gaura drummondii*, and their corresponding responses to plant bouquets used as standard attractants during the respective series of experiments.

zea moth behavior (Fig. 6). Differential responses of feral males to natural plant volatiles compared to blanks were consistently greater and slightly more uniform during the three periods illustrated than were responses to the synthetic chemical attractants compared to blanks; however, seasonal variations in moth response were apparent for both types of attractants. Specific factors contributing to the observed seasonal variations in moth response behavior are yet to be identified.

Use of the night-vision equipped video system has provided data for determining time-of-response patterns for moths responding to various plant attractants. For example, video data collected during the period of 6-13 August 1993 (Fig. 7) indicated that time-of-response for feral and laboratory-reared males to volatiles from *Gaura longiflora* (Spach) bouquets were similar, and that the majority of the moths from both sources responded during the early morning hours of the tests. Response patterns have varied somewhat with different volatile sources and different seasons of the year.

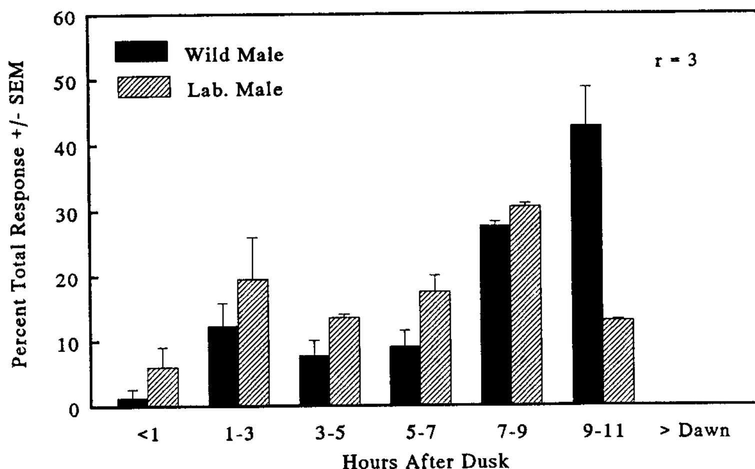


FIG. 7. Time-of-response comparison of feral and laboratory-reared male *H. zea* moths to volatiles from *G. suffulta* bouquets in 2-choice olfactometers determined by analyses of time-lapse video records.

The olfactometer system described has performed well for evaluating *H. zea* adult attractance responses to plant volatiles, and it may have applications for similar studies with other noctuid moths with foraging behavior similar to *H. zea*. Results of limited experiments with tobacco budworm, *Heliothis virescens* (Fabricius) and beet armyworm, *Spodoptera exigua* (Hubner) moths have indicated that these species responded well to plant volatile baits in this system; however, results from assays with soybean loopers, *Pseudoplusia includens* (Walker) have been quite variable (K.R.B., unpublished data), possibly indicating behavioral differences.

LITERATURE CITED

Beerwinkle, K. R., and I. L. Berry. 1975. Solid-state light intensity controller for biological research. ARS-S-77. Agricultural Research Service-USDA.

Beerwinkle, K. R., T. N. Shaver, and J. D. Lopez, Jr. 1993. Field observations of adult emergence and feeding behavior of *Helicoverpa zea* (Lepidoptera: Noctuidae) on dallisgrass ergot honeydew. Environ. Entomol. 22: 554-558.

Birch, M. C., and P. R. White. 1988. Responses of flying male *Anobium punctatum* (Coleoptera:Anobüdae) to female sex pheromone in a wind tunnel. J. Insect Behav. 1: 111-115.

Bryant, V. M. Jr., M. Pendelton, R. E. Murray, P. D. Lingren, and J. R. Raulston. 1991. Techniques for studying pollen adhering to nectar-feeding corn earworm (Lepidoptera: Noctuidae) moths using scanning electron microscopy. J. Econ. Entomol. 84: 237-240.

Budenberg, W. J., I. O. Ndiege, F. W. Karago, and B. S. Hansson. 1993. Behavioral and electrophysiological responses of the banana weevil *Cosmopolites sordidus* to host plant volatiles. J. Chem. Ecol. 19: 267-277.

Cardé, R. T., and T. E. Hagaman. 1979. Behavioral responses of the gypsy moth in a wind tunnel to air-borne enantiomers of disparlure. Environ. Entomol. 8: 475-484.

Charlton, R. E., and R. T. Cardé. 1990. Orientation of male gypsy moths, *Lymantria dispar* (L.), to pheromone sources: the role of olfactory and visual cues. J. Insect Behav. 3: 443-469.

Dethier, V. G. 1947. Chemical insect attractants and repellants. Blakiston, Philadelphia. 289 pp.

Finch, S. 1980. Chemical attraction of plant-feeding insects to plants, pp. 67-142. *In* T. H. Coaker [ed.], Applied Biology, Vol. V. Academic Press, New York.

Fitt, G. P. 1991. Host selection in Heliothinae, pp. 172-201. *In* W. J. Bailey and J. Riddell-Smith [eds.]. Reproductive Behavior of Insects: Individuals and Populations. Chapman and Hall, London.

Hartstack, A. W., J. A. Witz, and D. R. Bock. 1979. Moth traps for the tobacco budworm. J. Econ. Entomol. 72: 519-522.

Haynes, K. F., J. Z. Zhao, and A. Latif. 1991. Identification of floral compounds from *Abelia grandiflora* that stimulate upwind flight in cabbage looper moths. J. Chem. Ecol. 17: 637-646.

Heath, R. R., P. J. Landolt, B. Dueben, and B. Lenczewski. 1992. Identification of floral compounds of night-blooming jessamine attractive to cabbage looper moths. Environ. Entomol. 21: 845-859.

Hedin, P. A., F. M. Davis, J. C. Dickens, M. L. Burks, T. G. Bird, and A. E. Knutson. 1986. Identification of the sex attractant pheromone of the southwestern corn borer *Diatraea grandiosella* Dyar. J. Chem. Ecol. 12: 2053-2063.

Landolt, P. J., B. Lenczewski, and R. R. Heath. 1991. Lure and toxicant system for the cabbage looper (Lepidoptera: Noctuidae). J. Econ. Entomol. 84: 1344-1347.

Lingren, P. D., V. M. Bryant, Jr., J. R. Raulston, M. Pendelton, J. Westbrook, and G. D. Jones. 1993. Adult feeding host range and migratory activities of corn earworm, cabbage looper, and celery looper (Lepidoptera: Noctuidae) moths as evidenced by attached pollen. J. Econ. Entomol. 86: 1429-1439.

Lingren, P. D., J. R. Raulston, T. N. Shaver, and E. V. Wann. 1990. Mortality of newly emerged adult corn earworms following treatment with a feeding stimulant bait, pp. 128-132. *In* Proceedings 9th Annual Horticulture Industry Show, Tulsa, OK. Oklahoma Cooperative Extension Service, Oklahoma State Univ., Stillwater.

Lingren, P. D., J. R. Raulston, and E. V. Wann. 1987. Emergence and dispersal of adult corn earworms, pp. 73-77. *In* Proceedings 6th Annual Horticulture Industry Show, Tulsa, OK. Oklahoma Cooperative Extension Service, Oklahoma State Univ., Stillwater.

Lingren, P. D., W. B. Warner, J. R. Raulston, M. Kehat, T. J. Henneberry, S. D. Pair, A. Zvirgzdins, and J. M. Gillespie. 1988. Observations on the emergence of adults from natural populations of corn earworm, *Heliothis zea* (Boddie) (Lepidoptera: Noctuidae). Environ. Entomol. 17: 254-258.

Martin, W. R., Jr., D. A. Nordlund, and W. C. Nettles, Jr. 1990. Response of parasitoid *Eucelatoria bryani* to selected plant material in an olfactometer. J. Chem. Ecol. 16: 499-508.

McIndoo, N. E. 1926. An insect olfactometer. J. Econ. Entomol. 19: 545-571.Miller, J. R. and W. L. Roelofs. 1978. Sustained-flight tunnel for measuring insect responses to wind-borne sex pheromones. J. Chem. Ecol. 4: 187-198.

Miller, J. R. and W. L. Roelofs. 1978. Sustained-flight tunnel for measuring insect responses to wind-borne sex pheromones. J. Chem. Ecol. 4: 187-198.

Mitchell, E. R., F. C. Tingle, and R. R. Heath. 1991. Flight activity of *Heliothis virescens* (F.) females (Lepidoptera: Noctuidae) with reference to host-plant volatiles. J. Chem. Ecol. 17: 259-266.

Nettles, W. C., Jr. 1979. *Eucelatoria* sp. females: Factors influencing response to cotton and okra plants. Environ. Entomol. 8: 619-623.

Sanders, C. J. 1990. Wind-tunnel assays to determine the effect of minor pheromone components on the responses of male spruce budworm. Forestry Canada, Ontario Region. Infor. Report 0-X-404.

Shorey, H. H. 1970. Sex pheromones of lepidoptera pp. 249-284. *In* D. L. Wood, R. M. Silverstein, and M. Nakajima [eds.], Control of Insect Behavior by Natural Products. Academic Press, New York.

Teranishi, R., R. G. Buttery, S. Kent, G. Takeoka, P. D. Lingren, J. R.Raulston, and T. N. Shaver. 1991. Chemical composition of *Gaura drummondii* flower volatiles. J. Ess. Oil Res. 3: 287-288.

Tingle, F. C., and E. R. Mitchell. 1992. Attraction of *Heliothis virescens* (F.) (Lepidoptera: Noctuidae) to volatiles from extracts of cotton flowers. J. Chem. Ecol. 18: 907-914.

Tingle, F. C., E. R. Mitchell, and R. R. Heath. 1990. Preferences of mated *Heliothis virescens* and *H. subflexa* females for host and nonhost volatiles in a flight tunnel. J. Chem. Ecol. 16: 2889-2898.

Tingle, F. C., R. R. Heath, and E. R. Mitchell. 1989. Flight response of *Heliothis subflexa* (GN.) females (Lepidoptera: Noctuidae) to an attractant from groundcherry, *Physalis angulata* L. J. Chem. Ecol. 15: 221-231.

Trematerra, P., and A. Capizzi. 1991. Attracticide method in control of *Ephestia kuehniella* Zeller: Studies on effectiveness. J. Appl. Entomol. 111: 451-456.

SEASONAL RESPONSES OF BOLL WEEVILS[1] TO PHEROMONE TRAPS IN CROPPED AND ADJACENT UNCROPPED AREAS OF EAST-CENTRAL TEXAS

K. R. Beerwinkle, J. D. Lopez, Jr., and G. H. McKibben[2]

Areawide Pest Management Research, USDA, ARS, SPA, SCRL
2771 F&B Road, College Station, TX 77845

ABSTRACT

Boll weevil, *Anthonomus grandis grandis* Boheman, pheromone trapping studies were conducted in a large, relatively isolated area of contiguous row-crop farms (cropped area) and the surrounding uncropped area in the Brazos River Valley of Burleson and Brazos counties near College Station, TX, during 1990 and 1991. Results indicated that variations in the timing and magnitudes of the mean weekly trap captures in the cropped and uncropped areas were similar during the early and midseason periods until September when harvest of cotton began. Trap response patterns were characterized by an early-season period of peak weekly mean weevil captures of 20 or less during late April through May, followed by a midseason period of near-zero captures from early June through mid-July, and then a late-summer period of increasing captures that began in late July and peaked in early September at levels more than five times those obtained in the early season. During the late-summer period, weevil captures were uniform at relatively high levels, within-years, over the cropped area and the uncropped area out to distances of 20 km in 1990 and 13 km in 1991. After the beginning of harvest with the associated habitat destruction, the timing of variations in catch patterns for the traps in the cropped and uncropped areas remained similar; however, the magnitudes of mean weekly captures in the cropped area increased to levels of more than five times those obtained in the uncropped area. The magnitudes of mean weekly captures throughout the year in 1990 were less than half those obtained in traps at the same locations in 1991, probably because of the detrimental effects of abnormally cold temperatures in the area during December 1989.

INTRODUCTION

Studies of boll weevil, *Anthonomus grandis grandis* Boheman, movement and distribution in agricultural ecosystems of the Texas Rolling Plains showed consistent seasonal response patterns for feral weevils attracted to pheromone traps baited with live male weevils (Bottrell et al. 1970, Hardee et al. 1970, Ridgway et al. 1971). These authors found peak early responses of overwintering weevils occurred during late May to early June, followed by a mid-season period with minimal response from late June through early August. Weevil responses to pheromone traps began to increase about mid-August, reached peak late-season levels during September, and then declined through the fall. These studies indicated that pheromone traps located in or near cotton fields were very effective for surveying populations of overwintered and late-season boll

---

[1] Coleoptera: Curculionidae

[2] USDA-ARS, Boll Weevil Res. Unit, Mississippi State, MS

weevils, but that the traps were comparatively ineffective as survey aids during midseason because of increased competition from pheromone-producing males in fruiting cotton and decreased field-to-field movement of weevils during this period. Further, the studies indicated abrupt increasesof weevil migrations from early-maturing, heavily-infested cotton fields into adjacent areas during the latter part of midseason. Similar patterns of weevil attractance to pheromone traps relative to the phenological development of cotton in the area have been reported for studies in South Carolina (Roach et al. 1971, Roach and Ray 1972), Mississippi (Lloyd et al. 1972) and in the Lower Rio Grande Valley of Texas (Wolfenbarger et al. 1976).

In a later study in the Texas Rolling Plains, White and Rummel (1978) used pheromone traps baited with 3-mg grandlure synthetic pheromone baits to measure the dynamics of overwintered weevil emergence while they monitored entry of weevils into isolated cotton plots. They found that movement of overwintered boll weevils into cotton coincided with appearance of the first small squares (flower buds) and that the majority of weevils entered after appearance of squares (1/3-grown) that were large enough to support weevil reproduction. Weevil entry into cotton plots began 4-5 weeks after peak early-season weevil responses to traps, and trap responses subsequently declined while weevil colonization of cotton increased. They concluded that spring migration of weevils into cotton was a positive response to squaring cotton and that attractiveness of cotton was intensified by increased pheromone production of male weevils feeding on squares.

Carroll and Rummel (1985) found a strong correlation between time of emergence of boll weevils from overwintering in emergence cages and time of response of native overwintered weevils to grandlure-baited pheromone traps. They concluded that early-season response of overwintered weevils to pheromone traps is an accurate reflection of the emergence profile of boll weevils from overwintering habitat.

Lopez (1980) used grandlure pheromone baits in a trap-comparison study for monitoring seasonal trap responses of weevils in Burleson County, Texas. In this study, traps were placed adjacent to cotton fields, and he observed seasonal trap responses of boll weevils that were very similar to those observed in earlier studies in other cotton production areas. In an extension of the work of Lopez (1980), boll weevil trapping studies were conducted with grandlure-baited traps in a large, relatively isolated row-cropped area and the surrounding uncropped area in the Brazos River Valley of Burleson and Brazos counties of East-Central Texas during 1990 and 1991. The objectives of this study were (1) to compare the seasonal activity patterns of boll weevils using pheromone traps in the cropped area near cotton fields and in the surrounding uncropped areas at various distances removed from cultivated cotton, and (2) by means of this comparison, determine if there was evidence of delayed weevil emergence in the uncropped areas in comparison to the cropped area.

MATERIALS AND METHODS

In 1990, boll weevils were trapped during the period of 14 March to 21 December using Boll Weevil Scout® traps (Hercon Environmental Co., Emigsville, PA) positioned on stakes 0.8 m above ground level. The traps were baited with 10-mg grandlure Hercon laminated tape dispensers. The lures were changed every two weeks, and no toxicant strips were included in the traps. A total of 48 traps were located in an array along seven radials extending from a common center at the intersection of farm-to-market roads FM50 and FM60 in an intensively cropped (contiguous row-crop farms) area in the Brazos River Valley of Burleson County, Texas (Fig. 1). Individual traps were placed on the edges of public road right-of-ways and spaced at 3.2-km intervals in open areas along the radials. Eighteen traps were located in cropped areas and 30 traps were in surrounding uncropped areas. No effort was made to place the traps adjacent to any specific crop. The total test area was approximately circular in shape with a diameter of

about 48 km (1810 km^2) which included an elongated rowcrop area of about 335 km^2 (shaded area in Fig. 1) along the Brazos River. The river valley area had intensive irrigated and some dryland agriculture. The study area also included two small upland rowcrop areas of dryland farms. One area of about 10 km^2 was located in close proximity to the main cropland area near the origins of the radials F and E (Fig. 1). The other area of about 5 km^2 was located about 12 km west of the main crop area near the symbol G (Fig. 1). Cultivated row-crops in all cropped areas included cotton, corn, sorghum, and soybeans, with cotton predominating in irrigated areas.

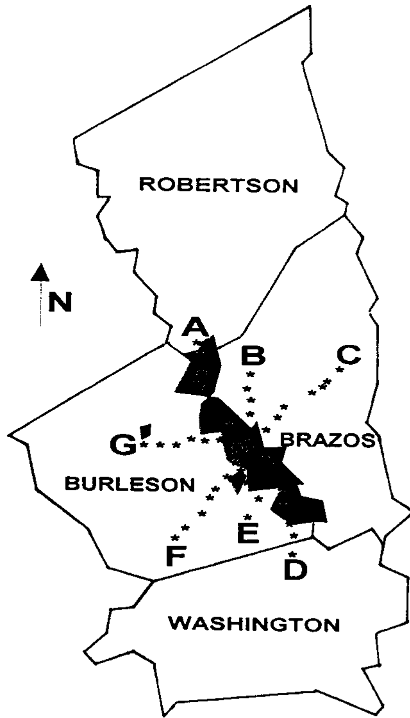


FIG. 1. Geographical location of the study area primarily in Burleson and Brazos counties of Texas with boll weevil trap locations designated by asterisks. Row-cropped areas are indicated by shading.

The boundaries of cropped areas were established by determining the latitude and longitude coordinates at various vehicle-accessible locations around the perimeters of the areas using a GPS Pathfinder® instrument (Trimble Navigation, Sunnyvale, CA). The coordinates were then plotted using the Map Viewer 1.0 computer program (Golden Software Inc., Golden, CO). The points with available coordinates were connected with straight lines to estimate the peripheral boundaries of the three rowcrop areas. The GPS system was also used to determine the latitude and longitude coordinates of each individual trap location for mapping and distance determinations for data analysis. The locations of individual traps in the cropped area relative to fields of cultivated cotton ranged from a few meters to about 0.5 km with a mean distance of <0.1 km.

In 1990, trapped weevils were collected and counted three times per week through October in the uncropped area and through December in the cropped area. In 1991, the scope of the study was reduced, and traps were operated in only 25 of the 48 locations monitored in 1990. Trap locations included 15 in the main cropped area, eight in the uncropped area distributed around the periphery of the cropped area in the distance interval of 0-5 km along five radials (C,D,E,F, and G), and two in the distance interval of 5-13 km along radial C (Fig. 1). The traps were placed on 24 April and checked three times per week through October in the uncropped area and through December in the cropped area as in 1990. No attempt was made to evaluate morphological characteristics or determine the sex of the captured weevils.

Within-year seasonal patterns of mean weekly trap captures for the composite of traps located in cropped and uncropped areas, respectively, were compared for each year. For more detailed analyses, uncropped trap data for 1990 were partitioned according to trap location distances from the nearest edge of a cropped area and grouped for distance intervals of 0-5 (15 traps), 5-10 (9 traps), and 10-20 km (6 traps). Uncropped data for 1991 were grouped in distance intervals of 0-5 (8 traps) and 5-13 km (2 traps). The weekly mean capture data for the various areas during each year were further partitioned into discrete time periods (six periods in 1990 and five periods in 1991) based on the approximate annual timing for the occurrence of various phenological development stages of cotton grown in the cropped area. The partitioned data were compared by ANOVA for trap capture differences over areas within time periods and over time periods within areas. Mean differences were tested by the Least Significant Difference (LSD) method (Statgraphics, Version 5, Statistical Graphics Corp., Rockville, MD). Temperature and rainfall data for the area, obtained from historical weather records (NOAA 1989, 1990, 1991), were used to postulate relationships between these variables and some of the observed variations in yearly and seasonal boll weevil captures.

RESULTS AND DISCUSSION

The survey trap data were analyzed to compare the activity patterns of boll weevils in the large, relatively isolated cropped area and the surrounding uncropped area during discrete periods within years and between years. Mean weekly captures in both the cropped and uncropped areas during the spring and fall seasons of 1991 were about twice those obtained in comparable periods of 1990 (Fig. 2). Notable differences in weather conditions of temperature and rainfall during the respective overwintering periods preceding the years of the study, and variations of these variables during the respective trapping seasons likely contributed to the differences in trap captures observed. Weather conditions for December 1989 were very harsh for the area with extremely cold temperatures and below average rainfall. Minimum temperatures of $\leq 0°C$ were recorded on 16 days during the month, 14 of which occurred in a 15-day period that included a period of 65 consecutive hours with below freezing temperatures and two nights in succession with low temperatures of -12.8 and -16.7°C, respectively. The average daily minimum temperature for the month was 6.2°C below the normal average minimum of 5.3°C, and rainfall for the month was 2.46 cm which was 5.08 cm below normal (NOAA 1989). The detrimental effects of such extremely cold temperatures and dry conditions on overwintering boll weevil survival are well documented (Smith and Scales 1965, Pfrimmer and Merkl 1981, Price et al. 1985, Fuchs and England 1989, Fuchs and Minzenmayer 1990, Slosser et al. 1996). Spring emergence of weevils in 1990 may also have been reduced by the effects of high rainfall (17.1 cm) which occurred during the 2-wk period of 20 April to 4 May (NOAA 1990). Price et al. (1985) reported that excessive rainfall (>10.0 cm) near the time of spring emergence in the Texas Rolling Plains appeared to be detrimental to survival, and Taft and Hopkins (1966) reported low overwintering survival of weevils in treatments with excessive moisture.

The harsh overwintering weather conditions preceding the 1990 trapping season may have had detrimental carryover effects on weevil captures in pheromone traps during the 1991

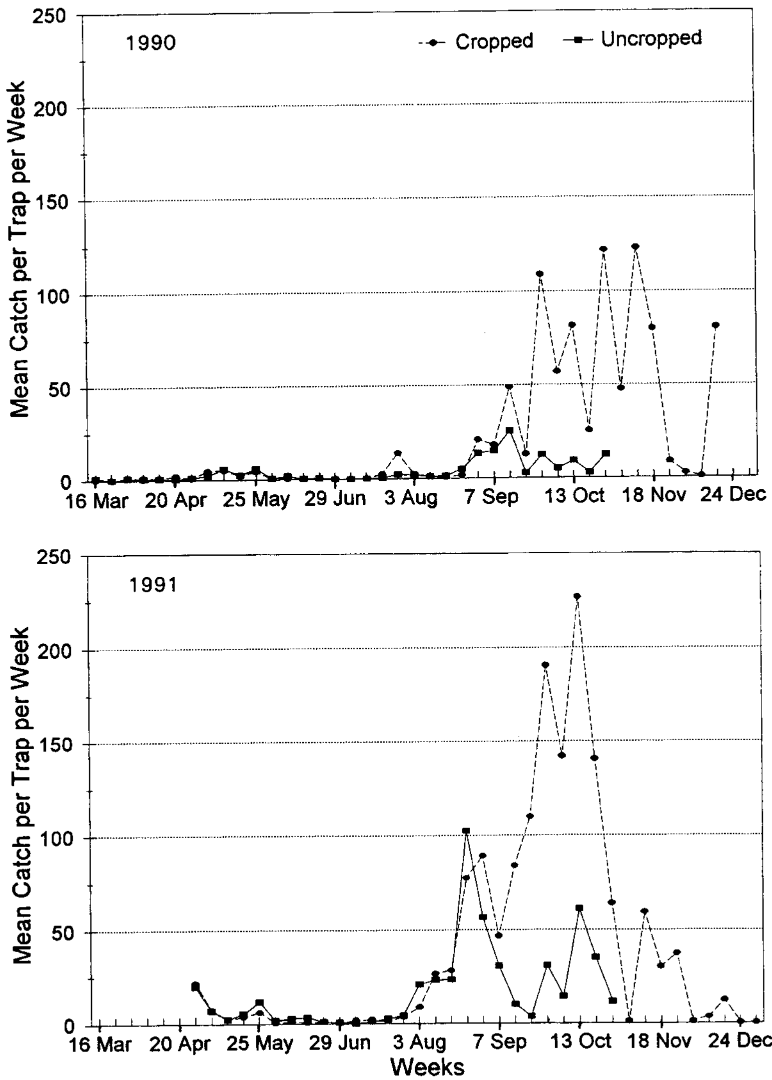


FIG. 2. Mean weekly boll weevil captures in pheromone traps located in the cropped and uncropped parts of the study area in 1990 and 1991.

season. Fuchs and England (1989) found a statistically positive linear relationship between numbers of weevils trapped in the Southern Rolling Plains of Texas during the spring and early summer of a given year and the number of years since a harsh winter. In addition, weevil captures during 1991 were likely depressed to some degree by the effects of weather conditions during the overwintering period immediately preceding the trapping season. Minimum temperatures of ≤0°C were recorded on 11 days during December 1990, five of which occurred in succession that

included 65 consecutive hours with below freezing temperatures and two successive nights with minimum temperatures of -8.9°C. The average daily minimum temperature for December 1990 was 1.5°C below normal and total rainfall for the month was 2.67 cm below normal (NOAA 1990). While the December 1990 weather conditions were not as severe as those occurring in December 1989, the conditions were likely harsh enough to have had some adverse effects on weevil survival. In addition, abnormally high rainfall totals of 76.3 cm (34.3 cm above normal) during the period of 1 January to 31 May 1991 (NOAA 1991) may also have had detrimental effects on overwintering weevil survival and subsequent spring emergence.

Though the direct effects of the noted occurrences of adverse weather during the respective overwintering periods preceding the two trapping seasons of the study cannot be quantified, they were important factors affecting weevil survival and their subsequent responses to pheromone traps during the ensuing trapping seasons. However, the relative severities of the overwintering weather conditions during the study likely had only minimal effects on the within-year spatiotemporal characteristics of the weevil trap-capture patterns in the cropped and uncropped areas which were of interest.

Seasonal patterns of mean weekly trap captures in the cropped area during the 2 years were temporally similar (Fig. 2), and both patterns were similar to the seasonal boll weevil activity patterns found with pheromone trapping studies in other cotton production areas by authors previously cited. Seasonal patterns of composite weekly mean captures in the uncropped area for the respective years were also temporally similar over the whole seasons. Within years, each of the uncropped-area patterns was remarkably similar, both in timing and magnitude variation, to the corresponding within-year cropped-area pattern through mid-September when harvest was underway (Fig.2). The abrupt increases in the levels of trap captures in the cropped area during the weeks following 21 September in 1990 and 7 September in 1991 corresponded to periods of intense harvest activities in the respective years. Differences in patterns of rainfall that occurred during the respective growing seasons were probably major factors contributing to the difference observed in harvest timing. In 1990, area rainfall of 28.8 cm was well distibuted over the period of 29 June to 21 September; whereas in 1991, only 5.9 cm occurred during a 9-week period from 29 June to 24 August, and then 20.7 cm occurred in the following three weeks (NOAA 1990, 1991).

Weekly mean trap captures through 21 September for both years are replotted on expanded vertical scales in Fig. 3 with the uncropped-area data for the respective years partitioned by trap location into discrete distance intervals from the periphery of the cropped area. For the 1990 data (Fig. 3) through 21 September, the calculated simple correlation coefficients between the weekly mean trap captures in cropped area and those in the uncropped area in the distance intervals of 0-5, 5-10, and 10-20 km were 0.93, 0.91, and 0.85, respectively. Similarly, for the 1991 data (Fig. 3) through 7 September, the calculated correlation coefficients between the cropped-area weekly mean trap captures and the corresponding uncropped-area captures in the distance intervals of 0-5 and 5-13 km were 0.91 and 0.81, respectively. All of the coefficients were positive and statistically significant ($P<0.005$, Student's *t* test) primarily because of the large uniform increases in trap captures in all of the partitioned areas during late August through early September of both years. However, the plots of Fig. 3 indicate that the magnitudes of mean weekly trap captures varied similarly in the various partitioned areas (including the cropped area) of the respective years, and they had high tendencies to increase and decrease proportionally with time through the late-summer seasons of both years.

The timing of periodic increases and decreases in trap captures in both the cropped and uncropped areas during both years were apparently associated with the timing of phenological development of cotton grown in the cropped area. For discussion purposes, the cotton production season was divided into six time intervals designated as periods A through F (Table 1) with the date intervals corresponding approximately to the various stages of cotton crop

phenological development. The time intervals associated with the first five stages (A through E) are delineated by arrows in Fig. 3. Results of ANOVA to evaluate the differences in mean weekly trap captures in the designated trap-location areas during each of the specified time intervals

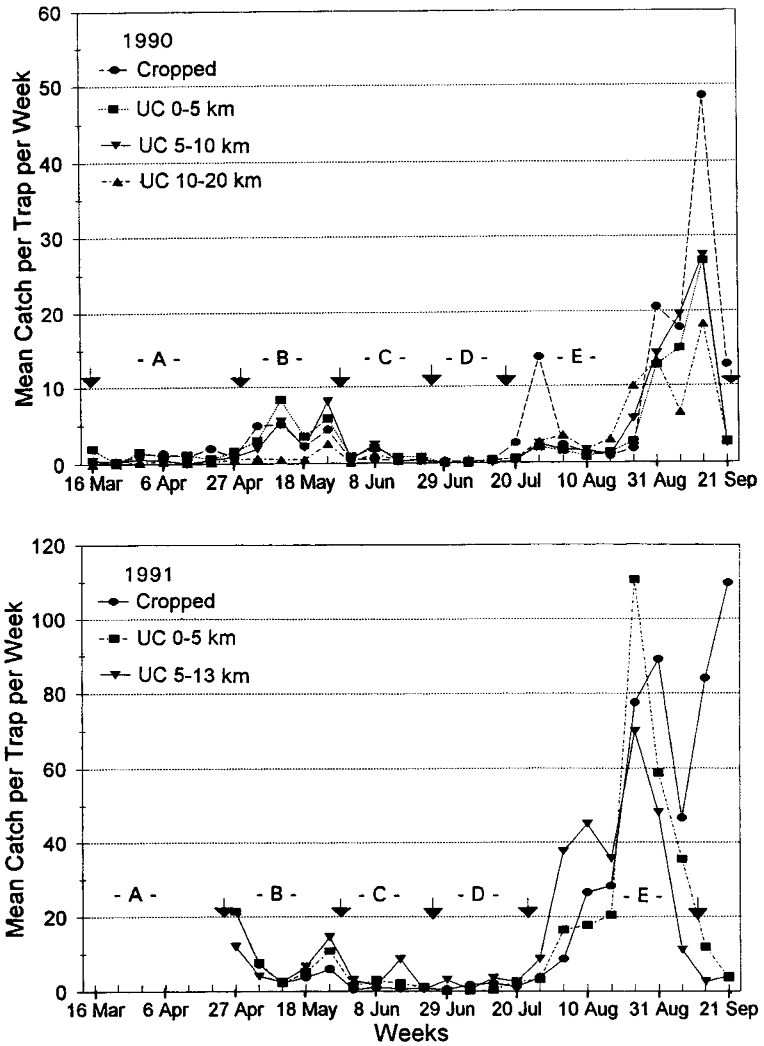


FIG. 3. Mean weekly boll weevil captures in pheromone traps located in the cropped and partitioned uncropped areas for the study periods in 1990 and 1991 through the week of 21 September of both years. Separate weekly mean values are plotted for those traps in the uncropped areas located within the intervals of 0-5, 5-10, and 10-20 km from the cropped area in 1990 and those located in the intervals of 0-5 and 5-13 km in 1991. The letter identified time intervals delineated by vertical arrows refer to the timing for various phenological stages of cotton development described in Table 1.

(rows) and to evaluate the differences in mean weekly trap captures within each trap-location area over the specified time intervals (columns) are presented in Tables 2 and 3 for 1990 and 1991, respectively.

TABLE 1. Approximate Annual Timing for Cotton Crop Phenological Development Stages in the Brazos River Valley of Burleson and Brazos Counties, Texas.

Period	Date	Phenological Stage
A	Mar -- Apr	Seedbed preparation -- planting
B	May	Cotyledon -- pin-head square
C	Early Jun -- Late Jun	1/3-grown square -- early bloom
D	Late Jun -- Late Jul	Peak bloom -- boll set
E	Late Jul -- Mid-Sep	Fruiting cutout -- early harvest
F	Mid-Sep -- Oct	Harvest -- stalk destruction

Previous research has shown that early season attractance to grandlure-baited traps is an accurate indicator of weevil emergence (Carroll and Rummel 1985), and that few newly-emerged weevils are attracted to presquaring cotton (White and Rummel 1978). Based on this premise, the uniformly low trap captures of about 1 weevil/trap-week over the 7 weeks of Period A in 1990 (Fig. 3, Table 2) indicated a low, but rather consisent rate of early-spring weevil emergence over most of the study area. The mean weekly capture in the uncropped area in the distance interval of 10-20 km was significantly lower than that in the interval of 0-5 km, and there were no significant differences among the other mean values. Traps were not placed until 24 April in 1991, so data are not available for Period A during that year.

TABLE 2. Mean Number of Boll Weevils Captured per Trap Week (± SE) in Traps Located in the Cropped Area (18 traps) and Those in the Uncropped Area in the Distance Intervals of 0-5 km (15 traps), 5-10 km (9 traps), and 10-20 km (6 traps), Respectively, from the Periphery of the Cropped Area During the Indicated Periods of 1990[a].

		Trap Locations			
Period	Date (Wks)	Cropped	0-5 km	5-10 km	10-20 km
A	16 Mar - 27 Apr	0.93 ± 0.35 ab, w	1.12 ± 0.31 a, w	0.42 ± 0.05 ab, wx	0.19 ± 0.08 b, w
B	7 May - 25 May	4.13 ± 1.69 a, x	5.08 ± 0.94 a, x	4.33 ± 0.52 a, y	1.04 ± 0.45 b, w
C	1 Jun - 22 Jun	0.39 ± 0.19 a, w	1.11 ± 0.19 b, w	0.72 ± 0.33 ab, x	0.50 ± 0.13 ab, w
D	29 Jun - 13 Jul	0.20 ± 0.10 a, w	0.16 ± 0.09 a, y	0.00 ± 0.00 a, w	0.17 ± 0.11 a, w
E	20 Jul - 21 Sep	12.0 ± 3.98 a, y	6.54 ± 1.61 a, x	7.60 ± 1.81 a, y	6.18 ± 1.30 a, x
F	28 Sep - 27 Oct	87.34 ± 23.9 a, z	13.7 ± 2.53 b, z	6.42 ± 1.84 bc, y	3.43 ± 1.32 c, y

[a] Individual trap mean weekly counts for the respective time intervals and location areas were transformed to log (count + 1) values prior to statistical analyses by ANOVA. Means for traps in the different locations in the same row followed by different letters (a, b, and c) and those for the different time intervals in the same column followed by different letters (w, x, y, and z) are significantly different (LSD test, $P \leq 0.05$).

TABLE 3. Mean Number of Boll Weevils Captured per Trap Week (± SE) in Traps Located in the Cropped Area (15 traps) and Those in the Uncropped Area in the Distance Intervals of 0-5 km (8 traps) and 5-13 km (2 traps) from the Periphery of the Cropped Area During the Indicated Periods of 1991[a].

		Trap Location		
Period	Date (Wks)	Cropped	0-5 km	5-13 km
B	27 Apr - 25 May	7.6 8 ± 1.22 a, w	10.2 ± 3.59 a, w	7.90 ± 1.30 a, wx
C	1 Jun - 22 Jun	0.73 ± 0.14 a, x	1.88 ± 0.58 b, x	3.38 ± 1.13 b, w
D	29 Jun - 20 Jul	1.49 ± 0.41 a, x	0.94 ± 0.26 a,x	3.00 ±1.00 a,w
E	27 Jul - 7 Sep	43.1 ± 12.4a, y	38.6 ± 3.17 a, y	36.4 ± 3.36 a,y
F	14 Sep - 27 Oct	133.8 ± 21.4 a, z	25.4 ± 4.26 b, z	13.0 ± 5.42 b, x

[a] Individual trap mean weekly counts for the respective time intervals and location areas were transformed to log (count + 1) values prior to statistical analyses by ANOVA. Means for traps in the different locations in the same row followed by different letters (a and b) and those for the different time intervals in the same column followed by different letters (w, x, y, and z) are significantly different (LSD test, $P \leq 0.05$).

The major period for spring emergence of overwintered weevils (Period B, Fig. 3; Tables 2 and 3) began in late April and continued through May of both 1990 and 1991. Trap captures during this period of 1990 were at significantly higher levels, as compared to Period A, in the cropped area and in the uncropped area out to a distance of 10 km. Trap captures in the uncropped interval of 10-20 km also increased, but the increase was not significant. The uniformity of trap captures in the respective partitioned trap-location areas during Period B of both years indicated that the within-year population densities of emerging overwintered weevils were highly similar in the cropped area and the partitioned uncropped areas out to a distance of about 10 km from the cropped area. In 1990, the only significantly different mean weekly trap captures among the partitioned areas during this period was for the lower value obtained in the uncropped-area distance interval of 10-20 km; whereas in 1991, there were no significant differences in the mean weekly captures for the cropped area and the two uncropped areas out to 13 km.

Trap captures decreased abruptly in all areas during the first week of June in both years (Period C, Fig. 3) at about the time 1/3-grown squares appeared in cotton growing in the cropped area, and they remained at the lower levels through late-June. The decrease in mean weekly captures during Period C, as compared to Period B, were statistically significant for all areas during both years except for the uncropped areas in the intervals of 10-20 km in 1990 and 5-13 km in 1991 (Tables 2 and 3). Reasons for this apparently synchronized, sharp decline of weevil captures in all study areas during both years is open to question. Other researchers have attributed similar observations to increased competition from pheromone-producing male weevils in fruiting cotton (Hardee et al. 1970, Boyd et al. 1973, Scott et al. 1974) or to depletion of overwintered weevil populations (Cherry 1974). Rummel and Bottrell (1976) concluded from their studies that abrupt declines in late-spring responses of weevils to pheromone traps was a seasonally-related phenomenon regulated by unknown factors, not by competition from pheromone-producing weevils in cotton, because similar responses were obtained for both traps located in or near cotton and those located remotely to cotton. The results of the present study support the conclusions of Rummel and Bottrell (1976).

During Period C of 1990 (Table 2), the mean weekly trap capture in the uncropped area in the distance interval of 0-5 km was significantly higher than the mean captures in the cropped area, but no other contrasts among the mean values for the various areas were significant . In 1991 (Table 3), capture levels in the two partitioned uncropped areas were not significantly different, but they were both significantly higher than those obtained in the cropped area. Thus, though they were all reduced in magnitude in comparison to Period B, the capture levels in these uncropped areas still provided evidence of continued low levels of overwintered weevil activity and some potential for migration from overwintering habitats to cultivated cotton through mid-to-late June of both years.

As cotton in the cropped area progressed to the peak bloom and boll-set stage during Period D of 1990 (Fig. 3; Table 2), mean weekly trap captures of weevils in all of the partitioned areas declined in comparison to their respective levels in Period C, but only decreases in the uncropped areas in the intervals of 0-5 km and 5-10 km were significant. Capture levels in all areas in 1991 during Period D compared to Period C were statistically unchanged (Table 3). Mean weekly weevil captures during Period D of 1991 were notably higher than those obtained in 1990 in all areas, but there were no significant differences in capture levels across areas of either year.

Boll weevil capture patterns obtained for periods B, C, and D during both 1990 and 1991 in this study are in general agreement with the capture patterns obtained by Coppedge et al. (1996) in similar trapping studies during similar time periods relative to plant phenology in three diverse cotton production areas of Texas. In general, more weevils were captured at sites remote to cotton out to distances of 10 km than were captured near cotton during each of the three periods, and the levels of trap captures declined in all areas as the season progressed. However, during periods equivalent to C and D, Coppedge et al. (1996) captured considerably greater numbers of weevils in areas remote to cotton in comparison to captures near cotton, especially at the Crockett and Uvalde locations, than were captured in the present study. Also, in the present study, there were no significant differences in the capture levels in any of the respective partitioned areas during Period D of either year as compared to the previous period, so these data provided no evidence of F_1 weevils moving out of cotton during periods of peak fruiting as was suggested by the results of their study. The apparent differences in the results obtained in the two studies may have been due to differences in overwintering weevil population levels, historical weather conditions, cropping systems, geographic locations, and other factors.

Trap captures began to increase area-wide each year during late July in coincidence with the beginning of fruiting cutout in dryland cotton (Period E, Fig. 3). This activity apparently signalled the beginning of major mid-to-late-season weevil migration periods that continued through the beginning of harvest operations. The abrupt, short-term increase in the cropped-area mean weekly captures for the week of 27 July 1990 (Fig. 3) was caused primarily by very high captures in a single trap that was located on on a fence row adjacent to dryland cotton in which fruiting cutout was occurring. Sharp increases in the levels of mean weekly weevil captures in all partitioned areas for Period E of both years, compared with their corresponding levels for Period D, were significant (Tables 2 and 3). Similarities of within-year trap capture patterns among partitioned areas and the lack of statistically significantly differences in mean weekly trap captures (Period E, Tables 2 and 3) among these areas indicated highly uniform distributions of immigrant weevils over the breadth of the study areas during this period of each year.

Similar mid-to-late season periods of increased attraction of weevils to pheromone-baited traps have been reported for other areas (Bottrell et al. 1970, Hardee et al. 1970, Ridgway et al. 1971, Roach and Ray 1972, Lloyd et al. 1972, Wolfenbarger et al. 1976). Increased trap captures during this period have been mostly attributed to intensified movement of weevils searching for more attractive reproductive habitat in nearby cotton (Hardee et al. 1970, Lloyd et al. 1972); however, Ridgway et al (1971) captured weevils at distances more than 9.7 km (6 miles) from cotton, and Roach and Ray (1972) captured weevils more than 32 km (20 miles) from source

areas. Rummel et al. (1977) found that weevils flew at significantly greater heights above ground during mid-to-late season migration periods than they did during their spring flights from hibernation areas, indicating that weevils flew longer distances during the late-summer migration period than they did during the spring emergence period. Further, in mark-recapture studies of late-season weevil migrations, Moody et al. (1993) found that weevils dispersed in all directions, and they captured marked weevils at distances of up to 16 km (10 miles) from source areas. In addition, they found that most of the weevils involved in late summer migration were in the reproductive state and that the percentage of weevils in diapause in the migrating population decreased as distance of migration increased. Based on their observations, they hypothesized that reproductive weevils dispersing from infested fields in late summer and early fall probably play a major roll in establishment of overwintered weevils in new areas. The results of the present study which indicated a uniform distribution of migrating weevils over the study area at distances of up to 20 km from cotton during the mid-to-late season migration period are compatible with the previous studies cited.

Sharp increases in weevil captures occurred in the cropped area during mid-September of both years (Fig. 2) in coincidence with the destruction of the weevil's cotton-field habitats as harvest operations were underway, and the high levels of captures continued through October. The increased levels of weevil captures in cropped areas were not tracked by captures in the peripheral partitioned uncropped areas. During this period of both years, capture levels decreased considerably with increased distance of trap locations from the cropped area, and mean weekly captures in the cropped area were more than five times those obtained in the nearest partitioned uncropped area (Period F, Tables 2 and 3). These results indicated the potential for much larger overwintering populations of weevils in areas close to cotton fields as compared to areas located at remote distances from them. These observations are compatible with the findings of Moody et al. (1993) whose studies indicated that the majority of mid-fall migrating weevils were in diapause and that the diapausing insects tended to enter overwintering habitats relatively near the field of origin.

Trapping studies were terminated in the uncropped areas at the end of October of both years, but were continued in the cropped area through December. Highly variable, apparently weather related, trap captures continued in the cropped area throughout this period of both years (Fig. 2).

Overall, the results of this study indicated that the seasonal activity patterns of boll weevils as measured with pheromone-baited traps in the Brazos River Valley area were very similar to the patterns observed in other cotton production areas of the United States with some apparent differences in timing due to location. Seasonal variations in trap capture patterns were apparently associated with the phenological development stages of cotton growing in the cropped area; however, the great similarities in magnitude variations and timing of captures in traps located near cotton and those located in areas remote to cotton from early spring through mid-September indicated that other seasonal environmental factors, as well as possible seasonal changes in weevil behavior, may also influence their responses to pheromone-baited traps. The detrimental effects of harsh winter conditions on overwintering survival of weevils were apparent, and there was some significant evidence of later, low-level emergence of overwintered weevils in the remotely-located uncropped areas as compared to the cropped areas. Knowledge gained in this study relative to the timing of the mid-to-late season migration period and the associated uniform distribution patterns of weevils over distances up to 20 km from cotton during this period may be beneficial to the future development of improved boll weevil management programs for the Brazos Valley area of Texas.

LITERATURE CITED

Boyd, F. J., Jr., J. R. Brazzel, W. F. Helms, R. J. Moritz, and R. R. Edwards. 1973. Spring destruction of overwintered weevils in West Texas with wing traps. J. Econ. Entomol. 66: 507-510.

Bottrell, D. G., R. E. Reeves, L. K. Almand, D. D. Hardee, and W. H. Cross. 1970. Studies of boll weevil populations and their movement in the High and Rolling Plains of Texas using male baited traps, 1968. Tex. Agric. Exp. Stn. MP-948.

Carroll, S. C., and D. R. Rummel. 1985. Relationship between time of boll weevil (Coleoptera: Curculionidae) emergence from winter habitat and response to grand-lure baited pheromone traps. Environ. Entomol. 14: 447-451.

Cherry, E. T., 1974. Monitoring boll weevil movements with pheromone traps. Univ. of Tenn., Tenn. Farm Home Sci. Prog. Rep. 90: 27 29.

Coppedge, J. R., G. D. Jones, T. M. O'Neil, J. R. Raulston, D. W. Spurgeon, and E. Salgado. 1996. Movement of boll weevils relative to cotton plant phenology, pp.972-976. *In* Proceedings, Beltwide Cotton Conferences, 9-12 January 1996, Memphis, TN. National Cotton Council of America, Memphis, TN.

Fuchs, T. W., and A. A. England. 1989. Winter habitat sampling for overwintered boll weevils as a component of an integrated pest management program. Southwest. Entomol. 14: 265-269.

Fuchs, T. W., and R. Minzenmayer. 1990. Ten year history of overwintered boll weevil emergence profile in the Concho Valley of Texas, 1980-89. Southwest. Entomol. 15: 497-498.

Hardee, D. D., W. H. Cross, P. M. Huddleson, and T. B. Davich. 1970. Survey and control of the boll weevil in West Texas with traps baited with males. J. Econ. Entomol. 63: 1041-1048.

Lopez, J. D., Jr. 1980. Comparison of two types of boll weevil pheromone traps to monitor seasonal response. J. Econ. Entomol. 73: 324-326.

Lloyd, E. P., M. E. Merkl, F. C. Tingle, W. P. Scott, D. D. Hardee, and T. B. Davich. 1972. Evaluation of male baited traps for control of boll weevils following a reproductive-diapause program in Monroe County, Mississippi. J. Econ. Entomol. 65: 552-555.

Moody, D. S., D. G. Bottrell, and D. R. Rummel. 1993. Late season migration of the boll weevil in the Texas Rolling Plains. Southwest. Entomol. 18: 1-10.

NOAA. 1989. Climatological Data- Texas. Vol. 94, Nos. 1-12. Nat. Climatic Center, Asheville, NC.

NOAA. 1990. Climatological Data- Texas. Vol. 95, Nos. 1-12. Nat. Climatic Center, Asheville, NC.

NOAA. 1991. Climatological Data- Texas. Vol. 96, Nos. 1-12. Nat. Climatic Center, Asheville, NC.

Pfrimmer, T. R., and M. E. Merkl. 1981. Boll weevil: winter survival in surface woods trash in Mississippi. Eviron. Entomol. 10: 419-423.

Price, J. R., J. E. Slosser, and G. J. Puterka. 1985. Factors affecting survival of boll weevils in winter habitat in the Texas Rolling Plains. Southwest. Entomol. 10: 1-6.

Ridgway, R. L., L. A. Bariola, and D. D. Hardee. 1971. Seasonal movement of boll weevils near the High Plains of Texas. J. Econ. Entomol. 64: 14-19.

Roach, S. H., and L. Ray. 1972. Boll weevils captured at Socastee, South Carolina, in 1970, in wing traps placed around fields with and without growing cotton. J. Econ. Entomol. 65: 559-560.

Roach, S. H., H. M. Taft, L. Ray, and A. R. Hopkins. 1971. Population dynamics of the boll weevil in an isolated cotton field in South Carolina. Ann. Entomol. Soc. Amer. 64: 394-399.

Rummel, D. R., and D. G. Bottrell. 1976. Seasonally related decline in responses of boll weevils to pheromone traps during mid-season. Environ. Entomol. 5: 783-787.

Rummel, D. R., L. B. Jordan, J. R. White, and L. J. Wade. 1977. Seasonal variation in the height of boll weevil flight. Environ. Entomol. 6: 674-678.

Scott, W. P., E. P. Lloyd, J. O. Bryson, and T. B. Davich. 1974. Trap plots for suppression of low density overwintered populations of boll weevils. J. Econ. Entomol. 67: 281-283.

Slosser, J. E., R. Montandon, D. R. Rummel, L. T. Wilson, and T. W. Fuchs. 1996. Survival of diapausing and nondiapausing boll weevils (Coleoptera: Curculionidae) subjected to freezing temperatures. Environ. Entomol. 25: 407-415.

Smith, G. L., and A. L. Scales. 1965. The effect of air and ground surface temperature on boll weevil winter survival. J. Econ. Entomol. 58: 174-175.

Taft, H. M., and A. R. Hopkins. 1966. Effect of different hibernation environments on survival and movement of the boll weevil. J. Econ. Entomol. 59: 277-279.

White, J. R. and D. R. Rummel. 1978. Emergence profile of overwintered boll weevils and entry into cotton. Environ. Entomol. 7: 7-14.

Wolfenbarger, D. A., H. M. Graham, R. D. Parker, and J. W. Davis. 1976. Boll weevil: seasonal patterns of response to traps baited with grandlure in the Lower Rio Grande Valley. Environ. Entomol. 5: 403-408.

USING SPATIAL INFORMATION TECHNOLOGIES FOR DETECTING AND MAPPING WHITEFLY AND HARVESTER ANT INFESTATIONS IN SOUTH TEXAS

J. H. Everitt, D. E. Escobar, K. R. Summy,
M. A. Alaniz, and M. R. Davis

United States Department of Agriculture
Agricultural Research Service
2413 East Highway 83
Weslaco, TX 78596-8344

ABSTRACT

This paper describes the application of airborne videography with global positioning system (GPS) and geographic information system (GIS) technologies for detecting and mapping whitefly (*Bemesia* sp.) infestations in cotton (*Gossypium hirsutum* L.) and harvester ant (*Pogonomyrmex barbatus* F. Smith) infestations in pasture lands in the Lower Rio Grande Valley of Texas. Plant canopy reflectance measurements made on cotton plants with no whitefly-produced deposits of sooty mold fungus (*Capnodium* sp.), those with low-moderate levels, and those with high levels showed that plants with low-moderate and high levels of sooty mold generally had lower visible and near-infrared reflectance than plants with no sooty mold. Whitefly infestations could be detected in color-infrared and black-and-white near-infrared video imagery based on the presence of whitefly-produced deposits of sooty mold fungus on the cotton foliage, which gave the cotton a dull magenta to gray-black image tonal response. Harvester ant infestations could be easily distinguished in black-and-white red video imagery where the ant mounds had a distinct light gray to white image response. The integration of the GPS with the video imagery permitted latitude-longitude coordinates of whitefly and harvester ant infestations to be recorded on each image. The GPS latitude-longitude coordinates were entered into a GIS to map whitefly and harvester ant infestations in cotton fields and pasturelands, respectively.

INTRODUCTION

Silverleaf whitefly, (*Bemesia argentifolii* Bellows and Perring) (formerly known as sweetpotato whitefly, *B. tabaci* Gennadius) has become increasingly destructive to cotton, vegetables and numerous ornamental plants during the past decade. Whitefly outbreaks in several areas of the United States during the 1991 growing season caused an estimated $200 million in losses to producers nationwide (Henneberry 1993). Damage caused by whiteflies to cotton in the Lower Rio Grande Valley (LRGV) of Texas during the 1991 outbreak approached $22 million in actual losses, and produced an overall impact of $73.4 million on the local economy (Norman et al. 1992). More recently, a general outbreak of whiteflies on LRGV cotton during the 1995 growing season caused extensive damage to the small percentage of cotton plantings that had survived an earlier onslaught of beet

armyworm [*Spodoptera exigua* (Hubner)] and other lepidopterous pests (Summy et al. 1996, Norman et al. 1996).

Damage caused by whiteflies of the *Bemesia* species complex involves a combination of factors, including the transmission of several important plant pathogens (Brown and Bird 1992) and a general reduction in plant vigor as a result of feeding activities (Byrne et al. 1990). Moreover, feeding nymphs excrete copious quantities of honeydew which may contaminate cotton lint and commonly promote the growth of associated sooty mold fungi (*Capnodium* sp.) (Hendrix and Wei 1992). Heavy sooty mold deposits on the plant foliage, while detrimental in the sense that they impede photosynthesis, are highly visible and distinct, and may be used to advantage in the detection of insect infestations for use in damage assessment surveys and research activities.

Past research showed that color-infrared (CIR) aerial photography could be used to remotely detect sooty mold deposits on citrus foliage caused by the honeydew producing insects, brown soft scale (*Coccus hesperidum* L.) and citrus blackfly (*Aleurocanthus woglumi* Ashby) (Hart and Myers 1968; Hart et al. 1973). Although the CIR photography provided only indirect evidence of insect infestation, it was shown to be practical for use in regional surveys (Hart et al. 1973). More recently, Everitt et al. (1994) used airborne multispectral videography to detect sooty mold deposits on citrus caused by the citrus blackfly.

The harvester ant (*Pogonomyrmex barbatus* F. Smith) is a pest affecting rangeland and pastures, other turf areas, and a variety of crop plants in the southwestern United States (Little 1972). Harvester ants are particularly damaging to pastures where they turn up the soil in mound-building activities and feed heavily on grass seeds, thereby interfering with the reseeding of pastureland (Myers et al. 1983). Mound size can vary from approximately 1 to 4-5 m in diameter. In south Texas, many ranges and pastures have heavy infestations of harvester ants that reduce the carrying capacity of the land. Aerial CIR photography has been used successfully to detect harvester ant infestations on grazing lands (Hart et al. 1971, Myers et al. 1983). Everitt and Nixon (1985) reported that aerial videography also distinguishing infestations of harvester ants.

Over the past few years, remote sensing techniques have been integrated with GPS and GIS technologies to assist natural resource managers in developing sound management strategies. Aerial videography and GPS technology have been merged and shown to be useful tools for detecting and monitoring insect activity over forested areas (Bobbe and Isikawa 1992, Myhre 1992). The latitude-longitude data provided by the GPS were entered into a GIS to georeference forest pest problems (Myhre 1992). Richardson et al. (1993) entered aerially obtained GPS coordinates into a GIS to map the distribution of undestroyed cotton fields in a regional management program for boll weevil (*Anthonomus grandis* Boheman) in south Texas (Summy et al. 1988, Summy and King 1992). Everitt et al. (1994) integrated aerial videography with GPS and GIS technologies to map citrus blackfly infestations over a large agricultural area.

Little information is available on the potential of using remote sensing techniques for detecting whitefly infestations. Although CIR photography has been used to detect harvester ant infestations, there is a scarcity of information on using videography for detecting infestations of this pest. To our knowledge no research has been conducted on the merging of remote sensing data with GPS and GIS technologies for mapping infestations of these two pests. The objectives of this study were: (1) to evaluate the potential of aerial multispectral videography for detecting whitefly infestations in cotton fields and harvester ant infestations in grazing lands in the LRGV of Texas, and (2) to demonstrate the incorporation of videography, GPS, and GIS technologies for mapping the distribution of whitefly and harvester ant infestations.

METHODS AND MATERIALS

These studies were conducted in the LRGV of south Texas. The whitefly study area was a 62 km^2 (4.8 x 12.9 km) agricultural area with a large number of cotton fields north of Weslaco in Hidalgo County. The experimental site for the harvester ant study was a 220 km^2 (9 x 24.5 km) agricultural area with a large number of pastures north of Elsa in Hidalgo and Willacy Counties. Aerial videography and ground truth observations were conducted for both studies. Plant canopy reflectance measurements were made for the whitefly study to help interpret the video imagery.

Video imagery was obtained with a three-camera multispectral digital video imaging system (Everitt et al. 1995). The system is comprised of three charge-coupled device (CCD) analog black-and-white video cameras, a computer equipped with an image digitizing board, a color encoder, and super(S)-VHS portable recorder. The cameras are visible/near-infrared (NIR) (0.4 - 1.1 μm) light sensitive. Each camera has a 12.5 mm focal length fixed lens. Two of the cameras are equipped with visible yellow-green (YG) (0.555- 0.565 μm) and red (R) (0.623 - 0.635 μm) filters, respectively, while the third camera has a NIR (0.845 - 0.857 μm) filter. The computer is a 486-DX50 system that has an RGB image grabbing board (640 x 480 pixel resolution). The NIR, R, and YG black-and-white image signals from the cameras are subjected to the RGB inputs of the computer digitizing board, giving a CIR composite digital image similar in color rendition to that of CIR film. The hard disk can store 1000 CIR composite images. In addition, the cameras' signals are also subjected to a color encoder that provides an analog CIR composite stored on the S-VHS recorder. The analog CIR imagery recording serves as a back-up in the event the computer malfunctions.

Imagery of the harvester ant study site was obtained at altitudes ranging from 600 to 1800 m on 21-22 September 1994. Imagery of the whitefly experimental area was taken at altitudes ranging from 300 to 1500 m on 11, 12 and 19 July 1995. The lower altitude imagery was taken to acquire more detail of some ground sites. All imagery was acquired with a fixed-wing aircraft between 1130 and 1530 hours under sunny conditions.

A Trimble[1] Transpak II GPS was integrated with the video system. The GPS was equipped with a navigation system which constantly received data from GPS satellites and readily calculated and continuously displayed the flight direction (bearing), altitude, time, ground speed, and latitude-longitude coordinates of the aircraft above the ground. Two modes were used simultaneously to obtain the GPS data for the whitefly and harvester ant surveys. In one mode an interphaser (Compix model LP-701) was used with the GPS which permitted the transfer and recording of this continuous information on the last two lines of the R-filtered camera, which in turn is also superimposed on the composite image. The latitude/longitude coordinates on the video correspond to approximately the center of each scene. The second mode was the "way point" mode in which "bull's eye" coordinates were manually entered and stored in the GPS memory (a storage capacity of 999 coordinate points). With this mode the coordinate data were entered directly into the computer. The accuracy of the GPS was approximately ±100 m from the center coordinates of each video scene, which was adequate for detecting the whitefly and harvester ant infestations. A differential GPS can provide submeter accuracy, but these systems are costly.

Personal computer ATLAS-GIS software was used to generate maps of Hidalgo and Willacy Counties based on the 1990 post census TIGER/Line files for the State of Texas.

---

[1] Mention of company name is for the reader's benefit and does not constitute endorsement of a particular product by the U. S. Department of Agriculture over others that may be commercially available.

The U.S. Census Bureau developed and trademarked a machine readable referenced map base called TIGER (Topologically Integrated Geographic Encoding and Referencing) for the 1990 census. The TIGER map-based system was constructed using USGS 1:100,000 scale digital line graph maps. The GIS map provided greater detail of the study areas such as highways, roads, hydrography and other landmarks. The map was produced to geographically map the whitefly and harvester ant infestations using the airborne GPS data obtained from the surveys.

Radiometric plant canopy reflectance measurements were made in two cotton fields near Weslaco, Texas during July 1995. Measurements were made in field 1 on 12 July, whereas field 2 measurements were conducted on 20 July. Reflectance measurements were made on cotton plants with no sooty mold deposits on the leaves (control), plants with light to moderate sooty mold deposits, and those with heavy deposits. Control cotton plants had typical green leaves, plants with light to moderate sooty mold deposits had a light to moderate gray leaf appearance, and leaves from those with heavy deposits had a dark gray to black cast. Reflectance measurements were made on 12 randomly selected plant canopies of each of the three categories of cotton plants at each field with a Barnes modular multispectral radiometer (Anonymous 1980). Measurements were made in the visible green (G) (0.52 to 0.62 μm), visible R (0.63 to 0.69 μm), and NIR (0.76 to 0.90 μm) spectral bands with a sensor that had a 15-degree field-of-view placed approximately 1 m above each plant canopy. Reflectance measurements were made between 1100 and 1500 hours under sunny conditions. Radiometric measurements were corrected to reflectance at a common solar irradiance reference condition (Richardson 1981).

Ground truth surveys were made of the study sites to verify the presence of whitefly and harvester ant infestations. Observational data recorded were plant species, density, cover, and soil type, and ground photographs were taken to help interpret the aerial video imagery.

Reflectance data were subjected to an analysis of variance. Duncan's multiple range test was used to test statistical significance among means at the 0.05 probability level (Steel and Torrie 1980).

RESULTS AND DISCUSSION

Whitefly. Fig. 1 shows leaves from cotton plants with no sooty mold fungus deposits (A), and those with low to moderate levels (B), and high levels (C) caused by whitefly infestations. The sooty mold fungus develops on the honeydew deposits excreted by whitefly nymphs and ultimately, can inhibit the cotton plants ability to carry on photosynthesis and contaminate the lint (Hendrix and Wei 1992).

Mean canopy reflectance values of cotton plants with no sooty mold deposits on the foliage, those with low to moderate levels, and those with heavy levels for two cotton fields are given in Table 1. For field 1, plants with low to moderate and high levels of sooty mold had lower visible G reflectance values than those with no sooty mold. At the visible R wavelength, plants with low to moderate levels of sooty mold had lower reflectance than those with no sooty mold. However, the visible R reflectance of cotton plants with high levels of sooty mold did not differ from that of plants with no sooty mold deposits. The inability to separate plants with high sooty mold levels from those with no sooty mold was attributed to soil background reflectance which increased their R reflectance (Richardson et al. 1975, Everitt et al. 1986). At the NIR wavelength, cotton plants with low to moderate levels of sooty mold deposits had lower reflectance than plants with no sooty mold deposits. Plants with high levels of sooty mold had lower NIR reflectance than those with light to moderate levels and/or those with no sooty mold

deposits. The reflectance data obtained from field 2 followed a similar pattern to that shown for field 1, but there was a better separation among the visible reflectance values.

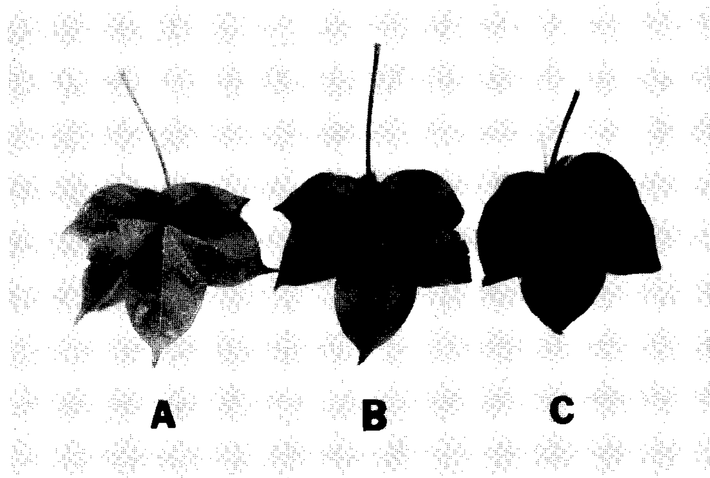


FIG. 1. Leaves from cotton plants with no sooty mold (A), low-moderate sooty mold levels (B) and high levels (C) of sooty mold caused by whitefly infestation.

Table 1. Canopy Reflectance of Non-infested and Whitefly-infested Cotton (as Determined by Deposits of Sooty Mold Fungus on the Foliage) at the Green, Red, and Near-infrared Wavelengths. Measurements Were Made in Two Cotton Fields Near Weslaco, Texas in July 1995.

Site and Date	Sooty Mold Level	Canopy reflectance(%) for three wavelengths		
		Green[a]	Red	Near-infrared
Field 1 12 July 1995	None	4.7a	2.9a	36.5a
	Low-Moderate	3.2b	2.2b	16.9b
	High	2.8b	2.5ab	9.0c
Field 2 20 July 1995	None	5.6a	3.0a	34.5a
	Low-Moderate	3.0b	2.1b	17.1b
	High	2.3c	2.0b	9.1c

[a] Means within a column at each date followed by the same letter do not differ significantly at the 0.05% probability level, according to Duncan's multiple range test.

The lower visible and NIR reflectance of cotton plants with sooty mold deposits on their leaves was attributed to the dark sooty mold fungus which absorbed a large percentage of the visible and NIR radiation (Gausman 1985). The more pronounced differences in the

NIR spectral region between plants with no sooty mold deposits and those with sooty mold deposits agrees with the findings of Gausman and Hart (1974).

Figs. 2-A, B, C, and D show CIR composite and NIR, R, and YG narrowband black-and-white digital video images, respectively, of a cotton field near Weslaco Texas, infested with whiteflies. The GPS data appears at the bottom of the CIR and R images. The imagery was obtained at an altitude of approximately 960 m. The arrow on the CIR composite points to the dark gray to gray-black image response of high levels of sooty mold fungus on the cotton foliage caused by whitefly infestation. Some of the gray-black image was attributed to in-canopy shadowing caused by loss of leaves due to whitefly devastation. Cotton plants with low to moderate levels of sooty mold fungus have a dull magenta to gray-brown response, while plants with no sooty mold deposits have a bright red tone. Sparsely vegetated and essentially bare soil areas have a green to light gray image. Most of the whitefly-infested plants can also be distinguished in the NIR black-and-white image. The sooty mold deposits generally could not be detected in the R and YG images. Analysis of the video imagery of the study area identified approximately 65 locations thought to be infested by whitefly. In several instances more than one infestation was recorded within large cotton fields. Ground reconnaissance of the study area verified the presence of whiteflies at all locations.

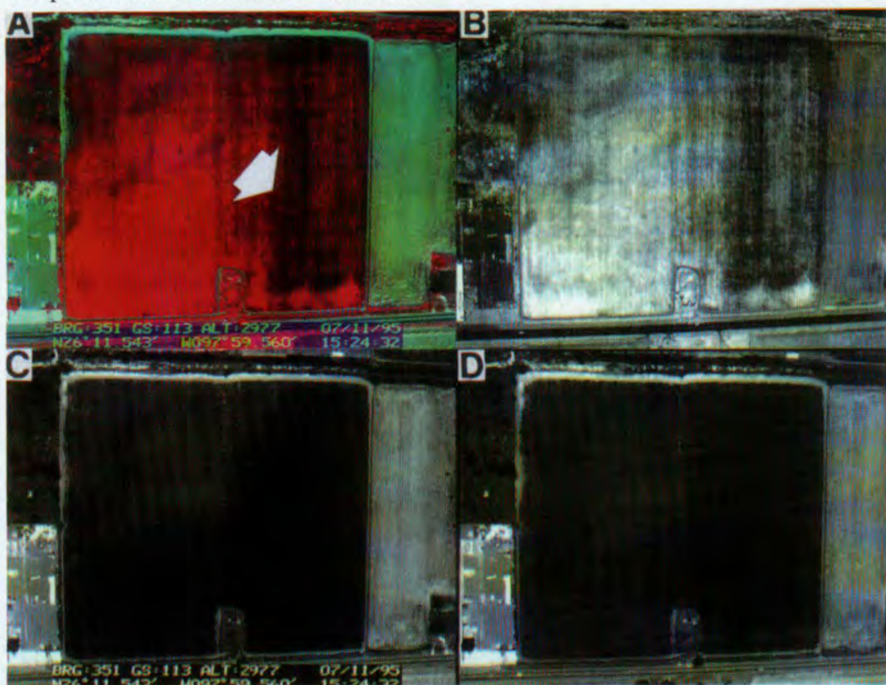


FIG. 2. Color-infrared (CIR) composite (A), and near-infrared (B), red (C), and yellow-green (D) black-and-white narrowband digital video images of a cotton field near Weslaco, Texas. The arrow on the CIR composite points to the dark gray to gray-black image response of high levels of sooty mold fungus on the cotton foliage caused by whitefly infestation. The infested area is also delineated in the near-infrared image. The global positioning system data shown at the bottom of the CIR composite and red images includes the bearing (direction), ground speed, altitude, date, north latitude, west longitude, and time.

The superiority of the NIR image over the YG and R images for distinguishing sooty mold deposits generally agrees with the canopy reflectance data, where the best differences among cotton plants with and without sooty mold deposits on the foliage occurred at the NIR wavelength interval (Table 1). These findings also concur with previous research on using videography for detecting citrus blackfly infestations (Everitt et al. 1994). Although the R and YG canopy reflectance data showed potential for spectrally separating between cotton plants with and without sooty mold deposits, these differences apparently were not great enough to be distinguished inthe R and YG video images.

The GPS data displayed at the bottom of the CIR and R video images of the whitefly-infested cotton field are useful to locate infestations and can be entered into a GIS. The GPS latitude-longitude data obtained from the "way point" mode was used with GIS technology to georeference whitefly infestations in the test site north of Weslaco. Fig. 3 (upper left) shows a GIS TIGER map of Hidalgo County with the experimental site denoted in the lower right portion of the map. The triangles depict GPS latitude-longitude coordinates for whitefly infestations within the area. Most of the locations are overlapped because of the small map scale.

Fig. 3 (lower right) shows a more detailed GIS map of the study area depicting the 65 locations (triangles) where whitefly infestations occurred. With this map, one can associate the general street/road addresses with the GPS marked location of each cotton field where whitefly infestations occur. The integration of the GPS with GIS technology enables the agricultural consultant to develop regional maps of a large agricultural area showing where insect infestations occur. The aerial video imagery can provide a means of determining the severity of infestations.

Harvester Ant. The R narrowband black-and-white digital video image of a pasture near Elsa, Texas with a heavy infestation of harvester ants is shown in Fig. 4. The arrow on the print points to the conspicuous light gray to white tonal response of a harvester ant mound. The generally circular shape of the ant mounds delineates them from the sparsely vegetated areas which have a similar image response. Grassland vegetation has a gray to dark gray response. The image was obtained at an altitude of approximately 1380 m. The GPS data are shown on the bottom of the image. The harvester ant mounds could also be easily detected in the CIR composite image and could generally be delineated in the YG image, but they could not be distinguished in the NIR image (other images not shown). Analysis of the video imagery of the 220 km^2 study area showed what appeared to be 44 ant infestations. Ground surveys confirmed the presence of harvester ants at every location.

The merger of the GPS latitude-longitude data obtained from the video survey of harvester ant infestations with a GIS is shown in Fig. 5. The lower portion of the figure shows GIS maps of Hidalgo and Willacy Counties with the study area delineated by a dotted boundary. The southern part of the experimental site was in Hidalgo County, whereas the northern portion was in Willacy County. The symbols (triangles) within the experimental area represented GPS latitude-longitude coordinates where harvester ant infestations occurred. Many of the locations are overlapped because of the small map scale. A more detailed GIS map of the study area is shown in the upper right portion of Fig. 5. A local site map showing even greater detail on the location of selected harvester ant infestations is shown in the upper left portion of Fig. 5. This capability of the GIS software is useful for obtaining more specific details on areas of interest.

These results demonstrate that aerial multispectral videography is a useful tool for detecting whitefly infestations in cotton and harvester ant infestations in grazing lands in the LRGV of Texas. The integration of videography, GPS, and GIS technologies are valuable for mapping the distribution of whitefly and harvester ant infestations over an agricultural producing area. Maps can be produced that would aid agricultural consultants and farm managers to depict where insect infestations occur over a large area. The video

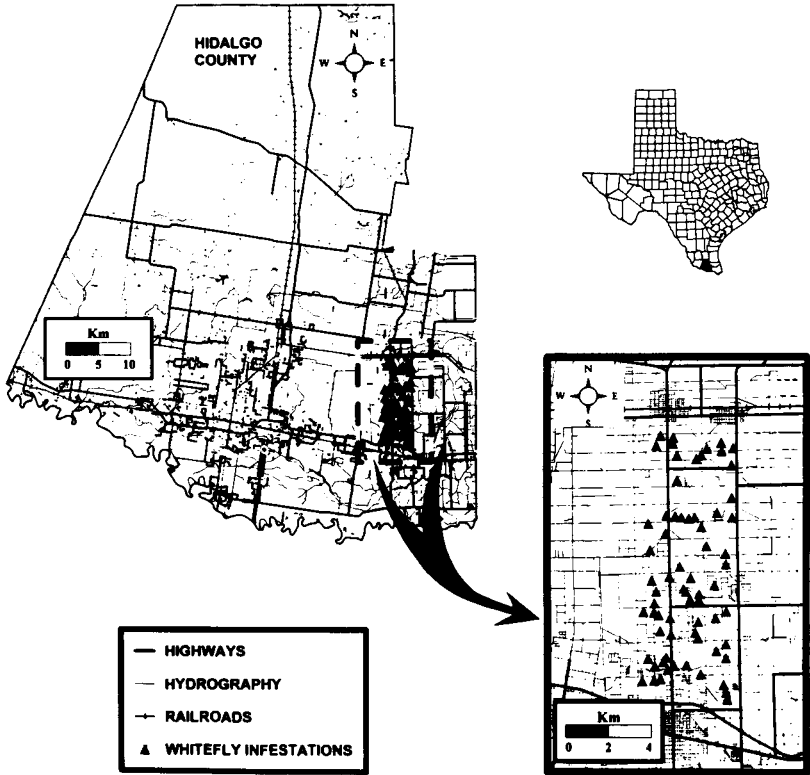


FIG. 3. Geographic information system (GIS) TIGER map (upper left) of Hidalgo County with the whitefly experimental site denoted in the lower right portion of the map. The symbols (triangles) within the study area represent global positioning system latitude-longitude coordinates of whitefly infestations in cotton fields. A detailed GIS map (lower right) clearly depicts the locations of the whitefly infestations.

FIG. 4. Black-and-white red narrowband video image of a pasture near Elsa, Texas infested with harvester ants. The arrow on the print points to the light gray-white response of a harvester ant mound. The global positioning system data shown at the bottom of the image includes the bearing (direction), ground speed, altitude, date, north latitude, west longitude, and time.

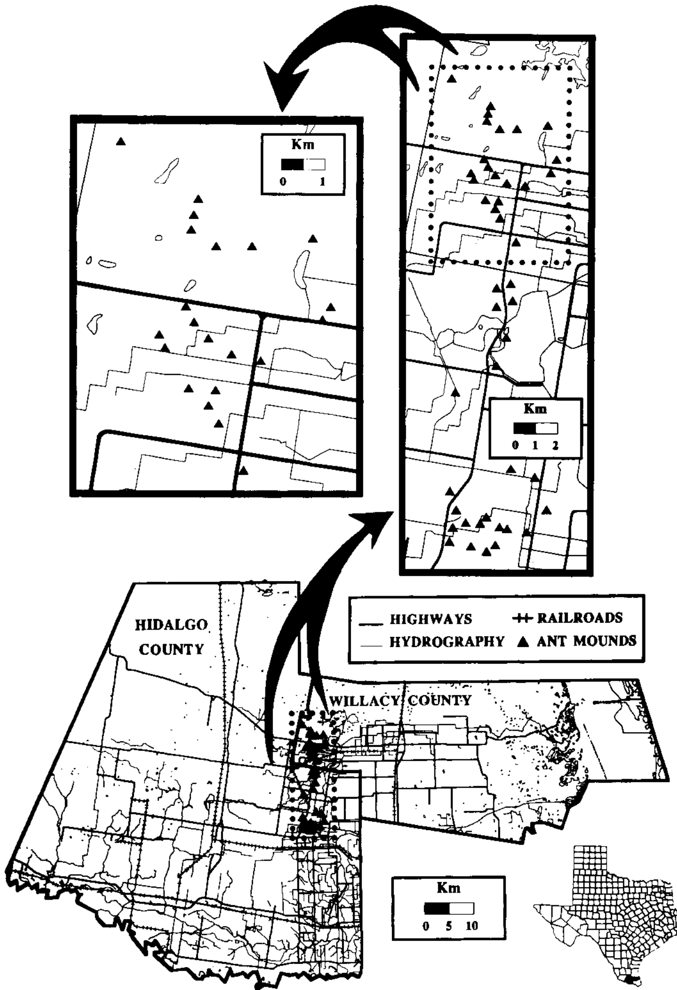


FIG. 5. Geographic information system (GIS) TIGER map (lower) of Hidalgo and Willacy Counties with the harvester ant study area delineated with a dotted boundary. The symbols (triangles) within the experimental site represent global positioning system latitude-longitude coordinates where harvester ant infestations occurred. Each symbol represents a single pasture with a harvester ant infestation. A detailed GIS map (upper right) clearly depicts the locations of the harvester ant infestations within the study area. The upper left map shows even greater detail of a portion of the study area.

imagery can also serve as a permanent geographically located image data base to monitor future contraction or spread of insect infestations over time.

ACKNOWLEDGEMENTS

Thanks are extended to Angie Cardoza for word processing and Buck Cavazos for preparation of illustrations.

LITERATURE CITED

Anonymous. 1980. Modular-multiband radiometer model 12-1000. Barnes Engineering Co., Stamford, CT. Bull. 12-1000A. 7 pp.

Bobbe, T. J., and P. Ishikawa. 1992. Real-time differential GPS: An aerial survey-Remote sensing application, pp. 108-116. *In* Proc. Resource Technol. 92 Sympos. Amer. Soc. Photogramm. and Remote Sens. Bethesda, MD.

Brown, J. K., and J. Bird. 1992. Whitefly-transmitted geminiviruses and associated disorders in the Americas and the Caribbean Basis. Plant Dis. 76:220-225.

Byrne, D. N., T. S. Bellows, and M. P. Parrella. 1990. Whiteflies in agricultural systems, pp. 277-281. *In* D. Gerling [ed.] Whiteflies: their bionomics, pest status and management. Intercept, Andover UK.

Everitt, J. H., D. E. Escobar, I. Cavazos, J. R. Noriega, and M. R. Davis. 1995. A three-camera multispectral digital video imaging system. Remote Sensing Environ. 54:333-337.

Everitt, J. H., D. E. Escobar, K. R. Summy, and M. R. Davis. 1994. Using airborne video, global positioning system, and geographic information system technologies for detecting and mapping citrus blackfly infestations. Southwestern Entomol. 19:129-138.

Everitt, J. H., and P. R. Nixon. 1985. Video imagery: a new remote sensing tool for range management. J. Range Manage. 38:421-424.

Everitt, J. H., A. J. Richardson, and P. R. Nixon. 1986. Canopy reflectance characteristics of succulent and nonsucculent rangeland plant species. Photogramm. Eng. and Remote Sensing 52:1891-1897.

Gausman, H. W. 1985. Plant leaf optical parameters in visible and near-infrared light. Graduate Studies Texas Tech University, No. 29. Texas Tech University Press, Lubbock, TX. 78 pp.

Gausman, H. W., and W. G. Hart. 1974. Reflectance of four levels of sooty mold deposits produced from the honeydew of three insect species. J. Rio Grande Valley Hort. Soc. 28:131-136.

Hart, W. G., S. J. Ingle, M. R. Davis, C. Mangum, A. Higgins, and J. C. Boling. 1971. Some uses of infrared aerial photography in entomology, pp. 99-1130. *In* Proc. 3rd Biennial Workshop Color Aerial Photography in the Plant Sciences. American Society of Photogrammetry, Falls Church, VA.

Hart, W. G., S. J. Ingle, M. R. Davis, and C. Mangum. 1973. Aerial photography with infrared color film as a method of surveying for citrus blackfly. J. Econ. Entomol. 66:190-194.

Hart, W. G., and V. I. Myers. 1968. Infrared aerial photography for detection of populations of brown soft scale in citrus groves. J. Econ. Entomol. 61:617-624.

Hendrix, D. L., and Y. Wei. 1992. Detection and elimination of honeydew excreted by the sweetpotato whitefly feeding upon cotton, pp. 671-673. *In* Proc. Beltwide Cotton Conf. National Cotton Council, Memphis, TN.

Henneberry, T. J. 1993. Sweetpotato whitefly - current status and national research and action plan, pp. 663-666. *In* Proc. Beltwide Cotton Confer. National Cotton Council, Memphis, TN.

Little, V. A. 1972. General and Applied Entomology. Harper and Row Publishing Co., New York. 527 pp.

Myers, V. I., M. E. Bauer, H. W. Gausman, W. G. Hart, J. L. Heilman, R. B. McDonald, A. B. Park, R. A. Ryerson, T. J. Schmugge, and F. C. Westin. 1983. Remote sensing in agriculture, pp. 2111-2228. In R. N. Colwell (ed.), Manual of Remote Sensing. American Society of Photogrammetry, Falls Church, VA.

Myhre, R. J. 1992. Use of color airborne videography in the U. S. Forest Service, pp. 145-152. *In* Proc. Resource Technol. 92. Sympos. Amer. Soc. Photogramm. and Remote Sens. Bethesda, MD.

Norman, J. W., Jr., A. N. Sparks, Jr., and D. Riley. 1992. Sweetpotato whiteflies in Lower Rio Grande Valley cotton, pp. 687-690. *In* Proc. Beltwide Cotton Conf. National Cotton Council, Memphis, TN.

Norman, J. W., Jr., A. N. Sparks, Jr., and B. D. Riley. 1996. Whiteflies in the Lower Rio Grande of Texas in 1995. Proc. Beltwide Cotton Conf. National Cotton Council, Memphis, TN. *In* press.

Richardson, A. J., 1981. Measurement of reflectance factors under daily and intermittent irradiance variations. Appl. Opt. 20:1336-1340.

Richardson, A. J., C. L. Wiegand, H. W. Gausman, J. A. Cuellar, and A. H. Gerbermann. 1975. Plant, soil, and shadow reflectance components of row crops. Photogramm. Eng. and Remote Sensing 41:1401-1407.

Richardson, A. J., K. R. Summy, M. R. Davis, A. Gomez, and D. W. Williams. 1993. The use of 1990 Tiger/Line™ Census files for monitoring the Rio Grande Valley cotton stalk destruction program, pp. 231-239. *In* Proc. Application Advanced Information Technol. Sympos.

Steel, R. G. D., and J. H. Torrie. 1980. Principles and Procedures of Statistics. McGraw-Hill Book Co., New York, NY. 481 pp.

Summy, K. R., W. G. Hart, M. R. Davis, J. R. Cate, J. W. Norman, Jr., C. W. Wofford, Jr., M. D. Heilman, and L. N. Namken. 1988. Regionwide management of boll weevil in southern Texas, pp. 240-247. *In* Proc. Beltwide Cotton Conf. National Cotton Council, Memphis, TN.

Summy, K. R., and E. G. King. 1992. Cultural control of cotton pests in the United States. Crop Protection 11:307-319.

Summy, K. R., J. R. Raulston, D. Spurgeon, and J. Vargas. 1996. Analysis of the beet armyworm outbreak on cotton in the Lower Rio Grande Valley of Texas during the 1995 production season, pp. 837-842. *In* Proc. Beltwide Cotton Conf. National Cotton Council, Memphis, TN. In press.

HELIOTHIS VIRESCENS (LEPIDOPTERA: NOCTUIDAE): INFLUENCE OF STERILE BACKCROSS RELEASES ON SUPPRESSION

M. L. Laster, D. D. Hardee and J. C. Schneider[1]

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

ABSTRACT

A pilot test to suppress a wild tobacco budworm, *Heliothis virescens* (F.), population by rearing and releasing insects with a sterile male trait was conducted during 1991-94. Backcross moths with the sterile trait were released in a 16.7-km square area in Washington and Sunflower Counties, Mississippi, in 1992 and in Bolivar County in 1993. Pheromone traps were used to monitor insect populations in both areas during both years and the non-release area served as the control. Moth releases were directed at the overwintered tobacco budworm emergence and were placed in the field 2 April to 15 May in 1992 and 12 April to 25 May in 1993. Approximately 69,000 moths per day were released in 1992 and 70,000 per day in 1993. A 3.0:1.0 released:wild ratio was achieved in 1992. This ratio dropped to 1.3:1.0 and 1.0:2.3 during June and July, respectively, due to moth movement into and out of the release area. Continued monitoring of this area in 1993 showed a 1.0:2.2 backcross:wild ratio (29.9%
[1]Department of Entomology, Mississippi State University, Mississippi State, MS 39762.
sterility) carried over the winter. This ratio dropped to 1.0:5.2 backcross:wild in June, increased to 1.0:4.7 and 1.0:3.4 during July and August, respectively. A 2.6:1.0 released:wild ratio was achieved for the Bolivar County release in 1993. This ratio declined to 1.0:1.6 in June, 1.0:3.6 in July and 1.0:4.0 in August. Continued monitoring in 1994 showed that male sterility in the overwintered populations across both release areas was 12.1%.

INTRODUCTION

The tobacco budworm, *Heliothis virescens* (F.), has demonstrated its adaptability as an agricultural pest by developing resistance to all insecticides used for its control (Harris et al. 1972, Elzen et al. 1992). The need for control methods other than insecticides has been recognized for some time. Knipling (1960) listed development and release of strains of an insect that possesses inferior or lethal genetic characteristics as one of four ways to use insects for their own destruction. He further stated that the production of sterile hybrids as a result of crossing two related species or races might be possible for some insects without changing their mating behavior or ability to find the females of the natural population. Laster (1972) reported male sterility of hybrids from the *Physalis* fruitworm, *H. subflexa* (Guenée) X *H. virescens*. Hybrid females from these crosses, when backcrossed to *H. virescens* males, produce sterile

[1]Department of Entomology, Mississippi State University, Mississippi State, MS 39762.

males in subsequent backcross generations (Hardee and Laster 1996). The sterile male trait has presently remained stable in the laboratory for 223 backcross generations. These backcross insects meet the criteria for two of the four ways proposed by Knipling (1960) for utilizing the autocidal principle of insect control: (1) males have been made sexually sterile; and (2) females perpetuate the sterile trait in the population. These factors make the sterile backcross ideal for autocidal control of the tobacco budworm because both sexes are detrimental to the population.

A pilot study was conducted on St. Croix, U.S. Virgin Islands,during 1977-81 (Proshold et al. 1982) to evaluate effects of releasing sterile backcross insects in the natural tobacco budworm population. During the release of 40,000 backcross insects per day in 1981, male sterility of the wild tobacco budworm reached 94.3%. When the 1981 native populations on St. Croix and Vieques, a neighboring island, were compared to previous years, suppression on St. Croix from the sterile backcross release was indicated.

Because the research conducted on St. Croix was essentially in a closed system (isolated by a 64 km span of open water), information was needed on performance of sterile backcross insects released in a typical agricultural production area. A pilot test was initiated in 1991 with releases in 1992 and 1993 in the central delta of Mississippi to study effects of released backcross insects on the wild *H. virescens* population. This paper reports release ratios and sterile:normal frequencies during and after the releases through July 1994.

MATERIALS AND METHODS

The 1992 release area was a 16-km square near Stoneville, Mississippi. An area of equal size, separated from the release area by 27 km, was established in Bolivar County as a control. Sampling in both areas was identical and the release and control areas were interchanged in 1993. Backcross pupae were placed in the field 2 April to 15 May in 1992 and 12 April to 25 May in 1993 during the overwintered tobacco budworm emergence period. Moths emerging from backcross pupae were released from 25 stations spaced approximately 3.2 km apart over the release area. Cardboard boxes containing the pupae were placed inside a wooden frame covered with bird netting to protect pupae and emerging moths from predatory birds. Each frame held four boxes of 16 plastic rearing trays (King et al. 1985) with 64 cells per tray. Plastic netting with 2.54- by 3.81-cm mesh size was placed between the trays to allow sufficient space for emerging moths to exit the tray cells. Each frame was supported by a metal post protruding about 1.5 m above ground level. One such frame was filled with boxes at each release station daily for 14 days in 1992 and 16 days in 1993. Boxes from the first frame were replaced with new boxes on day 15 in 1992 and day 17 in 1993 and the cycle was repeated. This procedure allowed pupae to remain up to 14 and 16 d in the field in 1992 and 1993, respectively, for moths to emerge. The last boxes were removed from the field on 1 June in 1992 and 7-9 June in 1993. Boxes were returned to the laboratory, and the old trays were discarded and replaced with new trays for their return to the field. Percentage emergence was obtained by counting live and dead pupae and pupal cases from all trays in two randomly selected boxes of each group of pupae removed from the field. Released insects were grown at the R. T. Gast Rearing Facility, Mississippi State, Mississippi, on a diet containing Calco Red® dye (Hendricks 1970) which served as an internal marker for identifying released moths.

Ramaswamy et al. (1985) found no significant differences in capture frequency between laboratory, field, and backcross genotypes of *H. virescens* in pheromone baited traps. Therefore, wire cone traps (Hollingsworth et al. 1978) baited with female sex pheromone (Scentry Inc., 610 Central Ave., Billings, MT 59102) were spaced at 1.6 km north-south and 3.2 km east-west throughout the release and control areas to monitor emergence, spatial distribution of released insects, and to determine released:wild ratios. Each trap had a 61-cm

diameter opening at the bottom of the cone and a 35-cm diameter opening in the center of the inner apron. Pheromone traps were monitored daily from 4 April to 1 June and twice weekly from 8 June to 2 August 1992. In order to determine the incidence of sterility in the overwintered tobacco budworm population following the 1992 release, traps were monitored daily five days per week from 30 March to 18 June 1993 and twice weekly from 21 June through August. Because the red marker is present only in released insects, a random sample of up to 10 males per trap per collection from 15 June to 2 August in 1992, 18 April through 31 August 1993, and 1 April through 1 August 1994 was combined in single pair matings with virgin females in the laboratory as described by Laster et al. (1988). After ovipositing, females were sacrificed and mating was determined by presence or absence of a spermatophore. Eggs were held an additional three days and male fertility was determined by the presence of a spermatophore and egg hatch. The percentage of sterile backcross and fertile (wild) moths was determined for each sample, and the total numbers of moths of each type captured on each sample date was estimated. These data were used to determine sterile:fertile ratios for June and July generations in 1992 and 1994, and June, July, and August in 1993.

Eggs and larvae were collected during July 1992 and 1993 from five concentric circles established at 4.8-km increments from the center of the release and control areas. Twelve cotton fields were randomly located within each circle. Tobacco budworm and bollworm, *Helicoverpa zea* (Boddie), eggs and larvae were collected weekly from 50 randomly selected plants in each of four randomly selected locations in the 12 cotton fields of each circle. Eggs and larvae were placed individually in cups with bollworm diet (King et al. 1985) and held in the laboratory for adult emergence. The adults were identified to species, and male tobacco budworm moths were paired with virgin females to determine the incidence of male sterility. The percentages of bollworm and tobacco budworm were determined from these collections.

RESULTS AND DISCUSSION

Emergence counts from the release boxes in 1992 showed 813 ± 106 ($\bar{x}$ ± SD, N = 85) of the 1,024 cells in the 16 rearing trays per box contained pupae. From these pupae, 690 ± 167 ($\bar{x}$ ± SD, N = 85) moths emerged from each box. Thus, 69,000 ± 16,700 ($\bar{x}$ ± SD, N = 85) moths emerged per day from the 100 release boxes placed at the 25 release sites. Percentage emergence for the first 21 days of the release period was 76.8 ± 17.6 ($\bar{x}$ ± SD, N = 21), but as temperatures increased during the remainder of the release period, percentage emergence increased to 92.4 ± 8.0 ($\bar{x}$ ± SD, N = 22).

Numbers of field collected eggs and larvae were low in all concentric circles around the release point and showed no gradient in populations. However, these collections did indicate that the species composition was 23.3% and 16.0% *H. zea* in the release and control areas, respectively, in 1992 and 35% and 21% in 1993.

The numbers of released (backcross) and wild males trapped in the release area during the release period in 1992, and the numbers of backcross and wild males trapped during the June and July generations following the backcross release are indicated in Table 1. Collections are not shown for the period from 8 June to 14 June because the moths were killed at the trap sites by fumigation and the collections contained no marked moths. The first wild moth was trapped in the release area 9 April. The first released moth with the red marking was captured 10 April; none were captured after 7 June. During this period, 28 traps positioned in the release area captured 6,386 released and 2,154 wild moths. Weekly released:wild moth ratios ranged from 1.0:2.9 to 4.2:1.0 with an overall ratio of 3.0:1.0 for the release period (Table 1). Ratios after 11 May are likely to be biased low since some of the males indicated as wild *H. virescens* could actually be sterile males of the first field generation.

TABLE 1. Numbers of Released Sterile Backcross and Wild Males Trapped in the Release Area (Sunflower and Washington Counties, MS) During the Release Period, and Numbers of Backcross and Wild Males Trapped During the June and July Generations Following the Backcross Release in 1992.

Date	No. backcross males trapped	No. wild males trapped[a]	Ratio backcross:wild
4/09-4/19	61	17	3.4:1.0
4/20-4/26	513	201	2.5:1.0
4/27-5/03	515	179	2.9:1.0
5/04-5/10	712	317	2.3:1.0
5/11-5/17	1,602	494	3.3:1.0
5/18-5/24	2,112	500	4.2:1.0
5/25-5/31	811	273	2.9:1.0
6/01-6/07	60	173	1.9:2.9
Total	6,386	2,154	3.0:1.0
6/08-6/14[c]	---	---	---
6/15-6/21	859 (57)	856 (57)	1.0:1.0
6/22-6/28	413 (150)	325 (118)	1.3:1.0
6/29-7/05	427 (92)	148 (92)	2.9:1.0
Total	1,699	1,322	1.3:1.0
7/06-7/12	105 (40)	111 (42)	1.0:1.1
7/13-7/19	2,892 (83)	5,017 (144)	1.0:1.7
7/20-7/26	2,008 (73)	5,464 (199)	1.0:2.7
7/27-8/02	772 (73)	2,549 (241)	1.0:3.3
Total	5,777	13,151	1.0:2.3

[a]6/15-8/02 estimated from the testing of a subsample of males for sterility; numbers of males demonstrated to be sterile are given in parentheses.
[b]6/15-8/02 estimated from the testing of a subsample of males for sterility; numbers of males demonstrated to be fertile are given in parentheses.
[c]Collections were killed at trap location by fumigation. They contained no marked moths and no sterile:fertile ratios were determined.

Trapping data for the first generation in the release area following the backcross release (15 June to 5 July) showed that the overall backcross:wild ratio declined from 3.0:1.0 during the release period to 1.3:1.0 (Table 2). Trapping data for the second generation (6 July to 2 August) in the release area showed a further decline in the backcross:wild moth ratio to 1.0:2.3 (Table 1). Movement of marked moths out of the release area during the June and July generations was probably a significant factor in this reduction (Schneider et al. 1989).

The numbers of backcross and wild males trapped in the control area during the release period and the number of backcross and wild males trapped during the June and July generations following the backcross release are indicated in Table 2. The first wild moth was trapped in the control area 8 April and four released moths were first captured 19 April (Table 2). No released moths were captured in the control area after 7 June. During the release period, 340 released moths and 1,780 wild moths were captured in the control area. Weekly

backcross:wild ratios recorded for the control area during the release period ranged from 1.0:2.4 to 1.0:24.7 with an overall ratio of 1.0:5.2 (Table 2).

TABLE 2. Numbers of Released Sterile Backcross and Wild Males Trapped in the Control Area (Bolivar County, MS) During the Release Period, and Numbers of Backcross and Wild Males Trapped During the June and July Generations Following the Backcross Release in 1992.

Date	No. backcross males trapped	No. wild males trapped[b]	Ratio backcross:wild
4/08-4/19	4	10	1.0:2.5
4/20-4/26	14	91	1.0:6.5
4/27-5/03	12	232	1.0:19.3
5/04-5/10	22	488	1.0:22.2
5/11-5/17	136	461	1.0:3.4
5/18-5/24	128	306	1.0:2.4
5/25-5/31	21	118	1.0:2.5
6/01-6/07	3	74	4.0:24.7
Total	340	1,780	1.0:5.2
6/08-6/14[c]	---	---	---
6/15-6/21	518 (39)	1,502 (113)	1.0:2.9
6/22-6/28	251 (45)	547 (98)	1.0:2.2
6/29-7/05	160 (51)	84 (27)	1.9:1.0
Total	929	2,133	1.0:2.3
7/06-7/12	39 (9)	99 (23)	1.0:2.5
7/13-7/19	186 (9)	908 (44)	1.0:4.9
7/20-7/26	448 (13)	2,823 (82)	1.0:6.3
7/27-8/02	190 (12)	2,539 (160)	1.0:2.3
Total	863	6,269	1.0:7.4

[a] 6/15-8/02 estimated from the testing of a subsample of males for sterility; numbers of males demonstrated to be sterile are given in parentheses.
[b] 6/15-8/02 estimated from the testing of a subsample of males for sterility; numbers of males demonstrated to be fertile are given in parentheses.
[c] Collections were killed at trap location by fumigation. The contained no marked moths and no sterile:fertile ratios were determined.

Trapping data for the first generation in the control area following the backcross release (16 June to 15 July) showed that the overall backcross:wild ratio increased from 1.0:5.2 during the release period to 1.0:2.3 (Table 2). This suggests that a net movement of progeny of released moths into the control area from the release area 27 km to the south may have persisted through June. However, trapping data for the July generation in the control area showed a decline in the backcross:wild ratio to 1.0:7.4 (Table 2). This is consistent with the progressive dilution of backcross moths and their progeny through their movement.

Emergence counts from the emergence boxes in 1993 showed 772 ± 130 ($\bar{x}$ ± SD, N = 92) of the 1,024 cells in the 16 rearing trays per box contained pupae. From these pupae, 663 ± 231 ($\bar{x}$ ± SD, N = 92) moths emerged from each box. Thus, 70,278 ± 24,486 ($\bar{x}$ ± SD, N = 44)

moths emerged from emergence boxes placed at release sites during the release period. Even though emergence boxes remained in the field a minimum of 16 days, low temperatures during the early part of the release period slowed pupal development and delayed emergence. Consequently, some pupae were taken from the field before the moths emerged. Percentage emergence for groups of pupae removed from the field from 27 April to 3 May was 47.1 ± 33.8 ($\bar{x}$ ± SD, N = 10). Environmental conditions were more favorable for pupal development during the remainder of the release period and the percentage emergence increased to 95.0 ± 1.9 ($\bar{x}$ ± SD, N = 36). Overall percentage emergence for the release period was 85.9 ± 1.3 ($\bar{x}$ ± SD, N = 36).

The numbers of released and wild backcross and *H. virescens* males trapped in the release area during the release period in 1993 and the numbers of backcross and *H. virescens* males captured during June, July, and August following the release are indicated in Table 3. The first released moth with the red marking was trapped in the release area 18 April; none were captured after 13 June. The first wild moth was trapped in the release area 26 April. During this period, 28 traps positioned in the release area captured 5,028 released (marked red) moths and 6,502 (3,315 backcross + 3,187 *H. virescens*) wild moths (estimated from the testing of a subsample of trapped males for sterility) with no marking (Table 3). These estimates indicate a 1.0:1.3 released:wild ratio. However, when the 3,315 wild backcross (immigrants from the 1992 release and some possible reproduction from the 1993 release) are combined with the 5,028 released backcross, overall backcross:*H. virecens* ratio was 2.6:1.0 (Table 3). Released and wild backcross captures are combined to show backcross:*H. virescens* ratios indicated in Table 3. Trapping data for the first generation in the release area following the backcross release (14 June to 4 July) show that the overall backcross:*H. virescens* ratio during the release period declined from 2.6:1.0 to 1.0:1.6 (Table 3). Backcross:*H. virescens* ratio further declined in the release area during July and August to 1.0:3.6 and 1.0:4.0, respectively. This declining ratio of backcross moths was consistent with the progressive dilution of backcross moths and their progeny through their movement and dispersion (Laster et al. 1993, Schneider et al. 1989).

The numbers of released and wild backcross males trapped in the control area during the release period in 1993 and the numbers of backcross and *H. virescens* males captured during June, July and August following the release are indicated in Table 4. The first wild moth was trapped in the control area 29 April and the first released moth with the red marking was trapped 17 May. No released moths were captured after 6 June. Estimates from the testing of a subsample of males for sterility showed that wild males trapped during the release period consisted of 1,935 backcross and 4,537 *H. virescens* (Table 4). This indicates that the overwintered population in the control area, where the backcross release was made in 1992, was 29.9% backcross. It is possible that some of these moths may have developed from released and/or overwintered backcross moths. The combined total (2,087) released and wild backcross indicated a 1.0:2.2 backcross:*H. virescens* ratio during the release period (Table 4). This ratio dropped to 1.0:5.2 backcross:*H. virescens* during June following the release and increased to 1.0:4.7 and 1.0:3.4 for July and August, respectively.

The numbers of wild backcross and *H. virescens* males trapped in the Bolivar County release area in 1994 following releases of backcross moths in 1993 are indicated in Table 5. Estimates from the testing of a subsample of males for sterility showed that wild males trapped during the overwintered emergence (1 April to 1 June) were 11.7% sterile. Male sterility dropped to 8.9% at the end of July, resulting in 9.7% for the entire trapping period.

The numbers of wild backcross and *H. virescens* males trapped in the Washington-Sunflower County release area in 1994 following releases of backcross moths in 1992 are indicated in Table 6. Estimates from the testing of a subsample of males showed that 12.4% of wild males trapped during the overwintered emergence (1 April to 1 June) were sterile. Male

TABLE 3. Numbers of Released and Wild Sterile Backcross and *Heliothis virescens* Males Trapped in the Release Area (Bolivar County, MS) During the Release Period and Numbers of Backcross and *H. virescens* Males Trapped in June, July, and August Following the Release Period in 1993.

Date	No. backcross ♂♂ trapped: Released	Wild[a]	Total	No. *H. virescens* ♂♂ Trapped[b]	Ratio backcross:*H. virescens*
4/18-4/25	2	0	2	0	--:--
4/26-5/02	49	0	49	1	49.0:1.0
5/03-5/09	990	179 (7)	1,169	77 (3)	15.2:1.0
5/10-5/16	1,039	599 (59)	1,638	400 (40)	4.1:1.0
5/17-5/23	1,089	1,100 (51)	2,189	937 (44)	2.3:1.0
5/24-5/30	1,091	767 (92)	1,858	938 (112)	2.0:1.0
5/31-6/06	730	594 (93)	1,324	725 (83)	1.8:1.0
6/07-6/13	40	76 (32)	116	109 (47)	1.1:1.0
Total	5,028	3,315(334)	8,345	3,187(663)	2.6:1.0
6/14-6/20	0	455 (137)		683 (205)	1.0:1.5
6/21-6/27	0	529 (90)		1,073 (183)	1.0:2.0
6/28-7/04	0	792 (104)		1,094 (143)	1.0:1.4
Total	0	1,776(331)		2,850 (531)	1.0:1.6
7/05-7/11	0	169 (38)		184 (41)	1.0:1.1
7/12-7/18	0	52 (14)		100 (27)	1.0:1.9
7/19-7/25	0	193 (46)		548 (128)	1.0:2.8
7/26-8/01	0	549 (56)		2,679 (279)	1.0:4.9
Total	0	963(154)		3,511 (475)	1.0:3.6
8/02-8/08	0	848 (55)		3,863 (252)	1.0:4.6
8/09-8/15	0	468 (59)		1,760 (219)	1.0:3.8
8/16-8/22	0	217 (39)		530 (95)	1.0:2.4
8/23-8/30	0	27 (11)		156 (62)	1.0:5.8
Total	0	1,560(164)		6,309(628)	1.0:4.0

[a] Estimated from the testing of a subsample of males for sterility; numbers of males demonstrated to be sterile are given in parentheses.
[b] Estimated from the testing of a subsample of males for sterility; numbers of males demonstrated to be fertile are given in parentheses.

TABLE 4. Numbers of Released and Wild Sterile Backcross and *Heliothis virescens* Males Trapped in the Control Area (Washington and Sunflower Counties, MS) During the Release Period and Numbers of Backcross and *H. virescens* Males Trapped in June, July, and August Following the Release Period in 1993.

	No. backcross ♂♂ trapped				
Date	Released	Wild[a]	Total	No. *H. virescens* ♂♂ Trapped[b]	Ratio backcross:*H. virescens*
4/26-5/02	0	7 (2)	7	7 (2)	1.0:1.0
5/03-5/09	0	120 (12)	120	233 (23)	1.0:1.9
5/10-5/16	0	430 (32)	430	447 (34)	1.0:1.0
5/17-5/23	15	546 (39)	561	1,826 (134)	1.0:3.3
5/24-5/30	66	359 (26)	425	1,202 (89)	1.0:2.8
5/31-6/06	71	431 (44)	502	766 (78)	1.0:1.5
6/07-6/13	0	42 (6)	42	56 (8)	1.0:1.3
Total	152	1,935(161)	2,087	4,537(368)	1.1:2.2
6/14-6/20	0	23 (6)		219 (59)	1.0:9.5
6/21-6/27	0	32 (13)		95 (39)	1.0:3.0
6/28-7/04	0	50 (8)		247 (38)	1.0:4.9
Total	0	105 (27)		551 (136)	1.0:5.2
7/05-7/18	0	68 (8)		111 (13)	1.0:1.6
7/19-7/25	0	16 (4)		107 (27)	1.0:6.7
7/26-8/01	0	233 (34)		1,266 (178)	1.0:5.4
Total	0	317 (46)		1,484 (218)	1.0:4.7
8/02-8/08	0	742 (30)		944 (239)	1.0:1.3
8/09-8/15	0	209 (28)		1,878 (255)	1.0:9.0
8/16-8/22	0	24 (7)		146 (43)	1.0:6.1
8/23-8/30	0	53 (17)		480 (158)	1.0:9.1
Total	0	1,028 (82)		3,448 (695)	1.0:3.4

[a] Estimated from the testing of a subsample of males for sterility; numbers of males demonstrated to be sterile are given in parentheses.
[b] Estimated from the testing of a subsample of males for sterility; numbers of males demonstrated to be fertile are given in parentheses.

sterility dropped to 8.5% for this area during June and July. Overall male sterility for the entire emergence period was 9.2%. Sterility in overwintered males across both release areas in 1994 was 12.1%.

TABLE 5. Numbers of Sterile Backcross and *H. virescens* Males Trapped in the Bolivar County Release Area in 1994 Following Release of Backcross Moths in 1993.

Date	Total moths trapped	No. *H. viescens* males[a]	No. backcross males[b]	Percentage male sterility
4/01-4/07	1[c]	1 (1)	0	0.0
4/08-4/14	4	2 (1)	2 (1)	50.0
4/15-4/21	18	18 (6)	0	0.0
4/22-4/28	142	117 (56)	25 (12)	8.5
4/29-5/04	278	252 (106)	26 (11)	9.4
5/05-5/11	759	662 (270)	97 (35)	12.8
5/12-5/18	654	572 (195)	82 (28)	12.5
5/19-5/25	475	430 (104)	45 (11)	9.5
5/26-6/01	109	101 (9)	8 (3)	7.3
Total[a]	2,440	2,155	285	11.7
6/02-6/08	493	467 (160)	26 (9)	5.3
6/09-6/15	1,283	1,162 (173)	121 (18)	9.4
6/16-6/22	1,358	1,188 (139)	170 (19)	12.5
6/23-6/29	169	156 (49)	13 (4)	7.7
6/30-7/06	73	73 (11)	0	100.0
7/07-7/13	423	392 (127)	31 (10)	7.3
7/14-7/20	825	752 (237)	73 (23)	8.8
7/21-7/31	1,784	1,647 (228)	137 (19)	7.7
Total	6,408	5,837	571	8.9
Combined Total	8,848	7,992	856	9.7

[a] Estimated from the testing of a subsample of males for sterility; numbers of males demonstrated to be fertile are given in parentheses.
[b] Estimated from the testing of a subsample of males for sterility; numbers of males demonstrated to be sterile are given in parentheses.
[c] Overwintered emergence; some early F_1 generation males may be included.

Previous models (Laster et al. 1976, Makela and Huettel 1979, Lewis and Parker 1983, Roush and Schneider 1985) indicate that the population density of tobacco budworm + backcross in an area of backcross release will be higher in the first generation following release than if the release had not been made. However, this was not true in the St. Croix study because the field increase for the first generation following initiation of the release was lower than before the release was made (Proshold et al. 1982, Proshold and Smith 1990). Also, data for the 1992 release showed no increase in the field population following the release because the population densities were similar in both the release and control areas.

Because of movement over the entire release area, a reduction in *H. virescens* populations due to the released insects was not shown. High overwintered survival of backcross insects with the sterile male trait could be a major contributing factor to successful suppression of *H. virescens* populations with area-wide releases of backcross insects. This factor combined with

an increased release ratio and successive releases for two or three years should effect a measurable reduction in the *H. virescens* populations. Successful reduction of *H. virescens* populations below damaging levels with this program would have several advantages over present insecticide control practices: (1) it would prevent releasing thousands of pounds of insecticides into the environment that contaminate streams and ground water; (2) it would circumvent the insecticide resistance problem with *H. virescens*; (3) releases would be directed at the overwintered emergence before crops are susceptible to injury; (4) released insects would not cause populations to be higher than normal during the first generation on cotton; (5) the release program would not interfere with normal production practices, nor wood these practices adversely affect the release program; and (6) because the release covers the entire area, *H. virescens* populations are suppressed on all crops simultaneously.

TABLE 6. Numbers of Sterile Backcross and *H. virescens* Males Trapped in the Washington-Sunflower County Release Area in 1994 Following Releases of Backcross Moths in 1992.

Date	Total moths trapped	No. *H. viescens* males[a]	No. backcross males[b]	Percentage male sterility
4/01-4/07	1[c]	1	0	0.0
4/08-4/14	84	8 (2)	0	0.0
4/15-4/21	89	57 (23)	32 (13)	36.0
4/22-4/28	201	167 (79)	34 (16)	16.9
4/29-5/04	378	323 (172)	55 (29)	14.6
5/05-5/11	811	719 (241)	92 (31)	11.3
5/12-5/18	1,055	953 (214)	102 (23)	9.7
5/19-5/25	414	359 (98)	55 (15)	13.2
5/26-6/01	56	51 (29)	5 (3)	8.9
Total[a]	3,013	2,638	375	12.4
6/02-6/08	310	277 (118)	33 (14)	10.6
6/09-6/15	964	839 (148)	125 (22)	13.0
6/16-6/22	831	777 (115)	54 (8)	6.5
6/23-6/29	252	198 (51)	54 (14)	2.4
6/30-7/06	76	76 (6)	0	0.0
7/07-7/13	1,707	1,579 (258)	128 (21)	7.5
7/14-7/20	5,048	4,707 (290)	341 (21)	6.8
7/21-7/31	3,934	3,559 (275)	375 (29)	9.5
Total	13,122	12,012	1,110	8.5
Combined Total	16,135	14,650	1,485	9.2

[a] Estimated from the testing of a subsample of males for sterility; numbers of males demonstrated to be fertile are given in parentheses.

[b] Estimated from the testing of a subsample of males for sterility; numbers of males demonstrated to be sterile are given in parentheses.

[c] Overwintered emergence; some early F_1 generation males may be included.

A successful sterile backcross release program would require an organized effort with backcross insects released over a large area. There are presently no rearing facilities sufficiently large to support a massive release program. Also, the program is not likely to be

adopted as long as insecticides effectively control *H. virescens* and their use continues. However, if a program of this nature should be needed in the future because of insecticide resistance or environmental regulations, the technology for implementation is available.

LITERATURE CITED

Elzen, G. W., B. R. Leonard, J. B. Graves, E. Burris, and S. Micinski. 1992. Resistant to pyrethroid, carbamate, and organophosphate insecticides in field populations of tobacco budworm. J. Econ. Entomol. 85: 2064-2072.

Hardee, D. D., and M. L. Laster. 1996. Current status of backcross sterility in *Heliothis virescens* (F.). Southw. Entomol. 21 85-100.

Harris, F. A., J. B. Graves, S. J. Nemec, S. B. Vinson, and D. A. Wolfenbarger. 1972. Insecticide Resistance. Southern Coop. Serv. Bull. 169: 17-27.

Hendricks, D. E. 1970. Oil soluble dye in larval diet for tagging moths, eggs, and spermatophores of tobacco budworms. J. Econ. Entomol. 63: 1019-1020.

Hollingsworth, J. P., A. W. Hartstack, D. R. Buck, and D. E. Hendricks. 1978. Electric and none-electric moth traps baited with synthetic sex pheromone of the tobacco budworm. USDA, ARS-S-173. 13 pp.

King, E. G., G. G. Hartley, D. F. Martin, and M. L. Laster. 1985. Large scale rearing of the tobacco budworm (Lepidoptera: Noctuidae). J. Econ. Entomol. 78: 1166-1172.

Knipling, E. F. 1960. Use of insects for their own destruction. J. Econ. Entomol. 53: 415-420.

Laster, M. L. 1972. Interspecific hybridization of *Heliothis virescens* and *H. subflexa*. Environ. Entomol. 1: 682-687.

Laster, M. L., E. G. King, and R. E. Furr. 1988. Interspecific hybridization of *Heliothis subflexa* and *H. virescens* (Lepidoptera: Noctuidae) from Argentina. Environ. Entomol. 17: 1016-1018.

Laster, M. L., D. F. Martin, and D. W. Parvin, Jr. 1976. Potential for tobacco budworm suppression by genetic sterilization. MAFES Tech. Bull. 82: 1-9.

Laster, M. L., D. D. Hardee, and J. C. Schneider. 1993. A pilot program to control the tobacco budworm, *Heliothis virescens*, by releasing sterile backcross insects into the natural population, pp. 874-876. *In* Proc. Beltwide Cotton Prod. Res. Conf., New Orleans, LA, January 10-14, 1993. National Cotton Council of America, Memphis TN.

Lewis, R. A., and M. W. Parker. 1983. Short run aspects of a genetic control program for *Heliothis virescens* (Lepidoptera: Noctuidae). Ecol. Med. 19: 213-220.

Makela, M. E., and M. D. Huettel. 1979. Model for genetic control of *Heliothis virescens*. Theor. App. Genet. 54: 225-233.

Proshold, F. I., M. L. Laster, D. F. Martin, and E. G. King. 1982. The potential for hybrid sterility in *Heliothis* management, pp. 329-339. *In* Proceedings of the International Workshop on *Heliothis* Management, Pantancheru, A. P. India. 1981.

Proshold, F. I., and J. W. Smith. 1990. *Heliothis virescens* (Lepidoptera: Noctuidae): Analysis of population responses following introduction of backcrossed insects against native populations on St. Croix, U.S. Virgin Islands. J. Econ. Entomol. 83: 405-411.

Ramaswamy, S. B., R. T. Roush, and W. F. Kitten. 1985. Release and recapture probabilities of laboratory-adapted and wild-type *Heliothis virescens* (F.) (Lepidoptera: Noctuidae) in pheromone baited traps. J. Entomol. Sci. 20: 460-464.

Roush, R. T., and J. C. Schneider. 1985. An analytical model for genetic control of *Heliothis virescens* incorporating the effects of sterile males. Theor. App. Genet. 71: 472-477.

Schneider, J. C., R. T. Roush, W. F. Kitten, and M. L. Laster. 1989. Movement of *Heliothis virescens* (Lepidoptera: Noctuidae) in Mississippi in the spring: Implications for area-wide management. Environ. Entomol. 18: 438-446.

IVERMECTIN[1] POUR-ON FOR CATTLE: EFFECTS ON SOME DUNG-INHABITING INSECTS

G. T. Fincher
Food Animal Protection Research Laboratory, USDA-ARS, 2881 F&B Road
College Station, Texas 77845

ABSTRACT

Dung from two steers treated with the recommended therapeutic dose of a pour-on formulation of ivermectin was bioassayed with the horn fly *Haematobia irritans* (L.) and the introduced dung beetles *Euoniticellus intermedius* (Reiche) and *Onthophagus gazella* (F.). Emergence of adult horn flies reared on dung from the treated steers was significantly reduced for 5-6 wk compared with emergence of flies reared on dung from an untreated steer. There were no effects on mean numbers of brood balls produced by either dung beetle species. However, emergence of adult *E. intermedius* and *O. gazella* from brood balls made with dung from treated steers was reduced for 1-2 wk and 2-3 wk, respectively.

INTRODUCTION

Ivermectin, a member of the avermectin family of drugs derived from an actinomycete isolated from soil in Japan, is a highly effective, broad-spectrum compound that kills certain parasitic roundworms and ectoparasites such as mites, lice, and certain insect pests of livestock (Miller et al. 1981, Benz 1985, Drummond 1985). Cattle do not metabolize ivermectin and most of it is excreted unaltered in the feces, regardless of the route of administration (Campbell et al. 1983). In cattle, it is effective against larvae of economically important dung-breeding flies (Meyer et al. 1980, Miller et al. 1981, Schmidt 1983). Because it is excreted in the dung of livestock, the possible impact of ivermectin on dung-inhabiting, nontarget insects such as dung beetles causes concern, both from a conservation standpoint and from a knowledge of the beneficial activity of these insects (Fincher 1981, 1991, 1992; Sommer et al. 1993). Ivermectin in dung may also cause undesirable environmental consequences by interfering with dung-inhabiting insects involved in the degradation of livestock dung on pasture (Wall and Strong 1987, Madsen et al. 1990). Concern about the impact of avermectins on dung-inhabiting insects and the potential effects on the degradation of dung and nutrient recycling on pasture has been expressed by scientists in several countries where avermectins are used to control livestock pests (Herd et al. 1993). The purpose of this study was to determine the effects of dung from cattle treated with a pour-on formulation of ivermectin on the reproduction of the horn fly *Haematobia irritans* (L.) and two introduced species of dung-burying beetles, *Euoniticellus intermedius* (Reiche) and *Onthophagus gazella* (F.).

[1]Mention of a trade name or product does not imply an endorsement or recommendation by USDA.

MATERIALS AND METHODS

Two steers (343 and 544 kg) were treated with an ivermectin pour-on formulation at the recommended therapeutic dose of 500 μg/kg body weight . A third untreated steer (479 kg) provided control dung. The ivermectin (Ivomec© Pour-On for Cattle; MSD AGVET, Division of Merck & Company, Rahway, N.J.) was purchased locally from a commercial source. The steers were housed indoors in separate concrete-floored pens and fed alfalfa cubes and water. None of the steers had previously been treated with anthelmintics or insecticides. Dung was collected every 2 h on the scheduled day of collection and placed in separate 20-liter plastic buckets until a 6,000 g sample was available from each steer. The dung collected from each steer was throughly mixed in separate containers before use. Dung collections were made on the day of treatment, before the pour-on was applied, and weekly thereafter for 8 wk.

For the horn fly bioassay, mixed dung (100 g) from each steer was formed into a pat and placed on the surface of moist sandy loam soil (4-5 cm deep) in a round plastic container (12 cm diameter x 14 cm deep). Before the dung pat was added, the soil surface in each container was covered with a thin layer of peat moss to simulate ground cover in a pasture environment. Fifty horn fly eggs (≤4 h old) were placed on the top surface of each pat. Each container was then covered with a screen lid that was replaced after 6 d with a cone trap to collect adult flies. The flies were allowed to die in the traps before they were counted. Each treatment was replicated five times weekly for 8 wk and all containers were maintained between 30 and 34 C° and 50-80% RH under a photoperiod of 14:10 (L:D).

For the dung beetle bioassay, two pairs of *E. intermedius* and *O. gazella* were allowed to construct brood balls with dung from each steer. Plastic pails (21.3 cm diameter, 16 cm deep) were filled to ≈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 containers. Two male-female pairs of either *E. intermedius* or *O. gazella* (7-14 d old) were placed in each pail, and the pail was covered with a screen lid. Each treatment was replicated six times weekly for 7 wk and all pails were maintained under the same conditions as the horn fly bioassay test. 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 3-5 days to prevent soil desiccation and to encourage beetle progeny to emerge as soon as possible. Beginning 21 and 26 days after *E. intermedius* and *O. gazella* parents were first confined, respectively, fresh dung (30-40 g) from the control steer was again placed in the cup of each pail to attract and capture emerging progeny. The numbers of captured progeny were recorded daily and dung in the cups was replaced as needed. 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.

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). Those replicates in which the two pairs of parent beetles constructed less than five brood balls were not included in the analysis.

RESULTS AND DISCUSSION

Emergence of horn flies reared on dung from treated steers 1 and 2 was significantly less ($P \leq 0.05$) for 6 and 5 wk, respectively, compared with emergence of flies reared on dung from the untreated steer (Table 1). However, even though the dose level (500 μg/kg) given to the steers by the topical treatment in this study was 2.5 times greater, dung from cattle injected subcutaneously with the recommended therapeutic dose (200 μg/kg) of ivermectin reduced adult horn fly emergence for 8 wk (Fincher 1992). This trend of prolonged toxicity on cyclorrhaphan larvae after the subcutaneous injection treatment, compared with the pour-on treatment, was reported by Sommer et al. (1992) who concluded that, although the dose was higher, ivermectin treatment by pour-on did not result in prolonged elimination of dung that is toxic to dung-breeding insects. These authors also reported that the approximate half-lives for ivermectin in dung were 2.5 and 3.0 days for treatment by pour-on and subcutaneous injection, respectively, and that most of the higher dosage of ivermectin was excreted during the first five days after treatment due to a more rapid distribution to intestinal contents. Later fecal concentrations after the pour-on treatment were lower than those found after subcutaneous injection. (Sommer et al. 1992).

TABLE 1. Mean Numbers of Adult Horn Flies Reared from 50 Eggs Deposited on 100 g of Dung from Cattle Treated with Ivermectin Pour-On.

Wk post-treatment	Untreated steer	Treated steer # 1		Treated steer # 2	
	Mean[a]	Mean[a]	% Reduction	Mean[a]	% Reduction
0	38.6[a]	36.4[a]	--	39.6[a]	--
1	27.8[a]	0.0[b]	100.0	0.0[b]	100.0
2	28.0[a]	0.0[b]	100.0	0.0[b]	100.0
3	25.2[a]	0.0[b]	100.0	0.0[b]	100.0
4	23.6[a]	0.2[b]	99.2	1.0[b]	95.8
5	33.0[a]	15.4[b]	53.3	20.6[b]	37.6
6	34.6[a]	17.4[b]	49.7	34.0[a]	1.8
7	41.2[a]	37.6[a]	8.7	38.4[a]	6.8
8	35.2[a]	35.2[a]	0.0	33.4[a]	5.1

[a]Means within each row followed by the same letter are not significantly different at the $P = 0.05$ level.

There were no significant differences ($P \leq 0.05$) in mean numbers of brood balls constructed from dung of the treated steers when compared with mean numbers of brood balls made from untreated dung (Tables 2, 3). The number of brood balls constructed by a pair of dung beetles varies considerably (Blume and Aga 1975, Fincher 1991). Although young and apparently healthy male and female beetles are paired, sometimes very few or no brood balls are constructed because of death, injury, or unexplained causes. For this reason, all replicates in which the two pairs of parent beetles constructed less than five brood balls were not used in the analysis of data. There was only one replicate rejected from the *E. intermedius* bioassay (from treated steer no. 2); however, nine replicates were

discarded in the *O. gazella* bioassay (one from treated steer no 1, two from treated steer no. 2, and six from the untreated steer).

TABLE 2. Mean Numbers of Brood Balls Constructed by Two Pairs of *Euoniticellus intermedius* from Dung of Cattle Treated with Ivermectin Pour-on, and Percentage Adult Eclosion.

Wk post-treatment	Untreated steer			Treated steer # 1			Treated steer # 2		
	Mean[a]	Range	% Eclosion[b]	Mean[a]	Range	% Eclosion[b]	Mean[a]	Range	% Eclosion[b]
0	21.3	13-32	76.6[a]	19.7	8-27	87.3[a]	19.3	5-26	74.1[a]
1	19.3	6-28	92.2[a]	27.0	19-30	0.0[b]	23.8	13-35	0.0[b]
2	27.2	15-38	75.5[a]	20.7	16-27	3.2[b]	26.2	22-29	65.0[a]
3	34.8	28-41	77.0[a]	38.8	32-48	74.7[a]	33.3	21-55	65.5[a]
4	36.2	31-44	75.1[a]	30.5	27-37	85.2[a]	33.7	29-39	69.3[a]
5	35.5	24-45	79.8[a]	32.7	25-37	66.3[a]	28.7	24-33	79.7[a]
6	23.5	17-28	81.6[a]	29.3	22-33	75.0[a]	23.2	12-33	69.8[a]
7	22.8	12-37	76.6[a]	27.3	24-31	82.3[a]	27.0	24-32	80.2[a]

[a]Means within each row under the mean columns are not significantly different at the $P = 0.05$ level.
[b]Values within each row followed by the same letter are not significantly different at the $P = 0.05$ level.

TABLE 3. Mean Numbers of Brood Balls Constructed by Two Pairs of *Onthophagus gazella* from Dung of Cattle Treated with Ivermectin Pour-on, and Percentage Adult Eclosion.

Wk post-treatment	Untreated steer			Treated steer # 1			Treated steer # 2		
	Mean[a]	Range	% Eclosion[b]	Mean[a]	Range	% Eclosion[b]	Mean[a]	Range	% Eclosion[b]
0	30.7	17-45	75.5[a]	23.3	8-53	55.7[a]	21.2	15-25	70.8[a]
1	39.7	27-48	76.5[a]	29.8	8-45	0.0[b]	31.0	24-42	0.006[b]
2	35.2	26-46	68.2[a]	27.2	19-41	0.0[b]	26.2	15-33	8.3[b]
3	29.0	18-38	69.8[a]	39.5	24-48	32.9[b]	31.2	24-40	76.5[a]
4	22.2	7-33	87.4[a]	31.0	15-41	78.0[a]	27.2	14-46	89.0[a]
5	43.5	29-54	77.8[a]	31.8	5-47	79.9[a]	27.7	13-52	80.7[a]
6	33.5	5-58	76.1[a]	36.3	7-46	80.3[a]	20.0	5-51	87.5[a]
7	40.8	24-55	87.8[a]	31.8	15-49	76.4[a]	37.5	20-48	77.3[a]

[a]Means within each row under the mean columns are not significantly different at the $P = 0.05$ level.
[b]Values within each row followed by the same letter are not significantly different at the $P = 0.05$ level.

There were significant differences ($P \leq 0.05$) in percentage adult eclosion of *E. intermedius* for 1-2 wk and of *O. gazella* for 2-3 wk when reared on dung from the treated steers (Tables 2, 3). For comparsion, the lower dose level (200 μg/kg) of the ivermectin injectable formulation caused a reduction in percentage eclosion of *E. intermedius* and *O. gazella* for 1 and 2 wk, respectively (Fincher 1992). Similar results from bioassays of dung from cattle treated with the injectable formulation of ivermectin have been reported for *O. binodis* Thunberg and *E. fulvus* (Goeze) (Ridsdill-Smith 1988, Lumaret et al. 1993).

In a previous experiment (Fincher 1992), two steers (292 and 383 kg) on the same diet as that used in the present study were treated with the injectable formulation of ivermectin and their dung was pooled and throughly mixed before it was bioassayed. Therefore, no differences in toxicity of dung from the two treated animals could be determined in that bioassay. In the present study, dung from each of the treated steers was bioassayed separately. Dung from treated steer no. 1 (343 kg) reduced the number of adult horn flies for 6 wk compared with dung from treated steer no. 2 (544 kg) which reduced horn fly emergence for only 5 wk. Dung from treated steer no. 1 also reduced the number of adult *E. intermedius* and *O. gazella* by 2 and 3 wk, respectively; whereas, dung from treated steer no. 2 reduced adult *E. intermedius* and *O. gazella* emergence by 1 and 2 wk, respectively. The apparent additional toxic persistence of one week in the dung of the smaller animal, weighing 201 kg less than the larger steer, may have been caused by physiological differences between the two animals, which varied the rate of ivermectin excretion.

The concentrations of excreted parasiticides in livestock dung over time are dependent on the mode of admininstration, especially if a sustained-release bolus is used that slowly releases the active compound over several months. This type of delivery system prolongs the release of toxic drug concentrations in livestock dung compared with the more conventional delivery methods such as injections, drenches, or pour-ons. Insecticides used for controlling horn flies and the system of delivery should be as compatible as possible with beneficial dung-inhabiting insects; otherwise, environmental problems could develop on pastures. If populations of beneficial dung-inhabiting insects are greatly reduced, dried cattle dung could cause a problem by fouling and smothering grasses, thereby reducing the effective grazing area of pastures.

ACKNOWLEDGMENT

I gratefully acknowledge Alton Aga, Eddie de la Rosa and James Smith for their technical assistance on this project.

LITERATURE CITED

Benz, G. W. 1985. Animal health applications of ivermectin. Southwest. Entomol. 7: 2-10 (suppl.).

Blume, R. R., and A. Aga. 1975. *Onthophagus gazella*: Mass rearing and laboratory biology. Environ. Entomol. 4: 735-736.

Campbell, W. C., M. H. Fisher, E. O. Stapley, G. Albers-Schonberg, and T. A. Jacob. 1983. Ivermectin: a potent new antiparasitic agent. Science 221: 823-828.

CoStat Statistical Software. 1986. User's manual, pp. 79-110. CoHort Software, Berkeley, Calif.

Drummond, R. O. 1985. Effectiveness of ivermectin for control of arthropod pests of livestock. Southwest. Entomol. 7: 34-42 (suppl.).

Fincher, G. T. 1981. The potential value of dung beetles in pasture ecosystems. J. Ga. Entomol. Soc. 16 (suppl. No. 1): 316-333.

Fincher, G. T. 1991. Sustained release bolus for horn fly (Diptera: Muscidae) control: Effects of methoprene and diflubenzuron on some nontarget species. Environ. Entomol. 20: 77-82.

Fincher, G. T. 1992. Injectable ivermectin for cattle: Effects on some dung-inhabiting insects. Environ. Entomol. 21: 871-876.

Herd, R. P., L. Strong, and K. Wardhaugh. 1993. Environmental impact of avermectin usage in livestock. Vet. Parasitol. 48: 1-344.

Lumaret, J. P., E. Galante, C. Lumbreras, J. Mena, M. Bertrand, J. L. Bernal, J. F. Cooper, N. Kadiri, and D. Crowe. 1993. Field effects of ivermectin residues on dung beetles. J. Appl. Ecol. 30: 428-436.

Madsen, M. B., Overgaard Nielsen, P. Holter, O. C. Pedersen, J. Brochner Jespersen, K. M. Vagn Jensen, P. Nansen, and J. Gronvold. 1990. Treating cattle with ivermectin: Effects on the fauna and decomposition of dung pats. J. Appl. Ecol. 27: 1-15.

Meyer, J. A., J. S. Simco, and J. L. Lancaster, Jr. 1980. Control of face fly larval development with ivermectin, MK-933. Southwest. Entomol. 5: 207-209.

Miller, J. A., S. E. Kunz, D. D. Oehler, and R. W. Miller. 1981. Larvicidal activity of Merck MK-933, an avermectin against the horn fly, stable fly, face fly, and house fly. J. Econ. Entomol. 74: 608-611.

Ridsdill-Smith, T. J. 1988. Survival and reproduction of *Musca vetustissima* Walker (Diptera: Muscidae) and a scarabaeine dung beetle in dung of cattle treated with avermectin B1. J. Aust. Ent. Soc. 27: 175-178.

Schmidt, C. D. 1983. Activity of an avermectin against selected insects in aging manure. Environ. Entomol. 12: 455-457.

Sommer, C., B. Steffansen, B. Overgaard Nielsen, J. Gronvold, K. M. Vagne Jensen, J. Brochner Jespersen, J. Springborg, and P. Nansen. 1992. Ivermectin excreted in cattle dung after subcutaneous injection or pour-on treatment: Concentrations and impact on dung fauna. Bull. Entomol. Res. 82: 257-264.

Sommer, C., J. Gronvold, P. Holter, and P. Nansen. 1993. Effects of ivermectin on two afrotropical dung beetles, *Onthophagus gazella* and *Diastellopalpus quinquedens* (Coleoptera: Scarabaeidae). Vet. Parasitol. 48: 171-179.

Wall, R., and L. Strong. 1987. Environmental consequences of treating cattle with the antiparasitic drug ivermectin. Nature 327: 418-421.

COMPARATIVE SUSCEPTIBILITY OF AFRICANIZED HONEY BEES[1] FROM SOUTH TEXAS TO INFESTATION BY *ACARAPIS WOODI*[2]

Robert G. Danka and José D. Villa

Honey Bee Breeding, Genetics and Physiology Laboratory
Agricultural Research Service, U.S. Department of Agriculture
1157 Ben Hur Road, Baton Rouge, LA 70820

ABSTRACT

The proportion of Africanized honey bees (*Apis mellifera scutellata* Lepeletier hybridized with European subspecies) that became infested with honey bee tracheal mites (*Acarapis woodi* [Rennie]), the number of female mites per infested bee, and the number of immature mites produced per each test bee were more similar to those of resistant European bees than susceptible European bees when groups of young bees were exposed to mites in infested colonies. Mean mite reproduction in the three bee types did not differ. Thus the mite resistance of Africanized bees was founded on a disruption of the migratory phase but not the reproductive phase of the mite life cycle, as has been found previously when resistant bees were compared with susceptible bees. Although Africanized bees from the southern United States somewhat resist infestation by *A. woodi*, this protection does not exceed that of commercially available European stocks.

INTRODUCTION

Africanized honey bees (*Apis mellifera scutellata* Lepeletier hybridized with European subspecies) now are established in the United States in southern portions of Texas, New Mexico, Arizona and California. The range of Africanized bees continues to expand, especially in the far western areas, and these bees are expected to pose apicultural and public health problems for at least the near future.

Acarapis woodi (Rennie), the honey bee tracheal mite, is an endoparasite that causes weakening and mortality of severely infested bee colonies (Eischen et al. 1989, Otis and Scott-Dupree 1992). Since infestation first was detected in the United States in 1984, it has become apparent that problems in North America are most acute in northern areas where winters are protracted (Otis 1990). Beginning in 1987 (Gary and Page 1987), several types of European-stock bees comparatively resistant to tracheal mite infestation have been identified in the United States and Canada (e.g., Clark et al. 1990, Szabo et al. 1991, Danka et al. 1995, Lin et al. 1996).

The effects of tracheal mites on Africanized bees are largely unknown. Africanized bees infested with the mite have been reported from Brazil (Lenhart et al. 1974) and Costa Rica (Otis et al. 1988). This bee type thus is not immune to parasitism, but mite associated problems seem to be uncommon in the Neotropics. We assessed the susceptibility of southern U.S. Africanized bees to infestation by tracheal mites by directly comparing several features of infestation in

[1]Hymenoptera: Apidae
[2]Acari: Tarsonemidae

Africanized bees with those in two types of European bees: one stock known to be resistant to mite infestation, and one known to be susceptible. Adult female *A. woodi* leave the tracheae in which they developed and migrate into the tracheae of young adult bees to reproduce; female progeny of these foundress mites reach adulthood beginning about 11-14 days after host bees are infested (Bailey and Ball 1991, Pettis and Wilson 1996). Based on this biology, we measured several parameters to evaluate potential differences in infestation between bee types during the migratory and reproductive phases of the life cycle of the parasite.

MATERIALS AND METHODS

The relative susceptibilities of the three bee types was assayed during February-March 1995 by exposing uninfested young adult worker bees from test colonies to tracheal mites in infested inoculation colonies, following procedures similar to those of Gary and Page (1987). Africanized colonies were derived from swarms captured in the Rio Grande Valley of Texas and maintained in two isolated apiaries near Rio Grande City, Texas. Ten test colonies were selected from this pool after morphometric multivariate analyses (Rinderer et al. 1993) indicated a high probability of Africanization [P(A)]: all test colonies had $P(A) > 0.95$, and five had $P(A) > 0.99$. Resistant European colonies were of a stock (Buckfast) imported into the United States from the United Kingdom in 1990. Susceptible European colonies were of a stock selected in Louisiana beginning in 1990. These two stocks have been maintained and homogenized by closed breeding. They have shown differential susceptibilities to tracheal mite infestation in field tests (Danka et al. 1995) and in short term evaluations using the bioassay employed here (Danka and Villa 1996). Four colonies of each of the two European bee types were used.

Combs with sealed worker brood were transported either in fiberglass windowscreen bags (Africanized bees from Rio Grande City) or inside holding colonies (resistant and susceptible bees from Baton Rouge, LA) to the USDA-ARS Honey Bee Research Unit at Weslaco, Texas. All adult bees were removed and the combs were held, grouped by colony source, in screen bags inside incubators at 35°C and 50-80% RH.

Newly emerged (0-24 h old) bees were identified as to colony source with coded single or double enamel paint marks (about 1 mm diameter) on abdominal tergites IV or V. Fifty-bee cohorts from each test colony were placed into the broodnests of four inoculation colonies having 43, 48, 68 and 83% of adult bees infested with *A. woodi*. After 10-11 days, marked bees were retrieved into flasks which were kept on crushed ice and filled with CO_2 to immobilize bees and mites; samples later were stored frozen. The numbers of foundress mites and immature mites (progeny) in each trachea of each bee were determined by excising the prothoracic tracheae (spiracle to first bifurcation) from thawed bees and examining them at 30-60X with a dissecting microscope. Male mites occasionally mature in bees as young as 11 days old (Pettis and Wilson 1996); the few males observed thus were classified as progeny. Female mites rarely mature this soon, but if they occurred in our samples this would have led to slight overestimation of foundress populations and underestimation of reproductive rates. Of 72 samples from individual test colonies, 35 were based on 40 bees, 18 had 30-39 bees, 17 had 20-29 bees, and two had 14-17 bees.

Four mite infestation parameters were compared among the three bee types by analysis of variance. Parasitological terminology follows that of Margolis et al. (1982). Prevalence is the percentage of infested bees within a cohort of test bees. Foundress intensity is the number of foundress mites parasitizing individual infested bees Mean fecundity is the average number of offspring produced per foundress mite in each infested bee. Progeny abundance is the number of first generation mites per bee for all bees in a cohort; this measure indicates the immediate future mite threat to a colony. The experiment was analyzed as a split plot design with bee type tested as the whole plot (using colony within bee type as the error term) and

inoculation colony as the subplot (using the interaction of inoculation colony × colony within bee type as the error term). Means of bee type responses within inoculation colonies were separated using least significant differences calculated with weighted error terms that used both whole plot and subplot errors (Cochran and Cox 1957).

RESULTS AND DISCUSSION

The prevalence, foundress intensity and progeny abundance of *A. woodi*, but not the mean fecundity of the parasite, differed among bee types (Table 1). In three of four inoculation colonies, resistant bees and Africanized bees generally had significantly lower tracheal mite prevalences, foundress intensities and progeny abundances than susceptible bees (Fig. 1). These trends were qualitatively similar but not statistically significant in the fourth inoculation colony. When averaged across all inoculation colonies, Africanized bees had 6% proportionally greater prevalence than resistant bees and 22% proportionally lesser prevalence than susceptible bees. Relative differences in foundress intensity among the bee types closely followed differences in prevalence among bee types. Overall foundress intensity in Africanized bees (1.7 ± 0.1 [$\bar{x}$ ± SEM]) was intermediate between that in resistant bees (1.5 ± 0.1) and that in susceptible bees (2.0 ± 0.1). Progeny abundance in Africanized bees on average was 16% more than in resistant bees and 40% less than in susceptible bees.

The different inoculation colonies used in the four trials influenced the response for each infestation parameter (Table 1). Overall, there was a weak trend for infestations to be greater when prevalences in inoculation colonies were greater. However, infestations in test bees also were affected by other, unmeasured factors within inoculation colonies (e.g., perhaps the age demographics and absolute numbers of resident bees). Statistical interactions of the effects of bee type and inoculation colony occurred for the parameters mean fecundity and progeny abundance. Responses for mean fecundity were variable and difficult to interpret. For progeny abundance, the interaction apparently arose because of disproportionately increasing differences in means at greater inoculation colony prevalences.

Africanized honey bees from south Texas were comparatively resistant to infestation by the honey bee parasite *A. woodi*. These bees were less susceptible to infestation than were bees of a susceptible U.S. stock, but were somewhat more susceptible than bees of a stock known to be resistant from previous studies (Danka et al. 1995, Lin et al. 1996). The measures of progeny

TABLE 1. Results of Analysis of Variance of Effects Influencing Four Parameters of Tracheal Mite Infestation When Africanized, Resistant and Susceptible Honey Bees Were Bioassayed.

Variance source	Prevalence *F*; df; *P*	Foundress intensity *F*; df; *P*	Mean fecundity *F*; df; *P*	Progeny abundance *F*; df; *P*
bee type	6.51; 2, 15; 0.009	7.72; 2, 15; 0.005	2.67; 2, 15; 0.102	10.67; 2, 15; 0.001
inoc. col.	39.30; 3, 45; <0.001	9.39; 3, 45; <0.001	13.13; 3, 45; <0.001	51.51; 3, 45; <0.001
bee type × inoc. col.	2.00; 6, 45; 0.086	0.84; 6, 45; 0.542	2.52; 6, 45; 0.034	4.43; 6, 45; 0.001

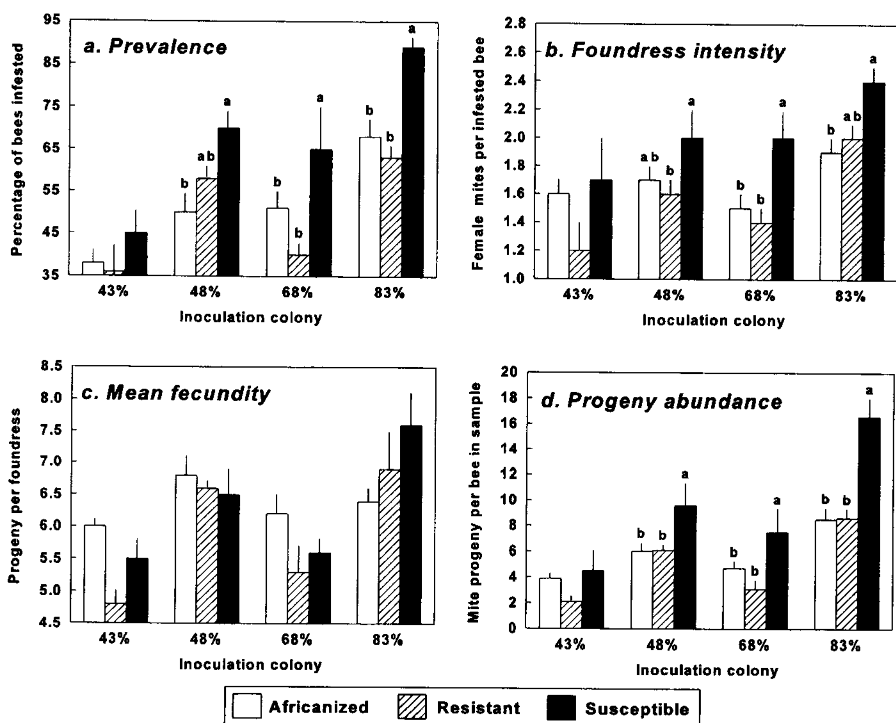


FIG. 1. Means of responses for four parameters of tracheal mite infestation in Africanized, resistant and susceptible honey bees. Error lines at tops of bars indicate one SEM. For each parameter, means within each inoculation colony that are unlettered or share the same letter do not differ at $P > 0.05$.

abundance suggested that comparable initial infestation pressure would be about one-sixth greater of a threat to Africanized colonies than to resistant colonies. However, our study did not assess the proportions of mite progeny that mature and reproduce. The longevity of adult Africanized bees is less than that of European bees in the tropics (Winston 1987) (comparative measurements have not been made in temperate regions). As pointed out by Roubik and Reyes (1984), this factor could further diminish the virulence of infestations in Africanized colonies because mites would have less time to mature.

The general means of resistance of Africanized bees paralleled that found previously for resistant European bees (Danka and Villa 1996). Relative to susceptible bees, tracheal mite migration and establishment (measured as prevalence and foundress intensity, respectively) in resistant bees was suppressed, but reproduction by foundress mites was not. The specific mechanisms regulating this differential mite transfer in bee stocks have yet to be determined.

A. m. scutellata had been under no selection pressure from tracheal mites prior to being exported to Brazil. Indeed, the mites were found only recently in South Africa (Matheson 1996), one of the two African countries of origin of Africanized bees, and still are unknown from Tanzania. Africanized bees, however, apparently have been under some selection pressure from the parasite during much of their existence in the Americas. Tracheal mites were found in Argentina in 1948 (Dyce 1955), Brazil in 1970 (Nascimento 1970), Colombia in 1979

(Menapace and Wilson 1980) and Mexico in 1980 (Wilson and Nunamaker 1982), and were reported from Venezuela in 1982 (Delfinado-Baker and Baker 1982). Most countries of Central America (Belize, Costa Rica, El Salvador, Honduras and Nicaragua) may have become infested with tracheal mites as Africanized bees expanded northward, as these countries were reported to be free of tracheal mites in 1982 (Nixon 1982) but infested in 1993 (Matheson 1993). Otis et al. (1988) suggested that Africanized bees probably vectored the mites into Costa Rica. Thus, Africanized bees may have had sufficient exposure to the mites to result in some resistance being expressed in the bee population expanding northward. However, observations that the parasite is much less of a problem in the relatively low elevation, warmer areas where Africanization is greatest both in the Neotropics (in Brazil for example, Shimanuki et al. 1990) and in the southern United States suggest that selection pressure may have been weak. A second explanation for resistance is that African bees may have been preadapted fortuitously against tracheal mite parasitism by factors such as enhanced grooming ability or cuticular chemistry that renders them less attractive to the mite. A third prospect is that recent hybridization with resistant European bees conferred resistance in the Africanized population we studied. Comparisons of Africanized and European bees existing at the margin of an Africanized zone would address this hypothesis; we did not test local European bees from south Texas.

The practical consequences of Africanized bees being somewhat resistant to tracheal mites are difficult to estimate, but it appears that this parasite will not significantly suppress the feral Africanized bee population of the southern United States. If resistance in Africanized bees is founded on mechanisms currently not widely found in U.S. bees, then the influx of Africanized germplasm may enhance survivorship of feral colonies threatened by tracheal mites. From an apicultural standpoint, the degree of resistance does not exceed that found in European stocks commercially available in North America. Given the notable management difficulties with Africanized bees, these bees should not be considered for use to counter tracheal mites.

ACKNOWLEDGMENT

We thank James Baxter, Arturo Cavazos, Rey Garza (USDA-ARS, Weslaco, TX), Gary Delatte, Lilia de Guzman and James Pursiful (USDA-ARS, Baton Rouge, LA) for assistance in field activities, Chuy Maldonado (USDA-ARS, Weslaco, TX) for morphometric analyses of Africanized colonies, and Anita Collins (USDA-ARS, Weslaco, TX) for use of colonies and facilities. This research was completed in cooperation with the Louisiana Agricultural Experiment Station.

LITERATURE CITED

Bailey, L., and B.V. Ball. 1991. Honey bee pathology, 2nd ed. Academic Press, London.

Clark, K.J., E. Huxter, N.J. Gates, and T.I. Szabo. 1990. Screening breeder honey bee stock for resistance to tracheal mites. Am. Bee J. 130: 800.

Cochran, W.G., and G.M. Cox. 1957. Experimental Designs., 2nd ed. Wiley and Sons, New York.

Danka, R.G., J.D. Villa, T.E. Rinderer, and G.T. Delatte. 1995. Field test of resistance to *Acarapis woodi* (Acari: Tarsonemidae) and of colony production by four stocks of honey bees (Hymenoptera: Apidae). J. Econ. Entomol. 88: 584-591.

Danka, R.G., and J.D. Villa. 1996. Influence of resistant honey bee hosts on the life history of the parasite *Acarapis woodi*. Exp. Appl. Acarol. 20: 313-322.

Delfinado-Baker, M., and E.W. Baker. 1982. Notes on the honey bee mites of the genus *Acarapis* Hirst (Acari: Tarsonemidae). Internat. J. Acarol. 8: 211-226.

Dyce, E.J. 1955. Beekeeping in Peru. Part 2. Am. Bee J. 95: 322-323.

Eischen, F.A., D. Cardoso-Tamez, W.T. Wilson and A. Dietz. 1989. Honey production of honey bee colonies infested with Acarapis woodi (Rennie). Apidologie 20: 1-8.
Gary, N.E., and R.E. Page, Jr. 1987. Phenotypic variation in susceptibility of honey bees, *Apis mellifera*, to infestation by tracheal mites, *Acarapis woodi*. Exp. Appl. Acarol. 3: 291-305.
Lenhart, T., A.S. Michael, and M.D. Levin. 1974. Disease survey of South American Africanized bees. Am. Bee J. 114: 338.
Lin, H., G.W. Otis, M.E. Nasr, and C.D. Scott-Dupree. 1996. Comparative resistance in Buckfast and Canadian stocks of honey bees (*Apis mellifera* L.) to infestation by honey bee tracheal mites (*Acarapis woodi* (Rennie)). Exp. Appl. Acarol. 20: 87-101.
Margolis, L., G.W. Esch, J.C. Holmes, A.M. Curris, and G.A. Schad. 1982. The use of ecological terms in parasitology (Report of an ad hoc committee of the American Society of Parasitologists). J. Parasitol. 68: 131-133.
Matheson, A. 1993. World bee health report. Bee World 74: 176-212.
Matheson, A. 1996. World bee health update 1996. Bee World 77: 45-51.
Menapace, D.M., and W.T. Wilson. 1980. *Acarapis woodi* mites found in honey bees from Colombia, South America. Am. Bee J. 120: 761762, 765.
Nascimento, C.B. 1970. Pesquisa de endo e ecto-parasitos de *Apis mellifera* L, pp. 199-207. *In* 1° Congresso Brasileiro de Apicultura. Florianópolis, SC, Brazil [cited in Apic. Abstr., 1974, 25: 149].
Nixon. M. 1982. Preliminary world maps of honeybee diseases and parasites. Bee World 63: 23-42.
Otis, G.W. 1990. Results of a survey on the economic impact of tracheal mites. Am. Bee J. 130: 28-31.
Otis, G.W., J.B. Bath, and W. Ramirez B. 1988. Have Africanized bees brought honey-bee tracheal mites to Costa Rica?, pp. 541-545. *In* G.R. Needham, R.E. Page, Jr., M. Delfinado-Baker and C. E. Bowman [eds.], Africanized honey bees and bee mites. Ellis Horwood, Chichester, U.K.
Otis, G.W. and C.D. Scott-Dupree. 1992. Effects of *Acarapis woodi* on overwintered colonies of honey bees (Hymenoptera: Apidae) in New York. J. Econ. Entomol. 85: 40-46.
Pettis, J.S. and W.T. Wilson. 1996. Life history of the honey bee tracheal mite (Acari: Tarsonemidae). Ann. Entomol. Soc. Am. 89: 368-374.
Phelan, P.L., A.W. Smith, and G.R. Needham. 1991. Mediation of host selection by cuticular hydrocarbons in the honeybee tracheal mite *Acarapis woodi* (Rennie). J. Chem. Ecol. 17: 463-473.
Rinderer, T.E., S.M. Buco, W.L. Rubink, H.V. Daly, J.A. Stelzer, R.M. Riggio, and F.C. Baptista. 1993. Morphometric identification of Africanized and European honey bees using large reference populations. Apidologie 24: 569-585.
Roubik, D.W. and F. Reyes. 1984. African honey bees have not brought acarine mite infestations to Panama. Am. Bee J. 124: 665-667.
Shimanuki, H., D.A. Knox, and D. De Jong. 1990. Bee diseases, parasites and pests, pp. 283-296. *In* M. Spivak, D.J.C. Fletcher and M.D. Breed [eds.], The "African" honey bee. Westview, Boulder, CO.
Szabo, T.I., L.P. Lefkovitch, and K.J. Clark. 1991. Comparative resistance of honey bees from a closed population to infestation by tracheal mites. Am. Bee J. 131: 643-645.
Wilson, W.T., and R.A. Nunamaker. 1982. The infestation of honey bees in Mexico with *Acarapis woodi*. Am. Bee J. 122: 503-505, 508.
Winston, M.L. 1987. The Biology of the Honey Bee. Harvard University, Cambridge, Mass.

OCCURRENCE OF *LANGURIA MOZARDI*[1] IN SIX *TRIFOLIUM*[2] SPECIES

I. J. Pemberton, G. R. Smith, and J. V. Robinson

Texas A&M University Agricultural Research and Extension Center
Overton, TX 75684

ABSTRACT

In the spring of 1994, plots of arrowleaf clover in East Texas suffering stem damage were found infested with the clover stem borer (*Languria mozardi* Latreille). A field was planted the following fall to determine phenology of oviposition and adult emergence of the clover stem borer and to characterize host plant preference of the insect among six *Trifolium* species: rose, crimson, berseem, ball, arrowleaf, and red clovers. Plants were collected regularly, stems sectioned and examined for insect damage. Degree of infestation, insect developmental stage, and plant growth parameters were recorded at each sampling. First observed on 27 March, oviposition frequency peaked from 19 April to 1 May in all six species of clover. A second egg-laying period in mid-June was observed in red clover, a perennial species. Evidence of successful larval development and pupation was observed in ball, red, crimson, and berseem clovers, and to a lesser extent, in arrowleaf clover. Few larvae and no pupae or adults were found in rose clover stems. Red and berseem clovers supported up to 38 individuals (eggs, larvae, and/or pupae) per plant. There was evidence that pathogenic organisms may have caused root rot and premature death of some plants.

INTRODUCTION

The clover stem borer, *Languria mozardi* Latreille, has been recognized as a clover pest for over 100 years (Lintner 1881). The species has been collected from alfalfa-producing areas across the southern and southwestern states, including Texas (Wildermuth and Gates 1920). Eggs usually are deposited singly in stems, with most oviposition occurring in the second through fourth internodes (Ellsbury and Baker 1989). Larvae feed in the pith, weakening the plant, reducing the quality of the hay, and producing an infection port for pathogens. The host range of this beetle is extensive, encompassing various members of the plant families Asteraceae, Fabaceae, Apiaceae, and Poaceae (Wildermuth and Gates 1920). More recently, the clover stem borer has been reported from *Trifolium vesiculosum* Savi (arrowleaf clover), *T. incarnatum* L. (crimson clover), *T. repens* L. (white clover), *T. pratense* L. (red clover), *T. hybridum* L. (alsike clover), *T. resupinatum* L. (persian clover), *T. striatum* L. (striate clover), *T. nigrescens* Viv. (ball clover), *T. subterraneum* L. (subterranean clover), and *T. alexandrinum* L. (berseem clover) in Mississippi (Ellsbury and Baker 1989; Knight et al. 1976).

---

[1]Order Coleoptera, Family Languriidae.
[2]This manuscript reports reserach conducted by the Texas Agricultural Experiment Station, The Texas A&M University System.

In the spring of 1994, a spaced-plant nursery of arrowleaf clover at Overton, TX, suffered damage by a boring insect. Stems were rotted inside and insect entry/exit holes were present. A larva found inside a stem was identified as a clover stem borer. This study was designed to determine the phenology of oviposition and adult emergence of the clover stem borer and to characterize its host plant preference among several clover species in East Texas.

MATERIALS AND METHODS

Six-week-old seedlings of one perennial and five annual clover species were transplanted to a prepared seed bed near Overton, TX, on 1 November 1994. The species tested were: *T. hirtum* All. 'Overton R18', *T. vesiculosum* 'Yuchi', *T. incarnatum* 'Dixie', *T. nigrescens* 'Common', *T. alexandrinum* 'OVB', and *T. pratense* 'Cherokee'. A total of 840 plants was arranged in 28 rows of 30 plants each. Each row of 30 plants consisted of five plants of six different species. Individuals of each species were grouped together in each row, and placement of species was staggered across rows. Beginning in mid-December, two randomly selected rows of plants (n = 4 to 10 plants per species) were collected every 2 to 4 weeks. Plants were cut off below the crown, brought to the laboratory, and examined for signs of damage by the clover stem borer. Plants were collected weekly after 20 March. If insect oviposition holes were found, stems were cut longitudinally and examined for eggs, larvae, pupae, and adults. Later in the spring when insects began pupating, a 5-cm section of the stem containing the pupa was excised and placed in a plastic petri dish with a piece of moist paper towel to observe adult emergence.

The number of infested plants, insects per plant, insect developmental stage, plant growth stage, and plant dry weight were determined at each sampling date. Growth curves for each plant species were constructed by regressing dry weight data on time. Plant growth stage was determined at each sampling date according to the following designations: rosette, stem elongation, flower bud, early flowering (approximately 10% flowering stems with open florets), full bloom, green seed, and mature seed/senescing plant. Presence of internal discoloration in stems and crowns was also noted.

RESULTS AND DISCUSSION

Evidence of clover stem borer activity was first observed on 27 March 1995 and occurred at all subsequent collection dates through 26 June. Oviposition sites (tiny, pin-sized holes) were found on stems. When cut open, a pale yellow egg was usually found inside. Eggs had begun hatching into pale yellow larvae by early April. We identified these as larvae of *L. mozardi* (Peterson 1960). Adult insects were occasionally found on foliage of clover plants collected for examination.

The clover stem borer deposited eggs in all six species of clovers in this study (Fig. 1). The number of infested plants in the samples increased greatly between the 10 April and 19 April sampling dates. Between 0 and 40% of all plants were infested on 10 April. Nine days later almost 90% of the plants of five species sampled were infested. Rose clover was only 50% infested at this time. Unintentional grazing of some plants by cattle in mid-May may have caused a decrease in recorded degree of infestation for the May 15 sampling date. However, by late May, almost 100% of the plants (excluding rose clover) were infested by *L. mozardi*.

Oviposition was first observed on 27 March, and its frequency peaked during late April and early May (Fig. 2). Eggs usually were deposited 5 to 15 cm above the base of the plant. Occasionally a second egg was found higher on the same stem. A second period of egg-deposition was observed on red clover in mid-June, soon after second generation

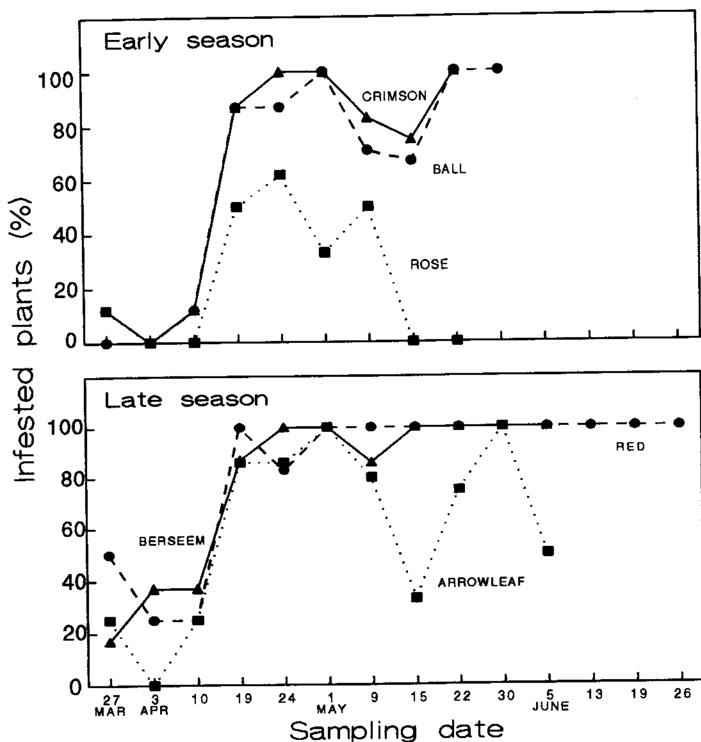


FIG. 1. Percentage plants infested by *Languria mozardi* for early and late season clovers during spring 1995 at Overton, TX.

adults had emerged. The largest numbers of eggs were deposited in arrowleaf clover, on which oviposition continued almost until plants were either mature or dead from virus and root rot disease in late May. Despite the high number of eggs, larval and pupal counts were low in arrowleaf clover. Evidence of successful larval development in the different clovers was indicated by the presence of pupae. Pupation was observed in ball, red, crimson, arrowleaf, and berseem clovers. The few larvae found in rose clover were smaller than those in other clover species, and obviously were not thriving. Some dead larvae were found inside the rose clover stems.

The average numbers of insects per plant differed greatly for the six species of clovers. By early May, when almost all the plants examined were infested, red and berseem clovers supported the largest populations of the borer. As many as 38 individuals (eggs, larvae, and/or pupae) were found per plant. Populations on crimson and ball clovers were slightly smaller, while numbers of insects on rose and arrowleaf clovers were the smallest.

Growth curves (Fig. 2) calculated from 1 March and subsequent sampling dates illustrate maturity differences and periods of peak growth for each species of clover. Rose, crimson, and ball clovers matured earlier than berseem, arrowleaf, and red clovers. Red clover, a perennial, continued new shoot growth development after the last sampling date, but at a much slower rate. The following events are noted on the growth curve: appearance of flower buds (B), 10% of stems flowering (F), and presence of mature seed (M). Red clover exhibited two flushes of flowering designated as 1 or 2 on the figure. Sampling was discontinued for each species of clover as plants senesced and mature seed developed. Most plants exhibited virus disease symptoms by the end of the study. Pupae collected from stems successfully completed development into adults of *L. mozardi* and these specimens were preserved.

Internal discoloration (browning) of stems and crowns was noted during this study. Crimson clover was the only species not exhibiting discoloration. Ball, arrowleaf, rose, berseem, and red clovers exhibited internal stem and/or crown discoloration among 19, 19, 33, 37, and 41%, respectively, of all the plants sampled during the growing season. Crown discoloration was noted before, as well as after, egg deposition began and therefore was not a result of injury caused by oviposition. However, internal stem discoloration originated at the oviposition site and spread distally. Continued browning within the stem was associated with larval feeding and tunnelling. When both types of discoloration occurred in the same plant, a section of healthy stem tissue was initially present between the two areas. As the season progressed and larvae tunnelled downward while feeding, the discolored areas sometimes merged together.

Disease symptoms were especially severe on arrowleaf clover. Leaves and stems of many plants became red, then necrotic. These plants wilted and died prematurely, some without setting seed. Dark lesions were observed on taproots of several uprooted arrowleaf clover plants. These symptoms suggested other factors such as root disease, rather than infestation by *L. mozardi*, were involved in the early decline of arrowleaf clover.

This study provided some insights into host plant preferences and the reproductive period of *Languria mozardi* in East Texas. Egg deposition on clover began in late March and peaked in early May. Primary flowering stems are preferred oviposition sites of the clover stem borer (Ellsbury and Baker 1989). All six clover species tested during this study had at least reached bud stage and most were starting to flower during the peak egg deposition period. Red and berseem clovers were preferred over the other clovers by *L. mozardi*, based on the degree of infestation recorded per plant. The high population on these two species may be a function of their later maturity and extended availability of succulent stems for egg deposition. This was certainly true for red clover which began a second phase of new shoot development, providing additional oviposition sites into late June. Ball clover matured slightly earlier than red and berseem clovers, but insect

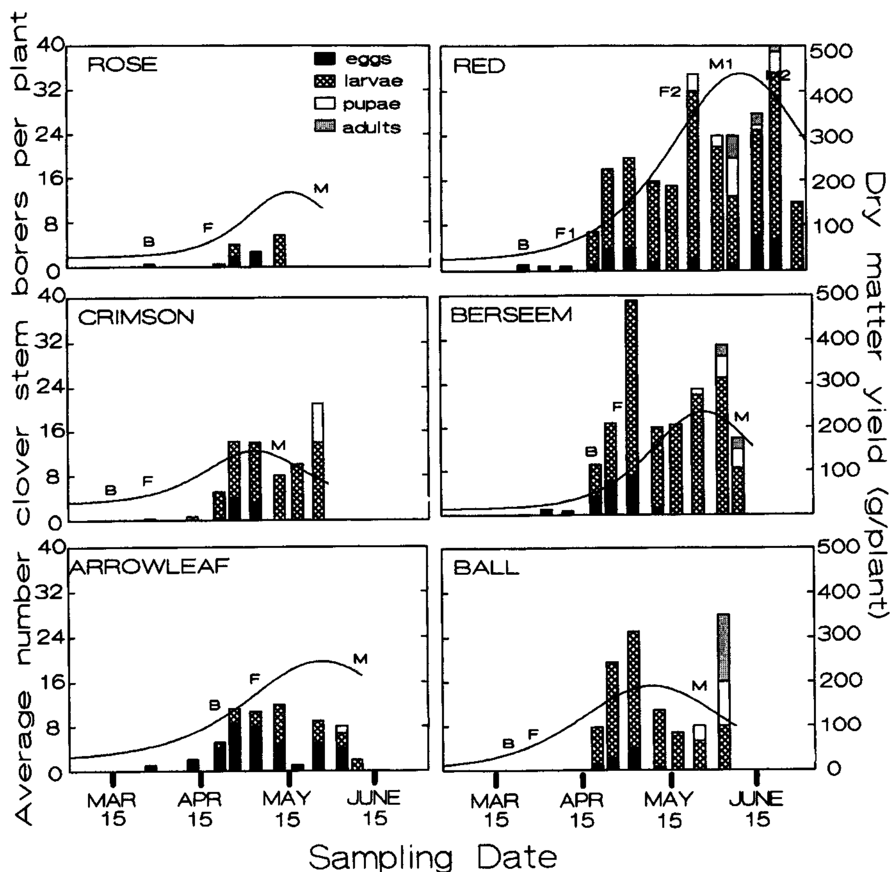


FIG. 2. Phenology of oviposition and larval development of *Languria mozardi* in relation to plant growth stages for six clover species at Overton, TX. Bars indicate average insect numbers per plant and insect developmental stage (left y-axis). Growth curves indicate average per-plant dry matter yield (right y-axis). Plant growth stages are indicated by B, flower bud; F, 10% flowering stems with open florets; M, mature seed/senescent plant.

development was successful on this species as well. Despite the variation in maturity dates of the clovers, the main egg deposition period was identical for all six species. Egg deposition on arrowleaf clover was extended, continuing through to the end of the sampling period.

Each oviposition site is a potential entry port for pathogenic organisms. The internal discoloration observed in stems may have resulted from the introduction of pathogenic fungi by ovipositing stem borers. While internal stem discoloration was associated with oviposition injury, crown discoloration was unrelated to infestation by *L. mozardi* and, along with root lesions, more likely was an indicator of root rot disease. Root rots and virus diseases were especially severe in arrowleaf clover in this study. Susceptibility to vascular diseases, and crown and root rots may contribute to the decline of plants attacked by *L. mozardi* because of compounded stress to the plants. Knight et al. (1976) reported an insect and disease complex on arrowleaf clover which included root-infecting fungi, viruses, and *L. mozardi*. Disease severity was reported to be higher in arrowleaf clover dually infected with bean yellow mosaic virus and *Phytophthora* root rot fungi than when either disease was present alone (Pratt et al. 1982). Bean yellow mosaic virus has been reported from Texas, and natural infections occur every year in clovers in this area (Pemberton et al. 1991). It is unclear how *L. mozardi* interacted with disease organisms in this study. Premature death of most arrowleaf clovers may have been due to a combination of virus and fungal diseases. Pupae were found in arrowleaf clover stems, indicating successful larval development on this species. The diseased condition of these plants may have been attractive to the clover stem borer female, indicated by the extended period of egg deposition. Premature plant death, however, eliminated arrowleaf clover as a source of food and shelter for the developing larvae.

Crimson and rose clovers were the two earliest maturing species in this study. Development time of *L. mozardi* is inversely proportional to temperature (Ellsbury 1991); therefore, early maturing clover species may escape extensive damage because stems are already senescent and larvae develop more slowly at lower temperature. Senescent stems may be unsuitable for oviposition or unpalatable to larvae. The two clovers differed considerably in degree of infestation, however. Ovipositing females showed the lowest preference for rose clover out of the six clover species tested. By the end of the study, infestation reached 100% for the other clovers, but fell to zero for rose clover. The few larvae found were either not thriving in the dry, woody stems, or were dead. Rose clover has not been reported as a host and appears to possess resistance to *L. mozardi* in the form of non-preference and/or antibiosis, as defined by Painter (1951). Presence of toxic or deterrent compounds cannot be ruled out as a protective mechanism for rose clover. Crimson clover also matured early, yet supported three times the number of insects as that found in rose clover. Thus, early maturity in itself did not provide as escape from infestation. Pupae were found in crimson clover stems, indicating that larval development was successful. Development of pupae into adults was not directly observed *in situ* since sampling of crimson clover was discontinued when plants had senesced. However, pupae removed from stems did complete development into adult beetles under laboratory conditions, and most likely the remaining stem stubble in the field could have provided adequate protection until adult emergence as well. Crimson clover exhibited tolerance of *L. mozardi* in its ability to flower and produce seed while supporting the insect.

Comparisons of dry matter yield for infested and noninfested plants within a clover species cannot be made due to the lack of an insecticide-treated control. However, rose, crimson, arrowleaf, and ball clovers had almost reached their growth plateaus by the peak oviposition period. Thus, the impact of oviposition injury on forage production was probably minimal on these four species. It is possible that subsequent pathogen introduction, if any, via oviposition sites might have reduced successful seed production

later in the season. Comparisons between clover species based solely on dry matter production cannot be made due to inherent differences in growth habit and yield potential among them (Smith et al. 1995). Although dry matter yield for rose clover was low compared to the other species in this study, this result is to be expected. There was no correlation between yield and degree of infestation in rose clover.

Because of the extended egg deposition period of this insect and the fact that clovers are a forage crop, chemical control is not recommended. Continuous reinfestation from nearby weed host plants would also lessen the impact of insecticide applications. Regular grazing or cutting may reduce populations of *L. mozardi* by destruction of eggs and larvae (Wildermuth and Gates 1920), but this practice may occur too late to prevent pathogen entry via oviposition sites. Despite the high infestation rate, many plants in this study completed their life cycle and produced at least some seed. Varying degrees of tolerance to *L. mozardi* are present in the six clover species in this study. Premature death of arrowleaf clover indicated greater susceptibility to some undetermined disease(s). Developing germplasm with resistance to crown and root rot diseases may allow arrowleaf clover to express greater tolerance to infestations of *L. mozardi*.

ACKNOWLEDGEMENT

We thank M. M. Ellsbury for critical review of this manuscript during preparation.

LITERATURE CITED

Ellsbury, M. M. 1991. Development of the immature stages of the clover stem borer (Coleoptera: Languriidae) at constant temperatures. Environ. Entomol. 20:1181-1186.

Ellsbury, M. M., and G. T. Baker. 1989. Biological and morphological aspects of the egg stage and oviposition of *Languria mozardi* (Coleoptera: Languriidae). Proc. Entomol. Soc. Washington 91:9-14.

Knight, W. E., O. W. Barnett, L. L. Singleton, and C. M. Smith. 1976. Potential disease and insect problems in arrowleaf clover. Amer. Soc. Agron., Southern Branch, Feb. 1-4, 1976, Mobile, AL, 3:7.

Lintner, J. A. 1881. The insects of the clover plant. Trans. New York State Agric. Soc. 32:187-207.

Painter, R. H. 1951. Insect Resistance in Crop Plants. The University Press of Kansas, Lawrence, KS. 520 pp.

Pemberton, I. J., M. R. McLaughlin, and G. R. Smith. 1991. Inheritance of resistance to virus-induced lethal wilt in arrowleaf clover. Phytopathol. 81:1001-1005.

Peterson, A. 1960. Larvae of Insects - An Introduction of Nearctic Species, Part II. Ohio State University, Columbus, OH. 416 pp.

Pratt, R. G., M. M. Ellsbury, O. W. Barnett, and W. E. Knight. 1982. Interactions of bean yellow mosaic virus and an aphid vector with Phytophthora root diseases in arrowleaf clover. Phytopathol. 72:1189-1192.

Smith, G. R., C. L. Gilbert, and I. J. Pemberton. 1995. Forage legume production in East Texas. Forage Research in Texas, 1994. TAES Publ. No. CPR-5252.

Wildermuth, V. L., and F. H. Gates. 1920. Clover stem-borer as an alfalfa pest. USDA Bul. No. 889.

REDUCTORIPODA ABSOLUTA GEN. ET SP. NOV. (ORIPODIDAE) AND A NEW *ALLOZETES* (CERATOZETIDAE) BERLESE, 1913 SPECIES FROM MEXICO (ACARI:ORIBATIDA)[1]

Sándor Mahunka[2] and José G. Palacios-Vargas[3]

ABSTRACT

Reductoripoda absoluta gen. and sp. nov. and *Allozetes lacandonicus* sp. nov. are described from Mexico. Both species belong to the canopy fauna. Eight original figures are included.

INTRODUCTION

The purpose to the present study, within the framework of the Hungarian-Mexican project, was to study the Mexican soil mites (Acari:Oribatida). Herein, we deal with the mite material found in soil samples presently deposited in UNAM, Laboratorio de Ecología y Sistemática de Microartrópodos, Depto. Biología, UNAM, Mexico City, and the collection of the Arachnoidea of the Hungarian Natural History Museum. The results of a preliminary taxonomoic investigation will be published in a series of short contributions (see Balogh and Palacios-Vargas 1996, Mahunka and Palacios-Vargas 1995).

Here we give the description of two new species, one of which also represents a new genus belonging to the family Oripodidae (see Aoki and Ohkubo 1974, Balogh and Balogh 1984). This genus also may lead to a better understanding of the family and to relationships within it because it shows specific structures of extremely reductive character.

When describing the new taxa, we follow the terminology already adopted in our previous works (e.g., Mahunka 1994)

DESCRIPTIONS OF THE NEW TAXA

ORIPODIDAE Jacot, 1925
Reductoripoda gen. nov.

Diagnosis: Family Oripodidae. Body surface well sculptured. Bothridium free, opening not covered by the humeral part of the notogaster. Lamellae normally developed, lamellar setae arising on their cusps. Notogaster with ten pairs of setae, four pairs of sacculi, and five pairs of lyrifissures. Gnathosoma typical for the family as are apodemes and epimeral borders. Epimeral setal formula: 3-1-3-3. Anogenital setal formula: 2-1-0-1. Lyrifissures *iad* long, located at the anterior corner of the anal opening. All legs tridactylous, heterodactily obvious. Lateral claws with anterobasal teeth. All femora well sculptured, the other joints smooth. Femora II-IV with blade-like formation. Normal leg chaetotaxy.

Type species: Reductoripoda absoluta sp. nov.

Remarks: The gradual reduction of the setae in the anogenital region is one of the most interesting characters of this family. The new genus represents one of the most strongly reduced types. We believe that it is the first Oribatid taxon without anal setae (Balogh and Balogh 1992). On this basis, it is easily distinguishable from related genera (i.e.,

[1]Project: Biodiversity of Mexican mites (see Borhidi et al. 1996). CONACyT, México-OMFB, Hungary.

[2]Department of Zoology, Hungarian Natural History Mueseum H-1088 Budapest, Baross u. 13, Hungary.

[3]Laboratorio de Ecología y Sistemática de Microartrópodos, Depto. Biología, Fac. Ciencias, UNAM. 04510 México, D.F.

Benoibates [Balogh 1958] and *Exoripoda* [Woolley 1961]), but it is questionable whether this feature is sufficient for the separation of the genus.

Derivatio nominis: After the strong reduction of setae in the anogenital region.

Reductoripoda absoluta sp. nov.
(Figs. 1-4)

Measurements: Length of body: 374 μm (male), 503 μm (female); width of body: 196μm (male), 217 μm (female).

Prodorsum: Its sculpture consists of foveolae, which are round and large anteriorly, but gradually becoming smaller and narrower toward the dorsosejugal region. Rostrum rounded, lamellae and prelamellae well developed. Rostral, lamellar and interlamellar setae distinct, rostral and lamellar setae unilaterally ciliate. All prodorsal setae slightly blunt at tip. Peduncle of the sensillus thin, its head large and wide, the surface of the latter barbulated.

Notogaster: Surface ornamented by foveolae, like the prodorsum, but these are elongated, mostly slit-like. Dorososejugal suture waved medially (Fig. 1), protruding significantly between the bothridia. Ten pairs of notogastral setae present, seven pairs long and setiform while three pairs in the posteromarginal position (setae *p*) are much shorter than the preceding ones and blunt at the tip. Sacculi hardly observable.

Lateral part of podosoma (Fig. 3): Lamellae continue in well developed prelamellae, the latter ending in sharp teeth. Lamellar setae arising on tubercles. Pedotecta I large, rounded anteriorly; pedotecta II-III very large, with a triangular lateral part (Fig. 4).

Ventral region (Fig. 2): Apodemes well developed, *ap. 2, ap. sej*, and the sternal ones connected with each other by a border framing the genital opening. The epimeral surface ornamented by foveolae, which are rounded anterolaterally, slit-like posteriorly, and absent medially. They compose small wrinkles on the ventral plate. Epimeral setal formula: 3-1-3-3, among them setae *3c* and *4c* hardly observable. Setae *1b* and *3b* the longest of all. Two pairs of short genital, one pair of similar aggenital and one pair of very long adanal setae arising in the anogenital region.

Legs: Tibia of leg I with a well developed process, solenidia *ø1* and *ø2* arising on it. Solenidium *ø1* conspicuously long on all legs. Leg setal formula: I: 0-5-2+1-4+2-16+2-3; IV: 1-2-2-3+1-10-3.

Material examined: Holotype (1530-HO-1995): Mexico, Chiapas State, Chajul Station. Single paratype from the same sample. Holotype deposited in the Hungarian Natural History Museum, Budapest, with identification number of the specimens in the Collection of Arachnida; paratype in the collection of the junior author.

Remarks: See remarks after the generic diagnosis.

Derivatio niminis: This species name refers to the complete lack of anal setae.

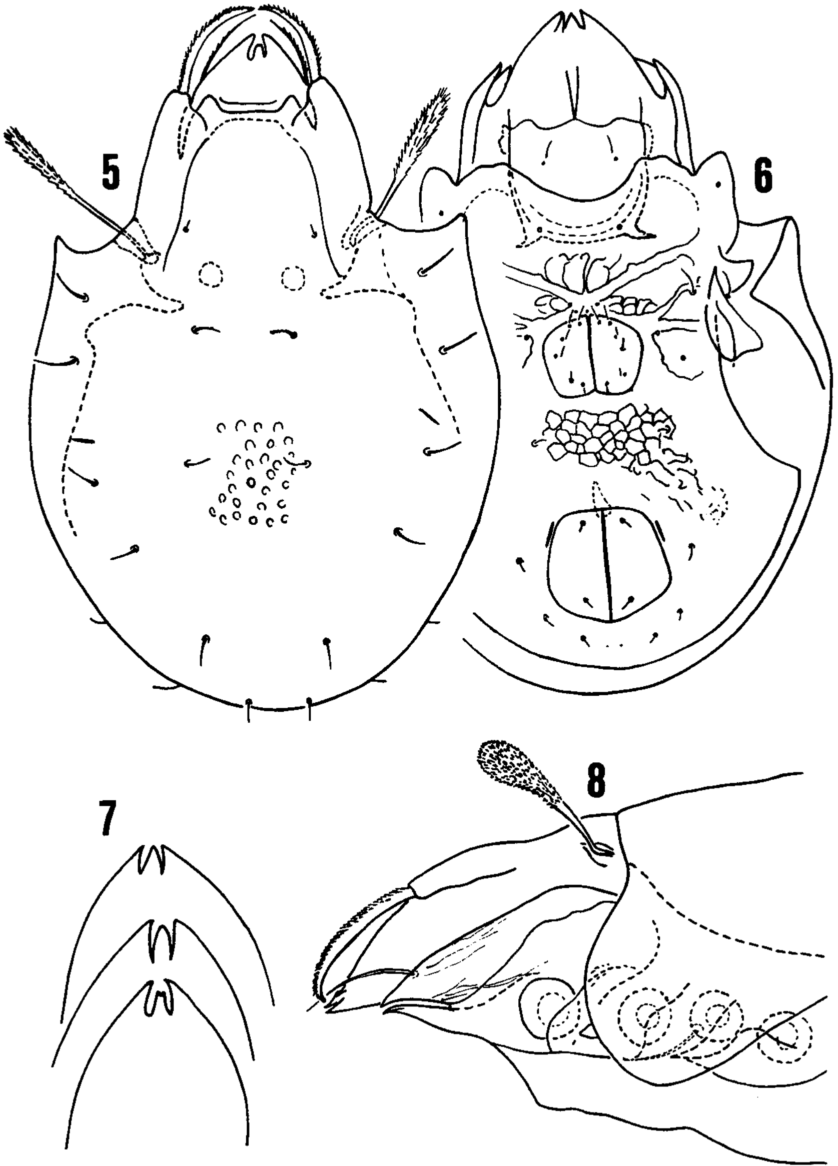
CERATOZETIDAE Jacot, 1929
Allozetes lacandonicus sp. nov.
(Figs. 5-8)

Measurements: Length of body: 246-267 μm; width of body: 172-183 μm (we have found only female specimens).

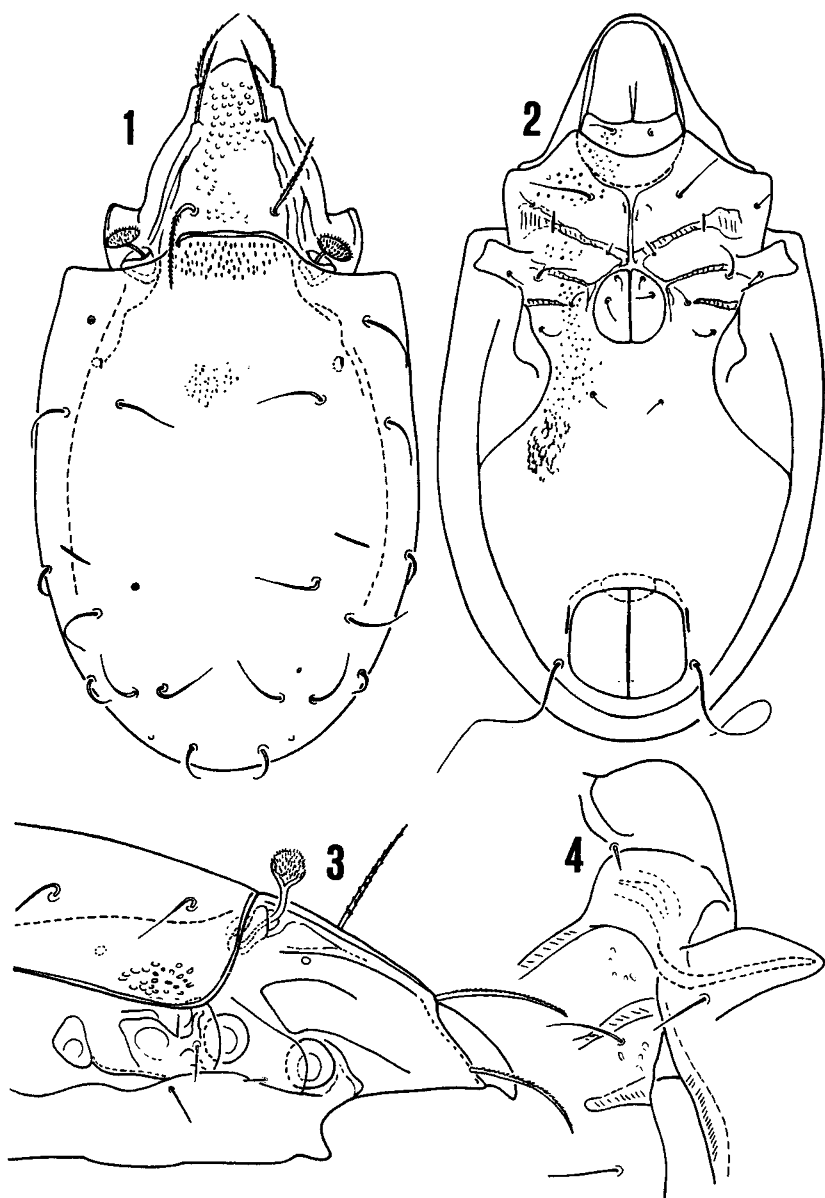
Prodorsum: Rostral apex clearly tripartite, divided by a deep incision. Median part always shorter than the lateral teeth; sometimes, it is slightly bifurcate or blunt at the tip (Fig. 7). Lamellae and translamella similar to those of the other species of the genus, a pair of deep incisions observable between the translamella and the lamellar cusps. Rostral setae thin, setiform, lamellar setae thick, distinctly and unilaterally barbed, interlamellar setae minute. Sensillus very long, slightly fusiform, well ciliated on its distal half.

Notogaster: Dorsosejugal suture absent. Pteromorphae large, directed downwards. Ten pairs of short notogastral setae, with their basal part slightly dilated, and four pairs of hardly discernible small sacculi present. The notogastral surface ornamented by foveolae, their diameter nearly as long as the distance among them (Fig. 5). A conspicuous median porus also visible.

Lateral part of podosoma (Fig. 8): Pedotecta I narrow, pedotecta II-III normal in size. Discidium very large, with a plate like widening. Custodium short but well observable, triangular.



FIGS. 5-8. *Allozetes lacandonicus* sp. nov. 5. body in dorsal aspect; 6. body in ventral aspect; 7. rostral apex variation in the types series; 8. podosoma in lateral aspex.



FIGS. 1-4. *Reductoripoda absoluta* gen. nov., sp. nov. 1. body in dorsal aspect; 2. body in ventral aspect; 3. podosoma in lateral aspect; 4. lateral portion of the coxisternal region.

Ventral region (Fig. 6): All apodemes interconnected in front of the genital opening forming a characteristic network. Epimeral surface with a weak polygonal pattern. Epimeral setae partly reduced or hardly observable. Only setae *1a*, *3b*, *3c*, *4a*, and *4b* located. Ventral plate with a polygonal sculpture medially, gradually dissolving toward the lateral margin. Anogenital setal formula: 6-1-2-4; all setae very short.

Legs: All legs monodactylous. Claws conspicuously large. Solenidium *ø2* of leg II arising on a narrowing projection. Seta *1"* on tibiae I and II short, spiniform; *ø1* of leg I very long; *ø1* of leg II, III and IV short and blunt.

Material examined: Holotype (1531-HO-1995): México, Chiapas State, Chajul Station, nine paratypes from the same sample. Holotype and four paratypes deposited in the collection of the Arachnoidea of the Hungarian Natural Histroy Museum, four paratypes in the collection of the junior author and one paratype in the Muséum de Histoire Naturelle, Genéve.

Remarks: The species of the genus *Allozetes* Berlese 1913 were discussesd recently by Mahunka (1988). Until now, this genus was known only from the Ethiopian and the Oriental Regions; this is the first species recorded from the Neotropics. (Balogh and Balogh [1992] erroneously recorded the presence of *Allozetes* species from the Nearctic and Neotropic Regions).

This new species is well distinguished from other *Allozetes* species by the tripartite rostrum and the sculpture of the notogaster and the ventral plate.

Derivatio nominis: This species is named after Selva Lacandona (Chiapas), the type locality.

ACKNOWLEDGMENT

The authors are indebted to Dr. Richard J. Elzinga of Kansas State University for the critical reading of the text and his valuable remarks.

REFERENCES

Aoki, J., and Ohkubo, N. 1974. A proposal of new classification of the family Oripodidae (*s. lat.*), with description of new species. Bull. Nat. Sci. Mus., Tokyo. 17:117-147.

Balogh, J., and Balogh, P. 1984. A review of the Oribatuloidea Thor, 1929 (Acari: Oribatei). Acta Zool. Hung. 30:257-313.

Balogh, J., and Balogh, P. 1992. The oribatid mites genera of the world, I-II. Hungarian Natural History Museum, Budapest, pp. 263+375.

Balogh, J., and Palacios-Vargas, J. G. 1996. Notes on the genus *Balazsella* Mahunka, 1983 with description of two new species (Acari: Oribatida). Acta Zool. Hung. In press.

Borhidi, A., Mahunka, S., and Palacios-Vargas, J. G. 1996. Report on the first year activity carried ou in the framework of the Hungarian-Mexican soil zoological co-operation: "Diversity of the Oribatid fauna of Mexico" 1995: Folia Ent. Hung. In press.

Mahunka, S. 1988. New and interesting mites from the Geneva Museum LXI. Oribatids from Sabah (East Malaysia) III (Acari: Oribatida). Revue suisse Zool. 95:817-888.

Mahunka, S. 1994. Oribatids of Madagascar II. (Acari: Oribatida). Revue suisse Zool. 101: 47-88.

Mahunka, S., and Palacios-Vargas, J. G. 1995. Two new Oribatid (Acari: Oribatida) species from the canopy of tropical dry forest in Mexico. Acta Zool. Hung. 41:295-300.

SELECTIVIDAD DE PRESAS DE TRES DEPREDADORES ACUATICOS DE LARVAS DE MOSQUITOS

S. M. Perez-Serna[1], H. Quiroz-Martinez[1 y 2], N. Ornelas-Nava[1], M. H. Badii[1], M. F. Suarez[3] y M. L. Rodriguez-Tovar[1]

RESUMEN

La selectividad o preferencia de presas de tres depredadores acuáticos fue evaluada de acuerdo a la metodología de la respuesta funcional. El notonéctido *Buenoa antigone* prefirió larvas del mosquito *Culex pipiens*; mientras que los copépodos depredadores *Mesocyclops longisetus* y *Macrocyclops albidus* no mostraron una clara preferencia.

ABSTRACT

Prey selectivity of three aquatic predators were evaluated according to functional response methodology. The notonectid *Buenoa antigone* preferred larvae of *Culex pipiens*; whereas no clear prey preference was observed with the predatory copepods *Mesocyclops longisetus* and *Macrocyclops albidus*.

INTRODUCCION

Para considerar un buen entomófago dentro de un programa de control biológico de plagas es necesario evaluar diferentes atributos ecológicos, uno de ellos es la preferencia o selectividad de presas de los depredadores hacia el organismo blanco de control. Aunque la investigación ha sido realizada en la agricultura, el interés por los depredadores acuáticos de larvas de mosquitos a aumentado gradualmente; por lo tanto, una buena selección de los mejores entomófagos es indispensable.

Bay (1974) mencionó que las relaciones depredador-presa en insectos acuáticos eran desconocidas. Desde entonces, la información cuantitativa del efecto regulatorio de estos depredadores ha sido desarrollada. En México, este conocimiento está basado en el uso de la respuesta funcional de copépodos, odonatos, hemípteros y coleopteros sobre larvas de mosquitos (Quiroz-Martinez, obs. pers.).

Sobre preferencia o selectividad de presas por diferentes especies de mosquitos con algunas especies de los nadadores de dorso, encontramos los siguientes estudios: Ellis y Borden (1970), así como Sih (1986), evaluaron la selectividad de *Notonecta undulata*. Mientras Gittelman (1975) realizó sus evaluaciones de preferencia de presas con *Martarega hondurensis* y *Buenoa antigone*. Posteriormente, Gittelman y Bergtrom (1977) reportaron la selectividad de presas para otros dos notonectidos, *Buenoa confusa* y *B. margaritacea*.

[1] Laboratorio de Entomología, Facultad de Ciencias Biológicas, U.A.N.L. Apdo. Postal 105-F, Cd. Universitaria; 66450 San Nicolás de los Garza, N. L. México.
[2] Programa de Graduados en Agricultura ITESM, Sucursal J de Correos, Monterrey, N. L.; CP 64849, México.
[3] Facultad de Salud, Sección de Inmunología, Apartado Aereo 25360 Cali, Colombia.

En los últimos años se han considerado a los copépodos como agentes de control biológico de larvas de mosquitos; algunos proyectos se han establecido para valorar su real potencial, aunque la información es muy escasa, destacando los estudios de Suarez et al. (1984), Marten (1990 a y b) y Brown et al. (1991). De las especies de copépodos presentes en alrededores de Monterrey, las más promisorias en biocontrol de larvas de *Aedes aegypti* son *Mesocyclops longisetus* y *Macrocyclops albidus*, de las cuales se obtuvo una alta capacidad de búsqueda o coeficiente de ataque (Quiroz-Martínez, obs. pers.).

El objetivo de este trabajo fue evaluar la preferencia o selectividad de presas de tres depredadores acuáticos sobre larvas de mosquitos, mediante la capacidad de búsqueda, es decir la capacidad del entomófago para buscar y localizar a la presa en el sistema de prueba, la cúal es el primer atributo de un buen agente de control biológico. Los depredadores fueron el nadador de dorso *B. antigone* y las especies de copépodos *Me. longisetus* y *Ma. albidus*.

MATERIALES Y METODO

Adultos del nadador de dorso *Buenoa antigone*, larvas del cuarto estadio de las presas, el mosquito *Culex pipiens* y el gusano de sangre *Chironomus* sp., fueron colectados en el Río Pesquería de Escobedo, N. L. Las chinches fueron colectadas con redes entomológicas en los recodos del río, principalmente en las orillas. Las larvas de *Cx. pipiens* fueron obtenidas por medio de cucharones, mientras que las del gusano de sangre con una pequeña red, removiendo el fondo del cuerpo de agua. La preferencia o selectividad del nadador de dorso fue evaluada en peceras de un litro de capacidad con 750 ml de agua declorada, con densidades de cada especie de presas de 1, 2, 3, 5, 7, 10, 20 y 30 en proporción 1:1 de larvas. Se realizaron cinco repeticiones, registrando el número de presas consumidas despues de 24 horas de exposición.

Los copépodos fueron obtenidos de crias de laboratorio, considerandose únicamente el adulto para las pruebas. Las presas fueron larvas del primer estadío de *Aedes aegypti*, obtenidas de una colonia de laboratorio. Además se uso como presa alternativa larvas del primer estadío de *Cx. pipiens* obtenidas de barquillas colectadas en el Arroyo Topo Chico de San Nicolás de los Garza, N. L. Las pruebas se llevaron a cabo en peceras de vidrio con 750 ml de agua declorada, exponiéndose las dos especies de copépodos individualmente a densidades 1, 2, 4, 8, 16 y 32 larvas de los mosquitos en proporcion 1:1 de cada especie. El depredador fué retirado a las 24 horas de exposición; las larvas vivas se colocaron en tubos de ensaye con un poco de agua, los cuales se calentaron para matar las larvas y determinar el número consumido de cada una de las especies presa.

Los resultados fueron analizados mediante los modelos de respuesta funcional de Holling (1959) y Rogers (1972), con los cuáles se obtuvo la capacidad de búsqueda o coeficiente de ataque (a'); Así como con los modelos de selectividad E de Ivlev (1961) y D de Jacobs (1974). Estos índices tienen valores extremos + 1 y - 1, interpretandose como una correlación, con el valor positivo como preferencia y el negativo como no preferencia.

RESULTADOS Y DISCUSION

El impacto de la depredación del notonectido *B. antigone* sobre las larvas de *Cx. pipiens* fue muy parecido en los sistemas de prueba (Tabla 1). Sin embargo, para *Chironomus sp.* el ataque disminuyó a casi la mitad en los sistemas con las densidades más altas. Además, la selectividad o preferencia de acuerdo a los modelos de respuesta funcional fue claramente hacia las larvas del mosquito; sin embargo, con los modelos de selectividad los índices estan cercanos al cero.

La preferencia del depredador *B. antigone* fue por larvas *Cx. pipiens* debido al mayor valor a' = 0.035335, comparada con a' = 0.021313 para las larvas de *Chironomus sp.*, de acuerdo a Holling (1959). Con el modelo de Rogers (1972), la capacidad de búsqueda fué a' = 0.042900 para las larvas del mosquito *Cx. pipiens* y para el gusano de sangre *Chironomus sp.* fué a' = 0.035682.

Tabla 1. Promedio de larvas consumidas en la selectividad de presas del nadador de dorso *Buenoa antigone* (5 repeticiones).

Densidad de Larvas	*Culex pipiens*	*Chironomus* sp.
1	1.0	0.8
2	1.6	1.2
3	2.2	1.2
5	3.8	2.6
7	2.8	0.6
10	4.8	2.6
20	9.0	5.0
30	10.2	6.2

Con los modelos de selectividad de Ivlev (1961), los coeficientes fueron negativos, pero el de *Cx. pipiens* cercano a cero con E = - 0.052631, mientras para *Chironomus* sp. E = - 0.333333. Además con el modelo de Jacobs (1974), la preferencia fue para *Cx. pipiens* con un valor positivo D = 0.092308, aunque tambien cercano al cero; para *Chironomus* sp. fue D = - 0.482052.

Existe una compleja variedad de factores los cuales determinaran la preferencia de un depredador hacia una presa en particular, el grado en que el entomófago responda determinará la presa preferida (Lee 1967). La preferencia por larvas de mosquitos por el nadador de dorso concuerda con lo reportado por Ellis y Borden (1970); biológicamente la razón de esa preferencia es porque las larvas del gusano de sangre forman pequeñas madrigueras en donde pasan la mayor parte del tiempo, localizándose en el fondo del agua o sobre algún sustrato. Estas presas están disponibles cuando llegan al cuarto estadio, previo a la aparición de la pupa.

La depredación ejercida por *Me. longisetus* fué con mayor voracidad que la de *Ma. albidus*. Pero para el primero, a pesar de la cantidad de presas que consumió, se apreció muy poca diferencia entre las especies de mosquitos. Sin embargo, con la segunda especie, fué menor el consumo, pero mas orientado hacia *Ae. aegypti* (Tabla 2).

Tabla 2. Promedio de larvas consumidas en la selectividad de presas por las dos especies de copépodos (5 repeticiones).

Densidad de Larvas	*Mesocyclops longisetus*		*Macrocyclops albidus*	
	Ae. aegypti	*Cx. pipiens*	*Ae. aegypti*	*Cx. pipiens*
1	0.6	0.8	0.6	0.0
2	1.6	1.8	0.2	0.0
4	2.2	3.2	1.4	0.4
8	5.6	4.4	4.8	0.4
16	11.2	9.6	6.2	0.2
32	22.6	21.2	15.4	0.6

El copépodo *Me. longisetus* mostró, de acuerdo a los modelos de respuesta funcional, una selectividad hacia *Cx. pipiens* con Holling de a' = 0.031768, mientras que Rogers fué a' = 0.065942. Estos valores se comparan con las obtenidas para *Ae. aegypti*, las cuáles fueron a' = 0.027385 y a' = 0.046595, respectivamente. La selectividad de presas de *Ma. albidus* fue para *Ae. aegypti*; bajo el modelo de Holling la capacidad de búsqueda fué de a' = 0.015612 y de acuerdo a Rogers a' = 0.022044; para *Cx. pipiens* estos valores fueron a' = 0.000295 y a' = 0.000308, respectivamente.

El índice de Ivlev calculados para los copépodos mostró que la preferencia de *Me. longisetus* fué para *Ae. aegypti* con E = 0.1633, comparado con E = 0.1298 obtenido para *Cx. pipiens*. Con el índice de Jacobs, la preferencia fué marcada por D = 0.3904 y D = 0.2952, respectivamente. Con la otra especie de copépodo depredador *Ma. albidus*, los índices de selectividad fueron negativos: mediante Ivlev, E = - 0.0482 para *Ae. aegytpi* y E = - 0.9033 para *Cx. pipiens*; de acuerdo a Jacobs, D = - 0.0920 y D = - 0.9492, respectivamente.

Lardeux (1992) mencionó que el impacto de los copépodos en tanques de agua, tambos y llantas cubiertas fue inconsistente, aparentemente debido a la disponibilidad de alimento para el crecimiento de estos crustaceos; además, interpretó que *Me. aspericornis* prefiere larvas de *Cx. quinquefasciatus* por la baja densidad en los sitios de sus pruebas. Nuestros resultados muestran que la preferencia o selectividad de *Me. longisetus* y *Ma. albidus* por larvas de mosquitos es controvertida, aunque existen reportes como los de Suarez et al. (1984) y Marten (1990 a y b), quienes los consideraron como muy promisorios en el control biológico de larvas de mosquitos. Esto puede deberse a que estas especies usadas en nuestro estudio, de copépodos depredadores y sus presas larvas de mosquitos, coevolucionaron en esta localidad.

Aunque la respuesta funcional ha sido usada para evaluar la capacidad depredadora de un entomófago, un sesgo que a esta metodología se le encuentra es que se expone un depredador a un tipo de presa. Como vemos en este trabajo, esta metodología tambíen puede usarse para determinar preferencia alimenticia. Consecuentemente, al hacer más complejos los sistemas de prueba obtendremos información mas cercana a las condiciones naturales en las cuáles se localiza un entomófago y de esta manera la decisión del mejor agente de control biológico podrá ser realizada.

Estos ensayos demuestran que, aún bajo las condiciones relativamente simples de laboratorio, aunado con inferencias lógicas, se puede llegar con modelos predictivos sobre lo que se espera pueda ocurrir en el campo y de esta manera avanzar en nuestro conocimiento bioecológico de los sistemas acuáticos, contribuyendo a un mejor aprovechamiento de los recursos bíoticos (entomófagos), en el combate biológico de las plagas en salud pública.

AGRADECIMIENTOS

Este manuscrito que forma parte del proyecto "Impacto de los depredadores acuáticos sobre larvas de *Culex pipiens* (Diptera: Culicidae)", convenio DGICSA No. 088-01-0175, la evaluación que correspondio del nadador de dorso; mientras que lo de los copépodos fue financiado por la Fundacion Rockefeller a travéz del proyecto "Control Biológico de *Aedes aegypti* con Copépodos". Al Dr. Luis O. Tejada del Programa de Graduados en Agricultura ITESM y Dr. Noor Tietze de Florida A & M University por las críticas y sugerencias para la realización de este escrito.

LITERATURA CITADA

Bay, E. C. 1974. Predator-prey relationships among aquatic insects. Ann. Review Entomol. 19: 441-453.

Brown, M. D.; B. H. Kay and J. K. Hendrikz. 1991. Evaluation of the Auatralian *Mesocyclops* (Copepoda:Cyclopidae) for mosquito control. J. Med. Entomol. 28:618-623.

Ellis, R. A. and J. H. Borden. 1970. Predation by *Notonecta undulata* (Heteroptera: Notonectidae) on larvae of the yellow fever mosquito. Ann. Entomol. Soc. Am. 63:963-972.

Gittelman, S. H. 1975. The ecology of some Costa Rica backswimmers (Hemiptera: Notonectidae). Ann. Entomol. Soc. Am. 68:511-517.

Gittelman, S. H. and G. Bergtrom. 1977. Depth selection in two species of *Buenoa* (Hemiptera: Notonectidae). Ann. Entomol. Soc. Am. 70:469-475.

Holling, C. S. 1959. Some characteristics of simple types of predation and parasitism. Can. Entomol. 91:385-398.

Ivlev, V. S. 1961. Experimental ecology of the feeding fishes. Yale University Press New Haven, CT 230 pp.

Jacobs, J. 1974. Quatitative measurement of food selection: A modification of the forage ratio and Ivlev's electivity index. Oecologia 14:413-417.

Lardeux, F. J. R. 1992. Biological control of Culicidae with *Mesocyclops aspericornis* and larvivorous fish (Poecillidae) in a village of French Polynesia. Med. Vet. Entomol. 6:9-15.

Lee, F. C. 1967. Laboratory observations on certain mosquito predators. Mosq. News 27:332.

Marten, G. G. 1990 a. Evaluation of cyclops for *Aedes albopictus* control in tires. J. Am. Mosq. Control Assoc. 6:681-688.

Marten, G. G. 1990 b. Elimination of *Aedes albopictus* from tired piles by introducing *Macrocyclops albidus* (Copepoda:Cyclopidae). J. Am. Mosq. Control Assoc. 6:689-693.

Rogers, D.1972. Random search and insect populations models. J. Anim. Ecol. 41:369-383.

Sih, A. 1986. Antipredator responses and the perception of danger by mosquito larvae. Ecology 67:434-441.

Suarez, M. F.; D. Ayala, M. J. Nelson y J. W. Reid. 1984. Hallazgo de *Mesocyclops aspericornis* (Daday) (Copepoda:Cyclopidae), depredador de larvas de *Aedes aegypti* en Apoima-Colombia (1). Biomedica 4:74-76.

VOL. 21 NO. 4 SOUTHWESTERN ENTOMOLOGIST DEC. 1996
SCIENTIFIC NOTE

NEW DISTRIBUTION RECORD FOR THE HAIRY MAGGOT BLOW FLY *CHRYSOMYA RUFIFACIES*

C. S. Martin, C. E. Carlton and C. L. Meek

Department of Entomology, Louisiana Agricultural Experiment Station, Louisiana State University Agricultural Center, Baton Rouge, Louisiana 70803

The hairy maggot blow fly, *Chrysomya rufifacies* (Macquart) is native to tropical regions of Australia and the Orient. It was first reported in the New World in Costa Rica by Jiron (1979) and later in the United States by Richard and Ahrens (1983) from specimens collected in several geographical sites in Texas. The only other distributional records are from southern California (Greenberg 1988), Arizona (Baumgartner 1986) and Florida (Mertins 1991), Steck and Butler 1991). *Chrysomya rufifacies* is an aggressive necrophagous fly whose larvae are facultatively predaceous on other carrion fly immatures. Not only can it be a serious primary and secondary myiasis-producing species, but its occurrence has important implications to the medico-legal application of forensic entomology. The biology of this species has been reviewed by Baumgartner (1993).

Here we document a range expansion of this species along the Gulf Coast Plain of the southern United States. A brief description of this field study is provided here to inform the reader of the circumstances leading up to the discovery of the hairy maggot blow fly populations in Louisiana. It is not intended to be a complete explanation of our research methods and results. A complete report of this law enforcement-oriented project regarding necrophilous insects on carcasses in special habitats (e.g., automobile trunks and burned passenger compartments) will be forthcoming.

In October 1995, a field study was begun at the Louisiana State University Firemen Training Center near the southern city limits of Baton Rouge (East Baton Rouge Parish, Louisiana) on La. Highway 30. The intent of the study was to document the succession and species composition of necrophagous insects on swine carcasses (*Sus scrofa*) placed in specialized habitats, notably, rear trunks and burned passenger compartments of automobiles. Previous research using swine carcasses in selected habitats indicated that such data were useful in estimating human post-mortem intervals when cadavers were found in similar situations during police investigations (e.g., homicides, suicides or unwitnessed suspicious human deaths).

The portion of the 1-year study reported here used carcasses of six pigs (avg. 16 kg each). Three animals were individually placed in three closed automobile trunks and, as a standard, paired with the remaining three pigs located in wire cages on the ground. Daily sampling of insect activity on each carcass was conducted. After the fly larval masses had dispersed, the adults emerged and the carcasses became dry (Smith 1986), a subsequent study was conducted using the same methodologies. This time, a fresh swine carcass was placed in

[1] Diptera: Calliphoridae
[2] Manuscript approved for publication by the Director of the Louisiana Agricultural Experiment Station as manuscript number 96-17-0246.
[3] Approved by the LSU Agricultural Center's Institutional Animal Care and Use Committee.

each wire cage and in the passenger compartment of each automobile instead of the rear trunk. Subsequently, each vehicle was set on fire by professional firefighters and allowed to burn for four minutes. The October 1995 study was repeated in the spring and summer of 1996.

Field experiments to document necrophagous insect succession and species composition on carcasses in East Baton Rouge Parish and surrounding parishes have been ongoing annually since the early 1980's (Grisbaum et al. 1995, Tessmer et al. 1995, Tessmer and Meek 1996, and other unpublished data). *Chrysomya rufifacies* has never been collected from any of these field experiments including more than 50 police-investigated homicides scenes in Louisiana where insect evidence was collected and submitted to the forensic entomology laboratory in the LSU Agricultural Center.

This study marks the first record of *C. rufifacies* larvae in Louisiana. The initial collection date of *C. rufifacies* immatures was 10 October 1995, and the immatures were recovered from two carcasses in wire cages as mentioned previously. Regardless of location (e.g.., rear trunk, passenger compartment or on the ground), every carcass became infested (>50%) predominantly with *C. rufifacies* larvae within three days during the October study. Field studies in April and in July 1996 failed to record the presence of *C. rufifacies*.

The authors wish to thank Dr. J.F. Butler and Jason Byrd at the University of Florida in Gainesville for confirming the identification of our *C. rufifacies* specimens, Arlen Gross and his firefighting staff at the LSU Firemen Training Center in Baton Rouge for providing a field site and assisting in the study, Dealer's Wrecker of Baton Rouge for providing and transporting the automobiles used at the site and the marksmanship of Patrick Lane, Louisiana State Police Crime Lab, for assisting also with the pig carcasses.

LITERATURE CITED

Baumgartner, D. L. 1986. The hairy maggot blow fly, *Chrysomya rufifacies,* confirmed in Arizona. J. Entomol. Sci.21: 130-132.

Baumgartner, D. L. 1993. Review of *Chrysomya rufifacies* (Diptera: Calliphoridae). J. Med. Entomol. 30: 338-352.

Greenberg, B. 1988. *Chrysomya megacephala* (F.) (Diptera: Calliphoridae) collected in North America and notes on *Chrysomya* species present in the New World. J. Med. Entomol. 25: 199-200.

Grisbaum, G. A., J. W. Tessmer, and C. L. Meek. 1995. Effects of initial post-mortem refrigeration of animal carcasses on necrophilous adult fly activity. Southwest. Entomol. 20: 165-169.

Jiron, L. F. 1979. On the calliphorid flies of Costa Rica (Diptera: Cyclorrhapha). Brenesia 16: 221-223.

Mertins, J. 1991. Blow fly infestation detected in new geographic areas, pp. 1-2. *In* Dx Monitor (Animal Health Report), American Association Veterinary Laboratory Diagnosticians, U.S. Animal Health Assoc. and USDA-APHIS, Winter issue.

Richard, R. D., and E. H. Ahrens. 1983. New distribution record for the recently introduced blow fly *Chrysomya rufifacies* (Macquart) in North America. Southwest. Entomol. 8: 216-218.

Smith, K. G. V. 1986. A Manual of Forensic Entomology. Cornell Univ. Press, Ithaca, N.Y.

Steck, G., and J. Butler. 1991. Insect detection: *Homo sapiens* (man). Triology 30: 5-6.

Tessmer, J. W., C. L. Meek, and V. L. Wright. 1995. Circadian pattern of oviposition by necrophilous flies (Diptera: Calliphoridae) in southern Louisiana. Southwest. Entomol. 20:439-445.

Tessmer, J. W., and C. L. Meek. 1996. Dispersal and distribution of calliphorid immatures from animal carcasses in southern Louisiana. J. Med. Entomol. 33: 665-669.

VOL. 21 NO. 4 SOUTHWESTERN ENTOMOLOGIST DEC. 1996
SCIENTIFIC NOTE

MATING BEHAVIOR AND PEAK MATING ACTIVITY OF THE PECAN WEEVIL *CURCULIO CARYAE*[1] (HORN)

Justin K. Collins, Phillip G. Mulder, Michael W. Smith[2], and Raymond D. Eikenbary

Oklahoma State University, Department of Entomology
Stillwater, OK 74078

The pecan weevil, *Curculio caryae* (Horn), causes significant reductions in yields throughout the pecan [*Caryae illinoinensis* (Wangengheim) K. Koch] growing regions of the U.S. Damage is caused by feeding of male and female weevils on fruit during the water and gel stages, oviposition by females during the dough stage, and feeding on developing pecan fruit by the larval stage (Calcote 1975). Upon completing development in the fruit, larvae exit through a small hole bored through the shell and shuck, and burrow into the soil. Prepupae remain 1-2 years in the soil before pupating into a pharate adult that remains an additional year in the soil before emerging (Raney and Eikenbary 1968a and Van Cleave and Harp 1971).

Hatfield et al. (1982) indicated that during mating, male pecan weevils assume the mating posture and begin tapping on lateral margins of the thorax of females using metathoracic legs. In their study, 15 mating pairs were used in four, four hour time blocks. Once mating was initiated, the pairs were separated and placed into holding containers. Based on observations described in this paper, period of peak mating seemed questionable since only 15 replicates were used at four, 4-h time intervals. In laboratory observations of pecan weevil mating for another behavior study on this insect, males were found to tap mesothoracic legs on lateral margins of female abdomens. To adequately analyze mating behavior, more replications were needed. The objective of this research was to elucidate behavioral patterns for peak mating periods.

Pecan weevils used in this study were collected from the OSU Horticulture research station in Stillwater, OK. Using 150 cone traps (Raney and Eikenbary 1968b) from 25 July to October 15, 1995, 1,800 adult weevils were captured.

To determine which legs were used to tap females, male behavior was observed for all mating periods studied. Day-old male and female pecan weevils were collected, separated, and fed for 24 h on immature pecan fruit. Each mating pair of weevils were placed in a 100cm Petri dish. Fifty mating pairs were used for each 1-h time block. Petri dishes were placed in front of a window on a dark surface. The window was used to

[1]Coleoptera: Curculionidae

[2]Oklahoma State University, Department of Horticulture and Landscape Architecture Stillwater, OK 74078

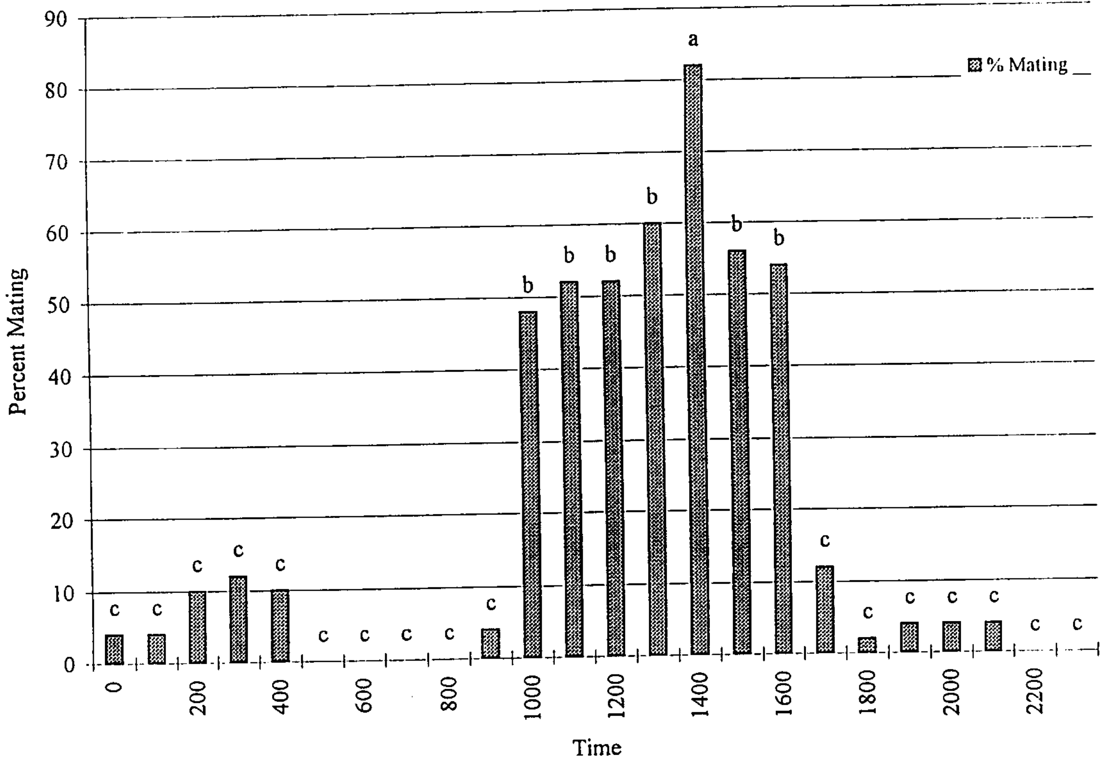


FIG. 1. Percentage of pecan weevil mating over a 24 hour time course

simulate natural lighting while the dark surface of the table simulated pecan tree bark. Temperature was 25 °C ± 5. For each 1-h time interval, during the 24-h period of this study, 50 mating pairs were used for a total of 1,200 pairs in a completely randomized design. When mating began the time was recorded. If no mating occurred within 1 h, weevils were removed, pairs separated, and weevils placed in holding chambers for subsequent studies. Weevils were not used again in this mating study. This process allowed determination of the exact time when mating frequency peaked. If mating occurred, data on leg tapping were recorded to determine if male weevils tapped prothoracic, mesothoracic, or metathoracic legs before insertion of the aedeagus. Data were analyzed using SAS and separated with an LSD (SAS Inc. 1987).

The peak mating period occurred at 1400 hr with 82% of the weevils mating at this time (LSD = 12.2, P=0.05). Percentage mating response from 1000 h until 1300 h and from 1500 h until 1600 h were similar, but were significantly different from 1400 h and other time periods (Fig. 1). Mating activity sharply decreased from 1600 h (54%) to 1700 h (12%) with activity continuing to decline after 1700 h. Data from this study indicates that mating behavior of pecan weevils is diurnal under these controlled conditions.

Results from 209 mating pairs indicated males tapped the mesothoracic leg against the lateral sides of female pecan weevils during mating. The male approaches the female from behind, assumes a mating posture if the female is receptive, and taps his mesothoracic legs for 3-5 seconds before inserting his aedeagus. In each observed mating, males tapped females with their mesothoracic legs. Males did not tap metathoracic or prothoracic legs at any time before or during mating.

Results from this study broaden the knowledge of pecan weevil biology and provides a background for testing pecan weevil pheromones in the laboratory. Additional work on the biology of this pest to determine feeding requirements for oviposition, host, and feeding preferences is needed.

LITERATURE CITED

Calcote, V.R. 1975. Pecan weevil: Feeding and initial oviposition as related to nut development. J. Econ. Entomol. 68:4-6.

Hatfield, L.D., J.L. Frazier, and W.W. Neel. 1982. Mating behavior of the pecan weevil, *Curculio caryae* (Horn) (Coleoptera: Curculionidae) J. Georgia Entomol. Soc. 17:275-279.

SAS Institute. 1987. SAS/STAT guide for personal computers, version 6 ed. SAS Institute, Cary, N.C.

Raney, H.G. and R.D. Eikenbary. 1968a. Investigations on flight habits of the pecan weevil, *Curculio caryae* (Horn) (Coleoptera: Curculionidae). Can. Entomol. 100:1091-1095.

Raney, H.G. and R.D. Eikenbary. 1968b. A simplified trap for collecting adult pecan weevils. J. Econ. Entomol. 62:722-723.

Van Cleave, H.W. and J.J. Harp. 1971. The pecan weevil: Present status and future prospectives. Proc. Southeast Pecan Growers Assoc. 64:99-111.

SCIENTIFIC NOTE

EFECTO DE *BACILLUS THURINGIENSIS* EN LA DEPREDACIÓN DE *BUENOA ANTIGONE* SOBRE LARVAS DE *AEDES AEGYPTI*.

H. Quiroz-Martinez, M. A. Herrera-Delgadillo y M. H. Badii
Laboratorio de Entomología, Facultad de Ciencias Biológicas,U.A.N.L.; Apdo. Postal 105 - F, San Nicolas de los Garza, N. L. México. CP 66450

La longevidad y el potencial de reproducción han sido afectadas en algunos entomófagos por *Bacillus thuringiensis* (Croft y Brown 1975), resultando letal para dípteros de las familias Dixidae, Chironomidae y Simuliidae, pero sin efecto sobre depredadores y excavadores (García et al. 1980; García y Sweeney 1986). *B. thuringiensis* var. *israelensis* (Bti) fue altamente tóxico sobre larvas del depredador *Toxorhynchites rutilus rutilus* (Lacey y Dame 1982). Igualmente el uso de *Gambusia affinis* y *Bti* ha resultado en un mejor control de *Culex tarsalis* que por si solos, sin mortalidad del pez (WHO 1984).

En el mercado se encuentran formulados de *Bt*, aunque algunas de las variedades han sido producidas en forma artesanal, por ejemplo *Bt* var. *kurstaki* en Guatemala y evaluado por Tejada (com. pers.), con buenos resultados sobre *Trichoplusia ni* en brocoli. En Perú, las comunidades rurales han producido *Bti* en cocos para el control biológico de larvas de *Anopheles spp.* y considerado como muy efectivo contra mosquitos (Ventosilla com. pers.).

Los hemípteros de la familia Notonectidae son considerados como unos de los mayor potencial como agentes de control biológico de larvas de mosquitos, pero poco conocimiento se tiene del efecto secundario de *Bti*, por lo cual se planteó como objetivo del estudio, evaluar el efecto de *Bti* en la depredación del notonectido *Buenoa antigone* sobre larvas de *Aedes aegypti*.

Adultos de *Buenoa antigone* fueron colectados en el Río Pesquería de Escobedo, Nuevo León; se colocaron individualmente en recipientes de vidrio con 750 ml de agua declorada, donde se expusieron a las densidades de 10, 15, 20, 30, 40, 45, 50, 60 y 70 larvas del cuarto estadio de *Aedes aegypti*, registrando la mortalidad después de 24 horas; además se realizo una prueba con larvas del mosquito tratadas por separado con 80 mg de *Bt* cepa GM-10, que representa la CL_{50} (Rodriguez-Tovar com. pers.). Los resultados fueron analizados con los modelos de respuesta funcional de Holling (1959) y Rogers (1972), obteniéndose con ellos la capacidad de búsqueda (a'), comparándose esta característica del entomófago para conocer el efecto de *Bt* sobre la depredación del notonectido.

La depredación del notonectido disminuyo ligeramente cuando se dio el tratamiento a las larvas, aunque no existió efecto letal de *Bt* sobre los notonectidos (Tabla 1); contrario a lo reportado para el sistema *Buenoa sp.-Culex pipiens*, donde aumento notoriamente la capacidad de búsqueda (Ortegon-Martínez y Quiroz-Martinez 1990).

Mediante el modelo de Holling, la capacidad de búsqueda aumentó ligeramente con las larvas tratadas con *Bt*, de a' = 0.0419 a a' = 0.0491; mientras que en el modelo de Rogers esta cualidad del depredador disminuyo cuando se trataron las larvas del mosquito de a' = 0.2877 a a' = 0.1086. La diferencia entre las tendencias de estos dos valores se debe al metodo usado para evaluar el parametro capacidad de búsqueda (a'); el primero de ellos se basa en la mortalidad de las presas, mientras que el segundo se basa en la sobrevivencia de ellas, siendo esta última mas cercana a la realidad, debido a que le proporciona una oportunidad de escape (Badii, obs. pers.).

Tabla 1. Larvas consumidas por adultos *Buenoa antigone* expuestos individualmente sobre larvas del cuarto estadio de *Aedes aegypti* sin y con tratamiento de *Bacillus thuringiensis* después de diez repeticiones.

Densidad larvaria	Sin Tratamiento	Con Tratamiento
10	10.0	8.2
15	13.6	12.0
20	19.0	14.2
30	21.8	15.2
40	23.0	15.0
45	15.2	12.8
50	25.8	17.0
60	19.8	17.8
75	34.6	20.6

Después de analizar los resultados podemos concluir que *Bacillus thuringiensis* cepa GM-10 no tuvo efecto letal sobre el notonectido *Buenoa antigone*, cuando este depredó larvas del mosquito *Aedes aegypti* afectadas por la bacteria entomopatógena; sin embargo la capacidad de búsqueda no mostró una clara tendencia ya que con un modelo de análisis estadístico aumento y con el otro disminuyo. Un aspecto relevante de este estudio es que ambas estrategias de control pueden ser usadas de una manera compatible, fijado así las bases para un manejo integrado de esta especie de mosquito vector.

Este trabajo se desarrolló dentro del proyecto "Estrategias de Manejo Integrado de Larvas de Mosquitos", convenio CONACYT M920/D0746; Dr. Luis O. Tejada del programa de Graduados en Agricultura del ITESM por la revisión y críticas para este trabajo.

LITERATURA CITADA

Croft, B. A. and W. A. Brown. 1975. Responses of arthropod natural enemies to insecticides. Ann. Rev. Entomol. 20:255-333.

Garcia, R.; B. A. Frederici, I. M. Hall, M. S. Mulla and H. Schafer. 1980. BTI- a potent new biological weapon. California Agriculture 34:18-19.

Garcia, R. and A. W. Sweeney. 1986. The use of microbial pathogens for the control of mosquitoes. Agric. Ecosys. and Environ. 15:201-228.

Holling, C. S. 1959. Some characteristics of simple types of predation and parasitism. Can. Entomol. 91:385-398.

Lacey, L. A. and D. A. Dame. 1982. The effect of *Bacillus thuringiensis* var. *israelensis* on *Toxorhynchites rutilus rutilus* (Diptera:Culicidae) in the presence and absence of prey. J. Med. Entomol. 19:593-596.

Ortegon-Martinez, J. J. y H. Quiroz-Martinez. 1990. Efecto de la cepa GM-10 de *Bacillus thuringiensis* CL_{50} en la capacidad depredadora de *Buenoa sp.* (Hemiptera: Notonectidae), sobre larvas de *Culex pipiens quinquefasciatus* (Diptera: Culicidae). Folia Entomol. Mex. 79:197-205.

Rogers, D. 1972. Random search and insect population models. J. Anim. Ecol. 41:369-383.

WHO. 1984. UNDP/World Bank/ WHO Special Programme for Research and Training in Tropical Diseases. Report of the 7th. Meeting of the Scientific Working Group on Biological Control of Vectors.

AUTHOR INDEX TO VOLUME 21

Aguirre-Uribe, Luís Alberto, 349
Alaniz, M.A., 421
Al-Dosari, Saleh A., 49
Anderson, Roger M., 345
Antilla, L., 229
Aranda, E., 83
Arnold, Don C., 303
Arrendondo-Bernal, Hugo César, 349
Badii, M.H., 83, 471, 483
Bay, D.E., 337
Beerwinkle, K.R., 395, 407
Biggerstaff, Michael I., 341
Borboa-Flores, J., 75
Buckner, James, 377
Burd, John D, 145, 167
Burke, R.A., 357
Burris, E., 59
Buschman, Lawrent L., 181
Cambra T, Roberto A., 205
Camino-Lavín, M., 325
Carlton, C.E., 477
Carroll, Becky L., 303
Castrejón-Ayala, F., 325
Castrejón-Gómez, V., 325
Chandler, L.D., 189
Cheary, Becky S., 303
Chen, Andrew C., 43
Chu, Chang-chi, 377
Clark, William H., 213
Cocke, Jr., J., 1
Cohen, Allen Carson, 377
Collins, Justin K., 479
Cortez-Rocha, M.O., 75
Cranshaw, Whitney S., 49, 135
Crocker, R.L., 317
Danka, Robert G., 451
Davis, M.R., 421
Eikenbary, Raymond D., 303
Eikenbary, Raymond D., 479
Ellington, J.J., 153
Elliott, Norman C., 145, 237
Ellison, Sherry, 387
Elzen, G.W., 127
Engle, C.,E. 283
Ervin, R. Terry, 105
Escobar, D.E., 421
Everitt, J.H., 421
Fincher, G.T., 445
Flint, H.M., 229
Flores, A.E., 83
Flores, S., 83
Freeman, Thomas, 377
Gandolfo, Daniel E., 117
Garza-Gonzáles, Enrique, 349
Goolsby, John, 13
Graves, J.B., 59
Hardee, D.D., 86, 433
Harris, Marvin K., 225
Henneberry, Thomas J., 357, 377
Hernéndez, E., 83
Herrera-Delgadillo, M.Aa., 483
Hooper, Douglas A., 135
Hoxie, Robert P., 167
Jech, L. Forlow, 357
Jiménez, M.L., 331
Jiménez-Pérez, A., 325
Johnson, T.D., 237
Khalema, Tieiso M., 105
Kidd, P.W., 293
LaRock, D.R., 153
Laster, M.L., 86, 433
Legaspi, Jesusa Crisostomo, 13, 33
Legaspi, Jr., Benjamin, C., 13, 33
Leggett, J.E., 229
Leonard, B.R., 59
Lingren, P.D., 23, 395
Lopez, Jr., J.D., 277, 407
Mahunka, Sándor, 465
Martin, C.S., 477
Martin, S.H., 59
McIntyre, Julie A., 135
McKibben, G.H., 407
Meagher, Jr., Robert L., 369
Meek, C.L., 477
Meola, S.M., 337
Michels, Jr., G.J., 237
Micinski, S., 59
Moore, T.R., 1
Mott, Dale A., 341, 345
Mulder, Phillip G., 479
Munroe, Paul D., 173
Nalon, Jr., W.T., 317
Natwick, E.T., 283
Nava-Camberos, Urbano, 225
Nelson, Dennis, 377
Newton, W.H., 1

Nordlund, Donald A., 33
Ornelas-Nava, N., 471
Palacios-Vargas, José G., 465
Palumbo, J.C., 283
Parks, N.J., 229
Paxton, Douglas W., 141
Peairs, F.B., 255
Pemberton, I.J., 457
Pendleton, Bonnie L., 345
Perales-Guitiérrez, Miguel Agustín, 349
Perez-Serna, S.M., 471
Peterson, Gary C., 105
Phillips, Jr., Sherman A., 173
Powell, J.D., 59
Powell, J.D., 59
Puterka, Gary J., 167
Quintero A., Diomedes, 205
Quiros-Martinez, H., 471, 483
Raulston, J.R., 23, 395
Ree, Bill, 225
Reed, David K., 145
Rice, Natasha R., 303
Richman, David B., 117
Rieske, L.K., 255
Rigueroa-Brito, R., 325
Rigueroa-Brito, R., 325
Riley, David G., 265
Roberson, J., 59
Roberson, J., 59
Robinson, J.V., 457
Rodríguez-del-Bosque, Luis, 55, 209, 317
Rodriguez-Tovar, M.L., 471
Romero, R.L., 237
Ruberson, J.R., 189
Rummel, D.R., 293
Schleider, Paul G., 43
Schneider, J.C., 433
Schweissing, Frank C., 49
Shaver, T.N., 23, 277, 395
Shiferaw, Asrat, 303
Sloderbeck, Phillip E., 181
Slosser, J.E., 1
Slowik, T.J., 247
Smith, C. Wayne, 369
Smith, G.R., 457
Smith, Michael W., 479
Smith, W.J., 369
Spurgeon, D.W., 23
Suarez, M.F., 471
Summy, K.R., 421
Tan, Wei-Jia, 265
Teetes, George L., 105, 341, 345
Tejas, A., 331
Thorvilson, Harlan G., 141, 173, 247, 293
Villa, José D., 451
Vinson, S. Bradleigh, 387
Webster, James A., 167
Wei, X., 317
Wellso, Stanley G., 167
White, S.L., 337
Witt, Merle D., 181
Wolfenbarger, Dan A., 265
Wong-Corral, F., 75

SUBJECT INDEX TO VOLUME 21

Acarapis woodi
infesting Africanized honey bees, 451

Allozetes lacandonicus
new species from Mexico, 465

Anomala flavipennis
pupation and adult longevity of, 55

Anomala foraminosa
pupation and adult longevity of, 55

Anthonomus grandis
responses of to pheromone traps, 407
susceptibility of to insecticides in Louisiana, 59

Aphis gossypii
effect of cyhalothrin on in Texas, 293

Apis mellifera scutellata
susceptibility of to tracheal mites, 451

Bacillus thuringiensis
effects of on predators of mosquito larvae, 483

Bemisia argentifolii
bifenthrin resistance in, 265
behavioral cues to cotton leaf surface features, 377
effects of imidacloprid on, 283
predation on by *Chrysoperla* spp., 33

Bemisia tabaci
exotic parasitoids of, 13

Chrysomya rufifacies
new distrubition record for, 477

Chrysoperla spp.
predators of *Bemisia argentifolii*, 33

Cotton
abaxial surface and emulsified leaf pH of, 369
infestation by pink bollworm, 229
leaf surface features as behavioral cues to silverleaf whiteflies, 377
spatial information technologies for detecting whiteflies, 421

Culex pipiens
as prey of aquatic predators, 471

Curculio caryae
mating behavior of, 479

Cydia caryana
larval response to low temperatures, 225

Cylindrocopturus adspersus
gustatory and olfactory responses to plant spp., 255

Diuraphis noxia
effects of aphicides on feeding behavior of, 145
effect of on carbohydrate content in wheat seedlings, 167

Dung beetles
effects of ivermectin pour-on for cattle on, 451

Empoasca bitubera
biology of in New Mexico, 117

Eoreuma loftini
activity patterns of in sugarcane, 23

Erythroneura spp.
ovipositon by on grape leaves in western Texas, 141

Franklinella occidentailis
deterred from entering greenhouses, 135

Grapes
ovipositon by *Erythroneura* spp on, 141

Haematobia irritans
SEM of ovipositor, 337

Helicoverpa zea
larvae affected by subfreezing temperatures in subtropics, 209
olfactometer bioassay for evaluating attractiveness of plant volatiles, 395
ovipostional response of to chickpea, 277

Heliothis virescens
backcross sterility in, 86
influence of sterile backcross releases on suppression, 433
ovipositional response of to chickpea, 277

Languria mozardi
occurrence of in six species of clover, 457

Onion
control of thrips on, 49

Pecans
IPM for aphids, 153
phytophagous and saprophagous arthorpods in, 303

Pectinophora gossypiella
infesting transgenic cotton, 229

Phyllocnistis citrella
native parasitoids of in Mexico, 349

Phyllophaga crinita
duration of pupal stage and adult longevity of, 55

Phyllophaga spp.
flight periods of parasites of in Texas, 317

Pogonomyrmex barbatus
spatial informtion technologies for detecting in pasture lands, 421

Pogonomyrmex tenuispina
predation on by spiders in Mexico, 213

Reductoripoda absoluta
new species from Mexico, 465

Schizaphis graminum
effects of seed treatment of sorghum hybrids, 181

Solenopsis geminata
comparative dessication rate of, 173

Solenopsis invicta
comparative dessication rate of, 173
polygyny in, 387
subcuticular iron-containing tissue in, 247

Solenopsis xyloni
comparative dessication rate of, 173

Sorghum
aphidophagous coccinellidae in, 237
effect of seed treatment of on greenbug, 181
residue destruction of on sorghum midge abundance, 345
resistance to sorghum midge

Spiders
seasonal variation of in Mexico, 331

Spodoptera exigua
tolerance to insecticides and response to IGR's, 127
toxicity of four insecticides to, 189

Stenpdiplosis sorghicola
effect of sorghum residue destruction on, 345
overwintering survival, 341
resistance to by sorghum hybrids, 105

Stomoxys calcitrans
excretion in, 43

Sugarcane
activity patterns of Mexican rice borer in, 23

Tabanus abactor
effect of trap design and color in evaluating activity of, 1

Thrips tabaci
control of with pesticides, 49

Timulla centroamericana
parasitoid of *Liris*, 205

Traps
new for Scarabidae, 325

Wheat
abundance and distribution of insects in stored grain, 75

Statement of Ownership, Management, and Circulation

(Required by 39 U.S.C. 3685)

1. Publication Title	2. Publication No.	3. Filing Date
SOUTHWESTERN ENTOMOLOGIST	3 2 4 - 0 5 0	September 30, 1996

4. Issue Frequency	5. No. of Issues Published Annually	6. Annual Subscription Price
Quarterly (March, June, September, October)	4	$10 Individual $20 Institution

7. Complete Mailing Address of Known Office of Publication *(Street, City, County, State, and ZIP+4) (Not Printer)*

Southwestern Entomological Society, c/o Allen Knutson
17360 Coit Road, Dallas, TX 75252

8. Complete Mailing Address of Headquarters or General Business Office of Publisher *(Not Printer)*

Same as above

9. Full Names and Complete Mailing Addresses of Publisher, Editor, and Managing Editor *(Do Not Leave Blank)*

Publisher *(Name and Complete Mailing Address)*

Southwestern Entomological Society, Department of Entomology
Texas A&M University, College Station, TX 77843

Editor *(Name and Complete Mailing Address)*

D. E. Bay
Department of Entomology, Texas A&M University, College Station, TX 77843

Managing Editor *(Name and Complete Mailing Address)*

10. Owner *(If owned by a corporation, its name and address must be stated and also immediately thereafter the names and addresses of stockholders owning or holding 1 percent or more of the total amount of stock. If not owned by a corporation, the names and addresses of the individual owners must be given. If owned by a partnership or other unincorporated firm, its name and address as well as that of each individual must be given. If the publication is published by a nonprofit organization, its name and address must be stated.) (Do Not Leave Blank.)*

Full Name	Complete Mailing Address
Southwestern Entomological Society	Department of Entomology, Texas A&M University College Station, TX 77843

11. Known Bondholders, Mortgagees, and Other Security Holders Owning or Holding 1 Percent or More of Total Amount of Bonds, Mortgages, or Other Securities. If none, check here. ☐ None

Full Name	Complete Mailing Address
None	N/A

12. For completion by nonprofit organizations authorized to mail at special rates. The purpose, function, and nonprofit status of this organization and the exempt status for federal income tax purposes: *(Check one)*

☒ Has Not Changed During Preceding 12 Months
☐ Has Changed During Preceding 12 Months
(If changed, publisher must submit explanation of change with this statement)

PS Form **3526**, October 1994 *(See Instructions on Reverse)*

13. Publication Name	14. Issue Date for Circulation Data Below	
Southwestern Entomologist	June 1996	
15. Extent and Nature of Circulation	Average No. Copies Each Issue During Preceding 12 Months	Actual No. Copies of Single Issue Published Nearest to Filing Date
a. Total No. Copies *(Net Press Run)*	525	525
b. Paid and/or Requested Circulation (1) Sales Through Dealers and Carriers, Street Vendors, and Counter Sales *(Not Mailed)*	0	0
(2) Paid or Requested Mail Subscriptions *(Include Advertisers' Proof Copies/Exchange Copies)*	500	486
c. Total Paid and/or Requested Circulation *(Sum of 15b(1) and 15b(2))*	500	486
d. Free Distribution by Mail *(Samples, Complimentary, and Other Free)*	6	9
e. Free Distribution Outside the Mail *(Carriers or Other Means)*	0	0
f. Total Free Distribution *(Sum of 15d and 15e)*	6	9
g. Total Distribution *(Sum of 15c and 15f)*	506	495
h. Copies Not Distributed (1) Office Use, Leftovers, Spoiled	19	30
(2) Return from News Agents	0	0
i. Total *(Sum of 15g, 15h(1), and 15h(2))*	525	525
Percent Paid and/or Requested Circulation *(15c / 15g x 100)*	98%	98%

16. This Statement of Ownership will be printed in the Dec. 1996 issue of this publication. ☐ Check box if not required to publish.

17. Signature and Title of Editor, Publisher, Business Manager, or Owner — Allen Knutson — Date: Sept 30, 1996

I certify that all information furnished on this form is true and complete. I understand that anyone who furnishes false or misleading information on this form or who omits material or information requested on the form may be subject to criminal sanctions *(including fines and imprisonment)* and/or civil sanctions *(including multiple damages and civil penalties)*.

Instructions to Publishers

1. Complete and file one copy of this form with your postmaster on or before October 1, annually. Keep a copy of the completed form for your records.
2. Include in items 10 and 11, in cases where the stockholder or security holder is a trustee, the name of the person or corporation for whom the trustee is acting. Also include the names and addresses of individuals who are stockholders who own or hold 1 percent or more of the total amount of bonds, mortgages, or other securities of the publishing corporation. In item 11, if none, check box. Use blank sheets if more space is required.
3. Be sure to furnish all information called for in item 15, regarding circulation. Free circulation must be shown in items 15d, e, and f.
4. If the publication had second-class authorization as a general or requester publication, this Statement of Ownership, Management, and Circulation must be published; it must be printed in any issue in October or the first printed issue after October, if the publication is not published during October.
5. In item 16, indicate date of the issue in which this Statement of Ownership will be printed.
6. Item 17 must be signed.

Failure to file or publish a statement of ownership may lead to suspension of second-class authorization.

PS Form **3526**, October 1994 *(Reverse)*