COTTON APHID[1] RESPONSE TO NITROGEN FERTILITY IN DRYLAND COTTON

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ABSTRACT

Cotton aphid, *Aphis gossypii* Glover, response to nitrogen fertility and planting date was investigated at the Texas Agricultural Experiment Station at Chillicothe in 1992-1994. Nitrogen was applied at 0, 32, 62, and 88 lb./acre just prior to planting cotton in late April, late May, and late June. Aphid density and percentage leaf nitrogen were estimated once a week in top- and bottom-half leaves. There was a significant, positive linear correlation between aphid density and fertility level in the late May planting date only. In multiple regression analyses, planting date was the most important variable affecting aphid density during August. Nitrogen fertility levels of 62-88, 32-62, and 0-32 lb/acre are recommended for cotton planted in late April, late May, and late June, respectively; these levels provided acceptable yields in each planting date without increasing severity of cotton aphid infestations.

INTRODUCTION

The cotton aphid, *Aphis gossypii* Glover, became a serious problem on cotton in the Texas Rolling Plains in 1975. Population densities returned to low levels from 1976 to 1980, but since then, with the exception of 1983, infestations have fluctuated from moderate to heavy, with high densities occurring in 1981, 1990, 1991, 1993, and 1995 (Boring 1975-1995). This general trend of increasingly high infestation levels since the mid-1980's has occurred throughout West Texas (Leser et al. 1992, Rummel et al. 1995).

While yield reductions caused by the cotton aphid in the Texas Rolling Plains generally have been low, this pest reduced yields an estimated 5.94% in 1991 (Head 1992). When populations exceed 50 aphids per leaf for 3 wk, yield reductions exceeding 100 lbs lint per acre occur in irrigated cotton (Fuchs and Minzenmayer 1995).

Entomologists realized that cotton aphids were less susceptible to insecticidal control because higher rates than previously used were required by 1989, and resistance to organophosphate insecticides was suspected in 1991 (Leser et al. 1992). Cultural control strategies were developed to lessen reliance on insecticides. For example, Slosser et al. (1992) reported that cotton aphid populations were reduced by planting in late May in the Texas Rolling Plains. Rummel et al. (1995) found that cultivars with smooth leaves and

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planting densities of 3-6 plants per row ft reduced infestation levels.

Several reports have linked severity of cotton aphid infestations with amount of nitrogen applied to cotton. McGarr (1943) found that aphid numbers were positively correlated with percentage of nitrogen fertilizer when cotton was treated with calcium arsenate. Isely (1946) reported that the number of nymphs per female and length of reproductive period were higher for cotton aphids reared on plants given complete soil nutrients compared with aphids reared on plants deficient in nitrogen. In irrigated cotton, Villamayor (1976) reported that cotton aphid numbers were greater on cotton receiving nitrogen compared to numbers on cotton without nitrogen. Cotton aphid numbers were positively correlated with quantity of nitrogen fertilizer applied, and cotton irrigated at 10-day intervals and fertilized with 60 kg N/f had greater cotton aphid numbers than cotton irrigated at 20-day intervals and fertilized with 30 kg N/f (El-Fattah 1975).

Although planting date is known to significantly affect cotton aphid population dynamics (Slosser et al. 1992), the effect of nitrogen fertility level on cotton aphid populations in dryland cotton has not been studied in detail. Therefore, the objective of this study was to determine the influence of planting date and the interaction with nitrogen fertility on cotton aphid populations.

MATERIALS AND METHODS

Experimental Design. This study was conducted at The Texas Agricultural Experiment Station at Chillicothe in 1992, 1993, and 1994. 'Paymaster 145' cotton was planted each year in north-south row directions on 40" row spacings. Cotton was grown dryland each year. A 3 X 4 factorial experiment, with treatments arranged in a randomized complete block design with three replications, was utilized. Factor A was three planting dates: 27 April, 22 May, and 25 June, 1992; 26 April, 28 May, and 24 June, 1993; and 6 May, 31 May, and 24 June, 1994. In subsequent discussions, these are referred to as late April, late May and late June plantings. Seeds were planted at 6.7, 4.2, and 4.2/row ft in late April, late May, and late June plantings, respectively. Factor B was four nitrogen fertility levels of 0, 32, 62, and 88 lbs N/acre applied just prior to each planting. Phosphorous and potassium were omitted from the fertilizer. Planting date and fertility level treatments were kept in the same locations throughout the experiment. Individual plots were 20 rows wide by 100 ft long (0.15 acre).

A large population of overwintering boll weevils, *Anthonomus grandis* Boheman, colonized the 27 April 1992 planting, numbering 523/acre. This planting was treated with azinphosmethyl at 0.25 lb AI/acre on 16 and 19 June and 2 July. Treatments were terminated before first blooms, which appeared on 9 July. These were the only insecticides applied during this study.

Aphid Sampling. Weekly sampling for cotton aphids was initiated at approximately pinhead square stage in each planting date. However, only the data collected from 28 July to 9 September 1992 (n = 7), 28 July to 27 September 1993 (n = 10), and 27 July to 30 August 1994 (n = 6) are summarized herein. These dates represent the period of rapid aphid population increase and decrease each year. Sample size was 20 top-half and 20 bottom-half leaves in each plot, but as aphid numbers increased in mid-August, sample size was reduced to 10 top and 10 bottom leaves (coinciding with densities of several hundred aphids per leaf), and finally to 5 top and 5 bottom leaves (coinciding with numbers exceeding 800-1000/leaf). Aphids were individually counted until populations reached about 100/leaf, after which numbers were estimated by counting groups containing 5 or 10 aphids per group. A leaf was picked from the plant, and examined immediately, every 2 to 3 steps along a selected row. Rows were randomly selected, and all top-half leaves were taken from the same row, and the sampler then selected a different row for bottom-half

leaves.

Plant phenology data, including numbers of squares and bolls, were taken once each week from 3 ft of row in each plot.

Leaf Nitrogen Determinations. On each sample date for aphids, 30 top-half and 30 bottom-half leaves were picked, minus the leaf petiole, from each plot. Rows were randomly selected as discussed previously for aphid sampling. Top-half and bottom-half leaves were placed in separate Ziplock® plastic bags and labelled with collection date, plot identification, and leaf location. These bags were immediately placed in an ice chest. Upon return to the laboratory at Vernon, Tex., leaves were stored at 15°F in a chest freezer for later analysis.

Percentage leaf nitrogen content was determined with a near infrared spectrophotometry (Technicon Infra Analyzer, Model 450)(NIRS) technique developed in our laboratory (WEP, unpublished data). The NIRS estimates of percentage nitrogen were based on a multiple regression and correlation analysis of the relationship between NIRS predicted N and actual Kjeldahl N (AOAC 1990). The model is based on four wavelengths (1818, 2139, 2180, and 2348 nm), and $R^2 = 0.955$ ($n = 107$, $SE = 0.026$) with an estimated prediction error of 0.263. Quality control procedures in our laboratory for Kjeldahl nitrogen determinations utilize a coefficient of variation of 2.00%. Expressed as a percentage, the estimated prediction error of the NIRS model was 1.64%.

Yield. Yield data were obtained by hand-picking two, 13.1 row ft sections in each plot after plant growth had been terminated by a killing freeze. Samples were taken on 30 November 1992 and 1994 and on 8 November 1993. A small laboratory gin was used to separate the lint and seed.

Data Analyses. Aphid counts and percentage nitrogen values were averaged over all sampling dates each year for analysis. Data were analyzed by analysis of variance for a 3 X 4 factorial experiment, arranged as randomized complete blocks. Mean squares and F-ratios were calculated as defined by McIntosh (1983) for experiments combined over years. Analyses (MSTAT Development Team 1988) were performed using the FACTOR and RANGE programs of MSTAT-C, and means were separated using protected least significant difference (LSD) ($\alpha = 0.05$). When only two means were compared, LSD was used. Linear and multiple regression analyses were conducted using the REGR and MULTIREG programs of MSTAT-C.

RESULTS AND DISCUSSION

Aphid Populations. The main effects for year and planting date significantly affected cotton aphid density (Table 1). Aphid numbers were significantly higher in 1993

TABLE 1. Average Number of Cotton Aphids per Leaf in Relation to Year and Planting Date. Chillicothe, TX.

Planting Date	Year[a] 1992	1993	1994	Average
Late April	26.1 Bb	112.0 Ba	15.2 Bb	51.1 B
Late May	23.7 Bb	75.4 Ca	16.8 Bb	38.6 B
Late June	47.6 Ac	144.3 Aa	100.7 Ab	97.5 A
Average	32.5 b	110.5 a	44.2 b	

[a]Values followed by a common lowercase letter within planting dates and by a common uppercase letter within years are not significantly different (P>0.05, LSD).

compared with numbers in 1992 and 1994, and numbers were significantly higher in cotton planted in late June compared with numbers in cotton planted in late April and late May. In 1993, the late May planting had the lowest numbers of aphids. The relationship between aphid numbers and planting date agrees with the earlier report by Slosser et al. (1992) for the years 1988 and 1989. These 5 years of data (1992-1994 reported herein plus the 1988 and 1989 data) show that a late June planting, in the Texas Rolling Plains, is very susceptible to development of high aphid populations.

The main effect for nitrogen fertility level at planting was not significant, indicating that there was no relationship between fertility level and subsequent aphid density (Table 2), and the planting date by fertility level interaction was not significant. However, there

TABLE 2. Average Number of Cotton Aphids per Leaf in Relation to Planting Date and Nitrogen Fertility at Planting. Chillicothe, TX.

Planting Date	Lbs. N Applied at Planting[a]				
	0	32	62	88	Average
Late April	50.1	56.1	44.0	54.1	51.1 B
Late May	32.6	38.3	38.5	45.2	38.6 B
Late June	88.2	85.1	113.8	103.0	97.5 A
Average	57.0 a	59.8 a	65.4 a	67.4 a	

[a]Values followed by a common lowercase letter within planting dates and by a common uppercase letter within years are not significantly different (P>0.05, LSD).

was a significant, positive linear correlation between fertility level at planting and aphid density in the late May planting date ($r = 0.946$, $P = 0.054$). Linear correlations were not significant for the late April ($r = -0.019$, $P > 0.300$) or late June ($r = 0.717$, $P = 0.283$) plantings.

The differential effects of nitrogen fertility level on aphid population development, as indicated by linear regression and correlation, may be related to plant phenology at the time aphid populations increase. The percentages of fruiting forms (squares plus bolls) that were bolls during early August, when aphid populations began to increase, were about 61, 38, and 1% for the late April, late May, and late June plantings, respectively. Thus, the late April cotton had a high boll load and was maturing during August, while the late June cotton was immature and just beginning to develop flowers. The late May cotton was intermediate between these extremes and was flowering heavily.

Although the main effect for leaf location on the plant (plant half) was not significant, the interaction between leaf location and year of study did influence aphid numbers (Table 3). When averaged over all 3 planting dates, there were no differences in aphid density between top and bottom leaves in 1992. In 1993 there were more aphids on bottom leaves than on top leaves, while in 1994 there were more aphids on top leaves than on bottom leaves.

The interaction between leaf location and planting date significantly influenced aphid numbers (Table 4). There were more aphids on bottom leaves than on top leaves in cotton planted in late April, while there were more aphids on top leaves than on bottom leaves in the late June planting. There were no differences between top and bottom leaves in cotton planted in late May. These trends are not similar to those reported by Slosser et al. (1992).

TABLE 3. Average Number of Cotton Aphids per Leaf and Average Percentage Leaf Nitrogen in Relation to Year and Plant Half. Chillicothe, TX.

Year	No. Aphids per Leaf[a]		% Leaf Nitrogen[a]	
	Top Half	Bottom Half	Top Half	Bottom Half
1992	34.8 a	30.1 a	4.19 a	3.95 a
1993	95.3 b	125.8 a	4.24 a	3.43 b
1994	57.4 a	31.0 b	4.23 a	3.37 b
Average	62.5 a	62.3 a	4.22 a	3.58 b

[a]Values are compared between top and bottom halves of the plant within years, and values with a common letter are not significantly different (P>0.05, LSD).

TABLE 4. Average Number of Cotton Aphids per Leaf and Average Percentage Leaf Nitrogen in Relation to Planting Date and Plant Half. Chillicothe, TX.

Planting Date	No. Aphids per Leaf[a]		% Leaf Nitrogen[a]	
	Top Half	Bottom Half	Top Half	Bottom Half
Late April	45.5 b	56.7 a	4.10 a	3.39 b
Late May	38.1 a	39.1 a	4.05 a	3.40 b
Late June	103.9 a	91.1 b	4.51 a	3.96 b
Average	62.5 a	62.3 a	4.22 a	3.58 b

[a]Values are compared between top and bottom halves of the plant within planting dates, and values with a common letter are not significantly different (P>0.05, LSD).

These data (Tables 3 and 4) indicate that both year of study and planting date influence aphid abundance on top- or bottom-half leaves. At present, the influence of year can not be predicted, and the influence of planting date is not consistent (data in Table 4 and Slosser et al. 1992). These results do not agree with those of Hardee et al. (1994) who reported that more aphids occurred in lower plant canopy in Mississippi. O'Brien et al. (1993), also reporting from Mississippi, found higher levels of aphids in the middle of the plant canopy compared to numbers in the upper or lower canopy. There does not seem to be any consistency among years, planting dates, or geographic location pertaining to aphid abundance within the plant canopy. Therefore, aphids should be sampled from 2 to 3 strata within the plant to obtain reliable estimates of population density.

Leaf Nitrogen. The main effects for year and planting date influenced percentage leaf nitrogen (Table 5). Leaf nitrogen was greater in cotton planted in late June than in cotton planted in late April or late May, which agrees with the 1989 data for leaf nitrogen reported by Slosser et al. (1992). Nitrogen levels were greater in 1992 than in 1993 or 1994. The year by planting date interaction was significant, an indication that trends in leaf nitrogen levels were not consistent from year to year.

The main effect for nitrogen fertility level at planting significantly influenced leaf nitrogen levels during August (Table 6). Highest levels of leaf nitrogen occurred when cotton was fertilized with 88 lb N/ac at planting and lowest levels occurred at 0 lb N/ac. There were significant linear correlations between nitrogen fertility levels at planting and percentage leaf nitrogen levels during August in the late April ($r = 0.918$, $P = 0.082$) and

TABLE 5. Average Percentage Leaf Nitrogen in Relation to Year and Planting Date. Chillicothe, TX.

Planting Date	Year[a] 1992	1993	1994	Average
Late April	3.76 Cb	3.90 Aa	3.58 Bc	3.74 B
Late May	4.07 Ba	3.62 Bb	3.48 Bc	3.72 B
Late June	4.39 Aa	3.97 Ab	4.35 Aa	4.24 A
Average	4.07 a	3.83 b	3.80 b	

[a]Values followed by a common lowercase letter within planting dates and by a common uppercase letter within years are not significantly different (P>0.05, LSD).

TABLE 6. Average Percentage Leaf Nitrogen in Relation to Planting Date and Nitrogen Fertility at Planting. Chillicothe, TX.

Planting Date	Lbs. N Applied at Planting[a] 0	32	62	88	Average
Late April	3.61 Bc	3.75 Bb	3.73 Cbc	3.88 Ba	3.74 B
Late May	3.24 Cc	3.75 Bb	3.92 Ba	3.98 Ba	3.72 B
Late June	4.02 Ab	4.30 Aa	4.35 Aa	4.27 Aa	4.24 A
Average	3.62 c	3.93 b	4.00 ab	4.05 a	

[a]Values followed by a common lowercase letter within planting dates and by a common uppercase letter within nitrogen fertility levels are not significantly different (P>0.05, LSD).

late May ($r = 0.934$, $P = 0.066$) planting dates. The linear correlation was not significant for the late June planting date ($r = 0.733$, $P = 0.267$). Thompson et al. (1976) reported that leaf nitrogen concentrations at the end of the season were directly related to initial application rates. This supports our findings for the late April and late May plantings because cotton is rapidly maturing during August in these planting dates. Thompson et al. (1976) also reported that early season leaves have uniformly high levels of nitrogen regardless of initial application rate, which is what we found during August for cotton planted in late June.

There was a positive linear correlation between aphid density (Table 2) and percentage leaf nitrogen (Table 6) during August only in the late May ($r = 0.877$, $P = 0.123$) planting date. The linear correlations were not significant for the late April ($r = 0.380$, $P > 0.300$) and late June ($r = 0.550$, $P > 0.300$) planting dates. The range of leaf nitrogen percentages was relatively small in the late April planting date, with a low value of 3.61 and a high of 3.88 (Table 6). Perhaps the average leaf nitrogen percentages were too low and the range too narrow to elicit an aphid density response, resulting in uniform aphid densities in the late April planting date (Table 2). In the late June planting date, the range in leaf nitrogen percentages was small, with a low value of 4.02 and a high of 4.35. However, average percentages exceeded 4.0% in all fertility levels. Although aphid numbers were high, there were no density responses to initial fertility levels (Table 2).

Timing of population increase has consistently occurred during August, in all three

planting dates, for seven consecutive years (1988- 1994), with peak population densities occurring between mid-August and mid-September. The data reported herein indicate that nitrogen fertility at planting influences ultimate aphid density in dryland cotton planted in late May only. However, highest aphid densities during August have always occurred in cotton planted in late June, and late June cotton is just beginning to flower during August. O'Brien et al. (1993) reported that populations increase rapidly at onset of flowering in Mississippi. In our study, average nitrogen levels in leaves exceeded 4% in late June-planted cotton, which indicates that an immature plant, with a low boll load and high leaf nitrogen content during August, is most susceptible to aphids.

Percentage leaf nitrogen was higher in top-half leaves, as compared to percentages in bottom-half leaves, in all three years (Table 3). Percentage leaf nitrogen was higher in top-half leaves in all three planting dates (Table 4). The interaction between fertility level and leaf location was not significant. However, highest aphid densities did not occur consistently in top-half leaves (Tables 3 and 4). Apparently, there are other environmental or biological factors, in addition to percentage leaf nitrogen, that regulate aphid abundance within the plant canopy in dryland cotton.

Multiple Regression Analyses. The variables affecting aphid density that were investigated included year, planting date, percentage leaf nitrogen levels within the four fertility levels applied at planting, and plant half. The main effect for year was the most important variable in the analysis of variance, accounting for 39.9% of the total variation in aphid density. The main effects for planting date, fertility level, and plant half were responsible for 21.7, 0.6, and 0.0003% of the total variation.

Multiple regression analyses were conducted using these four factors as independent variables and aphid density as the dependent variable. When the data for all years were used (Equation 1, Table 7), planting date was the only significant variable. Within individual years (Equations 2, 3, and 4; Table 7), planting date consistently influenced aphid density, and it was the only significant variable in 1992. In 1993, planting date, plant half, and percentage leaf nitrogen significantly influenced aphid density, and these three variables explained 71.2% of the variation. In 1994, planting date and percentage leaf nitrogen were significant. When averaged over all 3 years (Equation 5, Table 7), planting date, plant half and percentage leaf nitrogen were significant. Percentage leaf nitrogen was a significant variable in two of three years (1993 and 1994) which explains the significance of this variable in the average model (Equation 5, Table 7). Plant half was a significant variable in 1993 only, but aphid populations were very high in 1993 (Table 1), and the average model (Equation 5, Table 7) was probably influenced by the relationship in 1993. These regression analyses indicate that planting date is one of the most important variables affecting aphid density during August, and in some years (environments) plant half and percentage leaf nitrogen influence aphid densities, also.

Yield. The main effects for planting date and nitrogen fertility level at planting significantly influenced yield (Table 8). Yields decreased as planting was delayed after late April; this is the same trend reported by Bevers and Slosser (1992) for dryland cotton. Yields increased with increasing nitrogen from 0 to 62 lb N/ac, but significant increases were not obtained at 88 lb N/ac. The planting date by fertility level interaction was not significant. Average yields for 1992, 1993, and 1994 were 612.3, 545.4, and 294.9 lb lint per acre, respectively (LSD = 48.2, α = 0.05)

Pettit et al. (1994) suggested that manipulation of fertility level could be a useful cotton aphid management strategy, provided that the nitrogen level selected to reduce aphids did not reduce yields also. In this context, Villamayor (1976) reported that nitrogen fertilized cotton plants suffered more insect damage, but the fertilized plants produced higher yields than did unfertilized plants. The interaction LSD = 96.4 lbs lint/acre (α = 0.05, data in Table 8) is useful for selecting nitrogen application levels within each planting

TABLE 7. Multiple Regression Analysis of Relationship Between Average Number of Cotton Aphids per Leaf and Planting Date, Leaf Location on Plant, Percentage Leaf Nitrogen, and Year of Study. Chillicothe, TX.

	Equation[a]	n	a	b_1x_1	b_2x_2	b_3x_3	b_4x_4	R2	$S_{y.1...k}$
1.	All Years	72	-101.53	17.58 (PD)	14.35 (Half)	22.91 (%N)	8.94 (Y)	0.184	45.78
	s_b			7.73	14.95	16.30	6.96		
	probability			0.026	0.340	0.164	0.203	0.008	
2.	1992 Only	24	3.91	9.59 (PD)	-3.80(Half)	3.71 (%N)	-	0.454	10.98
	s_b			4.03	5.01	9.34	-		
	probability			0.026	0.455	0.695	-	0.006	
3.	1993 Only	24	-407.43	12.89 (PD)	102.19(Half)	88.48 (%N)	-	0.712	22.12
	s_b			5.56	15.99	16.28	-		
	probability			0.030	<0.001	<0.001	-	<0.001	
4.	1994 Only	24	-196.47	25.37 (PD)	12.23 (Half)	45.14 (%N)	-	0.770	23.65
	s_b			8.79	17.35	16.86	-		
	probability			0.008	0.488	0.013	-	<0.001	
5.	All Years Averaged	24	-201.20	10.96 (PD)	31.46 (Half)	49.86 (%N)	-	0.676	16.89
	s_b			5.57	11.62	14.74	-		
	probability			0.061	0.013	0.003	-	<0.001	

[a]Equation format: $y=a+b_1x_1....b_kx_k$ where y = average number of aphids per leaf, a=intercept, x_1=planting date (PD coded as 1, 2 or 3 for April, May and June, respectively), x_2=plant half (Half coded as 1 or 2 for top or bottom half, respectively), x_3=leaf nitrogen (%N in the four fertility levels at planting), and x_4=year of study (Year coded as 1, 2, or 3 for 1992, 1993 and 1994, respectively).

TABLE 8. Yield (lb lint per acre) in Relation to Planting Date and Nitrogen Fertility at Planting. Chillicothe, TX. 1992-1994.

Planting Date	Lbs. N Applied at Planting[a] 0	32	62	88	Average
Late April	688.1	644.5	719.7	774.8	706.8 A
Late May	514.7	592.5	643.3	580.0	582.6 B
Late June	132.9	164.4	175.5	179.7	163.1 C
Average	445.2 b	467.1 ab	512.8 a	511.5 a	

[a]Values followed by a common lowercase letter within planting dates and by a common uppercase letter within nitrogen fertility levels are not significantly different (P>0.05, LSD).

date for dryland cotton not treated with insecticides. For cotton planted in late June, nitrogen fertility ranging from 0 to 32 lb/acre (Table 8) provided acceptable yields while minimizing aphid densities (Table 2). For late May cotton, 32-62 lb N/acre (Table 8) produced the highest yields without aggravating aphid infestations (Table 2). For late April cotton, 62-88 lb N/ac produced the highest yields without increasing severity of aphid infestations (Table 2). These recommendations to reduce nitrogen inputs with planting dates after late April are probably justified because yields also decrease with later plantings.

ACKNOWLEDGMENT

This research was supported in part by grants from The Cotton Foundations (Project #III.5) and Cotton Incorporated, Texas State Support Committee (Cooperative Agreement No. 93-893TX). Additional support was provided by Texas Agricultural Experiment Station projects H-8136 and H-6819 (Vernon) and H-6035 (Lubbock). We appreciate the technical assistance of Bobby Idol, Jan Worrall and Jay Hunt.

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EFFECTS OF ENVIRONMENTAL CONDITIONS ON DIAPAUSE IN NATIVE POPULATIONS OF PINK BOLLWORM[1]

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ABSTRACT

Factors affecting induction and termination of diapause in pink bollworm, *Pectinophora gossypiella* (Saunders), were studied in several trials with field-collected animals under simulated field conditions from 1983 to 1994. Percentage diapause on 1 Oct had a significant correlation of 0.883 with chill units during the 2-wk period 8-21 Sept. In controlled environments, induction of diapause was delayed by higher temperature (32°C) combined with longer photoperiod (14:10 LD) and accelerated by cooler temperatures (30/15°C) combined with shorter photoperiod (10:14 LD). Additionally, larvae exposed to the modified and controlled environmental regimes for a longer period of time experienced a greater effect. One-, two-, or three-day exposure to the high temperature and longer days had no effect on larval survival 20 days after the beginning of exposure for diapausing larvae cultured at the lower temperatures and shorter days for almost four months and returned to the original culture conditions after exposure. However, exposure for 14 d at the high temperature and long days caused a marked reduction in percentage of larvae remaining in diapause. Results for 5-d exposure at the warm temperature and long photoperiod were intermediate. Moisture, combined with increased temperature and day length, caused earlier release from diapause than increased temperature and day length alone.

INTRODUCTION

Pink bollworm (PBW), *Pectinophora gossypiella* (Saunders), overwinter as diapausing, last-instar larvae in cotton seeds, cotton bolls, plant trash, and soil. The induction, maintenance, and termination of diapause provide synchronization of development and reproduction to favorable times and enhance survival during unfavorable periods within rhythmic seasonal cycles (Tauber and Tauber 1973). This capacity for diapause in PBW enables the pest to reinfest the next season's cotton crop in the desert Southwest.

Early researchers in many regions of the world, reviewed by Lukefahr et al. (1964), associated boll age and cool autumn

[1]Lepidoptera: Gelechiidae

temperatures with the induction of diapause. Lukefahr (1961), however, had previously established that diapause in the PBW was under photoperiodic control. Additional research indicated that photoperiod was the primary inductive stimulus but that the extent of response may be modified by temperature and boll age, the latter a result of changing nutrition of bolls as they mature (Adkisson et al. 1963, Adkisson 1964, Lukefahr et al. 1964, Crowder et al. 1975). In laboratory studies, Bull and Adkisson (1960) concluded that, regardless of PBW artificial diet, temperatures of 21°C or lower induced high percentages of larvae to enter diapause. Additional work in Texas demonstrated that photoperiod and moisture interacted to terminate PBW diapause (Wellso and Adkisson 1964). Lukefahr et al. (1964) found that a mean temperature of 21°C, compared to 27.5°C, partially overcame the diapause-averting effect of long photoperiods. Foster and Crowder (1976) used 20°C as the diapause-inducing temperature in their studies on the effects of dietary lipids. Gutierrez et al. (1981) concluded that the photoperiod response in PBW may be overridden as temperatures decrease, especially below 20°C. Bariola and Henneberry (1980) demonstrated a highly significant correlation between the occurrence of 29°C daily average temperatures and the beginning of diapause.

As noted by Barry and Adkisson (1966), diapause in an individual insect is an all or nothing response and is expressed phenotypically even though several genetic factors may interact to promote or avert the phenotypic change. The failure of pink bollworm larvae to pupate was used as the index for diapause in all papers referenced above. Recently, however, the titer of a diapause-associated protein, termed pectinophorin, has been used to (1) describe the induction, maintenance, and termination of PBW diapause (Salama and Miller 1992, 1993) and (2) determine whether individual PBW are in diapause (Miller et al. 1993). Miller and Fryxell (1993) concluded that there are three stages of PBW diapause which they termed early, mid, and late. At mid diapause, about 90 days after initiation of diapause, the titer of pectinophorin as a percentage of hemolymph protein reaches a maximum and declines thereafter. Disturbance of larvae in mid diapause, by a variety of means, caused extensive larval mortality. Using the pectinophorin assay, Miller and Salama (1992) suggested that even though the commitment to enter or break diapause may be made instantly, the ability of larvae to change biochemically is delayed up to three days.

During the years 1983-1994, a number of PBW diapause studies were conducted in California's Palo Verde Valley and in Riverside, California. Taking a cue from Bariola and Henneberry (1980), "that minimum temperature, number of hours per day at the minimum temperature or below a threshold temperature, and numbers of days in succession at an average temperature below a certain threshold" may be involved in the induction of PBW diapause, we related chill units to percentage diapause under ambient simulated field conditions in six of those years. Additionally, experiments were conducted to clarify the effects of crop termination regimes and of cotton species, on diapause induction in the PBW. We also conducted experiments using controlled environment chambers, as well as ambient simulated field conditions, to compare the effects of temperature/day length shifts on diapause induction, maintenance, and termination.

MATERIALS AND METHODS

Standard Methods. All studies involved the field collection of green cotton bolls infested with PBW. Collected bolls were randomly placed in screen-ventilated, incubation boxes with approximate dimensions of 30 x 23 x 13 cm (Fye 1976). Bolls rested on a mesh of hardware cloth elevated above the bottom of the incubation box by 1.5 cm^3 wooden blocks. The bottom of the box was covered with a paper towel layer which served as a site for larvae to spin their hibernacula. Numbers of bolls per box varied due to extent of PBW infestation and availability of appropriate bolls. Most often, boxes contained 100 bolls each but the number varied from 80 to 200. Unless stated otherwise, incubation boxes remained at ambient shaded conditions for two weeks at which time bolls were opened and all mature larvae, previously exited or removed from the bolls, were retrieved. Exited larvae were collected from the toweling, bract and calyx debris, and from wooden supports. In late fall, most mature larvae (estimate of 70-90%) were located within individual seeds, encased in their silken hibernacula. Unless specified otherwise, larvae were removed from their hibernacula.

Retrieved larvae were placed in 10 x 1.5 cm plastic petri dishes which contained previously cut-to-fit layer(s) of paper toweling or tissue. Petri dishes were maintained in the same ambient shaded conditions as were the bolls in the incubation boxes. Numbers of larvae per petri dish varied from about 10-20 depending on the experiment, quantity of larvae, availability of space, and time available for collection. If the only determination to be made was the number of adults or pupae versus larvae at the end of their culture period in petri plates, double layered paper toweling was sometimes used. Larvae sought confinement between layers to spin their encasements and separation of the toweling permitted rapid and accurate determination of the life forms present. Single layers of KimWipe® tissues were also satisfactory and were used often because they permitted improved observations through the bottom of the petri dishes without disturbing the larvae. Larvae that had not pupated in two weeks were considered diapaused. In situations requiring high numbers of larvae cultured for long periods of time, even single layers of KimWipe tissues did not always enable distinguishing between dead larvae, live or dead pupae, and dead adults that did not escape pupal cases or hibernacula. Frass and molds which invaded the diapaused larval sites sometimes prevented those precise determinations; most live PBW larvae were, however, easily observed through the bottom of petri dishes, even after many months of culture.

Study of Chill Units and Diapause Induction in PBW. The relationship between chill units and induction of fall diapause in PBW larvae was examined with data from six years (1983, 1985-1987, 1990, and 1993) for Upland cotton fields of similar maturity. Also similar in those years were: boll collections during September and October, two weeks incubation of bolls at outside temperatures in the shade, and two weeks of continued culture of larvae in petri dishes at the same outside location. The total numbers of larvae retrieved from bolls and used for the diapause studies in those years were 9,000, 2,282, 2,075, 3,289, 3,580, and 3,280, respectively. Points on figures which represent percentage diapause correspond to dates when bolls were collected in the fields.

Temperature values were obtained from Campbell Scientific CR-21 Microloggers at California Irrigation Management Information System stations 36 (north of Blythe) or 72 (south of Palo Verde) in the Palo Verde Valley. The University of California Statewide Integrated Pest Management Program's Degree-Day Utility User's Guide (Seaver et al. 1990) was utilized to calculate the number of degree days when temperatures were below a threshold value, also referred to as chill units. Four previously cited researchers selected either 20 or 21°C as a critical diapause-inducing temperature; on that basis we used 21°C. Daily computations for chill units used the single sine method, below 21°C, with no lower limit cutoff (Seaver et al. 1990). Total chill units were calculated for each of the seven weeks in the interval 18 Aug through 5 Oct. Correlations were calculated between percentage diapause on 1 Oct and chill units for each of the seven weeks, each of the six 2-wk periods, and each of the five 3-wk periods from 18 Aug through 5 Oct.

Studies on Effects of Crop Termination and Cotton Species on PBW Diapause Induction. In 1987, in addition to the data used in the correlation analysis of chill units and percentage diapause, we also collected data to examine the effect on PBW diapause induction of three crop termination regimes: 1) termination early/harvest early, 2) termination early/harvest late, and 3) termination late/harvest late. Dates of last irrigation, application of harvest aid chemicals (boll opener and defoliant), and first harvest defined earliness or lateness of the regimes (Table 1). Because the diapause responses over dates were curvilinear for the period of this study, September through the end of October, the percentage diapause was linearized by subtracting it from 101, then taking the natural logarithm (transformation to linearize an asymptotic regression). The three termination regimes were compared by fitting straight lines of the transformed diapause data versus date. Another experiment in 1987 used PBW infested bolls from two fields of similar age, one Upland and one Pima cotton, utilizing 1,830 and 1,960 larvae, respectively. The diapause

TABLE 1. Dates and Range of Dates of Crop Terminating Events, 1987.

Regime[a]	No. Fields	Last Water	®PREP[b]	®DEF[c]	First Pick	No. Larvae Used
TE-HE	4	17 Aug (14-18)	01 Sep	06 Sep (5-7)	17 Sep (15-21)	422
TE-HL	3	19 Aug (18-20)	02 Sep	07 Sep	26 Oct (26-28)	560
TL-HL	5	31 Aug (30-31)	20 Sep	29 Sep (28-30)	26 Oct (25-26)	884

[a] TE-HE = Termination Early-Harvest Early
TE-HL = Termination Early-Harvest Late
TL-HL = Termination Late-Harvest Late
[b] Ethephon, 2-Chloroethylphosphonic acid, a boll opener
[c] S,S,S - Tributylphosphorotrithioate, a defoliant

responses for Upland and Pima cottons were linear over the 1-month period of this study and were compared by fitting a straight line for the data points of percentage diapause versus date for each species. For both experiments, the standard methods of boll collection and incubation, and larval culture were used.

Studies to Compare PBW Diapause Induction under Field and Environmental Chamber Conditions. In the fall of 1990, an experiment was conducted which compared the extent of diapause induction when PBW infested bolls were held under outside ambient conditions and under two controlled environmental conditions. On each of five days, approximately every two weeks, beginning on 6 Sept and ending on 1 Nov 1990, green cotton bolls were collected from an unharvested cotton field heavily infested with PBW. Bolls were randomly placed in each of 15 incubation boxes. On each field collection day, five randomly selected boll boxes were placed under each of the following experimental conditions: (1) outside ambient conditions in the shade, (2) diapause-enhancing (30/15°C, 10:14 LD) and (3) diapause-averting (32°C, 14:10 LD). Temperatures and light regimes in the latter two conditions were maintained in environmental chambers. On the seventh day after incubation boxes were placed in the appropriate conditions, larvae that had exited the bolls were collected, placed in petri dishes at a rate of about 20 per dish, and returned to their respective culture conditions next to the bolls in the boxes. After an additional two weeks, numbers of larvae, pupae, and adults in the petri dishes were determined; those 1,548 larvae are referred to as short-term culture larvae. Two weeks after bolls were collected in the field and placed in incubation boxes, exited larvae were again obtained and combined with mature larvae found upon opening the bolls. All 2,032 larvae, referred to as long-term culture larvae, were placed in petri dishes and returned to the same culture conditions. Two weeks after those long-term larvae were placed in petri dishes, numbers of larvae, pupae, and adults were determined.

The treatment design in the above study was a three factor factorial with five boll collection dates, three environmental conditions, and two lengths of exposure to the environmental condition before placement of larvae in petri plates. Percentage diapaused larvae was calculated, excluding dead larvae, for each combination of treatments. For analysis of variance, percentage diapaused larvae was transformed to the arcsine of the square root of the proportion diapaused to homogenize the variances. The error term used for the analysis of variance was the error variance for binomial or percentage data transformed to the angular scale (about $821/n$, Snedecor and Cochran 1967). The numbers of larvae per treatment combination were variable, therefore the harmonic average, n, was used.

A similar experiment was conducted in 1993 except that PBW infested bolls were collected on only two dates, 29 Sept and 27 Oct.

Studies of Effects of Diapause-Enhancing and Diapause-Averting Conditions on Maintenance and Termination of PBW Diapause. Two studies of factors which might influence maintenance and termination of diapause were conducted in 1993/1994. The first of these compared the response of diapaused larvae to varying durations of exposure to diapause-averting conditions followed by a return to the original, diapause-enhancing conditions or to a colder regime. Bolls were

collected in the field on 27 Oct 1993 and placed in the diapause-enhancing conditions (30/15°C, 10:14 LD). Between 19-21 Feb 1994, bolls were opened and mature, diapaused larvae were transferred, 10 each, to 50 petri dishes. Dishes containing the larvae were returned to the same diapause-enhancing conditions overnight, allowing larvae to respin their hibernacula. With the exception of four dishes which stayed at diapause-enhancing conditions and four dishes which were moved to an even colder, constant temperature box (11°C, 11:13 LD photoperiod, hereafter referred to as "hard diapause"), all dishes were transferred the next morning to diapause-averting conditions (32°C, 14:10 LD). After 1, 2, 3, 5, and 14 d post transfer to the diapause-averting condition, four dishes per interval (three dishes at 14 d) were returned to the diapause-enhancing condition and four were placed in the hard diapause condition. One set of four dishes remained in the diapause-averting conditions. Dishes were examined on nine days between 8 Mar and 11 Jul. Numbers of live, diapaused larvae viewed through the bottom of the petri dishes were recorded. Final determinations were made on 10 Aug when plates were opened, tissues removed, and numbers of live and dead larvae, live and dead pupae, and adults were recorded. Effects of number of days at 32°C (six levels including 0 days at 32°C) and of "holding" temperature (two levels) plus the constant 32°C regime (13 treatments in all) were tested with an overall χ^2 test of independence in a 13 row by 2 column (outcome "yes" or "no") frequency table. If that test resulted in significant differences, individual comparisons were made with the χ^2 test of independence in 2 by 2 frequency tables.

The second experiment on diapause maintenance and termination was conducted using bolls collected on 29 Sept 1993, maintained at outside ambient conditions in the shade, and opened on 20-21 Oct. Thirty larvae, their hibernacula entwined with mature cotton fibers, were carefully removed and placed, still in their intact "Q-Tip" appearing structures, 10 each, in three petri dishes. They remained, undisturbed, at ambient conditions outside until 19 Jan 1994. On that date, before the three petri dishes were assigned to different environmental regimes, it was determined, by holding the dishes up to a light, that all 30 larvae remained alive and in the diapaused state. One dish was left at ambient conditions, one was transferred to the diapause-averting conditions (32°C, 14:10 LD), and one was transferred to the same diapause-averting conditions after wetting the fibers associated with the diapaused larvae. Water was added to the petri dish from a squeeze bottle; the excess water was poured off, leaving free water only associated with the fibers. Fibers in the latter dish were moistened again on 27 Jan and 10 Feb. Dishes were sealed with tape after the fibers were wet, or rewet, to reduce rapid drying. Dishes in the other culture conditions were also sealed, even though fibers remained dry. Numbers of live diapaused larvae were recorded, again by holding dishes up to light, at about weekly intervals into April but less frequently from then until 4 Aug. On 4 Aug, all encasements were opened and the numbers of live and dead PBW life stages recorded.

RESULTS AND DISCUSSION

Study of Chill Units and Diapause Induction in PBW. Although the relationship between the time of occurrence and magnitude of chill units below 21°C and an increase in

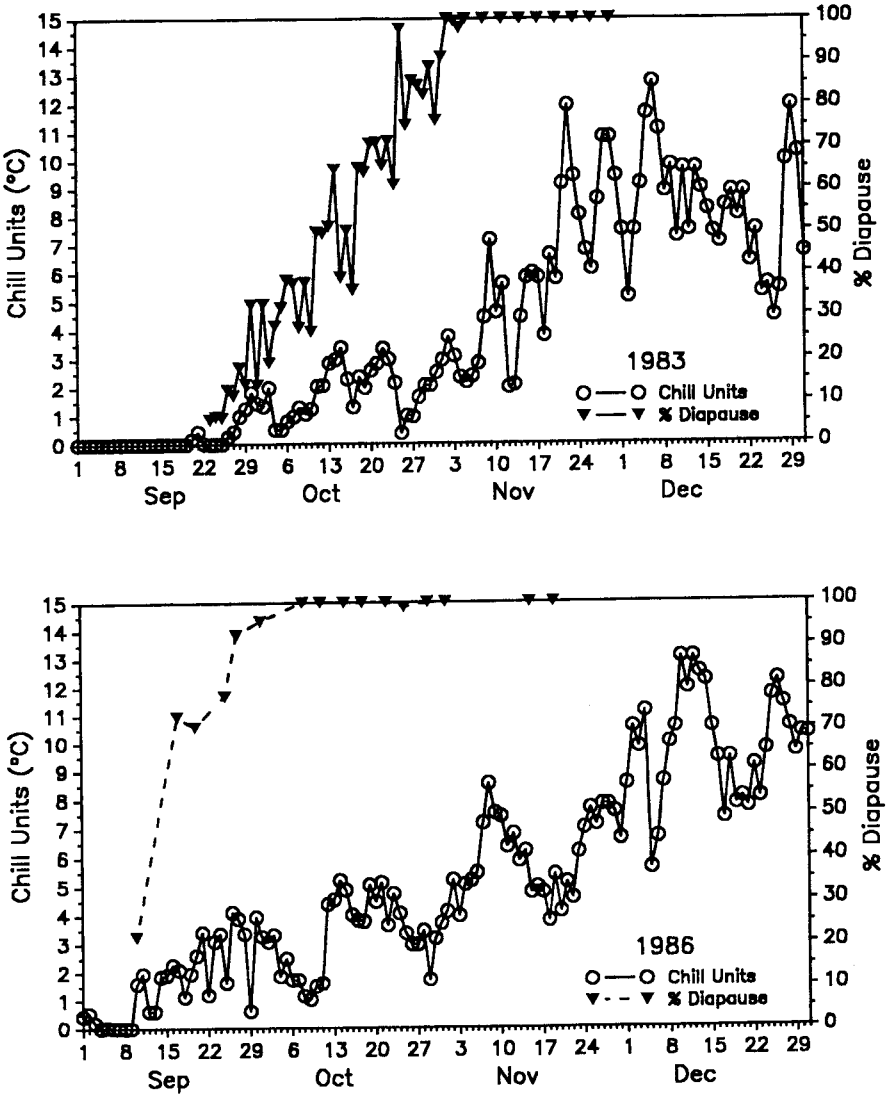

FIG 1. Chill units below 21°C and percentage pink bollworm diapause for 1983 (top) and 1986 (bottom); pink bollworm-infested green cotton bolls collected on the dates indicated and placed at ambient conditions in the shade; 9,000 and 2,075 larvae retrieved and placed into petri dish cultures at the same conditions, two weeks later, in 1983 and 1986, respectively.

percentage PBW diapause was more obvious in some years than others, trends were clear. The relationship between chill units, zero to nil or 2-4 units per day through September, and earliness of PBW diapause induction is shown for 1983 versus 1986 in Fig. 1. The leveling off of the diapause curve for larvae from bolls collected 18-25 Sept 1986 may be related to a sharp decrease in chill units 29 Sept and a general decrease during 4-12 Oct, about 2.5 weeks after boll collection and within the development period of some of those larvae. A similar shift in the rate of diapause was plotted by Adkisson (1964) and explained on the basis of temperature. Miller and Salama (1992) reported a decrease in pectinophorin titers in larvae, from bolls held under outside conditions, a few days after elevated min and max temperatures were recorded.

Correlations between weekly chill units from mid August to early October in six years and percentage PBW diapause are shown in Table 2. By 1 Oct, diapause was well established in all six years, the lowest was 23%, the highest was 96%. Chill units in weeks 4 and 5, taken singly, correlated most highly with diapause on 1 Oct (r = 0.732 and 0.796, respectively, $P \leq 0.05$) and together gave the highest 2-wk correlation (r = 0.883, $P \leq 0.01$). None of the three-successive-week correlations was as high. The relationship between percentage diapause and the mid September (weeks 4 and 5) chill units is shown in Fig. 2.

TABLE 2. Correlations Between Weekly Chill Units and Percentage Diapause on 1 October in six years (1983, 1985-1987, 1990, and 1993).

Chill Units for Weeks	Dates	Correlation[a]
1	18-24 August	-0.559
2	25-31 August	-0.287
3	01-07 September	0.406
4	08-14 September	0.732 *
5	15-21 September	0.796 *
6	22-28 September	0.586
7	29 Sept - 05 Oct	0.443
1 & 2	18-31 August	-0.410
2 & 3	25 Aug - 07 Sept	0.248
3 & 4	01-14 September	0.686
4 & 5	08-21 September	0.883 **
5 & 6	15-28 September	0.707
6 & 7	22 Sept - 05 Oct	0.606
1 to 3	18 Aug - 07 Sept	0.017
2 to 4	25 Aug - 14 Sept	0.704
3 to 5	01-21 September	0.829 *
4 to 6	08-28 September	0.857 *
5 to 7	15 Sept - 05 Oct	0.723

[a] 1-tailed test for $r > 0.000$; N = 6 years; critical r at $P \leq 0.05$ is 0.729 (*), $P \leq 0.01$ is 0.882 (**)

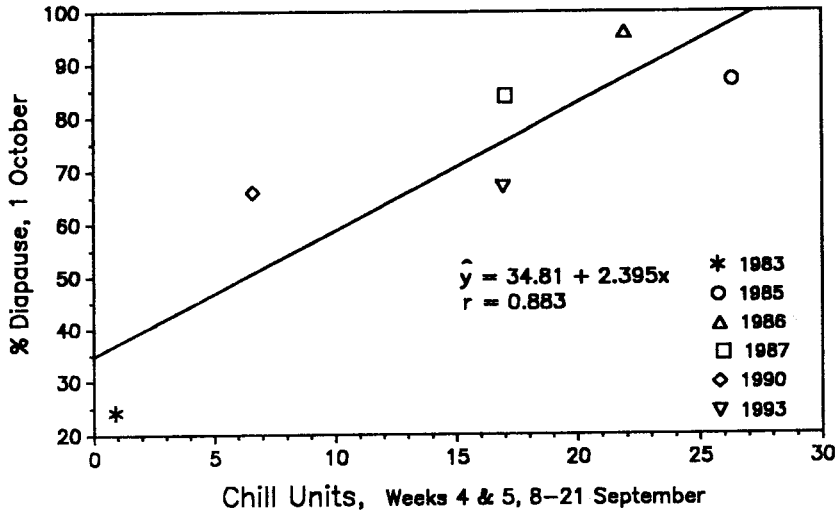

FIG 2. Relationship between percentage pink bollworm diapause on 1 October (larvae under ambient outside conditions in the shade) and chill units below 21°C in mid-September for six years. The correlation coefficient is significant at $P \leq 0.01$, one-tailed test. See text for definition of weeks.

The mid September chill units provided a measure of earliness (or lateness) of fall temperatures and hence correlated with level of diapause on 1 Oct.

Studies on Effects of Crop Termination and Cotton Species on PBW Diapause Induction. Timing of crop termination and harvest did not significantly impact the PBW diapause induction response over time in 1987 for the three situations studied (Table 1, Fig. 3). The response curves were linearized by transforming percentage diapause to log(101 - % diapause). Slopes of the resulting straight lines were not significantly different, nor were the levels of diapause at the mean date. Bariola and Henneberry (1980) found that plant stress due to irrigation cut off or chemical termination treatments did not significantly affect the initiation or incidence of PBW diapause and that percentage diapause was not related to cultivars within Upland cottons.

By mid September 1987, diapause was above 60% for PBW larvae from both Pima and Upland cotton (Fig. 4). The rate of increase in percentage diapause through October was not significantly different between the two species, although the level of diapause was significantly higher for Upland than for Pima on the mean date of 3 Oct (90.3 vs. 84.1%, $P \leq 0.01$). The sympodial growth habit of Pima cotton, which is less determinant than in Upland cotton, tends, in late season, to provide more bolls of a younger age at the top of the canopy in Pima than in Upland cotton. These results with Pima versus Upland cotton seem to mesh with the early conclusions of Adkisson et al. (1963) and Adkisson (1964) and later work by Crowder et al. (1975) which suggested that larvae in younger bolls are less

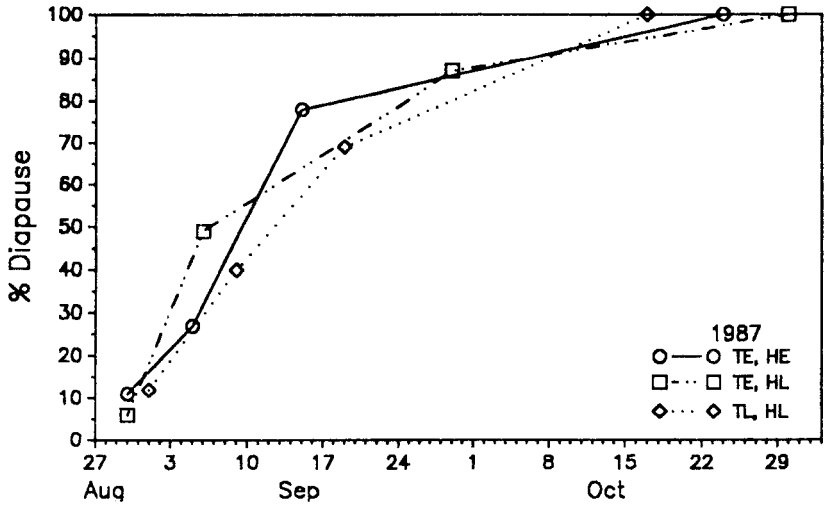

FIG 3. Percentage diapause of pink bollworm larvae from green cotton bolls in fields terminated and harvested early (TE, HE), terminated early and harvested late (TE, HL), and terminated and harvested late (TL, HL). See Table 1 for dates of termination and harvest. Pink bollworm-infested green cotton bolls collected on the dates indicated. Two weeks later, retrieved larvae were placed in petri plates at ambient conditions in the shade.

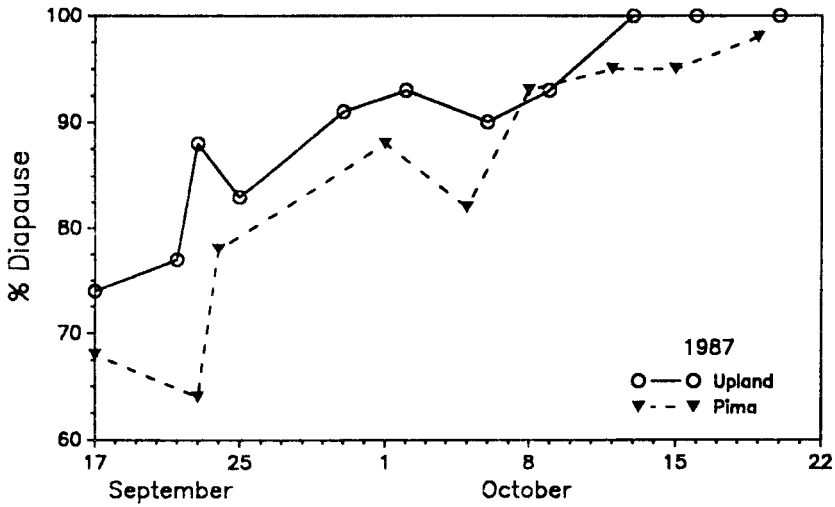

FIG 4. Percentage diapause of pink bollworm larvae from similar age Upland or Pima cotton fields. Pink bollworm-infested green cotton bolls collected on the dates indicated. Two weeks later, retrieved larvae were placed in petri plates at ambient conditions in the shade.

likely to diapause than larvae in older bolls. Crowder et al. (1975) also concluded that, although earlier maturing crops did possess a higher percentage of diapaused PBW larvae, because of their low number when the influence of nutrition was the greatest, the use of early crop termination as a control measure for the pest is still feasible.

Studies to Compare PBW Diapause Induction under Field and Environmental Chamber Conditions. Trials in 1990 were our first efforts to compare ambient outside conditions with those in controlled environmental chambers. The controlled environment diapause-enhancing conditions (30/15°C, 10:14 LD) were more or less enhancing than ambient conditions, depending on the date of larval collection (Figs. 5 and 6). Average temperatures and hours of light for the ambient conditions are shown in Table 3. Hours of daylight on the first date of boll collection, 6 Sept, were less than the 13 - 13.25 h of controlled light determined to be the critical threshold for diapause by Wellso and Adkisson (1964) and Adkisson (1964). When percentage diapause was analyzed for the factorial of boll collection dates, environmental conditions, and length of larval exposure, all main effects and interactions were significant ($P \leq 0.01$). Environmental conditions were compared by LSD ($P = 0.05$) for each collection date by exposure time combination. The harmonic average n for each date by time combination, rather than for all treatment combinations, was used for the error variance of $821/n$ for the LSD test. The effect of environmental condition was less for short-term culture larvae than it was for long-term culture larvae as shown by the vertical closeness of the diapause response curves in Fig. 5 compared to the greater separation in Fig. 6. For short-term exposure larvae, there were no significant differences between treatment conditions for the first three boll collection dates. On the fourth date, the rate of diapause was significantly higher for the diapause-inducing treatment than for the other two; on the fifth collection date the rate of diapause was significantly lower for the diapause-averting treatment than for the other treatments (Fig. 5). Rate of diapause was significantly greater in the diapause-enhancing treatment than in the other treatments for long-term exposure larvae on collection dates one, three, and four. The rate of diapause was significantly less in the diapause-averting treatment than in the other treatments on

TABLE 3. 1990 Average Maximum and Minimum Temperatures, by Week, for Four Weeks After Boll Collection and Hours of Light[a] on Those Dates.

Collection Date	Hours Light	°C Wk 1 Max	°C Wk 1 Min	°C Wk 2 Max	°C Wk 2 Min	°C Wk 3 Max	°C Wk 3 Min	°C Wk 4 Max	°C Wk 4 Min
6 Sep	12' 43"	40.4	22.1	38.5	21.7	35.3	15.8	32.5	15.2
20 Sep	12' 13"	35.3	15.8	32.5	15.2	33.7	13.7	32.3	10.0
4 Oct	11' 45"	33.7	13.7	32.3	10.0	31.0	10.9	32.5	8.9
18 Oct	11' 17"	31.0	10.9	32.5	8.9	24.1	6.7	28.6	5.0
1 Nov	10' 51"	24.1	6.7	28.6	5.0	25.3	7.1	23.1	4.9

[a] Sunrise to sunset, Blythe, CA; 33.6° latitude, 114.7° longitude determined from National Weather Service network program.

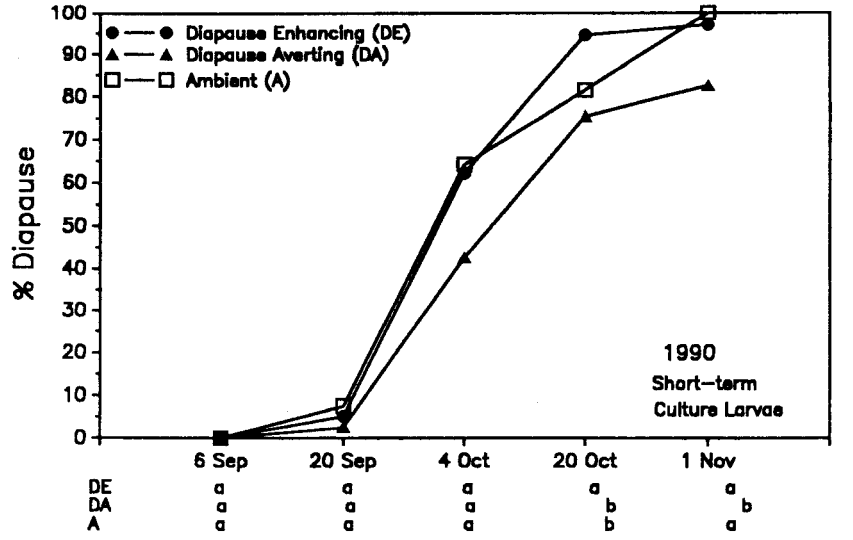

FIG 5. Percentage diapaused pink bollworm larvae exited from bolls during one week (short term larvae) at three environmental conditions and subsequently placed in petri plates at the same conditions for an additional 2-3 weeks. DE = 30/15°C, 10:14 LD; DA = 32°C, 14:10 LD; A = outside conditions in the shade. On each date, percentage diapause is significantly different between conditions with no letter(s) in common, LSD test, P = 0.05.

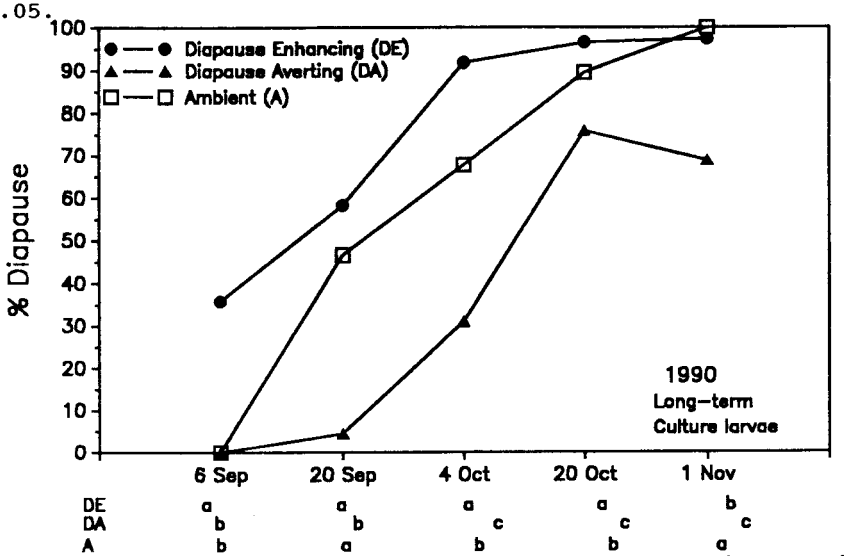

FIG 6. Percentage diapaused pink bollworm larvae exited during or removed at the end of a second week (long term larvae) at three environmental conditions and subsequently placed in petri plates at the same conditions for an additional 2-3 weeks. DE = 30/15°C, 10:14 LD; DA = 32°C, 14:10 LD; A = outside conditions in the shade. On each date, percentage diapause is significantly different between conditions with no letter(s) in common, LSD test, P = 0.05.

collection dates two through five (Fig. 6).

Results of a similar trial in 1993, with two instead of five boll collection dates, also showed that short-term culture larvae were significantly less affected by the diapause-averting conditions than were long-term culture larvae (data not shown).

The 1990 and 1993 studies on diapause-enhancing and diapause-averting conditions in the laboratory, as compared to outside ambient conditions, substantiated what most PBW researchers believe: i.e., "that the conditions throughout larval development may affect the induction of diapause" (Bariola and Henneberry 1980) and "the percentage of larvae actually entering diapause depends on conditions during incubation" (Miller et al. 1993). A commitment to diapause, as determined by the phenotypic assay, can be slightly modified in older larvae and greatly modified in younger larvae by exposure to enhancing or averting environments (Figs. 5 and 6). The older larvae received less exposure time (short-term larvae) whereas the younger larvae received longer exposure time (long-term larvae). The commitment to an all or nothing phenotypic change is modified by genetically determined physiological factors. That pectinophorin might be such a factor has not been proved or disproved although additional research efforts are certainly warranted.

Studies of Effects of Diapause-Enhancing and Diapause-Averting Conditions on Maintenance and Termination of PBW Diapause. Results from the 1993/1994 experiment wherein diapaused larvae were exposed to varying numbers of days at 32°C followed by placement at 30/15°C or 11°C are shown in Table 4. The rate of persistence of diapause on 15 Mar, about 3 wk after the beginning of the experiment, as measured by percentage live larvae, was not statistically different for larvae transferred for 0, 1, 2, or 3 days to 32°C before being moved back to 30/15°C. Similar results occurred for the larvae held at 11°C after their exposure to the same number of days at 32°C. These results may be related to those of Miller and Salama (1992), who used the pectinophorin assay, which suggested that the ability of larvae to change biochemically can be delayed up to 3 d after a commitment to enter or break diapause. Larvae exposed for 14 d had a rate of survival significantly lower than those exposed for 3 d or less and not significantly different from the rate for larvae held at constant 32°C. Larvae exposed for 14 d in the 32°C chamber, however, had adequate time for commitment to morphological change. Percentage larval survival was significantly higher for larvae held at 11°C than for those held at 30/15°C after 2-, 3-, and 5-d exposure, respectively, to 32°C. At the end of the experiment in August the extent of pupation in the chamber set for 11°C, 11:13 LD was not increased by up to 5 d at 32°C, 14:10 LD; numbers of adults were zero but substantial diapaused larvae remained. This was in contrast to very few diapaused larvae and many adults recorded at the end of the experiment in the chamber set for 30/15°C, 10:14 LD. These results indicate that the 30/15°C chamber afforded conditions only weakly suitable for maintenance of diapause and that the 11°C chamber afforded conditions that shut off diapause terminating processes after 1-5 d at 32°C even though conditions in the 32°C chamber were greatly promotive to pupation.

Although the numbers of larvae utilized per treatment were small (10) in the second diapause termination experiment conducted in 1993/1994, results from exposing undisturbed, diapaused larvae to diapause-averting conditions in the presence

TABLE 4. Percentage Pink Bollworm Lifeforms, (Larvae Originally Cultured at 30/15°C, 10:14 LD) Existing in Two Diapause Enhancing Conditions (30/15°C, 10:14 LD and 11°C, 11:13 LD) After Various Exposure Times at Diapause Averting Conditions (32°C, 14:10 LD).

Beginning Date: 02/23/94			Final Determination: 08/10/94		
Returned to	Days at 32°C	% Live Larvae 03/15/94	% Live of Remaining Larvae	% Pupated[a]	% Adult
30/15°C 10:14 LD	0[b]	78.4 abc[c]	31.2[d]	60.0 ab	30.0 c
	1	82.1 abc	0.0	82.1 a	48.7 abc
	2	65.0 cde	33.3	70.0 ab	52.5 abc
	3	62.5 cde	11.1	77.5 a	35.0 c
	5	42.1 ef	9.1	71.1 ab	50.0 abc
	14	23.3 f	20.0	83.3 a	67.7 ab
11°C 11:13 LD	0	87.5 ab	26.3 bc	2.6 c	0.0 d
	1	87.5 ab	37.5 ab	2.4 c	0.0 d
	2	92.1 a	37.8 ab	0.0 c	0.0 d
	3	87.5 ab	53.8 a	2.5 c	0.0 d
	5	71.1 bcd	15.0 c	0.0 c	0.0 d
	14	46.7 def	18.8 bc	46.7 b	40.0 bc
Left at 32°C 14:10 LD		35.0 f	0.0	80.0 a	72.5 a

[a] Live and dead pupae and adults.
[b] Approximately 40 larvae per regime, except only 30 for each 14 day regime.
[c] Percentages within a column with no letter(s) in common are significantly different at $P = 0.05$ using χ^2 test of independence in 2x2 frequency table.
[d] Returned to 30/15°C and left at 32°C not included in statistical analysis because numbers of remaining larvae were too low.

or absence of moisture were obvious (Fig. 7). Release from diapause was greatly enhanced by moisture and delayed by cool temperatures in the absence of moisture. On 5 Apr, for example, 90% of the larvae at cool, ambient temperatures remained in diapause (normal for field situations), whereas under diapause-averting conditions in the laboratory, only 50% of the larvae remained in diapause and, with the addition of moisture, all had been released from diapause by late February (90% a, 50% b, 0% c; LSD test, $P = 0.05$, analysis as for 1990 trial).

General Interpretations. Based upon literature reports and experience gained in this research program, some generalized interpretations are offered. In the fall and spring of each year, hours of daylight are consistently decreasing and increasing, respectively. Similarly, temperatures are decreasing and increasing, but not in a uniform manner. In the fall, cotton fields, varying in their extent of maturity, are completing their growth cycles. In the spring, soil moisture is varying in extent and time due to irrigation and

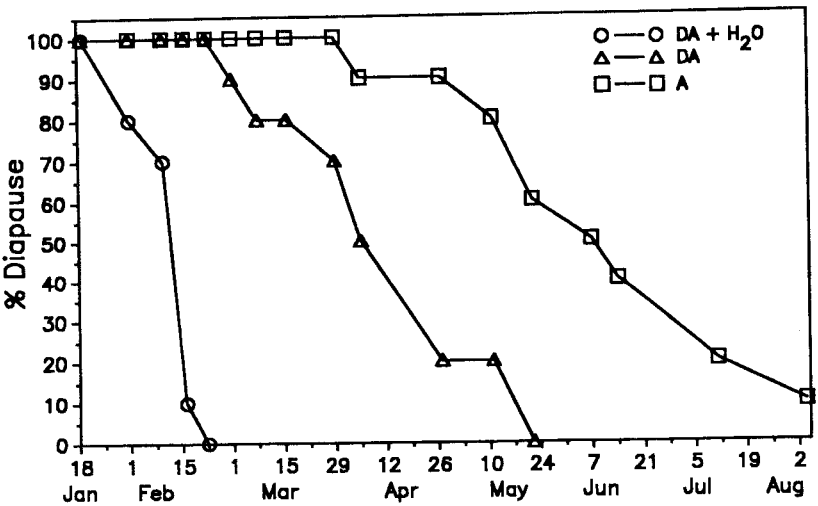

FIG 7. Percentage remaining in diapause, of diapaused pink bollworm larvae subjected to diapause averting (DA) conditions (32°C, 14:10 LD) ± addition of water on 19 and 27 January and 10 February 1994, or outside ambient conditions in the shade with no water, (A).

precipitation. Photoperiod, temperature, and PBW nutrient source are correlated with induction of diapause in the fall; photoperiod, temperature, and soil moisture are correlated with termination of diapause in the spring. If photoperiod is above about 13.5-14 h, the extent of insects in diapause is increased by temperatures below about 20-21°C. If temperatures are greater than those values, photoperiods below 13.5-14 h will increase the extent of diapause with the strength of diapause initiation increasing with decreasing photoperiod. Thus, either appropriately short photoperiods or adequately cold temperatures will induce diapause which suggests that both factors are generally of equal value. In the field, however, declining photoperiod is a constant and may be considered permissive with temperature being enhancing or averting for entrance into diapause. Although larval nutrition is recognized as having an impact on induction of diapause, because of methods of commercial cotton production in the desert Southwest, boll age (and thus larval nutrition) is considered only a minor factor at the time that photoperiod and temperature are impacting the large numbers of larvae having the capacity to diapause.

It is also suggested that similar photoperiod and temperature relationships, in reverse, interact to influence release from diapause. In the spring, increasing photoperiod is not generally permissive in the desert Southwest until planting dates are legally permitted (variable by region) and, even then, temperatures are usually not adequate for termination of diapause. Generally, however, temperatures are strongly enhancing by the first part of April but in some years they may remain averting well into the main spring emergence period of late April and early May. When photoperiod is permissive and temperatures are enhancing, soil moisture is also enhancing.

Lack of moisture, e.g., fallow ground following last year's cotton, is strongly averting to termination of diapause.

For many years, heat units have been used as a valuable predictive tool for estimating points along spring PBW moth emergence curves. It is not suggested that chill unit predictions for fall diapause induction are adequate to annually establish variable crop termination or plow down dates, however, refinement of the method might assist growers in the timing and logistics of fall cotton operations and ultimately lead to improvement in the establishment of host free limits in different areas. Just as those practical matters might be improved, additional research on the physiology and chemical nature and function of the diapause-associated protein, pectinophorin, may provide insight into methods for (1) altering the capacity of larvae to achieve the quiescent stage in the fall, (2) creating adverse cultural/microenvironmental disturbances during mid diapause, or (3) impacting larval ability to achieve the phenological shift to pupae in the spring.

ACKNOWLEDGMENT

The financial support of the California Cotton Pest Control Board is gratefully acknowledged. Thanks are also expressed to the USDA-ARS Western Cotton Research Laboratory for financial support in the early years of these studies, and especially to its Director, Thomas Henneberry for his encouragement and valuable suggestions. I extend a profound thanks to the numerous pest control advisors and growers in the Palo Verde Valley who assisted in locating, and who permitted the use of, cotton fields for these endeavors. In addition, I thank Thomas Miller for his cooperation and the use of laboratory facilities. In that regard, thanks are expressed to Sakuntala Sivasupramaniam and Doug DeAmbra for their laboratory assistance. I especially acknowledge and thank Carol Adams for the conduct of all statistical analyses and her valued assistance in the preparation of this paper.

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THE EFFECT OF TEMPERATURE AND HOST DENSITY ON THE REPRODUCTION OF *APHELINUS PERPALLIDUS*[a]

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ABSTRACT

The optimum rearing temperatures for *Aphelinus perpallidus* (Gahan), an arrhenotokous hymenopteran parasitoid of the blackmargined aphid, *Monellia caryella* (Fitch), in the laboratory were 24° and 28°C, with females producing 11.1 and 12.7 mummies, respectively, during a 48 hr period. Optimum densities were 0.75-1.0 aphid/cm^2. A significant interaction occurred between fertility and temperature and their effect on fecundity. The highest fecundity of unmated and mated females at 24° and 28°C was 14 mummies. Emergence of male and female progeny was significantly affected by temperature. Maximum male emergence (55-64%) occurred at 20°-28°C and was significantly higher than maximum female emergence which occurred at 24°C (21%) and 28°C (29%). Development time of *A. perpallidus* progeny decreased significantly as temperature increased and was similar at each temperature for male and female progeny. Male development time ranged from 13 days at 28°C to 31 days at 16°C while female development time ranged from 12 days at 28°C to 28 days at 16°C. Aphid density had no significant effect on male or female emergence or development individually or in interactions with other variables.

INTRODUCTION

Fecundity of hymenopterous parasitoids can be influenced by factors such as temperature and host density. The effect of temperature on fecundity of hymenopterous aphid parasitoids has been investigated by Force and Messenger (1964) for the spotted alfalfa aphid, *Therioaphis maculata* (Buckton); Jackson et al. (1974) on the greenbug, *Schizaphis graminum* (Rondani), corn leaf aphid, *Rhopalosiphum maidis* (Fitch), and oatbird cherry aphid,*Rhopalosiphum padi* (l.); and Giri et al. (1982) investigating the green peach aphid, *Myzus persicae* (Sulzer).

Force and Messenger (1964) examined the effects of temperature and host density on fecundity of *Praon palitans* Muesebeck, *Trioxys utilis* Muesebeck and *Aphelinus semiflavus* Howard, parasitoids of *T. maculata*. The optimum temperature for egg production by these parasitoids was 21°C. Females of all three species produced only males at temperatures approaching their upper thermal limit for survival (26.7°-32.2°C). Mean developmental period decreased as temperature increased from 10° to 32.2°C. Jackson et al. (1974) also found that temperature affected the fecundity of *Ephedrus plagiator* (Nees). More mummies were produced at

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21°C and emergence was highest at 21° and 27°C. The development period of *E. plagiator* varied inversely with temperature. Giri et al. (1982) investigated the effect of temperature on the fecundity of *Aphidius matricariae* Haliday, a parasite of *Myzus persicae* (Sulzer). The mean number of progeny produced was highest at 12.8° to 21°C and emergence was constant at 10° to 21°C, and declined significantly at 24°C and above.

The effect of host density on parasitoid fecundity has been investigated by Mackauer (1982, 1983) on *A. semiflavus* and *Aphidius smithi* Sharma and Subba Rao, parasitoids of the pea aphid, *Acyrthosiphon pisum* Harris; Shu-Sheng (1985) investigating *Aphidius sonchi* Marshall, a parasitoid of the sowthistle aphid, *Hyperomyzus lactucae* (L.); and Bueno and Stone (1987) who worked with *Aphelinus perpallidus* (Gahan), a parasitoid of *Monellia caryella* (Fitch).

Data on *A. semiflavus* (Mackauer 1982) indicate a dynamic relationship between host density and oviposition rate. Although the mean number of eggs oviposited daily was lower at 20 aphids compared to 60 aphids per test chamber, there was no significant difference in lifetime fecundity. Parasitoid fecundity at both densities was positively related to the total number of pea aphids available during the lifetime of each female. Mackauer (1983) found that the total number of eggs laid by *A. smiithi* on its host, *A. pisum*, was dependent on the number of aphids available per day of parasitoid life. Parasitoids exposed to five aphids daily deposited an average of 192 eggs per lifetime while those exposed to 100 aphids daily laid an average of 870 eggs. Shu-Sheng (1985) working with the parasite *A. sonchi* and its host, *H. lactucae*, found that parasitoid oviposition was reduced at fewer than 50 aphids/day. The daily reduction in oviposition resulted in a direct decrease in the total number of eggs laid during the parasitoid's lifetime. The total number of eggs laid was not affected at exposure rates greater than 50 aphids daily. Bueno and Stone (1987) found there was a significant positive relationship between the density of *M. caryella* and fecundity of unmated and mated *A. perpallidus* females. There was a significant difference in total emergence between densities with mated *A. perpallidus* females only. Results showed that mated *A. perpallidus* females must be exposed to aphid density levels of 0.50-0.75 aphids per cm^2 to obtain significantly higher parasitism. These studies like those on temperature show that maximum parasitoid fecundity is achieved at distinct aphid density levels. Above or below these densities factors such as superparasitism, host contamination and parasitoid interference cause a reduction of fecundity.

Aphelinus perpallidus has been reported to have limited to moderate impact on aphid populations in the U. S. (Tedders 1978; Edelson and Estes 1981; Watterson and Stone 1982; Bueno and Stone 1983, 1985). This natural enemy has potential for use in augmentation programs where parasitoid populations need to be increased in aphid infested locations. It was the objective of this study to determine the effects of temperature and aphid density on the fecundity, emergence, and development of *A. perpallidus*. These studies could lead to more effective rearing strategies not only for *A. perpallidus* but also for other hymenopteran parasitoids.

MATERIALS AND METHODS

Aphids (*M. caryella*) and parasitoids (*A. perpallidus*) were collected from Adriance pecan [=*Carya illinoensis* (Wang) K. Koch] orchard in College Station, TX and laboratory colonies initiated. The colonies were maintained at 24°C and 40%RH. Pecan seedlings were propagated by hand collecting nuts at Brison orchard in College Station.

Collection began at shuck split (mid-fall). Shucks were removed and nuts were stratified in moist vermiculite in a small box lined with a plastic bag at 4°C for approximately 1 month similar to Tedders et al. (1970). About 30 days before seedlings were needed, the nuts were planted in top soil in small, plastic basins. The basins were placed in a Rheem® environmental growth chamber and were maintained at 25°C, 80% RH, and 16hr:8hr light/dark cycle. About 3-4 weeks after germination occurred, the seedlings were transplanted into pots. Laboratory-reared aphids were transferred onto the experimental seedlings using a fine camel's hair brush. Preliminary studies showed that first and second aphid instars were preferred by *A. perpallidus*. Female parasitoids were exposed to four aphid densities (0.25, 0.50, 0.75, and 1.0 aphid per cm^2) at temperatures of 16, 20, 24, and 28°C, respectively, with a 16 hr:8 hr light/dark cycle in an Environator® growth chamber. Temperatures were chosen based on field observations in El Paso, TX (Bueno and Stone 1983, 1985) revealing that *A. perpallidus* populations were active at this temperature range. Density levels were chosen to complement the results from previous research (Bueno and Stone 1987). The leaf areas of twenty 1-month old Cheyenne seedlings was measured with a Li-Cor® leaf area meter. Leaflets from each seedling were excised and measured using an area meter. The mean area from seedlings determined the appropriate density of aphids for each seedling. The mean leaf area was $165 \pm 16 cm^2$. Seedlings used were approximately the same age as those that were sacrificed to determine mean leaf area. The number of aphids per seedling throughout this study was 41, 82, 124, and 165 aphids corresponding to 0.25, 0.50, 0.75, and 1.0 aphids/cm^2, respectively. When mated female parasitoids were required, individual females were placed with a male parasitoid in a 2-dram vial for 24 hr. A droplet of honey was placed at the bottom of the vial as a food source. Unmated female parasitoids were stored similarly. A mated or unmated female parasitoid was released and allowed to parasitize aphids for 48 hr. The test seedling was enclosed with an acrylic cylinder (30.5 cm high x 15.2 cm diameter) and exposed to the appropriate experimental conditions. Test seedlings were examined daily for mummies. Mummies were placed individually into gelatin capsules and placed in labeled petri dishes. Mummies were returned to the appropriate experimental conditions and observed for emergence. Four replicates of each density/temperature level (each temperature was run at each density) for unmated and mated females were performed. The number of mummies, dates of adult emergence and sex of the parasitoid were recorded.

A factorial experimental design was used where fecundity, emergence and development time of male and female progeny of mated and unmated *A. perpallidus* females were observed at each temperature/density level. A General Linear Model (GLM) was used to fit a single dependent variable in terms of a collection of independent variables. Since the independent variables were discrete (i. e., fixed rather than continuous measurements), a General Linear Model using an analysis of variance instead of regression was used to detect significant differences at $P<0.05$ caused by these variables and their interactions (SAS 1985). Interactions of variables were deemed significant if mean differences in responses for levels of one factor were constant across the levels of a second factor (Ott 1988). Scheffe's Mean Separation test (1953) was used to detect significant differences between means of individual variables. Data from significant interactions of the highest order will be discussed to explain trends which persisted throughout the study. If no significant interactions occurred between independent variables, data from the effects of individual variables will be used to explain the results.

RESULTS AND DISCUSSION

A significant interaction was found to exist between temperature, fertility of females, and fecundity (Table 1, Fig. 1). Generally, fecundity increased as temperature increased for both mated and unmated females and was similar for both groups except at 24°C where fecundity from unmated females was twice as high as mated females, 14 and 7 mummies, respectively. It is uncertain why this occurred. Fecundity of mated females ranged from 1.4 mummies at 16°C to 14.0 mummies at 28°C. Fecundity of unmated females ranged from 0.5 mummies at 16°C to 14.7 mummies at 24°C.

Fecundity was significantly affected by host density. Fecundity of *A. perpallidus* at 0.75 and 1.0 aphid/cm^2 was significantly higher (9.8 and 8.6 mummies, respectively) than at 0.25 aphid/cm^2 (3.7 mummies) (Table 2).

TABLE 1. General Linear Model Analysis of Experimental Variables and Their Interactions on the Mean Fecundity of *A. perpallidus*.

Source of Variation[a]	F-value[b]	Pr>F
Fertility[c]	0.33(1)	<0.01
Temperature	26.14(3)	<0.01
Density	6.18(3)	<0.01
Fert.xTemp.	4.63(3)	<0.01
Fert.xDen.	2.14(3)	0.10
Temp.xDen.	1.81(9)	0.08
Fert.xTemp.xDen.	1.76(9)	0.09

[a]n=128 observations.
[b]Signficant differences caused by variables and their interactions on the fecundity of *A. perpallidus* were determined at P<0.05. Degrees of freedom in ( ).
[c]Fertility of the parent female, unmated or mated.

TABLE 2. The Mean Fecundity of a Single *A. perpallidus* Female When Exposed to Different Densities of its Host, *M. caryella*, for 48 hr.

Density (aphids/cm^2)	No. of Mummies[a,b]	Standard Error
0.25	3.7b	0.86
0.50	7.8ab	1.22
0.75	9.8a	1.88
1.0	8.6a	1.55

[a]Means were tested by an analysis of variance of the General Linear Model. Means with the same letter are not signficantly different at P<0.05 by Scheffe's Mean Separation Test (1953).
[b]n=32 observations/density.

There was a significant interaction between fertility and temperature affecting the emergence of male progeny of *A. perpallidus* (Table 3, Fig. 2). Male emergence from mated females was lower at higher temperatures compared to male emergence from unmated females held at the same temperatures. Male emergence from mated females ranged from 32.8% at 16°C to 56.4% at 20°C decreasing thereafter. Male emergence from unmated females ranged from zero emergence at 16°C to 79.2% emergence at 24°C. Temperature was the only variable which significantly affected female emergence (Table 4). Emergence was significantly higher at 24° and 28°C (21 and 29%, respectively) compared to 16°C (2.5%). Male emergence was higher than female emergence at each temperature. Numerous females used in these studies were mated immediately upon eclosion and released with aphids. It is possible that sperm did not become available for fertilization until several hours

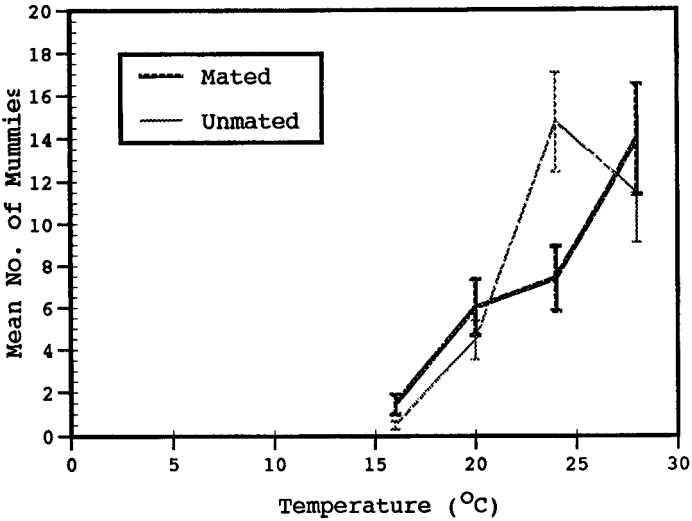

FIG. 1. Mean fecundity (±SE) of mated and unmated *A. perpallidus* females exposed to *M. caryella* at different temperatures for 48 hr.

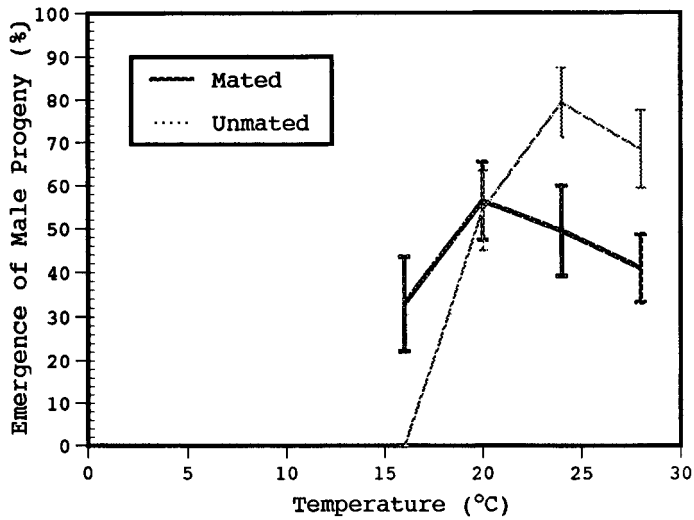

FIG. 2. Mean emergence (±SE) of male progeny of *A. perpallidus* from mummies when mated and unmated *A. perpallidus* females were exposed to *M. caryella* for 48 hr.at different temperatures.

after copulation (functionally virgin) (Mackauer 1976). This could have resulted in higher male emergence compared to female emergence. Also, when emergence of male and female progeny from mated females were combined at each temperature, emergence was similar to emergence from unmated females except at 16°C. This explains the large discrepancy in male emergence at 24° and 28°C between mated and unmated *A. perpallidus*.

TABLE 3. A General Linear Model Analysis of Experimental Variables and Their Interactions on the Mean % Emergence of Male and Female Progeny of *A. perpallidus*.

	F-value[b]		Pr>F	
Source of Variation[a]	Male	Female	Male	Female
Fertility[c]	0.84(1)	---	<0.01	---
Temperature	12.42(3)	4.09(3)	<0.01	0.01
Density	2.01(3)	0.94(3)	0.11	0.43
Fert.xTemp.	5.92(3)	---	<0.01	---
Fert.xDen.	0.52(3)	---	0.67	---
Temp.xDen.	1.06(9)	1.55(9)	0.40	0.16
Fert.xTemp.xDen.	1.13(9)	---	0.35	---

[a]n=128 and 64 total observations for male and female data, respectively.
[b]Significant differences caused by variables and their interactions on the emergence of progeny of *A. perpallidus* were determined at P<0.05. Degrees of freedom in ( ).
[c]Fertility is only a variable in male emergence since mated and unmated females produce male progeny; female progemy is only produced by mated females.

TABLE 4. The Effect of Temperature on Mean Emergence of Female Progeny of *A. perpallidus* When Its Host, *M. caryella*, was Exposed to a Single Parasitoid Female for 48 hr.

Temperature (°C)	Emergence (%)[a,b]	Standard Error
16	2.5b	1.71
20	5.7ab	3.15
24	20.6a	9.59
28	29.1a	7.78

[a]Means were tested by an analysis of variance of the General Linear Model. Means with the same letter were not significantly different at P<0.05 by Scheffe's Mean Separation Test (1953).
[b]These data were from aphids exposed to mated *A. perpallidus* females since unmated females only produced male progeny.

Temperature was the only variable that affected time of male and female emergence. There were no significant interactions among other variables (Table 5). Male emergence time significantly decreased as temperature increased. Emergence time ranged from 31.4 days at 16°C to 12.8 days at 28°C. Significant differences in female emergence time also occurred at each temperature. Emergence time ranged from 29.5 days at 16°C to 12.5 days at 28°C (Table 6). There were no data included from tests using unmated females since unmated females produced male progeny only. The numbers of parasitoids increased as temperature increased. Apparently these parasites are less tolerant to low temperatures.

Results indicate rearing *A. perpallidus* at the highest experimental temperatures (24°C and 28°C) produced the highest numbers of mummies. Other studies conducted on hymenopterous aphid parasites (Force and Messenger 1964, Giri et al. 1982, Cohen and Mackauer 1987) indicated that the optimum temperature was 21°C. Emergence of *A.perpallidus* progeny was higher as temperature increased indicating that this parasite has a higher temperature threshold compared to most

other parasite studies cited. Although emergence of female progeny of *A. perpallidus* from mated female parents did not exceed 30% at any

TABLE 5. A General Linear Model Analysis of Experimental Variables and Their Interactions on the Mean Emergence Time of Male and Female Progeny of *A.perpallidus*.

	F-value[b]		Pr>F	
Source of Variation[a]	Male	Female	Male	Female
Fertility[c]	0.42(1)	---	0.52	---
Temperature	168.52(3)	879.22(3)	<0.01	<0.01
Density	0.03(3)	2.12(3)	0.99	0.20
Fert.xTemp.	0.76(2)	---	0.47	---
Fert.xDen.	0.17(3)	---	0.92	---
Temp.xDen.	0.82(8)	2.85(4)	0.58	0.12
Fert.xTemp.xDen.	0.81(6)	---	0.57	---

[a]n=83 and 17 total observations for male and female data, respectively.
[b]Significant differences caused by variables and their interactions on the emergence time of progeny of *A. perpallidus* were determined at P<0.05. Degrees of freedom in ( ).
[c]Fertility is only a variable in male emergence time since mated and unmated females produce male progeny; female progeny is only produced by mated females.

TABLE 6. The Effect of Temperature on Mean Emergence Time of Male and Female Progeny of *A. perpallidus* When Its Host, *M. caryella*, was Exposed to a Single Parasitoid Female for 48 hr.

	Emergence Time(days)[a]		Standard Error	
Temperature(°C)	Male	Female[b]	Male	Female
16	31.4d	29.5d	0.92	0.50
20	21.2c	20.7c	0.33	0.54
24	16.3b	16.7b	0.24	0.30
28	12.8a	12.5a	0.46	0.13

[a]Means were tested by an analysis of variance of the General Linear Model. Means with the same letter were not significantly different at P<0.05 by Scheffe's Mean Test (1953).
[b]These data were from aphids exposed to mated *A. perpallidus* females since unmated females only produced male progeny.

temperature, female emergence exhibited an increasing trend from lower to higher temperatures. Other studies (Messenger and Force 1963, Force and Messenger 1964, Jackson et al. 1974) demonstrated that female parasitoid emergence decreased with increasing temperatures. Progeny of *A. perpallidus* apparently are more tolerant of higher temperatures compared to the other parasitoids studied.

Fecundity of *A. perpallidus* was higher at 0.75 and 1.0 aphid/cm^2 than at lower test densities. These data corroborate an earlier study by Bueno and Stone (1987) which showed that there was a strong positive correlation between fecundity of *A. perpallidus* and host density. It is also possible that fecundity of *A. perpallidus* could be increased even more by enclosing the aphid-parasitoid colonies in a larger cage or by releasing parasitoids in a greenhouse where many aphid-infested pecan seedlings are present. Messing and Aliniazee (1989) attempted to rear *Trioxys pallidus*, (Haliday), a parasitoid of the filbert aphid, *Myzocallis coryli* (Goetze) using different cage sizes and temperatures but very few parasitoids were recovered per cage. When a few parasitoids were released accidentally in an open greenhouse containing several hundred aphid-infested seedlings, subsequent generations yielded thousands of mummies. The mass rearing was carried out on open

greenhouse benches with natural photoperiods at 20°-30°C. Percentage emergence and development time of *A. perpallidus* progeny were not affected by host density.

Throughout the pecan growing season, *A. perpallidus* populations are decimated periodically by inclement weather, seasonal lack of hosts or insecticide treatments. Multiple insecticide treatments are usually applied during late season aphid population peaks. Since the damage impact of these populations is high, augmentation of *A. perpallidus* populations through inoculative releases may be an effective component for reducing aphid populations. These studies could lead to more effective rearing strategies of *A. perpallidus* in order to augment natural field populations. The integration of this research with current aphid management strategies in pecan could enhance more effective control of *M. caryella* in pecan orchards.

ACKNOWLEDGMENT

I would like to thank Dr. J. K. Olson for providing the facilities and equipment to conduct this research and Dr. R. A. Wharton for his meticulous critique of this research.

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THE EFFECT OF COLD STORAGE ON THE EMERGENCE OF *APHELINUS PERPALLIDUS*,[a] A PARASITOID OF *MONELLIA CARYELLA*[b]

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ABSTRACT

Cold storage studies revealed that emergence of *Aphelinus perpallidus* (Gahan) adults, a hymenopteran parasitoid of the blackmargined aphid of pecan, *Monellia caryella* (Fitch), was significantly affected by acclimation, mummy age, storage temperature, and storage period. Total emergence ranged between 61 to 72% for older mummies that were thermally acclimated to 4.4, 7.2, and 10°C stored for 7 days and 7.2°C stored for 15 days. Female and male emergence under similar acclimation ranged from 33 to 39% and 14 to 34%, respectively. Under these conditions, development time of *A. perpallidus* ranged from 18 to 22.5 days for both females and males. Altering the optimal storage conditions to further retard development decreased emergence rates.

INTRODUCTION

In biological control successful storage of parasitoids at low temperatures has practical importance. Cold storage not only prolongs survival of hymenopterous aphid parasitoids, but also delays emergence of stored immature parasitoids. Cold storage of immature stages of hymenopterous parasitoids in mummies (parasitized aphids) and as adults is advantageous in augmentive biological control programs because it allows: 1) accumulation of sufficient numbers of parasitoids from mummies for field release, 2) release of parasitoids at optimal weather conditions, 3) a means to keep parasitoids alive when not needed, and 4) transport of parasitoids over long distances, particularly during exportation (Hofsvang and Hagvar 1977). Mummies, particularly newly formed ones (Hofsvang and Hagvar 1977), are more suitable for storage than adults (Stary 1970). DeBach (1943) indicated that three causes of mortality of stored adults were cold, starvation, and dessication. Mummies are better suited for storage because the parasitoids are protected within them, mummies are easier to handle, and mummies can be stored considerably longer than adults. Procedures for cold storage have not figured prominently in classical biological control programs for aphid pests because successful programs have involved rapid introduction and establishment with no further need for augmentation. In cases where classical biocontrol has not been successful, however, cold storage may prove useful for breaking diapause

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and/or aligning physiological cues which trigger emergence in northern versus southern hemispheres.

Several studies on cold storage of insect mummies have been conducted on hymenopterous aphid parasitoids to determine the feasibility of this methodology for augmentative programs. Similar studies on cold storage have been conducted on *Lysiphlebus testaceipes* (Cresson) and *Aphelinus asychis* Walker, both parasitoids of *Schizaphis graminum* (Rondani) (Archer et al. 1973, Tyler and Jones 1974, Whitaker-Deerberg et al.1994); *Aphidius matricariae* (Haliday) and *Aphelinus flavipes* Kurdjumov used to control glasshouse aphid pests (Scopes et al. 1973); *Ephedrus cerasicola* Stary and *Aphidius colemani* Viereck, parasitoids of *Myzus persicae* (Sulzer) (Hofsvang and Hagvar 1975); and *Trioxys indicus* Subba Rao and Sharma, a parasitoid of *Aphis craccivora* Koch (Singh and Srivastava 1988).

These studies demonstated that parasitoid emergence was greater from younger mummies than from older ones. When the effects of temperature on parasitoids were analyzed, the optimal range for emergence was approximately 4-7°C. Emergence generally decreased as length of storage increased. Development time of parasitoids decreased as mummy age and temperature increased and increased as storage period increased. Acclimation (i. e., parasitoid response to temperature according to previous experiences) of mummies prior to storage increased emergence.

High populations of blackmargined aphid of pecan, *Monellia caryella* (Fitch), cause damage to pecan leaf tissue and remove cellular materials from tissue (Gentry et al. 1974, Durkin 1976). Furthermore, growth of sooty mold fungus on pecan leaves results from excretion of copious amounts of honeydew by *M. caryella* (Tedders and Smith 1976). High populations of aphids also result in tree defoliation and reduced flower production the following spring (Tedders 1978, Jackson and Payne 1980, Dutcher and Htay 1985).

Alternative control tactics, such as biological control, would aid in the control of *M. caryella*. *Aphelinus perpallidus* (Gahan), a hymenopterous parasitoid of *M. caryella*, has been reported to impact aphid populations in the US (Tedders 1978, Edelson and Estes 1981, Watterson and Stone 1982, Bueno and Stone 1983, 1985). This natural enemy has potential for use in augmentative releases where field parasitoid populations need to be increased in aphid infested locations. The objectives of this study were to determine the effects of acclimation and cold storage on survival and development of *A. perpallidus*. This information could aid in the release of viable parasitoids to augment field populations and help control *M. caryella* infestations in the field.

MATERIALS AND METHODS

Aphids and parasitoids were collected from Adriance pecan orchard in College Station, TX, and laboratory colonies were initiated. Mummies were collected from laboratory-reared parasitoid colonies. All mummies were placed individually in gelatin capsules at 1-3 days of age. One group of mummies constituted the age one group (1-3 days old); the age two group was held at laboratory conditions (24°C, 40% RH) until mummies were 4-6 days old. Mummies were subjected to either pre-acclimation (pre-storage conditions of 15°C in complete darkness for 24 hr), post-acclimation (post-storage conditions of 15°C in complete darkness for 24 hr), or non-acclimation (=control group). The acclimation temperature of 15°C was selected because it represented the mid-point between 10°C and laboratory temperature (24°C) and still below the parasitoid's normal temperature range (16 to 28°C) (Bueno and Van Cleave 1996). Cold

storage temperatures were 1.7, 4.4, 7.2 and 10°C with storage periods of 7, 15, and 30 days, respectively, in complete darkness. Since the majority of insects die as the result of tissue damage at tempeatures below freezing (Chapman 1982), the lowest temperature selected was 1.7°C. A factorial arrangement was used where adult emergence and development time were observed at each acclimation/age/temperature/storage period level.

Twenty mummies were assigned to each replication for non- and pre-acclimated samples and 25 mummies for the post-acclimated sample. Numbers of mummies in non- and pre-acclimated samples were reduced from 25 to 20 because sufficient numbers of pecan seedlings were unavailable. For this reason, data were converted and analyzed as a percentage.

The non-acclimated group was immediately placed in appropriate cold storage conditions. The pre-acclimated group was subjected to pre-storage conditions and afterwards placed in cold storage conditions. The post-acclimated group was subjected to post-storage conditions after exposure to appropriate storage conditions. Mummies were removed from control chambers and examined daily for adult emergence; non-emerged mummies were returned to control chambers until the storage period ended. Upon emergence, parasitioids were anesthetized and sex determined.

A general linear model (GLM) was used to fit a single dependent variable (mean percentage emergence and mean developmental time of a cohort) to a collection of independent variables. Since the independent variables were discrete (i.e., fixed rather than continuous meansurements) a GLM using an analysis of variance instead of regression was used to detect any significant differences ($P<0.05$) caused by these variables and their interactions (SAS 1985).

RESULTS AND DISCUSSION

Table 1 shows the effects of the experimental variables and their interactions.

TABLE 1. A General Linear Model (GLM) Analysis of Experimental Variables and Interactions on the Emergence of All *A. perpallidus* progeny[a].

Sources of Variation[b]	DF	F-value	Pr>F
Accl.	2	17.87	<0.01
Age	1	205.27	<0.01
Temp.	3	52.87	<0.01
S.P.	2	258.42	<0.01
Accl.x Age	2	4.14	0.02
Accl.x Temp.	6	7.90	<0.01
Age x Temp.	3	8.67	<0.01
Accl.x S.P.	4	7.29	<0.01
Age x S.P.	2	20.53	<0.01
Temp. x S.P.	6	3.67	<0.01
Accl.x Age x Temp.	6	3.33	<0.01
Accl.x Age x S.P.	4	2.33	0.06
Accl.x Temp.x S.P.	12	1.61	0.09
Age x Temp.x S.P.	6	2.46	0.02
Accl.x Age x Temp.x S.P.	12	1.28	0.23

[a]Significant differences caused by interactions of variables on the emergence of *A. perpallidus* progeny were determined at $P<0.05$ for 360 observations.

[b]Accl.=acclimation, age=mummy age, temp.=storage temperature, and s.p.=storage period.

Data indicate significant effects of individual variables and interactions between and among variables. Data from the highest order (three-way) significant interactions will be discussed to explain these trends.There was significant interaction among acclimation, mummy age and temperature. Emergence significantly increased as temperature increased and emergence was significantly higher from older mummies at each acclimation condition (Table 1). Fig. 1 shows the effects of mummy age and storage temperature on *A. perpallidus* emergence when mummies were subjected to different acclimation conditions.

Emergence from non-acclimated, older mummies ranged from 28% at 1.7° to 42.3% at 10°C. In contrast, emergence from younger mummies ranged from 6.3% at 1.7° to 20.3% at 10°C (Fig. 1A). Emergence from pre-acclimated, older mummies increased significantly from 30% at 1.7° to 48% at 10°C, whereas emergence from younger mummies was similar (23-26%) at 1.7, 4.4, and 7.2°C but increased significantly to 33% at 10°C (Fig. 1B). Emergence from post-acclimated, older mummies ranged from 15.5% at 1.7° to 49.3% at 10°C, whereas emergence from younger mummies ranged from 4.4% to 47.7% at 1.7° and 10°C, respectively (Fig. 1C). At 7.2°C, emergence decreased and was similar at 4.4°C in four of six observations; it is uncertain why this occurred. There was a marked increase in emergence from pre-acclimated younger mummies at each temperature compared to their non- and post-acclmated counterparts except at 10°C from the post-acclimated sample. Emergence was significantly higher from pre-acclimated mummies compared to their non-acclimated counterparts and especially from younger mummies at each temperature level. Emergence from pre-acclimated mummies overall was similar or higher than their post-acclimated counterparts at different age/temperature levels. Peak emergence for both groups was approximately 49% and occurred at 7.2 and 10°C.

The three-way interaction of mummy age, temperature, and storage period was significant. Fig. 2 shows the effect of mummy age and storage temperature on *A. perpallidus* emergence at different storage periods. Emergence at each age/temperature level for different storage periods was higher from older mummies and increased with temperature. Emergence from older mummies stored for 7 days ranged from 47.3% at 1.7° to 69.3% at 10°C, whereas, emergence from younger mummies ranged from 22.4% at 1.7° to 57% at 10°C (Fig. 2A). A storage period of 15 days resulted in an increase in emergence from older mummies from 18.4% at 1.7° to 54.1% at 10°C; whereas, emergence from younger mummies ranged from 9.2% at 1.7° to 27.5% at 10°C (Fig. 2B). Emergence from older mummies at 30 days storage ranged from 7.8% at 1.7° to 19.1% at 7.2°C; whereas, emergence from younger mummies ranged from 2.7% at 1.7° to 16.5% at 10°C (Fig 2C). Emergence at each age/temperature level was significantly higher from mummies at 7 days storage compared to 15 and 30 days storage. As in the previous interaction, it is uncertain why emergence at 7.2°C compared to 4.4°C decreased or was simliar in three of the six observations.

Table 2 shows that parasitoid developmental time was significantly affected by the individual variables and some of their interactions. Similar trends on the effects of these variables on development time persisted regardless of the number of variables interacting with each other. As with emergence, data from the highest order (three-way) significant interactions will be discussed.

The interaction of acclimation, mummy age, and storage period was significant ($P<0.01$). Fig. 3 shows the effect of mummy age and storage period on development time of *A. perpallidus* at different acclimation conditions. Development time for both age groups of mummies increased at each acclimation condition as storage period increased. In the non-

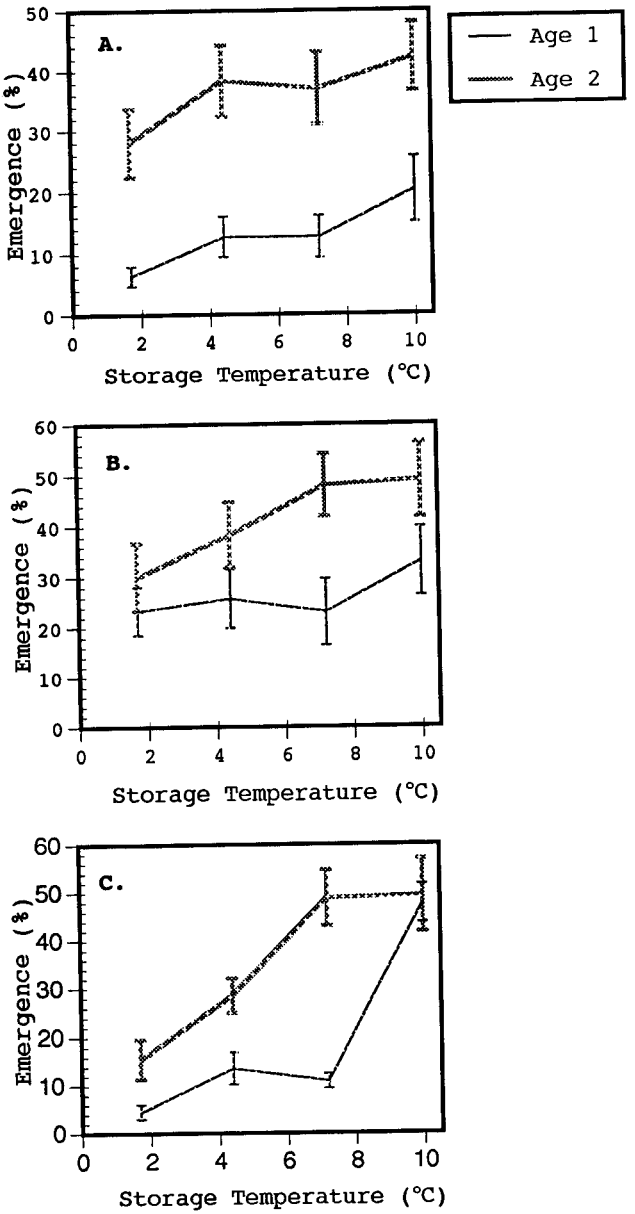

FIG. 1. The effect of mummy age and storage temperature on the mean emergence (±SE) of *A. perpallidus* progeny from non(A)-, pre(B)-, and post(C)-acclimated mummies.

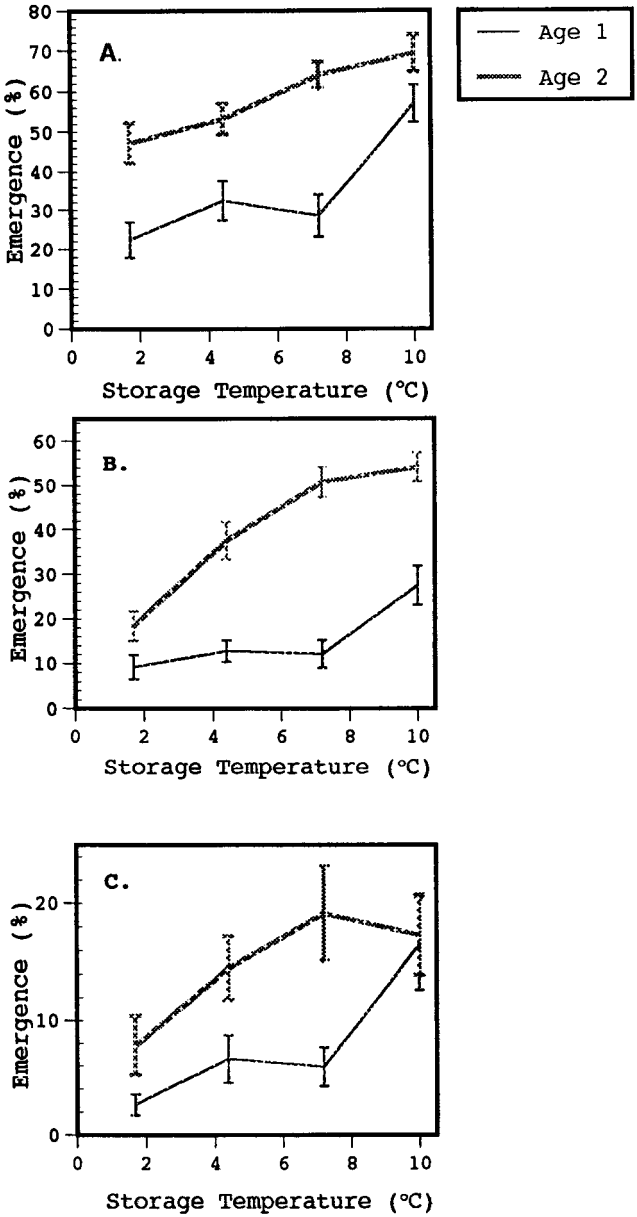

FIG. 2. The effect of mummy age and storage temperature on the mean emergence (±SE) of *A. perpallidus* progeny at 7(A), 15(B), or 30(C) days storage.

TABLE 2. A General Linear Model (GLM) Analysis of Experimental Variables and Interactions on the Development Time of All *A. perpallidus* Progeny[a].

Source of Variation[b]	DF	F-value	Pr>F
Accl.	2	4.32	0.01
Age	1	0.50	0.48
Temp.	3	4.17	<0.01
S.P.	2	64.96	<0.01
Accl.x Age	2	9.56	<0.01
Accl.x Temp.	6	1.88	0.08
Age x Temp.	3	1.17	0.32
Accl.x S.P.	4	0.52	0.72
Age x S.P.	2	3.67	0.03
Temp.x S.P.	6	0.70	0.65
Accl.x Age x Temp.	6	1.27	0.27
Accl. x Age x S.P.	4	6.90	<0.01
Accl.x Temp.x S.P.	12	1.11	0.36
Age x Temp.x S.P.	6	2.20	0.04
Accl.x Age x Temp.x S.P.	12	1.71	0.06

[a]Significant differences caused by interactions of variables on the development time of *A. perpallidus* progeny were determined at P<0.05 for 303 observations.

[b]Accl.=acclimation, age=mummy age, temp.=storage temperature, and s.p.=storage period.

acclimated group, development time of *A. perpallidus* was longer from younger mummies (35.7 and 46.3 days compared to 21 and 38.9 days from older mummies at 7 and 15 days storage, respectively) (Fig. 3A). Development time at 30 days was longer from older mummies than younger ones. Development time from pre-acclimated, younger mummies nearly doubled at each succeeding storage period (26.7, 40.8, and 82.8 days at 7, 15, and 30 days storage, respectively) and was longer than development times from older mummies at each storage period. (Fig. 3B). Parasitoid developmental time from post-acclimated mummies showed a reversal from pre-acclimated mummies (Fig. 3C). Development time at 7 days storage was 25 to 27 days for parasitoids emerging from younger and older mummies. Development time from older mummies was 38.5 and 63.3 days at 15 and 30 days storage, respectively, and longer than developmental time from younger mummies.

Generally, thermal acclimation did not lengthen parasitoid development time from younger or older mummies. Data indicate that pre-thermal acclimation of younger and older mummies shortened parasitoid development time at each storage period (except 30 days from younger mummies) compared to development from their non-acclimated counterparts, whereas, post-thermal acclimation of older mummies shortened parasitoid development time only at 30 days storage.

The interaction of mummy age, temperature, and storage period on development time was significant (P<0.05). Figure 4 shows the effect of mummy age and storage period on *A. perpallidus* development at different temperatures. Parasitoid development time for both age categories increased at each storage period for each temperature and generally was longer from younger mummies compared to their older counterparts. Development time at 1.7°C ranged from 27.5 days at 7 days storage to 91.6 days at 30 days from older mummies (Fig. 4A). Development time at 4.4°C from younger and older mummies was similar at 7 days storage, 22 to 23 days (Fig. 4B), but development time was longer in younger mummies than older mummies at 15 and 30 days storage. Development time at 7.2°C ranged from 18 days at 7 days storage from older mummies to 67 days at

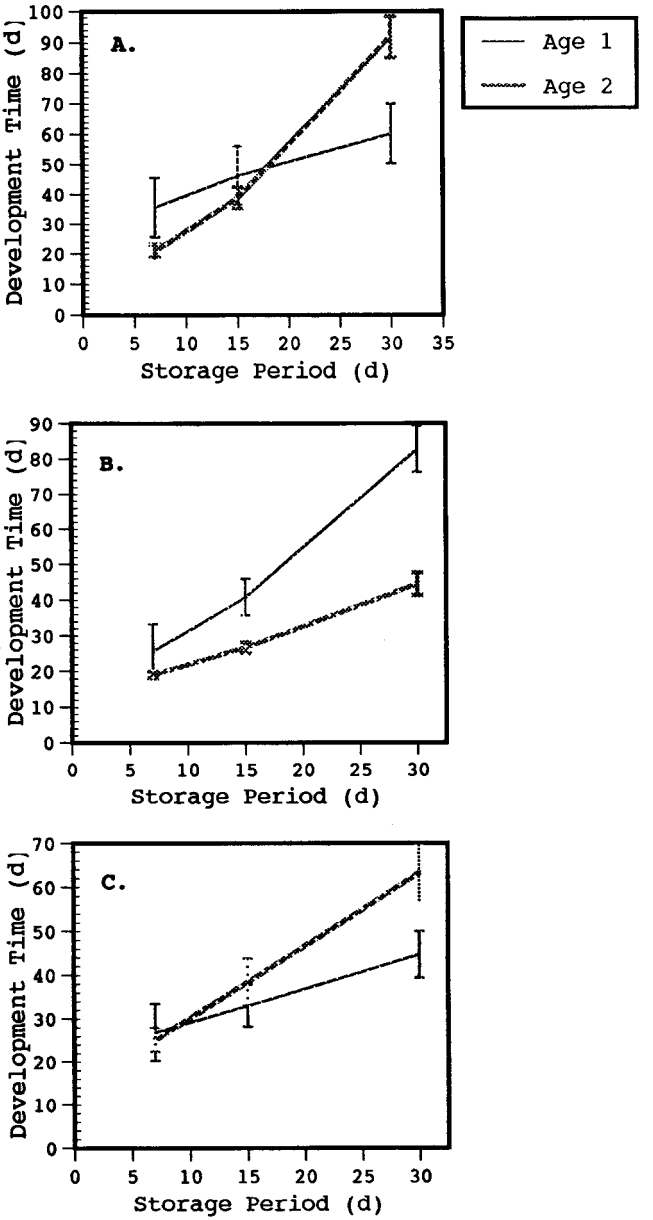

FIG. 3. The effect of mummy age and storage period on mean development time (±SE) of *A. perpallidus* from non(A)-, pre(B)-, and post (C)- acclimated mummies.

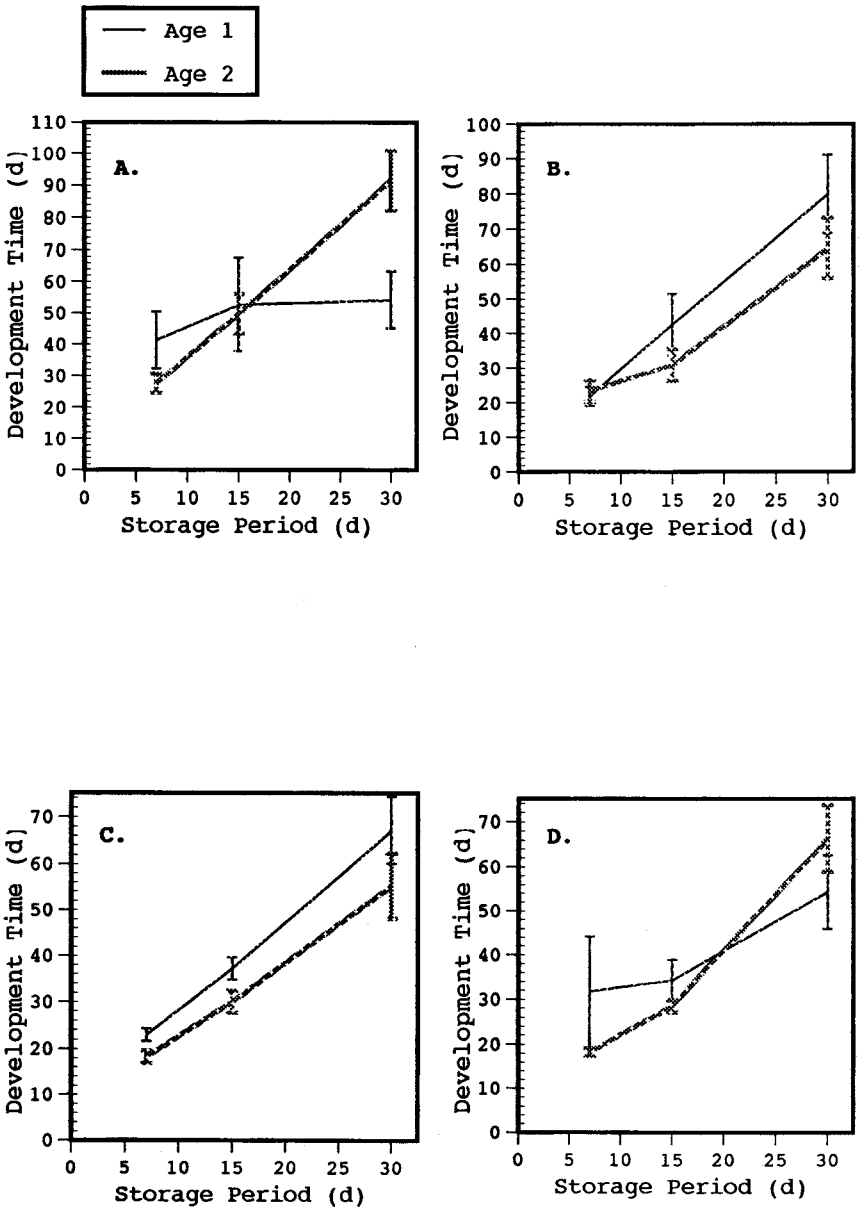

FIG. 4. The effect of mummy age and storage period on mean development time ($\pm$SE) of *A. perpallidus* progeny at 1.7°(A), 4.4°(B), 7.2°(C), and 10°C(D).

30 days storage from younger mummies (Fig. 4C). Development at 10°C was longest from younger mummies at 7 and 15 days storage (31.7 and 34.2 days, respectively), but was longer at 30 days from older mummies.(Fig. 4D).

Results from this research demonstrate that the experimental variables used usually impact parasitoid emergence in a consistent manner. Thermal acclimation increased emergence of *A. perpallidus*. In our study, subjecting *A. perpallidus* mummies to pre-thermal acclimation increased emergence at lower storage temperatures, while post-thermal acclimation increased emergence at higher temperatures. The higher percentage of mortality of immature stages in the non-acclimated group was due to the variation in temperature between the actual storage conditions and room temperature (Singh and Srivastava 1988). Our data agree with Chapman (1982) who concluded that parasitoids usually withstand low or high temperatures better after they have been thermally acclimated.

Emergence from older mummies was higher than from younger mummies. Older immature stages of *A. perpallidus* were more tolerant when exposed to cold storage conditions. Lower emergence from younger mummies could have been the result of higher mortality and/or the activation of diapause or quiescence; higher mortality could have been due to the lack of proper nutrients. Metabolic activity during the older larval and pupal periods is dependent upon nutritive material accumulated in the body during the immature larval stages; thus, using older mummies for storage will allow for this accumulation to occur and increase the probability of successful development. Based on results of this study, older *A. perpallidus* mummies are better suited to cold storage. In addition, prolonged storage at low temperatures results in depletion of nutrients necessary for the parasitoid to complete development and thus increases mortality at longer storage periods. Archer et al. (1973) reported that higher mortality at colder temperatures indicates a negative effect on metamorphosis and survival. Exposure of immature stages of *A. perpallidus* to low storage temperatures and longer storage periods may have resulted in the induction of diapause thus reducing emergence.

Female emergence is a major factor to consider in cold storage studies. Male parasitoids are necessary for mating purposes because fertilization of eggs is necessary to produce female progeny in the case of *A. perpallidus*. When considering all factors, thermal acclimation of older mummies exposed to storage temperatures of 4.4, 7.2, and 10°C for 7 days storage and 7.2°C for 15 days yielded the highest female emergence rate at 33-39%. Male emergence under these same conditions was 14-34%, and total emergence was 61-72%. Both male and female parasitoids were functional (i. e., progeny were produced when parasitoids were released with aphids). Total emergence under laboratory conditions was approximately 70%. These figures from cold storage studies for male and female emergence are conservative since total emergence was usually higher compared to combined male and female emergence. This descrepancy occurred because numerous emerged parasitoids were not sexed due to death and ensuing dessication and also to the occassional inability to distinguish between male and female parasitoids. Thus, it is certain that female and male emergence was higher than that recorded in this study.

At any acclimation condition, lower temperatures, and longer storage periods, the development of immature stages *of A. perpallidus* in younger mummies generally was extended over a longer period of time compared to immature stages in older mummies, although adult emergence rate was higher from older mummies. Storage conditions that yielded the

highest female emergence, as discussed above, usually resulted in minimal retarded parasitoid development. Under these conditions development time for female and male *A. perpallidus* ranged from 18 to 22.5 days. Post-emergence time (i. e., number of days required to emerge after storage) ranged from 3.5 to 11 days. The normal development time for *A. perpallidus* under normal laboratory conditions (24°C) was approximately 14 to 16 days. Parasitoid developmental time could be longer but storage conditions would have to be changed. Altering the optimal storage conditions to further retard development would decrease the emergence rate for *A. perpallidus*.

In conclusion, the rate of emergence and developmental time of *A. perpallidus* were highly variable depending on the storage conditions that parasitoids were subjected. The need for a specific number of parasites may vary as may the need to retard development, depending on the situation. Different requirements for parasitoid numbers as well as the extent of retarding development would dictate the appropriate storage conditions necessary to accomodate the need. The maximal build-up of laboratory *A. perpallidus* populations using acclimatiion and cold storage techniques is approximately 70% of mummies used.

Monellia caryella populations exhibit bimodal seasonal abundance patterns (Shepard 1973, Gentry et al. 1975, Polles and Mullenox 1977, Tedders 1978, Leser 1981, Edelson and Estes 1983). Early season infestations occur in May and June between the pollination and fruit enlargement phenological stages of the tree. Mid-season population densities during fruit enlargement to shell hardening are typically very low. Vigorous population growth from shell hardening to harvest produces the late season peak in population levels usually causing the most severe probelms (Dutcher 1985, Dutcher and Htay 1985). During this time, producers treat for aphids with multiple insecticide applications when maximal *A. perpallidus* populations are present. Because the damage impact of late season *M. caryella* populations is high and *A. perpallidus* populations reach maximal levels from shell hardening to harvest, this would be an appropriate time to augment parasite populations through inocualtive releases to help control aphid populations and possibly reduce insecticide treatments. Integration of this research with current aphid management strategies in pecan could help achive this goal.

ACKNOWLEDGEMENT

We would like to thank Dr. J. K. Olson for providing the facilities and equipment to conduct this research and Dr. R. A. Wharton for his meticulous critique of this research.

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ADULT DIETS FOR LABORATORY REARING OF THE MEXICAN RICE BORER (LEPIDOPTERA: PYRALIDAE)

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ABSTRACT

Longevity, oviposition profiles, fecundity, and egg fertility were compared when mated and unmated laboratory-reared adults of the Mexican rice borer, *Eoreuma loftini* (Dyar), were supplied water or 10% sucrose solution (w/v), or no diet at all. Male *E. loftini* survived longest when supplied water; longevity and fecundity of both mated and unmated females were also greatest when moths were provided water. Adult diet did not influence egg fertility. Adult diet influenced longevity of males more than that of females. Longevity of females was further reduced by access to a mate. Fecundity of mated females was greater, and more sensitive to adult diet, than that of unmated females. Impacts of the sucrose diet or absence of a diet on oviposition profiles of mated females were greatest after the second day of oviposition.

INTRODUCTION

The Mexican rice borer, *Eoreuma loftini* (Dyar), is the key pest of sugarcane, *Saccharum* spp., in the Lower Rio Grande Valley of Texas (Johnson 1984, Meagher et al. 1994). The ability to rear vigorous, healthy insects in the laboratory is an important aspect of biological investigations of this insect. We found the diet of Shaver and Raulston (1971) a satisfactory larval diet, and have experienced best results when this diet was finely shredded rather than presented as a poured diet (D.W.S. and J.R.R., unpublished data). However, preliminary rearing efforts indicated that nutrient or sugar solutions may not represent optimal adult diets.

van Leerdam et al. (1984) used a 20% glucose adult diet during oviposition experiments with *E. loftini*, but they did not indicate whether this diet was used in the maintenance of their colony. Brown et al. (1988) maintained adult *E. loftini* on a 10% sucrose diet. Our early efforts to maintain adults on sucrose solutions resulted in accumulations of large amounts of dark-brown liquid in the guts of adults after only a few days of feeding. This material was sufficiently conspicuous to interfere with dissections, and was absent from dissected field-collected moths. Although we have frequently observed *E. loftini* adults feeding on dew on sugarcane leaves and in grassy areas on field borders, nectar sources are absent within stands of sugarcane. Repeated efforts to observe *E. loftini* adults feeding on flowering plants near sugarcane fields also have been unsuccessful

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(D.W.S., unpublished data). These observations led us to hypothesize that water would be a more satisfactory adult diet than sugar solutions. Our objective was to evaluate the longevity, fecundity, and oviposition profiles, of adults of *E. loftini* given access to either water or a sucrose solution, or provided no diet at all.

MATERIALS AND METHODS

Eoreuma loftini larvae were obtained from a laboratory colony maintained under reversed photoperiod (14:10 [L:D] h) and thermoperiod conditions ranging from ≈22 to 33°C during the scotophase and photophase, respectively, (Spurgeon et al. 1994) for three or four generations at the USDA-ARS laboratory in Weslaco, TX. Larvae were individually reared in 29.5-ml plastic cups on finely shredded soybean-wheat germ diet of Shaver and Raulston (1971). Larvae were observed daily for pupation. Pupae were sexed and held individually in rearing cups containing ≈10 ml of moistened vermiculite. Female pupae were weighed to the nearest 0.01 mg on the day after pupation.

Pupae were observed daily for adult eclosion. At the time of eclosion, each moth was assigned a treatment (adult diet regime; paired with a mate or single) and identifying number. All moths or pairs of moths were confined separately in 473-ml cardboard ice cream cartons lined with oviposition substrate (a pleated, unbleached coffee filter), the edge of which was folded over the carton rim, covered with a sheet of clear plastic wrap, and held in place by the rim of the carton lid.

Diet treatment regimes were deionized water, 10% sucrose (w/v), or no diet. Fresh diets were supplied daily in a saturated cotton ball contained in a plastic rearing cup. A cup containing a water-saturated cotton ball was placed under the oviposition substrate of each moth receiving no diet to maintain a humidity within the rearing carton similar to that of other treatments. Five each of single males, single females, and male/female pairs were assigned to each diet treatment at a time. This procedure was repeated five times; observations were recorded on a total of 300 moths.

Oviposition substrates of females were replaced daily at the beginning of the photophase. Eggs were counted and the substrate of each mated female was retained individually in a plastic bag with a moistened paper towel to determine fertility. Eggs were classified as fertile if head capsules or larval stripes were visible after 5 d.

Size of the egg complement and fecundity are related to pupal weight (Spurgeon et al. 1995), so pupal weights of females were compared among treatment combinations by analysis of variance using the SAS procedure PROC GLM (SAS Institute 1988). Longevity, fecundity, fertility (total number of fertile eggs), and percentage of eggs that were fertile were also compared among treatments using analysis of variance. All interactions of main effects were considered in initial analyses. When interactions were not significant and were not contained in a signficant higher order interaction, they were absorbed by the error term providing their inclusion did not inflate the mean square error. Means were separated using the Ryan-Einot-Gabriel-Welsch multiple-range test (REGWQ option of PROC ANOVA).

RESULTS AND DISCUSSION

Longevity differed significantly among all adult diet regimes ($F=163.02$; df=2, 262; $P<0.01$). Mean longevity was greatest on water (12.9 d), intermediate on sugar solution (10.2 d), and least when no diet was supplied (6.1 d). Males lived longer (11.2 d) than females (8.28 d) ($F=91.77$; df=1, 262; $P<0.01$), presumably because of the greater energetic commitment by females to egg production. Both males and females also tended to live longer when held singly (10.5 d) than when held with a mate (9.0 d) ($F=24.87$;

df=1, 262; $P<0.01$).

A significant interaction between diet and sex ($F=39.15$; df=2, 262; $P<0.01$) indicated that differences in longevity among adult diet regimes were greater for males than for females (Fig. 1). A differential sensitivity to adult diet may have been observed because the energetic commitment to egg production represents a limitation to potential females longevity.

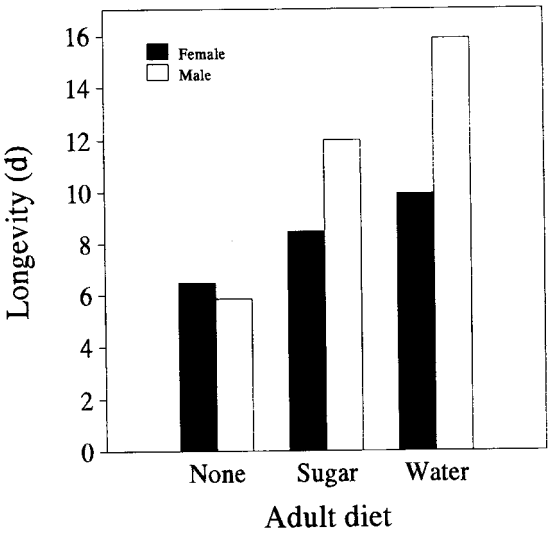

Fig. 1. Mean longevity of male and female *E. loftini* adults under different adult diet regimes.

The interaction between sex and mating status ($F=12.30$; df=1, 262; $P<0.01$) indicated that longevity of males held singly was similar to that of males held with a female, while unmated females lived longer than mated females (Fig. 2). The greater potential longevity of males may represent an adaptive advantage for the species under field conditions because these males would remain available as potential mates for subsequent females well after the initial mating of their female siblings.

Mean pupal weights of females were similar among treatment combinations ($F=1.16$; df=29, 120; $P=0.285$; overall model), ranging from 46.49 mg (water) to 48.93 mg (sucrose). Thus, differences in pupal weights did not greatly influence respective daily oviposition or fecundity estimates. Fecundity (mated and unmated pooled) differed significantly among all adult diets ($F=16.94$; df=2, 120; $P<0.01$). Mean fecundity was greatest when females were supplied water (252.5 eggs/female), intermediate when supplied sucrose solution (212.1 eggs/female), and least when no diet was supplied (149.3 eggs/female). Mean fecundity (pooled over all diets) of mated females (285.5 eggs/female) was much greater than that of unmated females (123.7 eggs/female) ($F=123.01$; df=1, 120; $P<0.01$). This increase in fecundity probably accounts for the reduction in female longevity associated with mating. The significant interaction between diet and mating status ($F=8.02$; df=2, 120; $P<0.1$) indicated that diet had a greater influence on fecundity of mated females than that of unmated females (Fig. 3). As diet suitability decreased, from water to no diet, oviposition by unmated females tended to occur earlier and the overall

oviposition period was shortened (Fig. 4). Daily oviposition by mated females was reduced after the first day of oviposition when no diet was offered, and after the second day of oviposition when sugar solution was offered, compared with moths supplied water (Fig. 5).

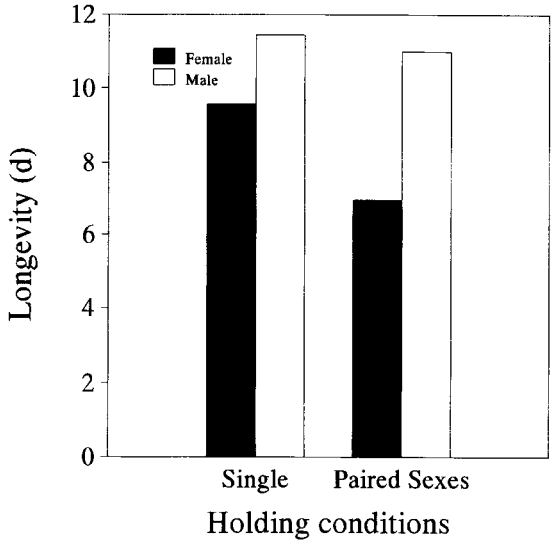

Fig. 2. Mean longevity of *E. loftini* males and females held singly or as paired sexes.

No differences in the percentage of egg that were fertile were detected among adult diet regimes or repetitions of the experiment ($F=1.47$; df=6, 68; $P=0.203$; overall model). Egg fertility ranged from 95.3% (sucrose solution) to 98.1% (no adult diet). Because the percentage of fertile eggs tended to be high in all treatments, effects of diet on fertility were similar to effects on fecundity. Mean fertility was highest when females were supplied water (350.7 fertile eggs/female), intermediate when supplied sucrose solution (286.2 fertile eggs/female), and lowest when no diet was supplied (186.8 fertile eggs/female) ($F=17.38$; df=2, 68; $P<0.01$).

The value of an adequate adult diet in rearing Lepidoptera is well documented for many species but requirements for individual species vary. Kira et al. (1969) reported that egg production of the European corn borer, *Ostrinia nubilalis* (Hübner), was greatly increased when water was supplied. Howell (1981) found that diets of water or sucrose solution increased longevity of the codling moth, *Cydia pomonella* (L.), compared with no adult diet, but did not appreciably increase fecundity because most oviposition occurred early in adult life. Sugar solutions have been shown to be superior to water as adult diets for the spruce budworm, *Choristoneura fumiferana* (Clemens), pink bollworm, *Pectinophora gossypiella* (Saunders), corn earworm, *Helicoverpa zea* (Boddie), tobacco budworm, *Heliothis virescens* (F.), and cotton leafworm, *Alabama argillacea* (Hübner) (Lukefahr and Griffin 1956, Lukefahr and Martin 1964, Miller 1987). Our data, combined with field observations, indicate that *E. loftini* is not adapted to a sugary diet. The caloric requirement in the *E. loftini* adult diet may be minimal because females are relatively short lived, contain a full complement of eggs at adult eclosion (Spurgeon et al. 1995), egg development is rapid and proceeds in the absence of mating, and most oviposition occurs early in adult life.

These data have ecological implications regarding the habitat requirements of *E. loftini* and in the use of novel control techniques such as adult attracticides. They should also be useful in future laboratory investigations of *E. loftini* basic biology.

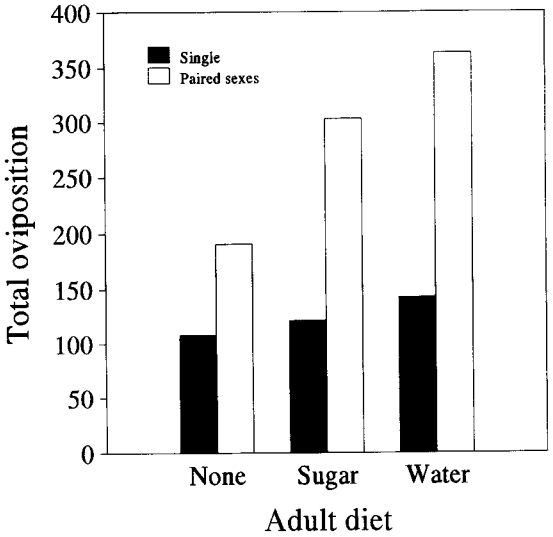

Fig. 3. Mean fecundity of unmated (single) or mated (paired sexes) *E. loftini* females under different adult diet regimes.

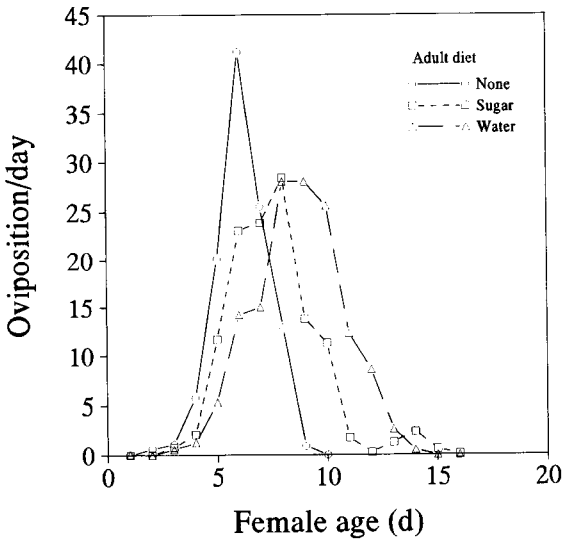

Fig. 4. Mean daily oviposition profiles of unmated *E. loftini* females under different adult diet regimes.

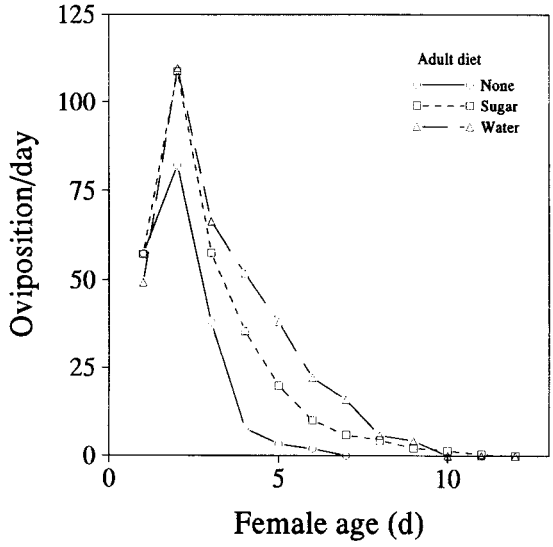

Fig. 5. Mean daily oviposition profiles of mated *E. loftini* females under different adult diet regimes.

ACKNOWLEDGMENT

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.

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CHANGES IN RESISTANCE TO INSECTICIDES IN TOBACCO BUDWORM[1] POPULATIONS IN MISSISSIPPI, 1993-1995[2]

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ABSTRACT

Strains of the tobacco budworm, *Heliothis virescens* (F.), collected in Mississippi in 1993 through 1995 were evaluated in bioassays to four classes of insecticides and *Bacillus thuringiensis* Berliner. High frequencies of resistance were found to cypermethrin, methomyl, and thiodicarb. Resistance to the organophosphorus insecticide profenofos was found in several strains. Significant resistance to *B. thuringiensis* Berliner was observed in one strain. Possible synergism of a pyrethroid in combination with the synergist piperonyl butoxide was observed. Imidacloprid, representing a new class of insecticide, was found to have ovicidal and larvacidal activity on *H. virescens*. Tolerance to imidacloprid was present in a field population resistant to carbamates but not to organophosphorus insecticides, suggesting the possibility of cross-resistance between carbamates and imidacloprid. Resistance to carbamates was clearly present in some populations susceptible to profenofos, indicating the presence of different mechanisms of resistance for the two classes of insecticides. Resistance to pyrethroids appears to be stabilized at a high level.

INTRODUCTION

Populations of the tobacco budworm, *Heliothis virescens* (F.), in the mid-South have developed resistance to pyrethroid, carbamate, organophosphorus, and cyclodiene insecticides (Elzen et al. 1990, 1992, 1994a, b; Martin et al. 1995). Initial resistance to pyrethroid insecticides in U. S. populations of *H. virescens* has been shown to be due to a kdr-like nerve insensitivity (McCaffery et al. 1989). Evidence for metabolic resistance also exists (Nicholson and Miller 1985; Graves et al. 1991; McCaffery et al. 1991; Clower et al. 1992; Ottea et al. 1993). Recent evidence has shown the expression of several

[1]Lepidoptera: Noctuidae

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

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mechanisms of resistance in field-collected populations in the U. S. (Ottea et al. 1995). However, target site insensitivity is probably the major mechanism of resistance to organophosphorus and carbamate insecticides (Kanga et al. 1994).

Currently, there are few alternatives to these classes of insecticides for control of *H. virescens*. Recently, resistance management strategies have placed greater emphasis on the use of biologicals (i.e. *Bacillus thuringiensis* Berliner) and further partitioning of chemical classes into "windows" (Baldwin and Graves 1991). Under this strategy, an attempt is made to avoid using all chemical classes on any given generation of insects, including specific recommendations for the use of certain classes of chemistry on specific generations of *H. virescens* based on resistance frequencies.

An investigation of generational and seasonal changes in insecticide tolerance may aid in formulating resistance management plans. The present study evaluates levels of resistance to four classes of insecticides and *B. thuringiensis* in strains of *H. virescens* collected in Mississippi in 1993 through 1995. In addition, results of tests with the synergist piperonyl butoxide (PBO) and an ovicidal bioassay with selected insecticides, including a representative of a new class, are also reported.

MATERIALS AND METHODS

The *H. virescens* strains evaluated and their collection dates are shown in Table 1. The Stoneville laboratory reference strain (STV-LAB) has been in culture at the Southern Insect Management Laboratory, Stoneville, MS, since collection from a Mississippi cotton field in 1984 (Elzen et al. 1992). The STV-LAB strain is annually infused with wild males captured in pheromone traps placed adjacent to cotton fields near Stoneville in an attempt to maintain genetic diversity. The susceptibility of the STV-LAB strain has not changed significantly in recent years (Elzen 1996). Field strains were collected from May (generation 1, G1) through August (generation 4, G4) from 1993 through 1995. All collections were made from the same areas around pheromone traps on similar dates (Table 1) in Washington County, MS. G1 was collected from geranium, *Geranium dissectum* L., and G2-G4 were collected from cotton, *Gossypium hirsutum* L. An additional strain (CAL) was collected from cotton in the hill area of northeast Mississippi (Calhoun County).

All *H. virescens* strains were reared in a similar manner. Adults were maintained in 3.8-liter cardboard cartons covered with cotton gauze as an ovipositional substrate and were fed a 5% sugar-water solution. Eggs were collected at least every other day and allowed to hatch at room temperature. Larvae were reared on a soybean flour-wheat germ diet (King and Hartley 1985).

Formulated insecticides tested were profenofos [Curacron 8 emulsifiable concentrate (EC); CIBA, Greensboro, NC], cypermethrin [Cymbush 3 (EC); ZENECA, Mountain View, CA], cyfluthrin [Baythroid 2 (EC); Bayer, Inc., Kansas City, MO], amitraz [Ovasyn 1.5 (EC); Agr Evo USA Co., Wilmington, DE], thiodicarb [Larvin 3.2 flowable (F); Rhone-Poulenc Agric. Co.,

Research Triangle Park, NC], methomyl [Lannate 2.4 liquid (LV); E. I. Dupont de Nemours & Co., Wilmington, DE], acephate [Orthene 90 soluble (S); Valent U.S.A. Corporation, Walnut Creek, CA], imidacloprid [Provado 1.6 (F); Bayer, Inc., Kansas City, MO], endosulfan [Thiodan 3 (EC); Agr Evo USA Co., Wilmington, DE], and *Bacillus thuringiensis* Berliner [Dipel (ES); Abbott Laboratories, North Chicago, IL].

The synergist tested was piperonyl butoxide [Butacide 8 (EC); ZENECA, Wilmington, DE].

TABLE 1. Location and Collection Date for 1993-95 Field Strains of Tobacco Budworm Tested in Bioassays. See Text for Additional Notes.

Strain	Location	Date
STV-LAB	Laboratory Reference	
	1993	
G1-93	Washington County, MS	28 May, 1993
G3-93	Washington County	24 July
G4-93	Washington County	29 August
	1994	
G1-94	Washington County, MS	9-13 May, 1994
G2-94	Washington County	10-15 June
G3-94	Washington County	11-15 July
G4-94	Washington County	15-19 August
	1995	
G1-95	Washington County, MS	8-17 May, 1995
G2-95	Washington County	13-16 June
G3-95	Washington County	18-24 July
G4-95	Washington County	17-22 August
CAL	Calhoun County, MS	11 August 1995

Spray Chamber Bioassay. The methods and materials used in this bioassay were previously described in detail (Elzen et al. 1992). This bioassay has been used to document changes in tolerance of *H. virescens* during the cotton growing season, within generations, and over several years (Elzen et al. 1992; Elzen 1994, 1995, 1996). Briefly, cotton ('DES 119') terminals (10-14 cm stem with three to four small leaves and buds) clipped from greenhouse grown plants were placed in floral piks (Dakota Plastics, Watertown, SD) containing water. 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 88 kg/cm^2 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 *H. virescens* (20 ± 3 mg) was placed on each terminal 30 min after spraying and each plant was covered 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°, 55-60% RH and a 14L:10D photoperiod. Treatment efficacy was determined after 72 h and numbers of dead (non-moving and black or shriveled) and moribund (non-responsive when prodded with a blunt probe) 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 arcsine transformed and analyzed by analysis of variance; means were separated by least significant difference [$P \geq 0.05$ (SAS Institute 1988)].

Ovicidal Activity Bioassay. Treatments were applied to cotton ('DES 119') grown in small plots at Stoneville, MS. Plot size was 9 m x 4 rows arranged in a randomized complete block with 4 replicates/treatment. Treatments were applied on 12 July by a high-clearance spray machine equipped with a compressed air spray system. Total spray volume was 56 liters per ha at 8 km/h at 102 kg/cm^2 pressure with two TX-10 nozzles/row. *Heliothis virescens* eggs were obtained from cultures maintained in the laboratory. Eggs from the STV-LAB strain and the G2-95 strain were treated separately. All eggs used were laid the previous night on tulle cloth material. Eggs were not washed or chemically treated before use and were easily removed by crumpling the material. Eggs treated in the field were placed on the upper surface of the last 3-4 expanded leaves in the terminal of 4 plants in each replication by brushing a weak solution of gum xanthan and water on the leaves and sprinkling eggs on the surface. Plots were sprayed 30 min after application of the eggs. Eggs remained in the field for 2 h after treatment and were then taken to the laboratory to determine mortality. The number of viable eggs was counted on each leaf in the laboratory 24 h after being sprayed. The number of eggs averaged 15.0 ± 5.5 (S.D.). Leaves were held in petri dishes on water-moistened filter paper at 29 ± 3°C, 55-60% RH, and a 14L:10D photoperiod. The number of unhatched eggs was determined at 48 and 72 h after eggs were applied. Eggs were considered dead when black and shriveled. Cumulative egg mortality (EM) 72 h after treatment is reported. Hatched larval mortality (LM) was observed at 48 and 72 h after treatment and is reported as cumulative larval mortality at 72 h after treatment. EM and LM were used to calculate total mortality (TM) at 72 h. Control mortality was never greater than 10%; data were corrected for control mortality using Abbott's (1925) formula. Percent mortalities were arcsine tranformed and analyzed by analysis of variance; means were separated by least significant difference [$P \geq 0.05$ (SAS Institute 1988)].

RESULTS AND DISCUSSION

Spray Chamber Bioassay. In general, no major changes occurred in the apparent nature of resistance in *H. virescens* in the strains tested from 1993 through 1995, with the notable exceptions of a possible increase in resistance to methomyl and the detection of resistance to *B. thuringiensis* in one strain in 1995 (Table 2). Levels of resistance remained similar to those observed in previous years (Elzen 1991, 1994; Elzen et al. 1992) with resistance levels from high to low as follows: pyrethroids > carbamates > organophosphates > *B. thuringiensis*. However, the following specific findings are worthy of note.

All strains tested in 1993 were significantly resistant to cypermethrin ($F = 62.16$; df = 3, 8; $P < 0.05$), thiodicarb ($F = 10.44$; df = 3, 8; $P < 0.05$), and endosulfan ($F = 10.57$; df = 3, 8; $P < 0.05$) compared with the STV-LAB strain. Only the G4-93

strain was significantly resistant to profenofos ($F = 13.67$; df = 3, 8; $P < 0.05$) and the G3-93 and G4-93 strains were significantly resistant to methomyl ($F = 13.80$; df = 3, 8; $P < 0.05$) compared with the STV-LAB strain (Table 2).

TABLE 2. Efficacy of Selected Insecticides Against 1993-1995 Field-Collected Strains of *H. virescens* in a Spray Chamber Bioassay Compared with a Laboratory Reference Strain[a].

Treatment	Kg(AI)/ha[c]	% Mortality[b], 72 h			
		Year			
		G1	G2	G3	G4
			1993		
Cypermethrin	0.09	33.3a	---	24.5a	22.2a
Thiodicarb	1.00	71.1b	---	53.3c	57.8c
Profenofos	1.12	84.5bc	---	100.0e	68.8c
Methomyl	0.50	94.4c	---	62.2d	53.3bc
Endosulfan	1.12	40.0a	---	40.0b	31.1ab
F; df = 4,10		16.67		91.97	6.61
			1994		
Cypermethrin	0.09	6.7a	33.5a	26.7a	35.5ab
Thiodicarb	1.00	53.3c	75.6b	64.5b	60.0bc
Profenofos	1.12	48.9c	95.6c	95.5c	91.1d
Methomyl	0.50	15.6ab	75.5b	62.2b	73.3c
B. thuringiensis	2.30	35.6bc	46.7a	57.8b	24.4a
F; df = 4,10		5.26	13.16	27.45	14.93
			1995		
Cypermethrin	0.09	22.2a	24.4a	17.8a	26.6a
Thiodicarb	1.00	66.7bc	66.7b	57.8b	44.4c
Profenofos	1.12	84.4c	95.5c	86.7c	86.6d
Methomyl	0.50	48.9b	57.7b	51.1b	40.0c
B. thuringiensis	2.30	22.2a	33.3a	44.4b	33.3b
F; df = 4, 10		19.44	26.77	21.73	149.52

[a]STV-LAB percent mortality: cypermethrin 95.5, thiodicarb 95.5, profenofos 95.5, methomyl 95.5, endosulfan 84.5, *B. thuringiensis* 40.0.
[b]Means within a column by year followed by the same letter are not significantly different ($P \geq 0.05$; least significant difference [SAS Institute 1988]).
[c]*B. thuringiensis* applied as liters of product/ha.

All strains tested in 1994 were significantly resistant to cypermethrin ($F = 24.28$; df = 4, 10; $P < 0.05$), thiodicarb ($F = 19.72$; df = 4, 10; $P < 0.05$), and methomyl ($F = 18.88$; df = 4, 10; $P < 0.05$) compared with the STV-LAB strain. The G1-94 strain had significantly lower ($P < 0.05$) mortality (6.7%) in response to cypermethrin than any strain tested. Likewise, the G1-94 strain had significantly lower ($P < 0.05$) mortality (15.6%) in response to methomyl than any strain tested. Only the G1-94 strain was significantly resistant to profenofos ($F = 2.73$; df = 4, 10; $P < 0.05$) compared with the STV-LAB strain. This strain was also significantly ($P < 0.05$) more resistant to profenofos than any other strain as was the G4-93 strain to profenofos. There is the possibility that the high level of resistance to profenofos shown in August 1993 (generation 4) carried through to influence the response of generation 1 collected in May 1994. No significant levels of resistance to

B. thuringiensis ($F = 3.82$; df = 4, 10; $P \geq 0.05$) were found among any strains tested (Table 2).

In 1995, significant levels of resistance were found in all strains to cypermethrin ($F = 51.70$; df = 4, 10; $P < 0.05$), thiodicarb ($F = 14.94$; df = 4, 10; $P < 0.05$), and methomyl ($F = 21.60$; df = 4, 10; $P < 0.05$) compared with the STV-LAB strain. Only the G1-95 strain was significantly resistant to profenofos ($F = 2.73$; df = 4, 10; $P < 0.05$) and *B. thuringiensis* ($F = 2.34$; df = 4, 10; $P < 0.05$) compared with the STV-LAB strain (Table 2). Percent mortality in response to *B. thuringiensis* in the G1-95 strain (22.2%) is similar to that of the G4-94 strain (24.4%) (Table 2).

From the above, it is apparent that multiple resistance is present and that cross-resistance exists among carbamates. It appears that pyrethroid resistance has stabilized at a high level.

Because the tests with and without PBO used reduced rates of insecticides in an attempt to detect any synergism (Table 3), responses cannot be compared directly with those in Table 2. However, the general levels of resistance follow the trend shown in Table 2 with effectiveness from low to high being pyrethroid < carbamate < organophosphorus insecticide).

Table 3. Efficacy of Selected Insecticides With and Without Piperonyl Butoxide (PBO) Against 1993 Field Strains of *H. virescens* in a Spray Chamber Bioassay.

Treatment	Kg(AI)/ha	% Mortality[a], 72 h	
		G1-93	G3-93
Cypermethrin	0.045	20.0b	6.7ab
Cypermethrin	0.045	33.3bc	24.4bc
+ PBO	0.28		
PBO	0.28	2.2a	2.2a
Thiodicarb	0.50	57.8d	11.1ab
Thiodicarb	0.50	46.7cd	22.2bc
+ PBO	0.28		
Profenofos	0.56	82.2e	64.4d
Profenofos	0.56	82.2e	44.4cd
+ PBO	0.28		
F; df		12.00; 6,14	12.43; 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]).

The increased level of mortality (4-fold) in the G3-93 strain (Table 3) in response to the addition of PBO to cypermethrin, while not significant ($P \geq 0.05$), may be an indication of metabolic resistance in this strain. Similar results have been previously found with field strains of *H. virescens* (Elzen et al. 1993, Martin et al. 1994). There was a decrease in mortality in response to cypermethrin in the G3-93 strain compared with the G1-93 strain; however, this difference was not significant ($F = 5.71$; df = 1, 4; $P \geq 0.05$).

No significant differences were found in percent mortality for thiodicarb with or without PBO in the G1-93 or G3-93 strains (Table 3). PBO might be expected to inhibit the activation of thiodicarb, resulting in reduced toxicity.

However, percent mortalities for thiodicarb with PBO ($F = 9.90$; $df = 1, 4$; $P < 0.05$) and without PBO ($F = 15.36$; $df = 1, 4$; $P < 0.05$) were significantly lower in the G3-93 strain compared with the G1-93 strain (Table 3) suggesting a role of selection pressure for increased resistance to thiodicarb during the cotton season. A similar decrease in percent mortality was also seen for thiodicarb in the G1-93 and G3-93 strains using the full rates of insecticide in the spray chamber bioassay (Table 2).

Bioassays of profenofos indicated no significant differences in toxicity with or without PBO in either strain (Table 3). Similar results were found by Kanga et al. 1994. Thus, enhanced metabolism is unlikely as a major mechanism of resistance in these strains. However, percent mortality was significantly lower in the G3-93 strain for profenofos alone ($F = 3.86$; $df = 1, 4$; $P < 0.05$) and profenofos plus PBO ($F = 36.80$; $df = 1, 4$; $P < 0.05$) compared with the G1-93 strain. This increase may again be a result of selection pressure during the cotton season.

The Calhoun strain showed a significantly high level of resistance to cypermethrin, a significant intermediate level of resistance to thiodicarb and methomyl, and no significant resistance to the organophosphorus insecticides profenofos and acephate, nor significant resistance to *B. thuringiensis* in comparison with the STV-LAB strain (Table 4). The cotton field from which this strain was collected had received six applications of a pyrethroid insecticide prior to collection of the larvae.

TABLE 4. Efficacy of Selected Insecticides Against a Laboratory-Susceptible and Field-Collected Strain of *H. virescens* in a Spray Chamber Bioassay.

Treatment	Kg(AI)/ha[b]	% Mortality[a], 72 h		
		STV-LAB	CAL	F; df
Cypermethrin	0.09	95.5bA	15.5aB	994.67; 1,4
Thiodicarb	1.0	95.5bA	75.6cB	17.33; 1,4
Profenofos	1.12	95.5bA	97.8dA	2.07; 1,4
B. thuringiensis	2.30	40.0aA	42.2bA	0.49; 1,4
Methomyl	0.50	95.5bA	64.4bcB	81.00; 1,4
Acephate	1.12	95.5bA	93.3dA	1.10; 1,4
F; df		95.60; 5,12	34.04; 5,12	

[a]Means within a column followed by the same lowercase letter or means within a row followed by the same uppercase letter are not significantly different ($P \geq 0.05$; least significant difference [SAS Institute 1988]).
[b]*B. thuringiensis* applied as liters of product/ha.

Ovicidal Activity Bioassay. Imidacloprid, which was developed primarily for its activity on sucking insects (Almand and Mullins 1991, Mullins and Engle 1993) and therefore targeted primarily against aphids, thrips, whiteflies, and plant bugs in cotton, was found to cause mortality of eggs and larvae of *H. virescens* (Table 5), a previously unreported finding. No significant differences in egg mortalities were found among treatments for the STV-LAB strain. However, amitraz and thiodicarb were significantly ($P < 0.05$) more

effective ovicides than imidacloprid or other treatments on the G2 strain. Significantly lower ($P < 0.05$) larval mortality resulted from amitraz treatment on the STV-LAB and G2-95 strains than was found for thiodicarb and cyfluthrin (Table 5). Lower egg mortality of cyfluthrin and thiodicarb on the G2-95 strain compared with the STV-LAB strain is an indication of possible resistance in the eggs. Elzen (1991) found significant reduction in the ovicidal activity of pyrethroid, carbamate, and organophosphorus insecticides on a field-collected strain of *H. virescens*. Leonard et al. (1990) had previously demonstrated similar results. There was also a reduction in the effect of thiodicarb and imidacloprid on larvae in the G2-95 strain compared with the STV-LAB strain (Table 5). A comparison with the field strain data in Table 2 further suggests a relationship between imidacloprid and carbamate resistance, but not between imidacloprid and organophosphorus insecticide resistance. This suggests that imidacloprid/carbamate resistance may involve the acetycholine receptor while organophosphorus insecticide resistance is more associated with acetylcholinesterase. Regarding total mortality, cyfluthrin and thiodicarb were significantly more effective than other treatments except imidacloprid at the high rate and cyfluthrin plus imidacloprid on the STV-LAB strain; cyfluthrin, amitraz, and thiodicarb were significantly more effective than other treatments on the G2-95 strain (Table 5).

TABLE 5. Efficacy of Selected Insecticides Against a Laboratory-Susceptible and Field-Collected strain of *H. virescens* in an Ovicidal Bioassay.

Treatment	Kg(AI)/ha	STV-LAB			G2-95		
		%EM[a,b]	%LM	%TM	%EM	%LM	%TM
Cyfluthrin	0.033	43.3a	85.8cd	65.5d	17.5a	75.8c	60.8c
Cyfluthrin + Imidacloprid	0.033 0.024	31.5a	63.5bc	52.5bc	23.5a	51.5bc	49.3bc
Imidacloprid	0.049	35.5a	36.5b	47.0bc	30.1a	21.5a	37.3ab
Imidacloprid	0.024	24.8a	45.3b	44.8a	20.5a	8.0a	23.8a
Amitraz	0.280	44.5a	8.8a	46.3ab	64.0b	10.5a	64.0c
Thiodicarb	0.280	31.3a	82.0cd	62.3cd	56.5b	36.8ab	63.0c
F; df = 5, 15		1.01	9.91	2.82	5.74	5.61	5.05

[a]EM = egg mortality; LM = larval mortality; TM = total mortality.
[b]Means within columns for each strain followed by the same letter are not significantly different ($P \geq 0.05$; least significant difference [SAS Institute 1988]).

Until recently, following the initial documentation of pyrethroid resistance (Plapp and Campanhola 1986; Leonard et al. 1987; Luttrell et al. 1987), major concerns had been primarily with pyrethroid resistance in *H. virescens*. Resistance to non-pyrethroids was documented in Mississippi and Louisiana in 1990 field populations (Leonard et al. 1991, Elzen et al. 1992). Resistance to all classes of insecticides was again found in Louisiana and Mississippi, and also Texas in 1991 (Elzen et al. 1994, Martin et al. 1995). The data presented herein document the continued presence of resistance to all classes of insecticides in *H. virescens*. However,

relatively few strains showed resistance to organophosphorus insecticides and resistance to *B. thuringiensis* was found in only one strain. Greater frequencies of resistance to organophosphorus insecticides have been shown using other bioassays (Elzen et al. 1992, Martin et al. 1995) and these issues should remain a concern. The finding of resistance to *B. thuringiensis* in a field population, likewise, should not be overlooked. In other cases, considerable variation in susceptibility of *H. virescens* to *B. thuringiensis* has been shown (Stone and Sims 1993). Therefore, the potential for development of widespread resistance to *B. thuringiensis* remains a possibility.

In 1995, the combination of high, sustained infestations of *H. virescens* and high levels of insecticide resistance in Mississippi resulted in the inability of producers in the most heavily infested areas to achieve control (Hardee and Herzog 1996). Multiple and cross-resistance in *H. virescens*, as well as continued development of resistance in other pests, could seriously jeopardize the cotton industry in the U. S. Resistance management plans should strongly emphasize strategies that involve conservation of all insecticides used against *H. virescens*. Increased use of biological/biorational insecticides, insect growth regulators, and the discovery and registration of new classes of insecticide chemistry for control of all pests should be emphasized.

ACKNOWLEDGMENT

Larry Adams (Southern Insect Management Laboratory, Stoneville, MS) reared insects, made field collections, and performed bioassays. The Calhoun County strain was provided by Blake Layton (Mississippi Cooperative Extension Service, Mississippi State, MS). The author thanks J. B. Graves (Louisiana State University, Baton Rouge, LA) and J. E. Mulrooney (USDA, ARS, Stoneville, MS) for critical review of the manuscript.

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EFFECT OF SHORT-TERM COLD STORAGE ON MUMMY-STAGE SURVIVAL IN TWO PARASITES OF THE RUSSIAN WHEAT APHID[1]

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ABSTRACT

We measured the effect of storage for 0, 2, 4, 7 and 14 days at 10°C on adult emergence from mummies in *Aphelinus asychis* (Walker) and *Aphelinus albipodus* (Hayat and Fatima) (Hymenoptera: Aphelinidae) stored in either waxed cardboard cartons or in gelatin capsules. For both species, no significant difference was observed in the proportion of adults emerged under either storage method. Adult emergence decreased significantly in both species when mummies were stored for 14 days compared to 0-7 days. No significant difference was observed in the size of adult *A. albipodus* parasites emerging from mummies stored for 14 days relative to those stored for shorter periods.

INTRODUCTION

Since the discovery of the Russian wheat aphid, *Diuraphis noxia* (Mordvilko), in Texas, U.S.A., during 1986 and its subsequent spread throughout North American grain growing regions, extensive collections and releases of exotic parasites have been made to combat this pest González Some of these parasites are currently being reared and shipped to field locations for release and evaluation.

Currently, Russian wheat aphid mass rearing and release efforts do not require storage of mummies to obtain sufficient numbers for synchronized adult emergence and release. Production of Russian wheat aphid parasites is maximized by collecting mummies when they have accumulated for 3-6 days, at which time many are in the late pupal stage (J. Bernal, pers. observ.). Thus, the high mortality normally associated with long-term cold storage is avoided. The objective of this study was to determine *Aphelinus asychis* (Walker) and *Aphelinus albipodus* (Hayat and Fatima) (Hymenoptera: Aphelinidae) mortality when stored for varying lengths of time at a low temperature (10°C) in shipping containers commonly used in insectary shipping programs.

MATERIALS AND METHODS

Parasites used in our experiments were obtained from colonies maintained at the University of California, Riverside. The parasites were reared on Russian wheat aphid on spring wheat (cultivar MIT). *Aphelinus asychis* and *A. albipodus* mummies were obtained 11-12 days after oviposition by adult parasites from colonies maintained at 22-25°C with a 14:10LD photoperiod and 30-60% RH.

Mummies were collected and subjected to either of two treatments. One group of 400 mummies was packed for shipping and storage in standard shipping cartons (450-ml waxed cylindrical cardboard cartons, each end replaced with 1-mm mesh plastic greenhouse shade cloth and secured by accompanying cardboard rings, 60 X 15 mm plastic petri dishes and a

[1] Homoptera: Aphididae

rubber band). Another group of 400 mummies were isolated individually in #1 clear gelatin capsules. Both groups of mummies were held in an unlighted chamber for 2, 4, 7, or 14 days at 10±1°C and 35% RH (i.e. cold storage). On days 2, 4, 7, and 14, 100 mummies of each group were removed from cold storage and incubated at 20±3°C for emergence of adults. Mummies stored in the standard shipping cartons were isolated individually in #1 clear gelatin capsules prior to incubation at 20°C. The average size of the emerged parasites from each group was estimated by measuring both the head capsule width and hind femur length of 20 *A. albipodus* females. Adult size is a reliable indicator of fitness in female parasites (Opp and Luck 1986, Shu-sheng 1985).

Comparisons between proportions of adults emerged from each group of mummies were made by examining for overlap among confidence intervals. Confidence intervals were estimated as $(t_{(1-[1-0.95])1/n,\ N})$, where n is the number of comparisons made (i.e., 10), and N is the sample size. The latter ensured that the comparisonwise error rate (10 comparisons) resulted in a 95% confidence level for the experimentwise error rate for a Type I error. We used analyses of variance to test for differences in the size of adult parasites among treatments. Means are presented followed by their standard error.

RESULTS AND DISCUSSION

A significant difference was not observed between the proportion of adults emerged from mummies isolated in gel caps prior to cold storage and those that were kept in the standard shipping cartons during cold storage for any number of days (Fig. 1). Thus, storage of mummies in standard shipping cartons may not result in decreased adult emergence compared to storage in other more protected conditions (i.e., gelatin capsule).

Although a significant difference was not observed between storage methods, a significant decrease ($p \leq 0.05$) in adult emergence was observed for mummies of both species held in cold storage for 14 days compared to those stored for shorter lengths of time (0-7 days) (Table 1). Table 1 shows data which have been pooled from both methods of cold storage (i. e., gelatin capsules and shipping cartons). Data were pooled because significant differences were not found between storage methods.

TABLE 1. Percentage of Adult Emergence in *Aphelinus asychis* and *A. albipodus* with Confidence Intervals (CI).

Days in cold storage	Percentage Emergence ±CI[a] *Aphelinus asychis*	Percentage Emergence ±CI[a] *Aphelinus albipodus*
0	85.0 ± 7.29 b	88.0 ± 6.63 b
2	79.0 ± 5.84 b	88.0 ± 4.66 b
4	74.5 ± 6.25 b	74.7 ± 6.26 b
7	75.3 ± 7.15 b	76.5 ± 6.08 b
14	53.3 ± 8.28 c	67.0 ± 6.74 c

a CI = 98.7 confidence interval; numbers followed by a same letter within column are not significantly different ($p < 0.05$)

The percentage of adult *A. asychis* emerging decreased from ca. 85% in mummies not held in cold storage to <55% when held in cold storage for 14 days. In the case of *A. albipodus*, adult emergence decreased from >85% in mummies not held in cold storage to <70% in mummies held in cold storage for 14 days. From these results, it appears that pupal stage *A. albipodus* may be better able to survive extended periods of cold storage

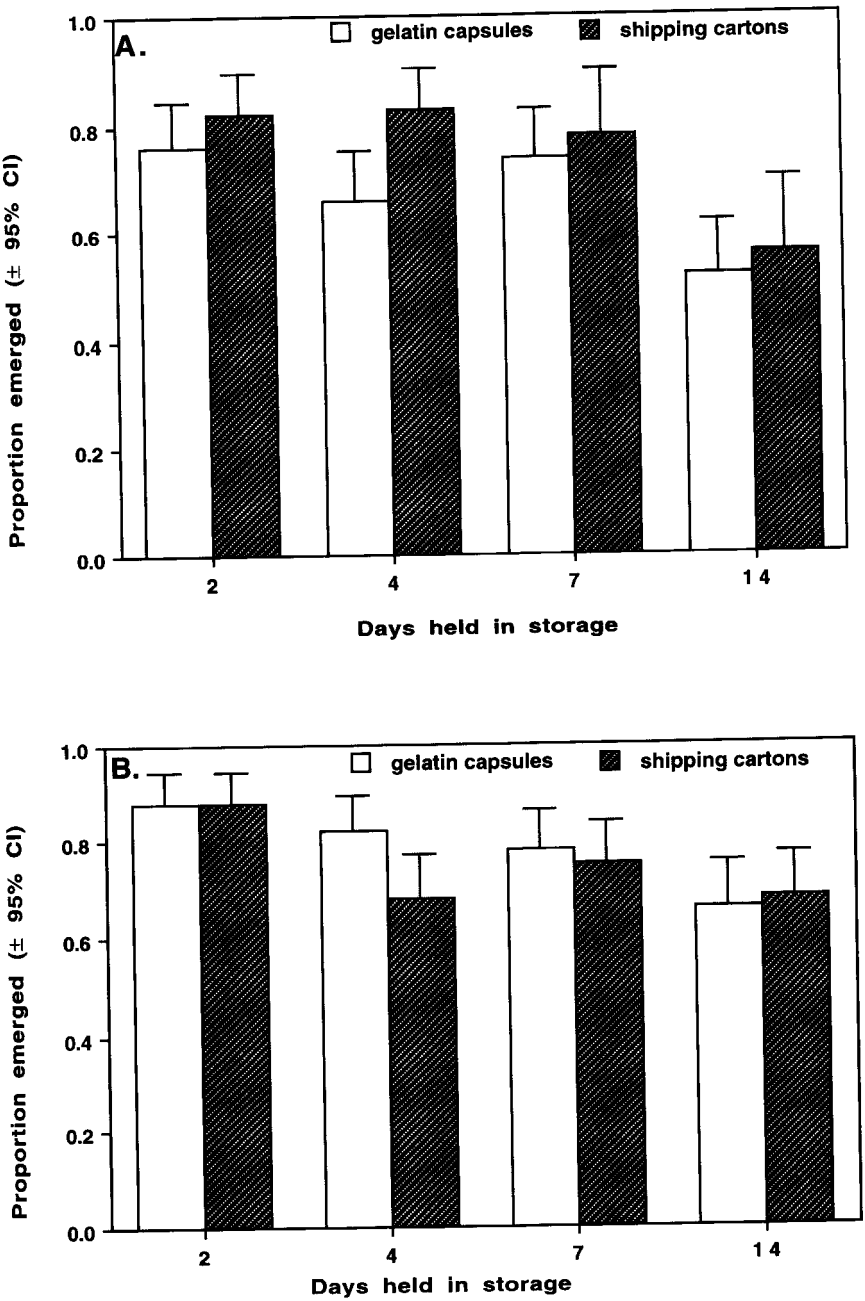

FIG. 1 Proportion of parasites emerged from standard shipping cartons vs. gelatin capsules after being held at 10°C A) *Aphelinus asychis*, B) *A. albipodus*.

than pupal stage *A. asychis*. For example, ca. 21% fewer *A. albipodus* adults emerged from mummies stored at 10°C for 14 days compared to mummies not stored at this temperature; ca. 32% fewer adults emerged in the case of *A. asychis* (Table 1).

Prior studies have assessed the effect of long term (more than two weeks) cold storage on aphid parasite mummies (Archer et al. 1973, Giri et al. 1982, Hagvar and Hofsvang 1991) and on adult parasites (Archer et al. 1976, Archer and Eikenbary 1973, Scopes et al. 1973) that are held for synchronized release in inundative release programs. Results of these studies were similar to our results. Long term cold storage was shown to significantly increase mortality (>25%) in mummies held for more than 15 days at 10°C while fecundity of surviving adults was not substantially affected (Archer and Eikenbary 1973). In addition, Archer et al. (1973) and Whitaker-Deerberg et al. (1994) showed that early-stage mummies, including 3-day-old prepupae, survived long-term storage better than more developed, pupal stage, mummies.

In this study, we measured head capsule width and hind femur length of *A. albipodus* females to assess potential effects of cold storage on fitness of the parasites used. Significant differences were not observed between size of parasites emerged from any of the treatments (femur: $F_{(4,174)} = 1.793$, $p = 0.132$; head width: $F_{(4,174)} = 0.408$, $p = 0.803$). This would indicate that the fitness of *A. albipodus* females held in cold storage was unaffected. However, development was practically concluded in the parasites used in this experiment so that storage at 10°C would not be expected to greatly affect their size. On the other hand, adults emerging from mummies stored for 14 days commonly died sooner than others, often within 24 hours. The reduced longevity of these parasites could translate into reduced fitness. Similarly, Archer and Eikenbary (1973) noted that female *A. asychis* that lived less than 7 days after cold storage failed to reproduce. Adult *A. asychis* emerged from mummies exposed to low humidity have a reduced longevity (M. Waggoner, pers. observ.). Thus, the reduced longevity observed in adults subjected to 14 days of cold storage during the mummy stage in this study may be due to desiccating effects of storage at 35% RH rather than an effect of cold on the parasites. Further experiments where parasites are maintained at low humidity but at moderate temperatures are required to confirm this hypothesis.

Based on the results of this study, we conclude that shipping late pupal stage mummies packed in standard shipping cartons stored for up to seven days at 10°C is not detrimental to the quality of *A. asychis* or *A. albipodus* reared on Russian wheat aphid. Storage under such conditions could allow the accumulation of parasites in cases where numbers of parasites needed for field releases exceed the production of a single cycle.

ACKNOWLEDGMENT

The authors would like to thank Dr. T. Bellows and Dr. R. Luck, University California Riverside, for review of the manuscript.

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NUEVAS ESPECIES DE *STERPHUS* (DIPTERA: SYRPHIDAE) DE COSTA RICA CON NOTAS SOBRE OTRAS ESPECIES PRESENTES EN COSTA RICA.

M. A. Zumbado[1] y F. C. Thompson[2].

ABSTRACT

Sterphus rufoabdominalis and *gamezi* are described. A new key to the species of *Sterphus* is given. Distribution notes are given for other Costa Rican species.

RESUMEN

Se describen las especies *Sterphus rufoabdominalis* y *gamezi*. Se presenta una nueva clave para las especies de *Sterphus*.

INTRODUCCION

Los sírfidos del género *Sterphus* se encuentran a lo largo de los trópicos del Nuevo Mundo. Se desarrollan en madera, ayudando en el reciclaje de nutrientes, los adultos son polinizadores. Debido a lo diverso de su ciclo de vida, el grupo es un buen indicador de la salud de los bosques tropicales. Este artículo describe dos especies previamente desconocidas para la ciencia. Esta información, junto con la clave de identificación incluída será de utilidad para científicos estudiando dípteros, y a nuestro entendimiento de la biodiversidad de Costa Rica.

Sterphus Rondani fue primero revisado en parte por Thompson (1973) y luego por Hippa (1978) quien describió 8 nuevas especies. Hippa y Thompson (1994) revisaron el grupo de especies *Sterphus cybele*. A pesar de este trabajo previo, nuevas especies aún siguen siendo descubiertas. Se describen aquí dos nuevas, junto con notas sobre las especies de Costa Rica. El género *Sterphus* contiene ahora unas 31 especies, las cuales se encuentran desde México hasta Chile y Argentina. Según la clasificación actual (Hippa 1978), estas especies están distribuidas en 3 subgéneros y 10 grupos de especies. De Costa Rica se conocen siete especies.

También se presenta una nueva clave para las especies del género. La terminología morfológica es la indicada por McAlpine (1981), exceptuadas las modificaciones introducidas por Thompson (1997). Todo el material está depositado en INBio, a menos que se indique otro sitio.

Sterphus coarctatus (Wiedemann)

Xylota coarctata Wiedemann 1830: 100.
Sterphus coarctatus: Thompson 1973: 229.

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2 Systematic Entomology Laboratory, ARS, USDA, NHB-168, Smithsonian Institution, Washington, D.C. 20560 USA.

Sterphus coarctatus fue descrita de Brasil, pero aparentemente presenta una distribución amplia a través del bosque tropical de tierras bajas. Los registros costarricenses son de Guanacaste, Cerro El Hacha, 300 m, 12 km SE de La Cruz, L-N 329600, 368000, V 1988, M. Espinoza (♀ INBIO CRI000672026); Guanacaste, Est. Maritza, 600 m, lado O Vol. rosi, L-N 326900, 373000, 28 II - 10 III 1992, C. Cano (♀ INBIO CRI000750613); Guanacaste, Est. Pitilla, 700 m, 9 km S Sta. Cecilia, L-N 330200, 380200, 22 IX a 14 X 1992, C. Moraga (♂ INBIO CRI000824924).

Sterphus gamezi Thompson, especie nueva.
Figuras 1-4

Descripción.

Cabeza: Negra; cara brillante excepto estrechamente blanco-polinosa lateralmente; gena brillante anteriormente, blanco-polinosa y pilosa posteriormente; lúnula frontal amarilla; triángulo frontal brillante, desnudo excepto por algunos pelos cortos posteriormente; triángulo vertical plateado-blanco-polinoso y desnudo anteriormente, pardusco-polinoso y amarillo-pardusco-piloso posteriormente; occipucio blanco-polinoso y piloso ventralmente, llegando a ser pardusco-polinoso y amarillo-pardusco-piloso en 1/3 dorsal; antena negra, negro-pilosa; arista parda; basoflagelomero alargado, aproximadamente 3 veces tan largo como ancho.

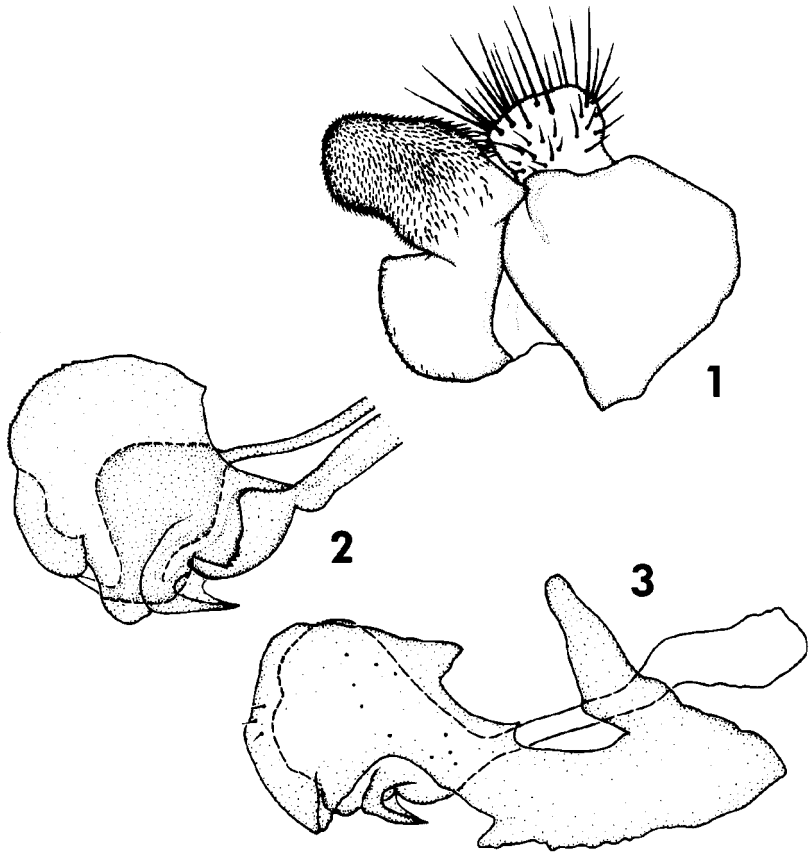
Tórax: Negro; postpronoto y escudo brillante excepto ampliamente negro-pardusco-polinoso lateralmente y estrechamente blanco-polinoso hacia postpronoto medial, corto-piloso negro y amarillo excepto más largo y denso negro-piloso dorsomedialmente al ala y más largo amarillo-piloso sobre notopleura y callo postalar; escutelo negro-pardusco-polinoso, amarillo-pardusco-piloso; pleura blanco-grisáceo-polinosa, amarillo-pilosa; calyptera negra; plúmula corta, negra; halterio blanco con capítulo negro. Patas: negras, extensivamente amarillo-pilosas; trocánter posterior con tubérculo pequeño; tibia posterior con dos dientes apicales; fémur posterior con fuertes pelos espinosos negros apicoventralmente; tarsos frontal y posterior extensivamente negro-pilosos; tarso medio con 3 tarsómeros apicales negro-pilosos. Ala: negra anteriormente, transparente posteriormente; coloración negra extendiéndose hasta y llenando celda BM, 1/3 anterobasal de celda DM y 1/4 posteroapical de celda R4+5; membrana cubierta por microtriquias excepto en 1/3 anterobasal de celda CuP, lóbulo anal anterior a vena A2 y basomedialmente sobre álula; álula alargada, casi tan ancha como celda BM.

Abdomen: Negro excepto amarillo en 2/3 basomediales de 2do tergo, peciolado, más angosto en base de 2do segmento y más ancho en unión de 3ro y 4to, casi 3 veces tan ancho como en su parte más angosta, con 2do tergo apicalmente casi el doble ancho de la base y casi 3 veces tan largo como ancho en su parte más estrecha; 1er tergo largo-piloso amarillo; 2do tergo largo-piloso amarillo sobre 2/3 basales y lateralmente, negro apicalmente; 3er tergo corto-piloso negro; 4to tergo corto-piloso amarillo excepto estrechamente negro-piloso lateral y apicalmente; 1er esterno negro, amarillo-piloso; 2do esterno amarillo, amarillo-piloso; 3er y 4to esternos negros, negro-pilosos; genitales del macho, opaco-polinosos, negro-pilosos.

@HEAD2 = Material examinado.

Holotipo: Costa Rica, Cartago, Quebrada Segunda Ref. Nac. Fauna Silv. Tapantí, 1250m, L-N 194000, 560000, IV 1992, R. Vargas (♂ INBIO CRI000459533). Holotipo en depósito con el USNM, Washington.

La frase "en depósito con ..." indica depósito condicional de un tipo como opuesto a "depositado en ..." lo que indica depósito permanente de un tipo. Los tipos son propiedad de la Ciencia, las organizaciones sólo sirven como custodios para la Ciencia. Los custodios más



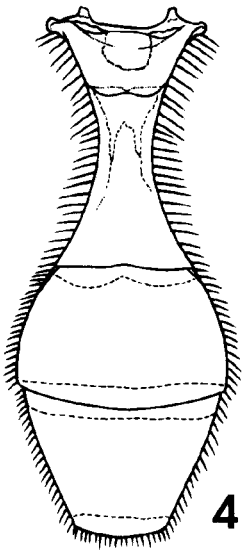
Figs. 1-3. *Sterphus gamezi* Thompson. 1, 3, Genitales del macho, vista lateral. 2. Edeago, vista lateral.

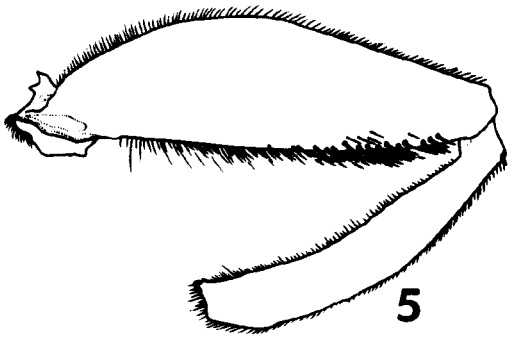
apropiados para tipos principales son organizaciones dentro del país de origen, siempre que esas organizaciones estén bien dispuestas y sean capaces de aceptar esta responsabilidad para con la Ciencia. Sin embargo, cuando un tipo principal es el único representante de una especie un lugar más apropiado para el tipo puede ser una colección mundial de testigos o comprobantes (Janzen 1993). Al ubicar al representante único en una colección mundial se incrementa la eficiencia y efectividad de los sistemáticos, al tener ellos en un solo lugar, una muestra más comprensiva de la diversidad del taxon. El United States National Museum ha acumulado una de las colecciones más comprensivas de sírfidos en el mundo y tiene un activo programa de investigación sobre la sistemática del grupo. Por lo tanto, el holotipo de *Sterphus gamezi* estará ubicado en esa colección hasta que sean obtenidos especímenes adicionales o nuestro conocimiento sistemático avance a tal punto que la necesidad del tipo en una colección mundial de testigos ya no sea esencial. Alcanzado ese punto el tipo deberá regresar al INBio.

Nos complace dedicar esta nueva especie costarricense a un gran maestro constructor para la biodiversidad, el Dr. Rodrigo Gámez Lobo, quien estableció el INBio.

Diagnosis:

Sterphus gamezi pertenece al grupo de especies *batesi* y probablemente es la especie hermana de *batesi* Shannon conocida del Amazonas. *Sterphus batesi* difiere de *gamezi* en lo





Figs. 4-5.*Sterphus*. 4, *Sterphus gamezi* Thompson, abdomen, vista dorsal. 5, *Sterphus rufoabdominalis* Zumbado, fémur posterior, vista lateral.

siguiente: 1) cara plateado-polinosa, no brillante medialmente; 2) escudo brillante, no extensivamente pardusco polinoso lateralmente; 3) escutelo brillante, negro piloso, no opaco polinoso y amarillo piloso; y 4) calyptera blanca, no negra.

Sterphus genuinus (Williston)

Xylota genuina Williston 1888: 284.
Sterphus genuinus: Thompson 1973: 223, 227.

Sterphus genuinus fue descrita de Brasil (Río de Janeiro), pero aparentemente está ampliamente distribuída a través del bosque tropical de tierras bajas. Los registros costarricenses son de: La Suiza de Turrialba, Cartago, IX 29, X 1, XI 13, Pablo Schild (3 machos, en USNM); Guanacaste, lado suroeste del Volcán Cacao, Estac. Cacao, 1000-1400 m, VI 1990, II Curso Parataxon (♂ INBIO CR1000247903); Cartago, Ref. Nac. Fauna Silv. Tapantí, 1150 m, L-N 194000, 559800, I 1992, G. Mora, F. Quesada (♂ INBIO CRI000529706); Cartago, P. N. Tapantí, 1250 m, L-N 194000, 560000, R. Vargas (♂ INBIO CRI000882984).

Sterphus janzeni Thompson

Sterphus janzeni Thompson en Hippa y Thompson 1994: 491

Sterphus janzeni fue descrita de Costa Rica y permanece conocida solo por aproximadamente treinta y dos especímenes, todos los cuales han sido recolectados en elevaciones medias (700-1800 m) en Costa Rica y Panamá (Chiriquí). Los nuevos registros costarricenses son de: Guanacaste, Est. Pitilla, 700 m, 9 km S Sta. Cecilia, P. N. Guanacaste, L-N 330200, 380200, III 1991, P. Ríos, (♂ INBIO CRI000657505); 31 III - 15 IV 1992, C. Moraga (♀ INBIO CRI000698445); P. Ríos (♀ INBIO CRI000860481); 27 VII a 14 VIII 1992, P. Ríos, (♂ INBIO CRI000778476); 19 V - 3 VI 1993, C. Moraga (♀ INBIO CRI001315383); Guanacaste, Río San Lorenzo, 1050 m, Tierras Morenas, Z. P. Tenorio, L-N 287800, 427600, IV 1992, F. Quesada (♀ INBIO CRI000930407); Puntarenas, Est. Biol. Las Alturas, 1500 m,

L-S 322500, 591300, X 1991, M. Ramírez (♀ INBIO CRI000632487); I 1992, M. Ramírez, G. Mora, F. Quesada (♂ INBIO CRI000573879); III 1992, M. Ramírez (♂ INBIO CRI000979989, ♀ INBIO CRI000979987, ♀ INBIO CRI000987276, ♀ INBIO CRI000987566); Puntarenas, Est. La Casona, 1520 m, Res. Biol. Monteverde, L-N 253250, 449700, IV 1993, N. Obando (5 ♀♀ INBIO CRI000793422, INBIO CRI000793462, INBIO CRI001181801, INBIO CRI001181808, INBIO CRI001181809); Cartago, Quebrada Segunda, P. N. Tapantí, 1250 m, L-N 194000, 560000, 18 a 28 II 1993, F. A. Quesada (♂ INBIO CRI001211420); Cartago, Q. Segunda, P. N. Tapantí, L-N 194000, 559800, X 1993, G. Mora (♀ INBIO CRI001128778)

Sterphus plagiatus (Wiedemann)

Xylota plagiata Wiedemann 1830: 98.
Sterphus plagiatus: Thompson 1973: 219.

Sterphus plagiatus fue descrita de Brasil, pero es una especie ampliamente distribuída en bosques tropicales de tierras bajas que ocurre desde México hacia el sur hasta Brasil. Los registros costarricenses son de Puntarenas, Estación Sirena en el Parque Nacional Corcovado (June, ♂ INBIO CRI000328944); Puntarenas, Golfo Dulce, 2 km west of Piedras Blancas, 200 m, IX-XI 1989, malaise trap, Paul Hanson (1 ♂ en USNM); Est. Pitilla, 700 m, 9 km S Sta. Cecilia, P. N. Guanacaste, L-N 330200, 380200, VII 1991, C. Moraga (♀ INBIO CRI000505775).

Sterphus rufoabdominalis Zumbado, especie nueva
Figuras 5-10

Descripción.

Macho.—Cabeza: negra; cara gris-plateado-polinosa excepto carina medial estrechamente brillante; gena brillante y desnuda anteriormente, escasamente gris-polinosa y blanco-pilosa posteriormente; lúnula frontal naranja-pardusca; triángulo frontal brillante y desnudo excepto blanco-plateado-polinoso y piloso lateralmente; triángulo vertical negro-polinoso, negro-piloso; occipucio blanco-plateado-polinoso y blanco-piloso en 2/3 ventrales, pardusco-negrusco-polinoso y negro-piloso en 1/3 dorsal. Antena: escapo y pedicelo negros, negro-pilosos; basoflagelomero negro-pardusco, casi el doble del largo que el ancho; arista naranja-pardusca.

Tórax: negro; postpronoto brillante apicolateralmente, gris-pardusco-polinoso posteriormente, negro-polinoso anterior y medialmente, amarillo-piloso con unos pocos pelos negros entremezclados; escudo negro-polinoso excepto por franja indistinta gris-polinosa medial y sublateral y gris-polinoso en notopleura, con pelos apretados negros y cortos, excepto pelos amarillos erectos más largos en notopleura; callo postalar sub-brillante, negro-polinoso, negro-piloso; escutelo sub-brillante, negruzco-polinoso, negro-piloso excepto fleco ventral amarillo; anepisternum y katepisternum gris-plateado-polinosos, blanco-pilosos; anepimeron con porción anterior sub-brillante, negruzco-polinosa, negro-pilosa; metasternum desnudo; tégula y basicosta negro-pilosas; calyptera blanco-grisacea excepto margen y fleco negros; plúmula negra; flecos espiraculares negros; halterio blanco; ala pardo-clara, enteramente cubierta por microtriquias. Patas: negras, blanco-pilosas excepto 3 tarsómeros apicales negros, fémures dorsalmente negro-pilosos, fémur posterior también negro-piloso en 1/3 apical; tibia anterior negro-pilosa ventral y dorsalmente, tibia media negro-pilosa anterior y ventralmente, tibia posterior negro-pilosa dorsobasalmente; trocánter posterior con un pequeño tubérculo apicolateral; fémur posterior engrosado, con setas espinosas ventrales en 1/2 apical, con área anterobasal de apretadas sétulas pardas; tibia posterior con carina ventromedial en 1/3 basal, apicalmente prolongada en dientes apical y posterior.

Abdomen: rojo, brillante, amarillo-piloso excepto por algunos pelos negros basolateralmente en segundo tergo; genitales rojos, amarillo-pilosos con algunos pelos negros

entremezclados.

Hembra. Similar excepto: cara con franja medial brillante más ancha; frente brillante en 1/2 ventral, negro-polinosa en 1/3 dorsal, blanco-plateado-polinosa medialmente, negro-pilosa en 1/2 dorsal; trocánter posterior sin tubérculo; quinto segmento rojo, amarillo-piloso.

Material examinado.

Holotipo: Costa Rica, Guanacaste, Est. Pitilla, 700 m. 9 km S Sta. Cecilia, P. N. Guanacaste. L-N 330200, 380200, 31 III - 15 IV 1992, C. Moraga (♂ INBIO CRI000698444. Depositado en el INBio, Santo Domingo.

Paratipos: Material depositado en el INBio: COSTA RICA. Guanacaste, P. N. Guanacaste, Est. Pitilla, 700 m, 9 km S Santa Cecilia, L-N 330200, 380200, 21 III - 21 IV 1989, GNP Biodiversity Survey (1 ♂ INBIO CRI000090346); V 1991, C. Moraga (♂ INBIO CRI000450685, ♂ INBIO CRI000450663, ♀ INBIO CRI000450758); 31 III - 15 IV 1992, C. Moraga (♂ INBIO CRI000698438, ♂ INBIO CRI000698441, ♂ INBIO CRI000698442, ♀ INBIO CRI698440); 2-15 V 1992, C. Moraga (♂ INBIO CRI000405431); VI 1991. P. Ríos (♂ INBIO CRI000546411); V 1991. P. Ríos (♂ INBIO CRI000327485, ♀ INBIO CRI000327717, ♀ INBIO CRI000327467); 31 III - 29 IV 1992. K. Taylor (♂ INBIO CRI000505577); 19 V - 3 VI 1993, P. Ríos (♂ INBIO CRI001355493); 19 V - 03 VI 1993, P. Ríos (♀ INBIOCRI001355501); 6-19 IX 1993, P. Ríos (♀ INBIOCRI001614280); 6-17 IX 1993, C. Moraga (2 ♀♀ INBIOCRI001614903, INBIOCRI001614933); IV 1994, C. Moraga (♀ INBIOCRI001789372, ♂ INBIOCRI001789371, ♂ INBIOCRI001789450); V 1994, P. Ríos (♂ INBIOCRI002046479, ♂ INBIOCRI002046480, ♀ INBIOCRI002046496); VI 1994, P. Ríos (♀ INBIOCRI001884023, ♂ INBIOCRI001884043); VII 1994, P. Ríos (♀ INBIOCRI002049347); VII 1994, C. Moraga (♂ INBIOCRI002001607); IX 1994, P. Ríos (2 ♀♀ INBIOCRI002005364, INBIOCRI002005365); Guanacaste, R. F. Cord. Guanacaste (Tenorio), R. San Lorenzo, 1050 m, L-N 287800, 427600, IV 1991, C. Alvarado (♂ INBIO CRI000443711); Guanacaste, A. C. Arenal, Río San Lorenzo, Tierras Morenas, 1050 m, L-N 283950, 424500, 10-30 IX 1993, G. Rodríguez (♀ INBIOCRI002079780); Alajuela, Est. San Ramón Oeste, 620 m, IV 1994, L-N 318100, 381900 (♂ INBIOCRI001779977); Alajuela, 2 km N Colonia Blanca, 800 m, P. N. Rincón de la Vieja, L-N 308800, 397800, 13 a 28 VI 1992, III Curso Parataxónomos. (♀ INBIO CRI000704026); Puntarenas, Est. Biol. Las Alturas, 1500 m, Coto Brus, L-S 322500, 591300, M. Ramírez (♂ INBIO CRI000631888); San José, Zurquí de Moravia, 1600 m, XI 1992, P. Hanson (♀ INBIO CRI001146851). Material depositado en el USNM: COSTA RICA. Guanacaste, Volcán Cacao, SW side, Estac. Cacao 1000-1400 m, L-N 323300, 375700, X 1989, R. Blanco & C. Chaves (♀ INBIO CRI000097599); Guanacaste, Estac. Pitilla, 9 km South of Sta. Cecilia, 700 m, L-N 330200, 380200, V 1990, II Curso Parataxónomos (♂ INBIO CRI000241914), V 1991, Petrona Rios (♂ INBIO CR1000327483), 31 III-29 IV 1992, K. Taylor (♀ INBIO CR1000376111); Limón, 16 km West Guápiles, 400 m, VIII-IX 1989, Paul Hanson (Malaise trap).

Diagnosis.

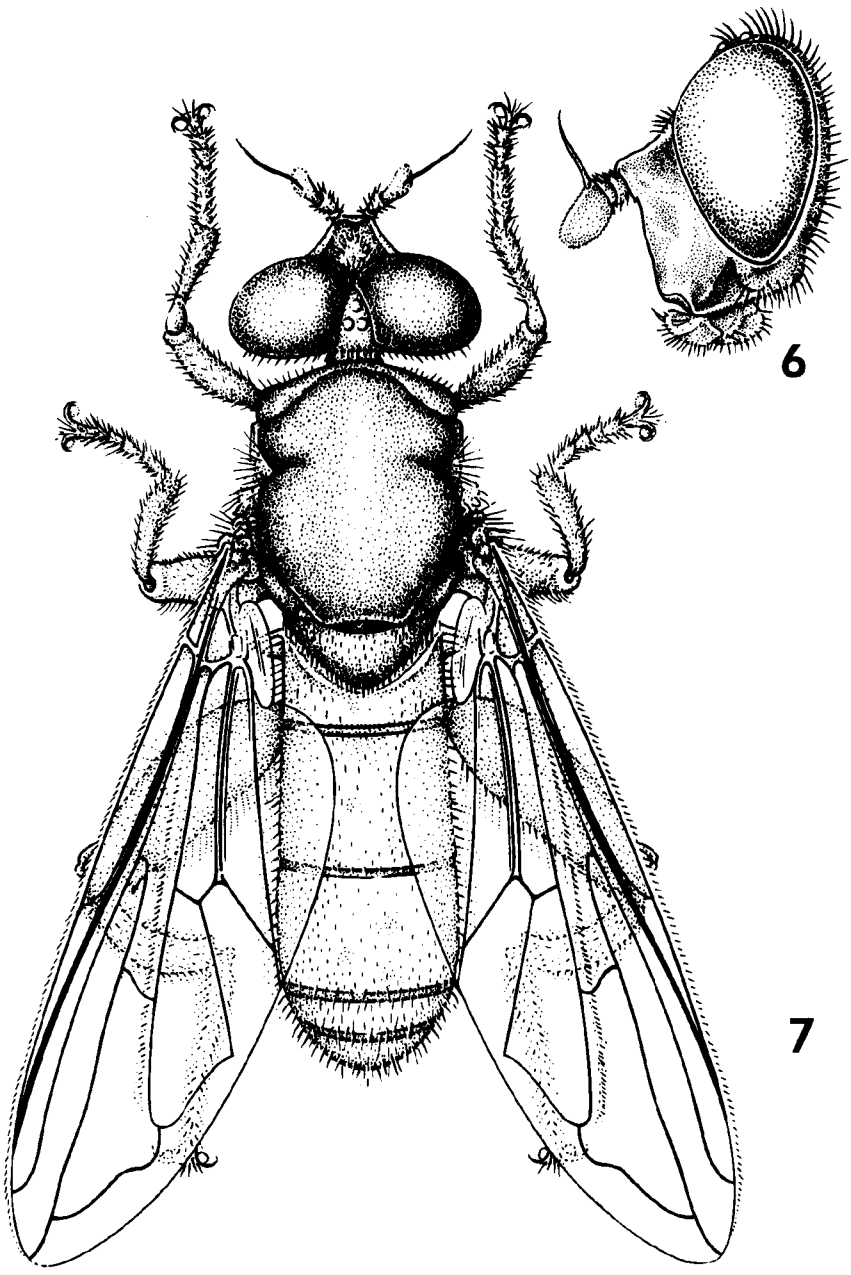
El nombre de la especie, *rufoabdominalis*, se refiere al carácter más distintivo de la especie, su abdomen rojo. Solo *Sterphus* (*Telus*) *telus* tiene el abdomen rojo como *rufoabdominalis*, pero se distingue fácilmente por los caracteres subgenéricos del fémur posterior y ala maculada.

Sterphus rufoabdominalis pertenece al grupo de especies *plagiatus*, pero difiere de todas las otras especies de este grupo en la carencia de banda transversa amarillo-polinosa en el mesonoto anterior.

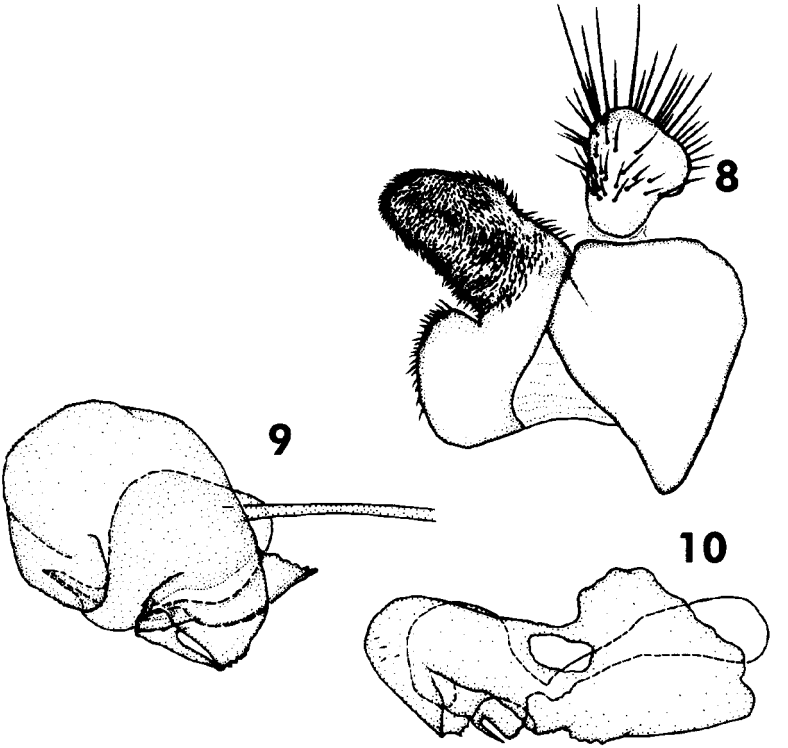
Sterphus stimulans Thompson

Sterphus stimulans Thompson 1973:206.

Sterphus stimulans fue descrita a partir de un único ♂ de Bolivia (Chipiriri). En Costa Rica



Figs. 6-7. *Sterphus rufoabdominalis* Zumbado. 6, Cabeza, vista lateral. 7, Hábito.



Figs. 8-10.*Sterphus rufoabdominalis* Zumbado. 8, 10, Genitales del macho, vista lateral. 9, Edeago, vista lateral.

la especie es conocida del bosque lluvioso montano a altas elevaciones (San José, Cerro de la Muerte, 20 km SE Empalme, P. Hanson (4 hembras, en USNM); Heredia, Braulio Carrillo N. P., Estación Barva, 2500 m, III 1990, A. Fernández (♂ INBIO CRI000108726), 1 ♂ INBIO CRI000169588 (en USNM)), II 1990, G. Rivera (1 ♀ INBIO CRI000202147 (en USNM)).

Sterphus tricrepis (Shannon)

Crepidomyia tricrepis Shannon 1926: 47.
Sterphus tricrepis: Thompson 1973: 223, 226.

Sterphus tricrepis fue descrito de Perú (Río Charape), pero es una especie ampliamente distribuída tanto en bosque tropical de bajura como de elevaciones medias, ocurriendo desde Cuba hasta Perú y Bolivia en el sur. Los registros costarricenses son de: Puntarenas, área de Monteverde, 1400-1700 m, 6-14 VI 1973, Erwin & Hevel, Central American Expt. (1 macho, en USNM); Puntarenas, Est. La Casona, 1520 m, Res. Biol. Monteverde, L-N 253250, 449700, IV 1993, N. Obando (♂ INBIO CRI001181788).

Clave para las especies de *Sterphus* Phillipi

1. Abdomen no peciolado, si ligeramente constreñido, entonces ancho mínimo nunca menor que 3/4 del ancho máximo (como en Fig. 7) 8
— Abdomen peciolado, ancho mínimo usualmente menos que 1/3 del ancho máximo (como

en Fig. 4) 2

2. Tibia posterior con espina apical 5
— Tibia posterior sin espina apical 3

3. Pata posterior naranja excepto coxa y 2 tarsómeros apicales parduscos, cara lisa (Brasil) . . . . . *tinctus* (Fluke)
— Pata posterior bicolor; coxa posterior, trocánter, fémur y tarsómeros apicales negros, tibia posterior y basitarsómero naranja; cara con protuberancia tuberculosa abajo 4

4. Tibias naranja unicolor; ala enteramente microtricosa (Venezuela) *incertus* (Thompson)
— Tibias bicolor, pardo-anaranjadas basalmente y pardo-oscuras apicalmente; ala microtricosa excepto en frente de vena anal (Colombia) . . . . . *nitidicollis* Hippa

5. Tibia posterior con 1 espina apical; peciolo abdominal corto, alrededor 1/4 del largo del segundo segmento (de Costa Rica a Brasil) . . . . . *coarctatus* (Wiedemann)
— Tibia posterior con 2 espinas apicales; peciolo abdominal largo, alrededor 3/4 del largo del segundo segmento 6

6. Celda anal desnuda excepto 1/4 apical con microtriquias; pata media naranja (Brasil) . . . . . *fulvus* Thompson
— Celda anal casi completamente microtricosa; pata media negra (Brasil) 7

7. Mesonoto brillante; 4to tergo abdominal negro-piloso . . . . . *batesi* (Shannon)
— Mesonoto extensivamente opaco, pardusco-polinoso lateralmente; 4to tergo amarillo-piloso . . . . . *gamezi* Thompson

8. Abdomen oscuro; ala transparente o casi completamente transparente 10
— Abdomen rojo 9

9. Fémur posterior ahusado, grandemente ensanchado en 1/2 apical, delgado en 1/2 basal y ápice; ala completamente oscura excepto por banda apical clara (Ecuador) . . . . . *telus* Thompson
— Fémur posterior uniformemente ensanchado (Fig. 5); ala uniformemente pardusca, sin banda apical clara (Fig. 7) . . . . . *rufoabdominalis* Zumbado

10. Metasterno piloso 28
— Metasterno desnudo 11

11. Cara brillante medialmente, polinosa lateralmente, mayormente negra, color de fondo nunca completamente amarillo o naranja 16
— Cara completamente polinosa, color de fondo naranja, amarillo o negro 12

12. Gena y frente negras 23
— Gena y mayor parte de frente naranja, todo el triángulo frontal del ♂ y 1/3 inferior de frente de la ♀ naranja 13

13. Patas naranja 15
— Patas oscuras 14

14. Pelos torácicos pardo rojizos; bases tibiales pardo rojizas (Is. Juan Fernández) . . . . . *aurifrons* Shannon
— Pelos torácicos blancos y negros; tibias completamente negras (Chile) . . . . . *coeruleus* (Rondani)

15. Mesonoto enteramente corto-piloso amarillo; celdas basales microtricosas; trocánter posterior con una espina ancha bien desarrollada; fémur posterior largo-piloso, con pelos más largos que ancho tibial; 4to tergo enteramente brillante (Colombia) . . *aureus* Hippa
— Mesonoto corto-piloso amarillo con pelos negros más largos entremezclados en 1/2

posterior; celdas basales parcialmente desnudas basomedialmente; trocánter posterior sin espinas; fémur posterior corto-piloso, con pelos más cortos que el ancho tibial; 4to tergo opaco-polinoso medialmente (Colombia) . . . . . *ochripes* Hippa

16. Mesonoto sin franja longitudinal de pelos dorados 18
— Mesonoto con franja longitudinal de apretados pelos dorados en mitad posterior . . . 17

17. Abdomen con bandas de pelos tomentosos dorados en 3er hasta 4to (machos) o 5to (hembras) tergos; ala difusamente pardo-amarillenta (Brasil) . . . . *shannoni* Thompson
— Abdomen con tergos sin bandas apicales de pelos dorados; ala con franja diagonal parda distintiva (de México a Brasil) . . . . . *plagiatus* (Wiedemann)

18. Trocánter y tibia posteriores sin espinas o procesos 3
— Trocánter y/o tibia posterior(es) con espinas o procesos distintivos 19

19. Sutura transversa dorado-polinosa; tibia posterior sin espina apical (de Costa Rica a Argentina) . . . . . Complejo *genuinus*
— Sutura transversa sin polinosidad dorada; tibia posterior usualmente con espina apical larga 20

20. Patas frontal y media con tibia y tarsos naranja (Colombia) . . . . . *fassli* Hippa
— Patas frontal y media más extensivamente oscuras, al menos con tarsómeros apicales negros 21

21. Mesonoto con pelos gruesos, dorado-plateado-opacos en márgenes anterior y lateral; triángulo frontal del ♂ negro brillante. Trocánter posterior del ♂ con espina simple (Venezuela) . . . . . *woodorum* Thompson
— Mesonoto negro y amarillo-piloso anterior y lateralmente, completamente negro-piloso sobre el ala; triángulo frontal del ♂ dorado-polinoso 22

22. Basoflagelomero circular; mesonoto brillante sobre disco; trocánter posterior con una espina simple; tibia posterior sin una espina apical larga ni carina apicoventral (de Colombia a Ecuador) . . . . . *nitidicollis* Hippa
— Basoflagelomero trapezoidal; mesonoto enteramente opaco polinoso; trocánter posterior del ♂ con una espina bilobulada; tibia posterior con una espina apical larga y una carina apicoventral (Bolivia) 23

23. Fémures frontal y medio oscuros solo en 1/2 basal, apicalmente naranja (Bolivia) . . . . . *andicus* Hippa
— Fémures frontal y medio oscuros en 4/5 basales o más 24

24. Cara naranja 26
— Cara oscura, parda a negra 25

25. Mesonoto con penachos de apretados pelos largos dorados sobre ala y en callo postalar; escutelo parcialmente amarillo-piloso; tibias frontal y media pardo-anaranjadas en 1/2 basal; fémures extensivamente blanquecino-pilosos (Bolivia) . . . . . *incarum* Hippa
— Mesonoto y escutelo enteramente negro-pilosos; tibias negras; fémures negro-pilosos (Venezuela) . . . . . *chloropygus* (Schiner)

26. Patas negras (Bolivia) . . . . . *stimulans* Thompson
— Patas frontal y media con tibia y tarsos naranja 27

27. Tergos 4 y 5 con pelos similares a los de otros tergos; tergos 2 y 3 más extensivamente brillantes, con mancha transversa azulado-metálica (Colombia) . . . . *sapphirifer* Hippa
— Tergos 4 y 5 con conspicuos pelos gruesos blanco-amarillentos opacos, contrastando fuertemente con esos de otros tergos; tergos 2 y 3 opacos, brillantes sólo estrecha y

lateralmente (Colombia) . . . . . *pilifer* Hippa

28. Fémures anterior y medio negros en 3/4 basales; cara brillante medialmente, sólo con franja polinosa lateral oblicua, con carina media ampliamente oscura en toda su longitud (Venezuela) . . . . . *venezuelaensis* Thompson
— Fémures anterior y medio amarillos; cara enteramente polinosa, enteramente pálida o con un parche triangular dorsomedial oscuro no alcanzando el borde superior de la boca . 29

29. Gena amarilla anteriormente (Bolivia) . . . . . *calypso* Hippa
— Gena enteramente negra 30

30. Tarso posterior oscuro, con pelos oscuros; 2da celda basal desnuda en mitad anterior basal (Costa Rica, Panamá) . . . . . *janzeni* Thompson
— Tarso posterior pálido, con pelos pálidos; 2da celda basal microtricosa 31

31. Esterno 4 parcialmente negro piloso; basoflagelomero ovalado (Colombia, Perú) . . . . . *cybele* (Hull)
— Esterno 4 enteramente amarillo piloso; basoflagelomero rectangular (Colombia) . . . . . *cydippe* Hippa

AGRADECIMIENTOS

Agradecemos a Paul Hanson, Universidad de Costa Rica (UCR), San José, por permitirnos estudiar material a su cargo [otros acrónimos de colecciones usados son USNM por United States National Museum (= the National Museum of Natural History), Washington e INBio por Instituto Nacional de Biodiversidad, Santo Domingo, Heredia, Costa Rica). Agradecemos también a los Drs. Jorge A. Jiménez, INBio; Patricia Gentili, Smithsonian Institution, Washington; Manya B. Stoetzel, Allen Norrbom, y M. Alma Solís, Systematic Entomology Laboratory, USDA, Washington; Daniel Gandolfo, South American Biological Control Laboratory, USDA, Hierlingham, Argentina; por sus revisiones críticas del manuscrito. Agradecemos a Elizabeth Roberts, Washington (Figs. 1-5 y 8-10) y a Silvia Troyo, San José (Figs. 6, 7) por las ilustraciones respectivas. Finalmente queremos agradecer a los parataxónomos del INBio, por el valioso aporte de su trabajo a esta publicación.

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SUSCEPTIBILIDAD DE ADULTOS DE *BEMISIA ARGENTIFOLII*[1] (BELLOWS & PERRING), A INSECTICIDAS DE USO COMUN EN BAJA CALIFORNIA SUR, MEXICO

R. Servín-Villegas[2], J.L. Martínez[3], E. Troyo-Diéguez[2] y A. Ortega-Rubio[2].

RESUMEN

Se determinó la toxicicidad de tres insecticidas comunes en Baja California Sur, México, paratión metílico, metamidofós y endosulfan, en muestras de mosquita blanca (*Bemisia argentifolii)* colectadas de una parcela cultivada, sometida a la acción de insecticidas (población CI), y de una población aislada sin presión de selección (población SI), criada en el Campo Experimental del Centro de Investigaciones Biológicas del Noroeste. El sitio de muestreo se localizó en la cuenca agrícola de La Paz, BCS, México. Para los bioensayos en laboratorio se utilizó el método de la película residual en frascos entomológicos de 20 ml, en los que se aplicaron al menos 5 dosis diferentes de los insecticidas mencionados, más un testigo, con 5 repeticiones por bioensayo. En la población CI, la mayor concentración CL_{50} correspondió a metamidofós, con valor de 673 µg/ml, le siguió endosulfan con un valor medio de 507 y paratión metílico con 349. La población SI, mostró una mayor susceptibilidad a endosulfan, siguiéndole paratión metílico y metamidofós, con CL_{50} de 180 µg/ml, 290 y 350, respectivamente. Los individuos CI no presentaron diferencias significativas (ANVA 95%) entre las medias del porcentaje de mortalidad al 50 y 95%, en los tres insecticidas evaluados. Sin embargo, la susceptibilidad de la población SI a los insecticidas ensayados fue ligeramente mayor a la mostrada por la población CI. La relación de resistencia entre ambas poblaciones fue de 2.73, 1.91 y 1.19 para endosulfan, metamidofós y paratión metílico, respectivamente.

ABSTRACT

The toxicity of the most common insecticides used in Baja California Sur, México, against a variety of agricultural pests was assayed on two stocks of white fly *(Bemisia argentifolii)*. Three insecticides were evaluated in laboratory: methyl-parathion, metamidophos and endosulphan. Two stocks of white fly were used for the assays. The resistant stock which is the population under insecticide pressure (CI) was obtained from a cultivated horticultural plot exposed to the action of theses insecticides for at least five years. An isolated population (SI) never exposed to the action of any insecticide was obtained from an experimental cultivated plot located at the Experimental Station of the Centro de Investigaciones Biológicas del Noroeste, S.C., 17 km West La Paz city, in the southern part of the Mexican Peninsula of Baja California. We applied five different dosages, plus a control, to individuals from both stocks. The experimental design included five replicates, using the residual film method. The

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required concentracions CL_{50} for the population CI were 673, 507, and 349 µg/ml for metamidophos, endosulphan, and methyl parathion, respectively. The population SI showed a higher susceptibility to these insecticides, requiring average concentrations (CL_{50}) of 180, 290 and 350 µg/ml for the 95% confidence interval of mortality-dosages, for the three insecticides assayed. Although the observed differences were not statistically significant (ANOVA 95%), nevertheless, the susceptibility to the assayed insecticides for the SI stock was slightly higher than the evidenced by the CI stock. The observed resistance relation between both populations was 2.73, 1.91, and 1.19, for endosulphan, metamidophos, and methyl parathion, respectively.

INTRODUCCION

En México, el grupo de plagas conocido como mosquita blanca se ha incrementado de manera excesiva en los últimos años (Ortega 1990, López 1993, López y Lupercio 1993). Dicho insecto es de importancia económica considerable ya que además de alimentarse de la savia de sus hospederos, es vector de diversos virus. De las especies conocidas como "mosquita blanca", *Bemisia tabaci* (Genn.) y *B. argentifolii* (Bellows & Perring) son particularmente importantes para las regiones tropicales y subtropicales del mundo (Muniyapa 1983, Perkins 1983, Ortega 1990, Byrne y Bellows 1991, Costa *et al.* 1991). La última especie mencionada fue descrita recientemente por Bellows *et al.* (1994) y corresponde al denominado biotipo "B".

Mundialmente el control químico ha sido el método principal para el combate de la mosquita blanca (Prabhaker *et al.* 1989, Rao *et al.* 1990, Rowland *et al.* 1991); sin embargo, ha tenido poco éxito ya que año con año la plaga resurge con mayor tolerancia a insecticidas. Lo anterior se debe a su capacidad para desarrollar resistencia a una gran variedad de compuestos químicos. Asimismo, habita en el envés de las hojas, lo que evita un contacto directo del insecticida con la plaga, debido a la ineficacia de los métodos de aplicación utilizados comúnmente para asperjar el producto químico sobre el follaje (Johnson *et al.* 1982, Prabhaker *et al.* 1988, Costa *et al.* 1991, Omer *et al.* 1992). *B. tabaci,* congénere de *B. argentifolii,* se alimenta y desarrolla en una amplia gama de plantas hospederas, silvestres y cultivadas (Russell 1963, 1977, Costa *et al.* 1991), manteniéndose activa a través del año.

El Estado de Baja California Sur se encuentra ubicado en una de las regiones más áridas y con menor precipitación de México, por lo que sus recursos acuíferos disponibles son escasos; tal situación origina una alta inversión económica para el desarrollo agrícola.

La existencia de plagas potenciales, como la mosquita blanca, aumenta de manera considerable los costos de producción. De igual manera, las zonas agrícolas del estado en general, se componen de agroecosistemas en forma de parches, en donde la mayoría de los agricultores no cuentan con programas adecuados para el control de las plagas agrícolas. Por lo anterior, es común observar migraciones de plagas de una parcela a otra, infestándose constantemente, aún en los cultivos donde se utilizan diversos insecticidas, ya sean sólos o mezclados. Frecuentemente en los campos agrícolas se realizan hasta tres aplicaciones por semana, y en ocasiones no se cumple con las fechas de siembra recomendadas. La situación anterior favorece el desarrollo de poblaciones resistentes, lo que impide un control eficaz (Rao *et al.* 1990, Omer *et al.*1992). El conocimiento de la respuesta de la mosquita blanca a la acción de insecticidas agrícolas es de suma importancia, ya que de ello depende el éxito de su control (Prabhaker *et al.* 1992).

Por lo anteriormente expuesto, el objetivo del presente trabajo es conocer los niveles de susceptibilidad que presentan poblaciones de mosquita blanca *B. argentifolii* sometidas a presión de selección, debida a la aplicación de insecticidas químicos de uso común en Baja California Sur, México (población CI), y poblaciones de la misma plaga aisladas del control químico (población SI).

MATERIALES Y METODOS

El presente estudio se realizó de enero a octubre de 1994, utilizando dos poblaciones de mosquita blanca, la primera población (CI), que estuvo sujeta a la acción de insecticidas, se obtuvo de un cultivo de col establecido en una parcela de la zona ejidal Chametla-El Centenario. Dicha localidad se ubica en la cuenca agrícola de La Paz, a los 24°05'N y 110°25'W, al nivel del mar, con un clima seco-cálido y una precipitación anual de 204 mm, concentrada principalmente en los meses de agosto y septiembre (García 1981), y con una aridez marcada hacia los meses de abril y mayo (Troyo-Diéguez *et al.*, 1990). La segunda población (SI) fue establecida desde 1992, en el Campo Experimental del CIBNOR, utilizando como sustrato alimenticio plantas de melón, por ser el cultivo de mayor aceptación por *B. argentifolii* en la localidad; dicha población se mantuvo fuera del contacto de plaguicidas durante dos años, previos al presente estudio.

Los insecticidas con grado de alta pureza (97%) usados en la presente investigación fueron: paratión metílico, metamidofós y endosulfan; como testigo y solvente se utilizó acetona. La metodología para los bioensayos se basó en los trabajos de Staetz y Boyler (1992), Satoh y Plapp (1993), y Servín *et al.* (1995). Se colocó 1 ml de la dosis requerida del insecticida, en frascos viales de 20 ml de capacidad, los cuales después de ser debidamente etiquetados, fueron colocados horizontalmente en un aparato giratorio, para distribuir en todas las paredes del recipiente el compuesto hasta el total secado del solvente. Posteriormente se llevaron al campo los frascos necesarios, colocando en ellos 20 adultos de mosquita blanca, colectados con un microaspirador diseñado especialmente para ello (Martínez 1994), con el debido manejo para no dañar los especímenes. Los frascos con los insectos se trasladaron al laboratorio dentro de recipientes a temperatura de 15°C, y después de tres horas se estimó la mortalidad contando los insectos muertos y con movimientos anormales. Para cada insecticida se utilizaron 5 dosis diferentes con 5 repeticiones, mediante un diseño experimental completamente al azar.

La mortalidad registrada en el testigo fue de 3% en promedio. Para los análisis estadísticos, los datos de porcentaje de mortalidad (%) fueron transformados a $(\%+0.5)^{1/2}$, con el fin de normalizarlos, de acuerdo con Steel y Torrie (1960). Para el análisis de la información de mortalidad, se utilizó el programa Probit; los diagramas de caja y de dispersión, ANVA's y comparaciones entre medias se realizaron con computadora mediante el programa Statgraphics ver. 6.0. En los diagramas de rectángulo de las Figuras 1 a 8, la línea que atraviesa la caja es la mediana, mientras que la longitud mayor (vertical) del rectángulo o caja está determinada por los valores de los cuartos inferior y superior; por su parte, las líneas en los extremos inferior y superior marcan los valores mínimo y máximo, si es que los hay (Salgado-Ugarte 1992).

RESULTADOS Y DISCUSION

Los resultados obtenidos se presentan en el Cuadro 1, donde se muestra la respuesta dosis-mortalidad CL_{50} de los insecticidas utilizados, el Intervalo de Confianza al 95%, la CL_{95} y la pendiente de la línea de regresión. Como puede apreciarse, el compuesto más tóxico para la población CI fue el paratión metílico, requiriéndose 349 µg/ml para eliminar el 50% de los individuos; para la misma población el menos tóxico fue metamidofós, ya que requirió 673 µg/ml, para eliminar la misma cantidad de individuos. Los valores de la pendiente de la línea de regresión indican que la población es, en cuanto a su susceptibilidad al insecticida, más homogénea para metamidofós con un valor de 3.95, siguiéndole en orden decreciente endosulfán y paratión metílico con 1.55 y 1.47 respectivamente (Cuadro 1). En general, los valores obtenidos sugieren escasa heterogeneidad en la población CI, lo cual puede deberse al aislamiento geográfico de la Península de Baja California, México. Dicho aislamiento podrá representar en el futuro, una de las causas para que se desarrollen mecanismos de resistencia en la plaga bajo estudio, como ha ocurrido en otras localidades (Prabhaker *et al.* 1992).

CUADRO 1. Valores de CL_{50}, I.C. al 95%, CL_{95} y pendiente de la línea de regresión para tres insecticidas comunes, en adultos de *B. argentifolii*, con insecticidas (CI) y sin los mismos (SI).

Población	Insecticida	CL_{50} µg/ml	I.C. al 95% para CL_{50}	CL_{95} µg/ml	Pendiente línea regr.	Relación de Resistencia
CI	Metamidofós	673.2	431.3-1041.5	1754.4	3.95	1.91
SI	"	351.6	319.2-382.2	696.7	5.53	1.00
CI	Endosulfan	507.4	401.0-627.0	5802.3	1.55	2.73
SI	"	186.0	131.9-243.3	4080.9	1.22	1.00
CI	P. metílico	349.7	280.3-424.6	4523.0	1.47	1.19
SI	"	293.6	258.9-329.1	912.1	3.42	1.00
		µ=393.6				

En un estudio similar, Prabhaker *et al.* (1985) encontraron una importante heterogeneidad genética en poblaciones de *B. tabaci* de diferentes localidades del sur de California, en respuesta a diversos compuestos insecticidas, empleándose organofosforados, DDT, piretroides y carbamatos. Los valores de la pendiente obtenidos por dichos autores sugieren que las aplicaciones evaluadas constituyen un potencial importante para el desarrollo de altos niveles de resistencia, lo cual aparentemente todavía no ocurre en las áreas agrícolas de la región bajo estudio.

En promedio, la población mantenida fuera del contacto con insecticidas (SI) presentó valores menores a los de la colonia bajo presión con insecticidas (CI), aunque las diferencias observadas no fueron estadísticamente significativas (α=5%). Para el caso de endosulfan, se requirieron 186 µg/ml para eliminar el 50% de la población SI, para paratión metílico 294, y para metamidofós 352 µg/ml, siendo la media general igual a 393.6 µg/ml. Los valores de la pendiente obtenidos sugieren que los efectos son más homogéneos para metamidofós (5.53), y menos homogéneos para paratión metílico y endosulfan (3.42 y 1.22, respectivamente) (Cuadro 1). Dicha pendiente representa el incremento de porciento de mortalidad en relació con el aumento de las dosis experimentales aplicadas.

Los diagramas de caja ilustrados en las Figuras 1, 3 y 5 muestran la escasa diferencia entre las medianas de la variable transformada de % de mortalidad, así como el amplio intervalo de respuesta de las muestras de ambas poblaciones estudiadas bajo el efecto de los tres insecticidas ensayados. Obsérvese como la mediana fue muy similar en ambas poblaciones para los tres insecticidas, excepto para el caso de metamidofós, al cual la población más susceptible (Pob. SI) respondió en una forma más evidente, aunque la diferencia no fue estadísticamente significativa, con α=5%. Lo anterior significa que las poblaciones evaluadas no han desarrollado una verdadera resistencia, evidenciando más bien diferentes niveles de susceptibilidad a los insecticidas bajo estudio (Cuadro 2). En todos los casos evaluados fue evidente que el valor de CL_{50} osciló alrededor de 350 µg/ml, correspondiendo al punto de inflexión de las curvas de dosis experimentales (Figuras 2, 4 y 6).

Por otro lado, en la Figura 8 se observa una menor dispersión de datos para el insecticida endosulfan aplicado a la población SI, siendo evidente en la misma una variabilidad más marcada para paratión metílico y metamidofós. A partir de este análisis gráfico se infiere que endosulfan resulta ser más efectivo para el control de la población SI, lo cual concuerda con los valores presentados en el Cuadro 1, donde se observa precisamente que endosulfan requiere la menor CL_{50} para el control de la población SI. Sin embargo, cabe aclarar que lo anterior es una tendencia, dado que las diferencias no fueron estadísticamente significativas, para un nivel de significancia α=5%.

CUADRO 2. Concentrado de datos de análisis de varianza para la variable transformada % de mortalidad[1], causada por los insecticidas paratión metílico, endosulfan y metamidofós, en dos poblaciones (CI y SI) de mosquita blanca (*Bemisia argentifolii*), en Baja California Sur, México.

INSECTICIDA	VARIANZA (poblaciones)	VARIANZA (error)	F. calculada
Paratión metílico	0.02725	11.284198	0.002 ns
Endosulfan	11.14213	8.833863	1.261 ns
Metamidofós	12.79648	13.719109	0.933 ns

Nota: [1] Transformada % de mortalidad = $(\%+0.5)^{1/2}$

[2] F requerida para $gl_{1,58,\alpha=0.05}=4.14$; ns: diferencias no significativas.

Comparando los valores de la CL_{50} de la colonia susceptible con los obtenidos por Ortega (1990) y por Prabhaker *et al.* (1989) en su población también susceptible, se puede concluir que la población bajo estudio requirió una dosis menor de metamidofós, endosulfán y paratión metílico para eliminar el 50% de los individuos. Lo anterior puede implicar que la población susceptible en el presente trabajo puede ser usada como línea base para realizar estudios similares con otros insecticidas y otros cultivos, lo cual es de enorme relevancia para la región, dada la escasez de información al respecto.

El factor de resistencia de la población de *B. argentifolii* sometida a los efectos de insecticidas [(CL_{50} de Pob CI)/(CL_{50} de Pob SI)] fue mayor que la unidad para los tres insecticidas. Para el insecticida paratión metílico se requirió 1.2 veces del producto para controlar la mosquita blanca (población CI), con respecto a la colonia susceptible; es decir, casi la misma cantidad. Para metamidofós se obtuvo un valor de 1.9, casi el doble, para controlar la población CI. Endosulfan fue el insecticida que presentó mayor relación de resistencia, requiriéndose 2.7 veces más del mismo para controlar la población CI. Los valores de relación de resistencia obtenidos sugieren que al momento del muestreo, paratión metílico resultó ser el producto más tóxico.

En comparación con otros autores (French *et al.* 1973, Prabhaker *et al.* 1985, Rowland *et al.* 1991), los resultados sugieren que la presión de selección ejercida por los insecticidas evaluados no fue suficientemente alta, para poder establecer conclusiones válidas en términos resistencia. El método del Diagrama de Caja fue de suma valía para el análisis de la dispersión y tendencia de los datos observados (Hartwig y Dearing 1979, Deleon y Anagnoson 1991). Dicho método de análisis exploratorio de datos, al ser complementario con las técnicas de análisis de varianza de uso común (Steel y Torrie 1960, Sokal y Rohlf 1981) y con el programa de cómputo Probit, resultó ser innovador y de amplia utilidad en pruebas de toxicología y evaluación de susceptibilidad a insecticidas.

Finalmente, los autores consideran que la metodología utilizada para la realización de los bioensayos descritos, fue eficiente para el análisis de susceptibilidad de la mosquita blanca en comparación con otros métodos, ya que la mortalidad del testigo fue de 3% en promedio. Por otra parte, la técnica empleada se podrá aplicar bajo condiciones de campo, sin requerir necesariamente mayor infraestructura ni equipamiento, lo cual permite actuar de manera rápida y eficiente para un programa de muestreo de la plaga y determinación de los niveles de susceptibilidad a insecticidas de uso común.

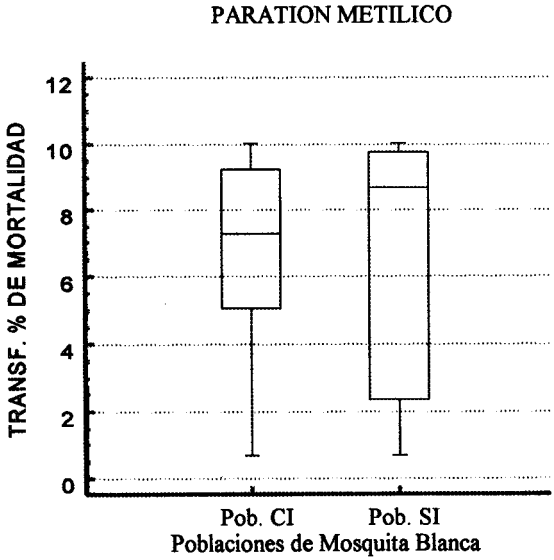

FIG. 1. Respuesta general de las poblaciones CI y SI de *B. argentifolii*, a la acción del insecticida paratión metílico; se emplean valores transformados de mortalidad.

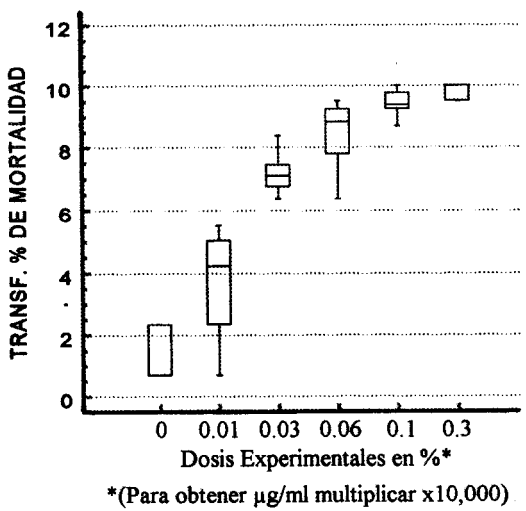

FIG. 2. Efectos de las dosis ensayadas de paratión metílico sobre ambas poblaciones de *B. argentifolii*; se emplean valores transformados de mortalidad.

ENDOSULFAN

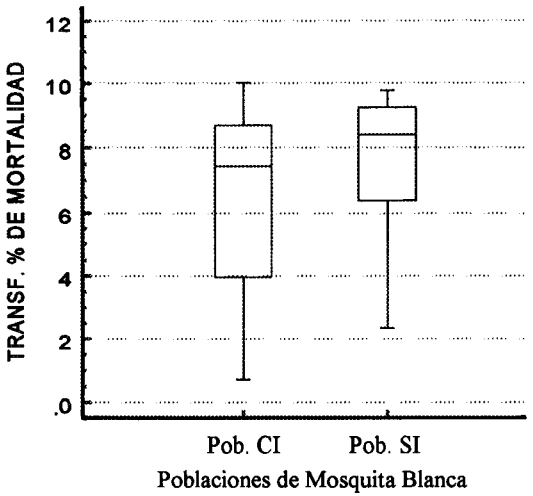

FIG. 3. Respuesta general de las poblaciones CI y SI de *B. argentifolii*, a la acción del insecticida endosulfan; se emplean valores transformados de % de mortalidad.

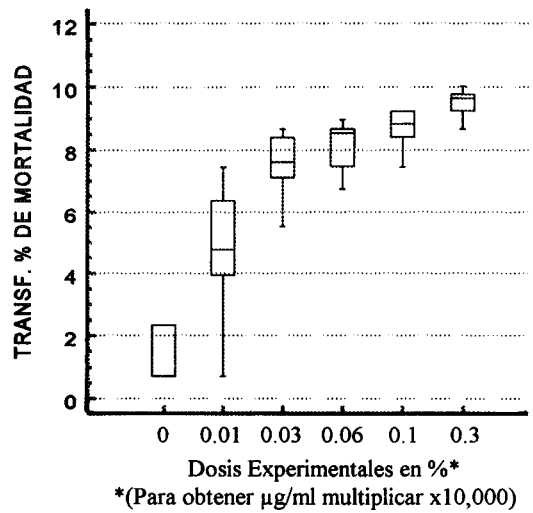

FIG. 4. Efectos de las dosis ensayadas del insecticida endosulfan sobre ambas poblaciones de *B. argentifolii*; se emplean valores transformados de % de mortalidad.

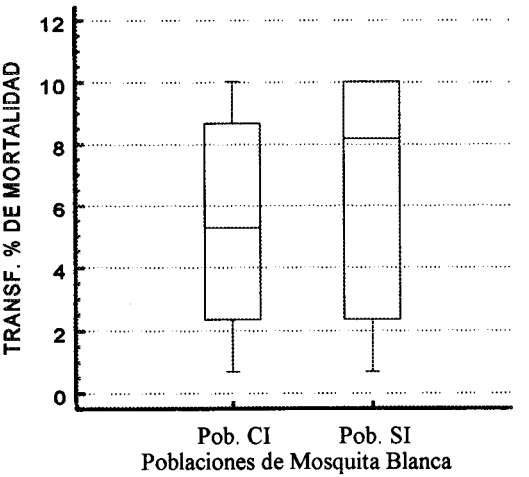

FIG. 5. Respuesta general de las poblaciones CI y SI de *B. argentifolii* a la acción del insecticida metamidofós; se emplean valores transformados de % de mortalidad.

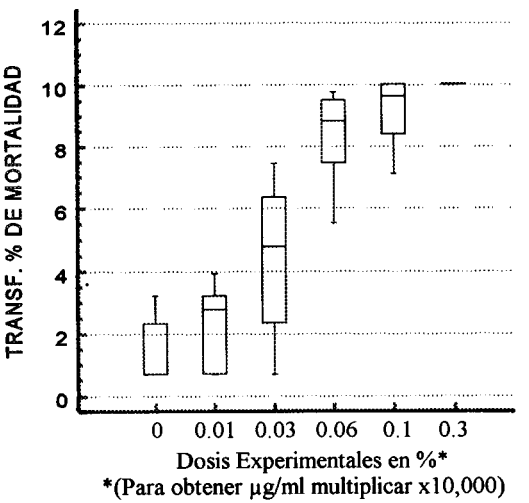

FIG. 6. Efectos de las dosis ensayadas del insecticida metamidofós sobre ambas poblaciones de *B. argentifolii*; se emplean valores transformados de % de mortalidad.

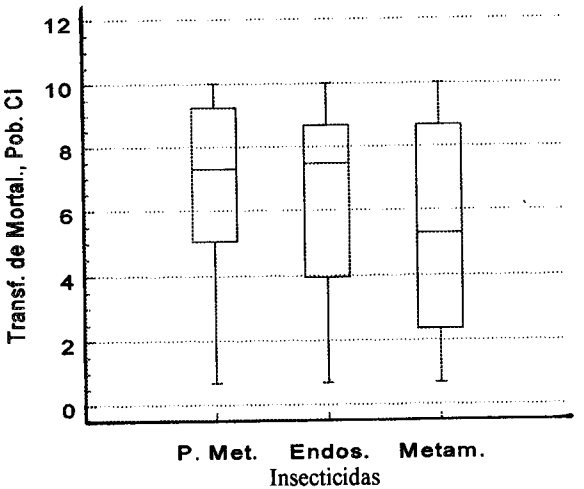

FIG. 7. Respuesta global de *B. argentifolii* sometida en campo al efecto de plaguicidas (población CI), a la acción de tres insecticidas en laboratorio, según el método de la película residual.

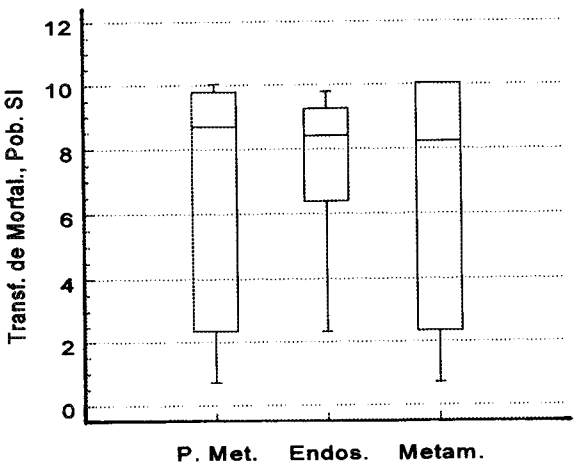

FIG. 8. Respuesta global de *B. argentifolii* aislada en campo del efecto de plaguicidas (población SI), a la acción de tres insecticidas en laboratorio, según el método de la película residual.

CONCLUSIONES

La población de mosquita blanca con un historial de aplicaciones de insecticidas, resultó ser todavía susceptible a los insecticidas utilizados en el presente estudio, los cuales son representativos del uso extensivo de plaguicidas que actualmente se realiza en las cuencas agrícolas del noroeste de México. Los amplios intervalos de los datos obtenidos del análisis global de la información (Figuras 7 y 8) nos indican la relativa susceptibilidad de la especie en la región a los insecticidas estudiados. La baja CL_{50} observada en promedio para los tres insecticidas, 394 µg/ml, con un valor mínimo de 186 para Endosulfan (población SI), puede brindar lineamientos para revisar y modificar, en su caso, las dosis de aplicación empleados en la región con los insecticidas evaluados. Lo anterior puede apoyar de manera importante los programas de control químico de plagas de relevancia económica, que se realicen en la región y en la localidad.

El método del bioensayo de película residual resulta ser eficiente y adecuado para este tipo de estudios, ya que garantiza que las concentraciones evaluadas en cada ensayo sean uniformes en los tratamientos de insecticida seleccionados y en todas las repeticiones efectuadas. Se comprobó la utilidad de dicho método para la evaluación del nivel de susceptibilidad de mosquita blanca, facilitando el seguimiento de las estrategias de uso y manejo de insecticidas que eventualmente sean establecidas en la región.

AGRADECIMIENTO

Los autores desean expresar su agradecimiento a Lidia Hirales L. y Franco Cota por su asistencia en el muestreo de la plaga, y al Biol. Ricardo Aguilar por su revisión crítica del manuscrito. El presente trabajo fue financiado por el Consejo Nacional de Ciencia y Tecnología, Secretaría de Educación Pública y por el Centro de Investigaciones Biológicas del Noroeste, mediante asignación financiera del proyecto AGR-3 "Biología y toxicología de plagas agrícolas en el noroeste de México".

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ACAROS FITOFAGOS PRESENTES EN ARBOLES FRUTALES DE BAJA CALIFORNIA SUR, MEXICO.

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ABSTRACT

The foliage of fruit orchards at three locations in southern Baja California Sur was sampled seasonally in 1990 to determine the presence and diversity of mites. A total of 9 families and 13 species of mites were collected. The Phytoseiidae family was the most diverse with three species, followed by Tetranychidae and Tenuipalpidae with two species each. The species *Euseius hibisi* (Chant) had the widest distribution, finding it in three locations and on all fruit trees sampled.

RESUMEN

Se determinó la presencia y diversidad de ácaros en el follaje de árboles frutales en tres localidades de Baja California Sur, mediante colectas estacionales de enero a diciembre de 1990. Se registró un total de 13 especies de ácaros incluidas en 9 familias. La familia Phytoseiidae con 3 especies fue la más diversa, seguida por Tetranychidae y Tenuipalpidae con 2 especies cada una. La especie *Euseius hibisi* (Chant) presentó la distribución más amplia ya que se encontró en las tres localidades y en todos los árboles frutales estudiados.

INTRODUCCION

Los ácaros que habitan en plantas de ecosistemas naturales forman poblaciones que se encuentran en equilibrio con sus enemigos, sin causar daño aparente a sus hospederos; en cambio, en la mayoría de los agroecosistemas, dichas poblaciones de ácaros se caracterizan por tener una densidad poblacional alta, llegando a constituir verdaderas plagas en una amplia variedad de cultivos, provocando grandes pérdidas económicas (Atkins 1978, Faragalla 1988, Castaño 1992). En México, son pocos los trabajos realizados sobre ácaros fitófagos (Tuttle *et al.* 1976, Orozco y Nuñez 1988, Estebanéz y Rodríguez 1989). Una de las regiones más pobremente estudiadas de nuestro país, por el aislamiento geográfico que presenta, es Baja California Sur. En esta localidad, a pesar de sus condiciones climáticas y su aridez (García 1981), existen áreas frutícolas importantes (S.A.R.H. 1990), las cuales podrían caracterizarse como "oasis" ya que se encuentran rodeadas de grandes extensiones de vegetación natural, donde varias especies encuentran las condiciones óptimas para su desarrollo. Entre los árboles frutales más cultivados en Baja California Sur, están: mango, aguacate, naranjo y algunas variedades de cítricos, los cuales no se encuentran distribuidos de manera uniforme. La fauna acarológica presente en estos cultivos es totalmente desconocida; por ello, el objetivo principal del presente trabajo fue identificar la diversidad específica de estos microartrópodos en dichos hospederos y su papel en la cadena trófica.

MATERIALES Y METODOS

El área de estudio está ubicada en la Región del Cabo, hacia el sur de la Península de Baja California. En ella se delimitaron tres localidades: una en el poblado de El Pescadero, ubicado en la Vertiente del Oceáno Pacífico; otra en el poblado de San Bartolo, localizado en la costa adyacente al Golfo de California, y una tercera en San José del Cabo. Las colectas se realizaron dos veces por cada estación del año, en huertos frutícolas de enero a diciembre de 1990. En cada colecta se seleccionaban al azar entre tres y cinco árboles de cada uno de los siguientes frutales: mango, aguacate, naranja, toronja, lima, guayaba y granada. Después se colectaron muestras de follaje, de la parte media de los árboles, una parte de este material fue golpeado sobre un tamiz, para colectar los ácaros allí presentes; el resto de la muestra fue colocado en embudos de Berlesse. Los ácaros obtenidos por ambos métodos se preservaron en alcohol al 70%; se clasificaron y aclararon para su posterior preparación en líquido de Hoyer. Finalmente, fueron identificados hasta especie (Krantz 1978), excepto aquellos determinados como nuevas especies. El análisis taxonómico, se hizo en microscopio de contraste diferencial de interferencia.

RESULTADOS Y DISCUSION

Se identificaron especímenes de las familias siguientes: Tetranychidae, Tenuipalpidae, Eriophyidae, Tarsonemidae, Phytoseiidae, Stigmaeidae, Cheyletidae, Ascidae y Winterschmidtidae (Tabla 1). La familia Tetranychidae estuvo representada por *Eutetranychus banksi* (Mc Gregor) y por *Oligonychus* (*Oligonychus*) sp. Berlese. La primera se encontró asociada a mango, naranja, toronja y lima; la segunda se encontró solamente en aguacate. Ambas especies fueron colectadas en Pescadero y San Bartolo. *E. banksi* ha sido reportada como una importante plaga en cítricos en EEUU (Van Der Geest 1985); en nuestro caso, podríamos decir que es una especie aparentemente de baja densidad, lo que puede deberse a las constantes aplicaciones de insecticidas que se realizan para el control de la mosca mexicana de la fruta (*Anastrepha* sp.), plaga de mango y cítricos en la porción sur de este estado. Esta especie puede ser considerada como poco selectiva para sus hospederos, ya que de los siete árboles estudiados, se presentó en cuatro, a diferencia de *Oligonychus* (*Oligonychus*) sp. la cual fue detectada solamente en aguacate. Este ácaro no pudo identificarse a nivel de especie por no contar con machos.

De la familia Tenuipalpidae, se determinaron las especies *Brevipalpus ovobatus* Donnadieu y *B. californicus* (Banks). La primera estuvo presente en árboles de naranja, lima y granada, en El Pescadero. *B. californicus* estuvo presente solamente en árboles de toronja en San Bartolo. De los resultados obtenidos, podemos considerar a *B. ovobatus* como una especie más generalista con sus hospederos que *B. californicus*. Ambas especies son consideradas como plagas importantes en cítricos (De León 1961, Baker *et al.* 1975); sin embargo, nosotros las encontramos poco representadas en el muestreo realizado.

De los eriófidos, la única especie encontrada fue *Eriophyes mangiferae* (Sayed), presente sólo en árboles de mango. Dicha especie, aparentemente ha tenido una introducción reciente en Baja California Sur, y está reportada como una seria plaga que causa marchitamiento y defoliación en árboles de mango (Keifer *et al.* 1982) acabando incluso con grandes cultivos. Este eriófido se encontró únicamente en San José del Cabo; sin embargo, sería importante tomar medidas adecuadas, antes de que dicho ácaro se distribuya a otras localidades.

De la familia Tarsonemidae, se encontró *Tarsonemus* sp., sobre árboles de mango, en San José del Cabo. El papel de estos ácaros es difícil de definir por ahora; sin embargo algunas de sus especies están reportadas como enemigos naturales de ácaros fitófagos.

TABLA 1. Familias y especies de ácaros fitófagos colectados en árboles frutales de Baja California Sur.

FAMILIA Especie	ARBOL FRUTAL	LOCALIDAD
ASCIDAE		
Proctolaelaps ca.*regalis*	A, N	P
CHEYLETIDAE		
Mexecheles aztecorum	A, M, N	P
ERIOPHYIDAE		
Eriophyes mangiferae	M	SJC
PHYTOSEIIDAE		
Amblyseius evansi	Gy, M, N	P, SB
Euseius hibisci	A, Gr, Gy, L, M, N, T	P, SB, SJC
Tiphlodromus g.*flumenis*	Gy, M, N	P, SB, SJC
STIGMAEIDAE		
Angistemus longisetus	Gy	P, SB
TARSONEMIDAE		
Tarsonemus sp.	M	SJC
TENUIPALPIDAE		
Brevipalpus ovobatus	Gr, L, N	P
Brevipalpus californicus	T	SB
TETRANYCHIDAE		
Eutetranychus banksi	L, M, N, T	P, SB
Oligonychus sp.	A	P, SB
WINTERSCHMIDTIDAE		
Czenspinskia transversostriata	A	P

Frutales: A=Aguacate, Gr=Granado, Gy=Guayabo, L=Lima, M=Mango, N=Naranjo, T=Toronja.
Localidades: P=Pescadero, SB=San Bartolo, SJC=San José del Cabo.

La familia Phytoseiidae estuvo representada por las especies: *Euseius hibisci* (Chant), *Amblyseius evansi* (Chant), y *Tiphlodromus* ca. *flumenis* (Parrot), la cual probablemente sea una nueva especie. De todas ellas la más abundante fue *E. hibisci*, encontrándose ampliamente distribuida en las tres localidades estudiadas y en todos los árboles frutales colectados. Dicho ácaro ha sido reportado como un excelente depredador de tetraníquidos (Mc Murtry 1985), lo cual puede ser muy importante para programas de control biológico. *A. evansi*, se colectó en árboles de mango, guayaba y naranja en El Pescadero y San Bartolo. Este ácaro también es considerado como un excelente depredador de ácaros fitófagos. *Tiphlodromus* sp., colectado en mango, naranja y guayaba, estuvo representado en toda el área estudiada; este género de distribución mundial, ha sido motivo de importantes estudios por su alta capacidad como depredador (Sabelis 1985).

La familia Stigmaeidae, la segunda en importancia como depredadores después de los fitoseidos, estuvo representada por la especie *Angistemus longisetus* Gonzalez, la cual fue colectada en árboles de guayaba en San Bartolo y El Pescadero. Especies de este género se alimentan de *E. banksi* y *B. ovobatus*, los cuales fueron colectados en el presente estudio.

De la familia Cheyletidae, se determinó la especie *Mexecheles aztecorum* De León en árboles de mango, aguacate y naranja en El Pescadero, la cual ha sido reportada como

depredadora de ácaros fitófagos; existen especies que se llegan a alimentar de 7 a 8 presas por día en condiciones de laboratorio, aunque se ha demostrado que pueden ser desplazados rapidamente por ácaros fitoseidos (Gerson 1985). La especie *Proctolaelaps* ca. *regalis* De León, de la familia Ascidae, se colectó en árboles de naranja y aguacate en El Pescadero. *Czenspinskia transversostriata* Oudemans, de la familia Winterschimidtidae, fue colectado en aguacate en El Pescadero, durante los meses fríos; se desconocen sus hábitos alimenticios. En la Tabla 2 se presentan datos sobre presencia/ausencia de las diferentes especies de ácaros colectados durante el año.

TABLA 2. Presencia durante el año de las diferentes especies de ácaros colectados en árboles frutales de Baja California Sur.

	meses del año*											
FAMILIA Especie	E	F	M	A	M	J	J	A	S	O	N	D
ASCIDAE												
Proctolaelaps ca. *regalis*	═	═	═	═							═	═
CHEYLETIDAE												
Mexecheles aztecorum	═	═	═								═	═
ERIOPHYIDAE												
Eriophyes mangiferae			═	═	═	═	═	═	═			
PHYTOSEIIDAE												
Amblyseius evansi	═	═	═								═	═
Euseius hibisci	═	═	═	═	═	═	═	═	═	═	═	═
Tiphlodromus ca. *flumenis*	═	═	═	═	═	═	═	═	═	═	═	═
STIGMAEIDAE												
Angistemus longisetus	═	═	═	═							═	═
TARSONEMIDAE												
Tarsonemus sp.			═	═	═	═	═	═	═			
TENUIPALPIDAE												
Brevipalpus ovobatus		═	═	═	═	═	═	═	═	═	═	
Brevipalpus californicus	═	═	═								═	═
TETRANYCHIDAE												
Eutetranychus banksi		═	═	═	═	═	═	═				
Oligonychus sp.		═	═			═	═	═	═	═	═	
WINTERSCHMIDTIDAE												
Czenspinskia transversostriata	═	═	═	═	═	═	═	═	═	═	═	═

* Las líneas representan la época del año en que están presentes las especies de ácaros estudiadas.

Del presente trabajo, se concluye que a pesar de las condiciones de aridez presentes en Baja California Sur, existe una fauna acarológica importante, representada por especies oportunistas que en agroecosistemas de desierto pueden desarrollarse favorablemente y llegar a ser plagas potenciales de importancia económica. Igualmente existe una variedad de depredadores, que bajo condiciones naturales aparentemente mantienen a los ácaros fitófagos bajo control.

AGRADECIMIENTO

Este trabajo fue realizado con el apoyo económico del Centro de Investigaciones Biológicas del Noroeste, S.C., del Consejo Nacional de Ciencia y Tecnología, y de la Secretaria de Educación Pública. Los autores agradecen al Téc. Franco Cota su ayuda en la colecta del material aquí descrito.

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GLANDULAS SALIVARES Y PLEURALES DE *TOXOTRYPANA CURVICAUDA*[1]

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ABSTRACT

Males of *Toxotrypana curvicauda* were dissected to determine whether they possess sex pheromone-producing glands like males of other Tephritid species. Abdominal portions of the salivary glands and other pheromone-producing tissues in males of different ages were studied by scanning electron microscopy and histology. Sexually-dimorphic salivary glands were found in males, containing cellular columns without lumen and corresponding to a type II (Nation 1974). Enlarged epidermic cells were found in the pleural region of the abdominal segments 3, 4, and 5, similar to the pleural glands in other Tephritid species. Unlike other Tephritids, *T. curvicauda* does not appear to possess anal glands and rectal diverticula.

RESUMEN

Se disectaron individuos machos de diferentes edades de *Toxotrypana curvicauda* para localizar la presencia de glándulas productoras de feromonas sexuales, similares a las descritas en otras especies de tefrítidos. Las glándulas salivares se estudiaron con el microscopio electrónico de barrido para observar el arreglo de las columnas celulares y se hizo histología de los órganos productores de feromonas. Se encontraron glándulas salivares sexualmente dimórficas en los machos que corresponden al tipo II descrito por Nation (1974). En la región pleural de los segmentos abdominales 3, 4 y 5, se observaron células epidérmicas agrandadas tal como las de las glándulas pleurales de otras especies de tefrítidos. No se encontró evidencia de glándulas rectales ni de divertículos anales.

INTRODUCCION

Las moscas de la fruta de la familia Tephritidae son uno de los grupos de insectos que en México requieren mayor atención por el daño que ocasionan a la fruticultura. Este es también uno de los grupos de insectos donde el comportamiento de cortejo, cópula y oviposición está regulado por, entre otros estímulos, feromonas sexuales.

En varias especies del género *Anastrepha*, se han descrito cuatro tipos de glándulas que alcanzan su máximo desarrollo aproximadamente al mismo tiempo que los machos comienzan a cortejar a las hembras; el desarrollo de estas glándulas está fuertemente relacionado con la habilidad para atraer a las hembras y en algunas especies se ha

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comprobado que estas glándulas son productoras de feromona sexual (Nation 1989). Estas son: a) un par de glándulas salivares sexualmente dimórficas; b) glándulas pleurales, consistentes de una capa de células epiteliales agrandadas en la región pleural de los segmentos abdominales 3, 4 y 5; c) glándulas anales; y d) divertículos rectales (Nation 1974, 1981, 1990).

Los machos de *Toxotrypana curvicauda* Gerstaecker producen una feromona sexual, la 2-metil-6-vinil pirazina o 2,6 MVP (Chuman *et al.* 1987), y presentan un comportamiento de llamado consistente en el hinchamiento de la región pleural del abdomen (Landolt y Hendrichs 1983) el cual es común en el comportamiento de apareamiento de otros tefrítidos e indicio de la liberación de la feromona. La feromona sexual de esta especie representa una posibilidad para el desarrollo de tecnologías de detección, verificación de niveles poblacionales y control, y se ha diseñado un sistema de trampeo basado en el empleo de feromonas y trampas pegajosas esféricas (Landolt 1993).

El objetivo de este trabajo fue describir la morfología del abdomen de los machos de *T. curvicauda* y de las glándulas probablemente involucradas en la producción de la feromona sexual.

MATERIALES Y METODOS

Los insectos se colectaron como larvas infestando papayas (*Carica papaya*), en una plantación en San Isidro, Yautepec, Mor. México. Larvas del último estadio se colocaron en tierra húmeda hasta la emergencia de los adultos.

Se hicieron disecciones en fresco, en solución fisiológica salina, de 20 machos y 10 hembras de entre 1 y 10 días de edad para localizar glándulas similares a las descritas en otras especies. La disección se inició por el extremo distal del abdomen, separando la cutícula abdominal a lo largo de la línea media ventral hasta localizar las glándulas salivares en su porción abdominal. Una vez localizadas éstas, se aplicó cristal violeta en solución fisiológica para teñir las glándulas y los conductos salivares pero no los demás tejidos, sobre todo las tráqueas, con las cuales es fácil confundir los conductos. La disección se continuó, siguiendo los conductos salivares a través del resto del abdomen y tórax, separando cutícula, órganos y músculos, y añadiendo más cristal violeta si era necesario, hasta el inicio de los conductos salivares en la cabeza. La porción abdominal de las glándulas salivares se fijó con Duboscq-Brasil (Pantin 1968), se deshidrató con una serie ascendente de etanol, se secó por el método de punto crítico con CO_2, se cubrió con una capa de oro puro de 70 nm de espesor y se estudió y fotografió con un microscopio electrónico de barrido JEOL 35-C, ubicado en la Unidad de Microscopía Electrónica del Colegio de Postgraduados, Montecillo, México.

Los últimos segmentos del abdomen del macho se maceraron durante 72 hrs. en KOH al 10% para buscar la parte cuticular de los divertículos rectales y de las glándulas anales.

Machos de 1, 3, 6, 8, y 10 días de edad y hembras de 6 días se usaron para hacer cortes histológicos. Estos se sacrificaron con acetato de etilo. Se siguió la técnica de inclusión en parafina para preparar, cortar y teñir los ejemplares como se describe a continuación: Los insectos se fijaron con Duboscq-Brasil (Pantin 1968) durante 72 hrs., se lavaron con etanol al 80% y se deshidrataron en etanol al 90, 96 y 100%, permaneciendo por lo menos 24 hrs. en cada uno. Una vez deshidratado, el material se desalcoholizó con benzoato de metilo, el cual se sustituyó por benzol, y éste finalmente por parafina. Luego se hicieron, con un microtomo de rotación, cortes transversales de 10 µm de espesor por el abdomen. Los cortes se fijaron en portaobjetos, se desparafinaron, se tiñeron mediante la técnica de hemalumbre-eritrosina y se incluyeron en bálsamo de Canadá.

RESULTADOS Y DISCUSION

Las glándulas salivares se inician en individuos de ambos sexos en un estrecho ducto común en el labio, se bifurcan a la altura del cuello y continúan por el tórax a ambos lados del esófago. En la hembra las glándulas salivares terminan en dos vesículas a los lados del buche (proventrículo), a la altura de los segmentos abdominales 1 ó 2. En los machos existen tales vesículas en la misma posición. Además, en la región de los segmentos abdominales 3 y 4, en ambos lados, se localiza una columna de células multienrollada sin bifurcaciones, que por su posición, anatomía e histología corresponden con las glándulas sexualmente dimórficas descritas en otras especies, es decir, son una porción abdominal de las glándulas salivares (Fig. 1). La histología de esta porción abdominal reveló que la glándula corresponde al tipo II de la clasificación de Nation (1974), la cual es formada por una columna de células que carecen de lumen y no se forma un tubo entre varias células (Fig. 2). Las células de la epidermis en la región pleural de los segmentos abdominales 3, 4 y 5 son columnares en machos desde 1 día de edad (Fig. 3). Estas células continúan presentando una forma columnar o globosa a la edad de 3, 6 y 8 días, hasta llegar a ser aplanadas en aquellos de 10 días (Fig. 4). En las hembras, las células epidérmicas de la misma región son siempre aplanadas (Fig. 5). Estas características en los machos corresponden a la descripción de las glándulas pleurales de otras especies de tefrítidos; sin embargo, consideramos que se necesita mayor evidencia para afirmar que en realidad se trata de tejido glandular. No se encontró indicio de la presencia de divertículos rectales ni de glándulas anales productoras de feromona sexual.

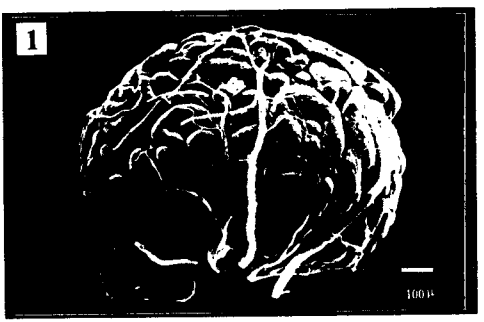

FIG. 1. Vista general en microscopio electrónico de barrido de la porción abdominal de las glándulas salivares en lost machos de *T. curvicauda.*

FIG. 2. Corte transversal por la porción abdominal de las glándulas salivares.

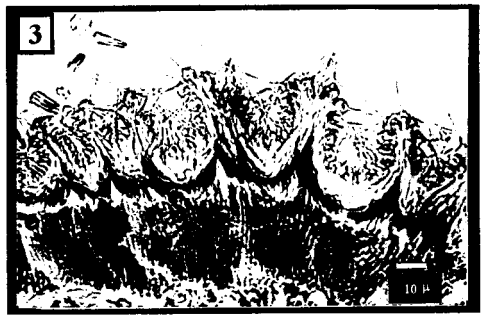

FIG. 3. Corte transversal por el abdomen del macho de un día.

FIG. 4. Corte transversal por el abdomen del macho de diez días.

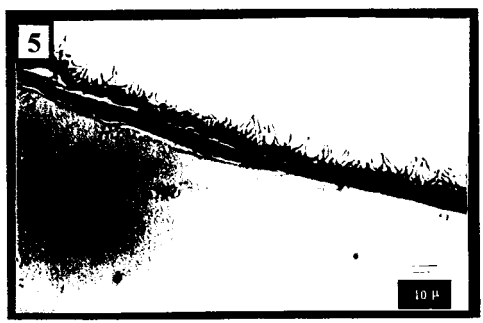

FIG. 5. Corte transversal por el abdomen de la hembra de seis días.

Estos resultados indican que las glándulas salivares sexualmente dimórficas y las glándulas pleurales no son características solamente de los géneros *Anastrepha* y *Ceratitis*, tal como lo manifestado por Nation (1989). Sin embargo, es necesario efectuar investigaciones tendientes a conocer su fisiología y la relación entre su desarrollo y el comportamiento sexual. El conocimiento de los aspectos básicos de la producción de feromonas sexuales, como identificar los órganos productores, es fundamental en el establecimiento de sistemas efectivos de trampeo y control de este grupo de insectos.

AGRADECIMIENTO

Este trabajo es parte del proyecto IPN-DEPI 945307; Federico Castrejón A. fue becario del CONACYT y del Instituto Politécnico Nacional durante su realización.

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SOUTHWESTERN ENTOMOLOGICAL SOCIETY

PERSPECTIVE

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

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BIOLOGY AND ECOLOGY OF *APHIS GOSSYPII* GLOVER (HOMOPTERA: APHIDIDAE)

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ABSTRACT

Aphis gossypii Glover is a destructive pest of over two dozen crops world wide. Damage to a few of these crops is due to direct feeding, but for most of these crops its impact is through its role as a virus vector. As expected, this has resulted in many articles dealing with methods of controlling this insect. The aphid has the ability to become resistant to many pesticides and there is growing concern over environmental impacts of pesticide use. As a result, manipulation of the agroecosystem will play an ever increasing role in the management of this insect.

Most aspects of the biology of this aphid are covered in this review. The recurrent theme centers around the importance of host plant influences on the biology of the aphid. In addition to examining the literature on the biology of the aphid, a large section is devoted to organisms which influence mortality in the aphid, and to aphid borne viruses transmitted by this aphid. This review covers the literature from 1912 to 1995, but articles on aphid management (e.g. pesticide efficacy, planting date) were omitted except where they deal with the aphid's biology or ecology.

Research on the biology of this aphid has been heavily skewed into several disparate categories. First, research has focused on the influence of host plant and temperature on the reproductive rate of this aphid. Second, research has focused on the cause for alate production such as nutritional stress, other nutritional factors, crowding, and temperature. Third, research has focused on the viral borne pathogens transmitted by this aphid. Three specific cases are highlighted: citrus tristeza, cucumber mosaic virus, and the potyviruses. Last, research has focused on the role of organisms which feed on this aphid. Due to the complexity of research possible in this area, very little research has explored the effect of these "beneficial organisms" on life history traits of this aphid.

INTRODUCTION

Aphis gossypii Glover is an important agricultural pest because it has a broad host range, and transmits many agriculturally important plant viruses. Damage is direct through feeding which can kill the host, but also productivity is reduced long before plant death (Andrews and Kitten 1989, Cartwright 1992). Damage is indirect through contamination with aphid honeydew and by vectoring viral pathogens. Honeydew causes economic loss through physical contamination and through providing a nutrient source for fungi that contaminate produce and reduce photosynthesis rates by blocking sunlight.

In the past, this aphid has been controlled with a wide array of insecticides. The growing concern over the use of pesticides is a major theme in much of agriculture due to environmental contamination and the

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economic impact of pesticide resistance. The response in the USA is to promote reduced dependence on pesticides, increased reliance on beneficial organisms, and increased government regulation of chemical use. To continue to control the pest under these conditions requires a detailed knowledge of pest biology and the pest's interactions with other organisms and the environment.

This review summarizes the literature on *A. gossypii* published from 1912 to 1995 with a few articles from 1996. The goal is to provide information to help future research, and provide a key into the literature. While this review is fairly comprehensive, articles on aphid management (e.g. pesticide efficacy, planting date, and seasonal differences) were omitted except where they provide insight or corroborative detail on the biology or ecology of the aphid. Especially in the case of seasonality, this should not be taken as an indication that the effect is unimportant (see Slosser 1993, Slosser et al. 1992). However, the critical elements of season which are the driving force in the relationship (light, temperature, rainfall, humidity, plant growth stage, etc.) are more carefully studied elsewhere.

Because host plants are an important aspect in the biology of this aphid, old taxonomic names were converted into modern equivalents. Where possible, the work by Huxley et al. (1992) was used along with the conventions therein. Tanaka (1976) was used if the plant was not found in Huxley et al. A dagger "†" after the scientific name indicates use of an updated scientific name. The work by Jones and Luchsinger (1986) was used as a source for the Cronquist system of plant evolution.

MORPHOLOGY

External morphological characters can be used to distinguish different life stages of this aphid. Cornicle length can separate instars of *A. gossypii* reared under fluctuating temperatures. There was considerable overlap between instars, but there was no overlap between nymphs and adults (Singh and Srivastava 1989). Other characters combined can provide greater separation between instars especially under constant temperatures (Inaizumi and Takahashi 1989a). First-instar nymphs are distinguished by having four antennal segments, while second-instar nymphs have five segments. Differences between second- and third-instar nymphs are fairly small but at constant temperatures they can be separated using a combination of characters (body length, cornicle length, and setal number on cauda and anal plate). Third-instar nymphs have no setae on the margin of the genital plate, while fourth-instar nymphs have such setae. Second-instar nymphs with developing wings appear to have shoulders, third-instar nymphs have small wing pads, and the developing wings are prominent on fourth-instar nymphs. Exact measurements are not provided as they will be different for different host plants, and morphological differences due to host plant are much larger than differences due to genetics (Wool and Hales 1996).

The following stages have been illustrated for this aphid from Japan: fundatrix, fundatrigeniae, alienicola, gynoparae, oviparous female, alate and apterous male, hibernating viviparae, virginandroparae, androparae, heteroparae, and androgynoparae. There are also individuals with partly developed wings from nearly apterous to nearly functionally winged (Inaizumi 1968, Inaizumi 1980, Inaizumi 1983) (for definitions of the life stages see [Miyazaki 1987, Moran 1992] but also see [Blackman 1994]). The following stages have been illustrated for this aphid from Iran: alate and apterous viviparae (Ghovanlou 1974).

The internal morphology of *A. gossypii* has received little attention. However, one paper describes the morphology of the brain of *A. gossypii* reared on *Brassica* sp. (Satija and Dhindsa 1968).

LIFE HISTORY

Host Range. The world wide distribution of *A. gossypii* is partly due to its broad host range. Table 1 organizes the recorded hosts according to the Cronquist system of plant classification (Jones and Luchsinger 1986). The families listed are ones where at least one plant species has been recorded as a host, but with no consideration of host suitability. The species listed are plants useful to man where the aphid is sufficiently abundant to require

TABLE 1: Plant Families With Members Serving As Hosts For *Aphis gossypii*. Hosts that are a source of food, fiber, or ornamental are listed under the appropriate family according to the Cronquist system of classification as found in Jones and Luchsinger (1986).

Family	Species	Common Name	Damage[a]	Citation
	Division: Pinophyta (Gymnosperms) Class: Coniferopsida			
Order: Coniferales				
Cupressaceae				35
	Division: Magnoliophyta Class Magnoliopsida (Dicots)			
Subclass: Magnoliidae				
Order: Magnoliales				
Annonaceae				51
	Annona muricata L.	Soursop		13
Order: Laurales				
Lauraceae	*Persea americana* Mill.	Avocado	F	61
Order: Piperales				
Piperaceae	*Piper betle* L.	Betelvine	F	48
	Piper methysticum Forest.	Kava	V	16
Order: Ranunculales				
Ranunculaceae				44
Subclass: Hamamelidae				
Order: Hamamelidales				
Hamamelidaceae				35
Order: Urticales				
Ulmaceae				51
Cannabinaceae				51
Moraceae				35,51
Urticaceae				51
Order: Casuarinales				
Casuarinaceae				35
Subclass: Caryophyllidae				
Order: Caryophyllales				
Nyctaginaceae				51
Cactaceae				13
Chenopodiaceae				51
Amaranthaceae				51
Portulacaceae				35
Caryophyllaceae	*Dianthus caryophyllus* L.	Carnation	V	56
Order: Polygonales				
Polygonaceae				51

Subclass: Dilleniidae				
Order: Plumbaginales				
Plumbaginaceae				35
Order: Theales				
Dipterocarpaceae				51
Theaceae				35
Clusiaceae				35
Order: Malvales				
Tiliaceae				51
Sterculiaceae	*Theobroma cacao* L.	Cacoa		13
Malvaceae				35,51
	Hibiscus cannabinus	Kenaf	N	42,43
	H. esculentus	Okra	F,V	33,34,49
	H. syriacus	Rose of Sharon		30
	H. rosa-sinensis	Chinese Hibiscus		46
	Gossypium hirsutum	Cotton	F	1,28
	G. hirsutum	Cotton	V?	14
	G. arboreum			15
Order: Lecythiadales				
Lecythidaceae				13
Order: Violales				
Violaceae				51
Passifloraceae	*Passiflora edulis* Sims.	Passionfruit		13
Caricaceae	*Carica papaya* L.	Papaya, Papaw		13
Cucurbitaceae				51
	Cucumis sativus L.	Cucumber	F,V	11,12
	Cucurbita pepo	cv. 'Michlo Lavan'	V	5
		Zucchini	V	7
	Cucumis melo L.	Muskmelon	V	7
Begoniaceae				8
Order: Capparales				
Capparidaceae				51
Brassicaceae				51
	Brassica campestris L.	Turnip, cv. Yorii	V	21
Order: Ericales				
Epacridaceae				13
Ericaceae				35
Order: Ebenales				
Ebenaceae	*Diospyros virginiana* L.	Persimmon	F	61
Subclass: Rosidae				
Order: Rosales				

Pittosporaceae				35
Crassulaceae				35
Saxifragaceae				35
Rosaceae				35,51
	Pyracantha sp.	Pyracantha	F	45
	Malus pumila Mill.	Apple	F	26,27
	Fragaria sp.	Strawberry cv.'Tufts'	N	63
	Pyrus communus L.	pear	N	19
	Eriobotrya japonica Lindl.	Loquat		13
Order: Fabales				
Mimosaceae				35
Caesalpiniaceae				51
Fabaceae				51
	Vigna unguiculata (L.) Walp.	Cowpea, line TVu54	V	6
	Phaseolus vulgaris L.	Bean cv. 'The Prince'	V	6
	Glycine max (L.) Merrill	Soybean	V	6
	Trifolium alexandrinum	Egyptian Clover	V	37
	Vigna radiata (L.) R. Wilcz.	Greengram	V	47
	Vigna mungo (L.)	Blackgram	V	9
	Maughania macrophylla		C	10
Order: Proteales				
Proteaceae	*Macadamia* sp.	Macadamia Nut	F	35,61
Order: Myrtales				
Sonneratiaceae				13
Lythraceae				51
Myrtaceae				35,51
	Psidium guava	Guava	N	19
Punicaceae				51
	Punica granatum L.	Pomegranite		13,67
Onagraceae				35
Combretaceae				35
Order: Celastrales				
Celastraceae				35
Order: Euphorbiales				
Euphorbiaceae				51
Order: Rhamnales				
Vitaceae				51
	Vitis vinifera L.	grape		13
Order: Sapindales				
Sapindaceae	*Litchi chinensis*	Litchi (=Lychee)		13
Burseraceae				51

Anacardiaceae				35
	Anacardium occidentale L.	Cashew nut		13
Rutaceae				35,51
	Citrus sinensis (L.) Osbeck	"Marrs" Sweet Orange	V	58
	Citrus aurantifolia (Christm.) Swingle	Mexican Lime	V	58
Order: Geraniales				
Oxalidaceae				51
Balsaminaceae				51
Order: Apiales				
Araliaceae				35,51
Apiaceae				51
	Apium graveolens L.	Celery		13
	Daucus carota L.	Carrot		13
Subclass: Asteridae				
Order: Gentianales				
Apocynaceae				35,51
Asclepiadaceae				51
	Calotropis procera		V	38
Order: Solanales				
Solanaceae				35,51
	Solanum melongena	Eggplant	F, V	17,54,64
	Capsicum annuum L.	Chilli	V	24,40,65
	Solanum tuberosum L.	Potato	F, V	41,57
	Nicotiana tabacum L.	Tobacco	V	24,25,60
Convolvulaceae				51
	Ipomoea batatas (L.) Poir.	Sweet potato	V	31
Order: Lamiales				
Boraginaceae				35,51
Verbenaceae				35,51
Lamiaceae				35,51
Order: Plantaginales				
Plantaginaceae	*Plantago ovata* Forsk.	Isabgol	F	52
Order: Scrophulariales				
Oleaceae				13
Scrophulariaceae				44
Myoporaceae				35
	Artocarpus altilis	Breadfruit		13
	Artocarpus communis	Breadfruit		13
Gesneriaceae				13

Acanthaceae				51
Pedaliaceae	*Sesamum indicum*	Sesame (=gingelly)	F	39
Bignoniaceae				35,51
Order: Campanulales				
Goodeniaceae				13
Order: Rubiales				
Rubiaceae				35,51
	Gardenia augusta (L.) Merrill	Gardenia	F	36
Order: Dipsacales				
Caprifoliaceae				35
Order: Asterales				
Asteraceae				35,51
	Dendrathema grandiflorum Kitam.	Chrysanthemum	F	2,3,23,66
	Helianthus annuus L.	Sunflower	V	62
	Zinnia elegans Jacq.	Zinnia	V	53
	Lactuca sativa L.	Lettuce cv. 'Iceberg'	N	29
	Division: Magnoliophyta Class:	Liliopsida (Monocots)		
Subclass: Arecidae				
Order: Arecales				
Arecaceae	*Cocos nucifera* L.	Coconut		13
Order: Arales				
Araceae				51
	Colocasia esculenta (L.) Schott	Taro		
Subclass: Commelinidae				
Order: Commelinales				
Commelinaceae				51
Order: Cyperales				
Poaceae				
	Saccharum officinarum L.	Sugarcane	V	32
	Zea mays L.	Corn	V	55,13
	Triticum aestivum L.	Wheat	N	20,13
Subclass: Zingiberidae				
Order: Zingiberales				
Musaceae	*Musa acuminata* Colla.	Banana	V	59
	Musa textilis Née	Abaca	V	50
Zingiberaceae				51
Cannaceae				51
Subclass: Liliidae				
Order: Liliales				

Liliaceae				35
	Lilium longiflorum Thunb.	Easter lily	F	18
	Allium sativum	Garlic	V	4
Iridiaceae				51
Dioscoreaceae	*Dioscorea batatas*	Chinese Yam	V	22
Order: Orchidales				
Orchidaceae				13

[a]V= virus vector. F= feeding Damage. C= competition. N= present, but nature of problem not directly stated (e.g., where authors use phrases like "injurious", or "subject to attack").

1) Abdel-Wahab and Rizk 1970; 2) Adams and Hall 1990; 3) Adams et al. 1990; 4) Ahlawat 1974; 5) Antignus et al. 1989; 6) Atiri et al. 1986; 7) Banik and Zitter 1990; 8) Batchelder 1927; 9) Benigno 1979; 10) Bhattacharya and Srivastava 1987; 11) Binnis 1971; 12) Brouwer and Dorst 1975; 13) Carver 1996; 14) Cauquil 1981; 15) Chakravarthy and Sindu 1986; 16) Davis et al. 1996; 17) Dhandpani and Kumaraswami 1982; 18) Doucette 1962; 19) El-Nagar et al. 1985; 20) Fagundes and Arnt 1978; 21) Fujisawa 1985; 22) Fukumoto and Tochihara 1978; 23) Furk and Vedjhi 1990; 24) Gahukar and Nariani 1982; 25) Gooding and Kennedy 1985; 26) Hameed and Dinabandhoo 1978; 27) Hameed et al. 1975; 28) Hassanein et al. 1971; 29) Hinsch et al. 1991; 30) Inaizumi 1980; 31) Kennedy and Moyer 1982; 32) Khurana and Singh 1972; 33) Kisha 1978; 34) Kishore and Rai 1982; 35) Leonard and Walker 1971; 36) Miller and Williams 1989; 37) Mishra et al. 1980; 38) Mohan and Sharma 1987; 39) Muralidharan et al. 1977; 40) Nandanwar et al. 1976; 41) Nderitu and Mueke 1986; 42) Norman and Sutton 1969; 43) Norman et al. 1972; 44) O'Brien et al. 1993; 45) Pinnock et al. 1974; 46) Pospisil 1972; 47) Ramakrishnan et al. 1973; 48) Raut and Bhattacharya 1987; 49) Regupathy and Jayaraj 1972; 50) Retuerma 1982; 51) Roy and Behura 1983; 52) Sagar and Jindla 1984; 53) Sastry et al. 1973; 54) Seth and Raychaudhuri 1977; 55) Shaunak and Pitre 1973; 56) Singh and Singh 1989; 57) Singh et al. 1984; 58) Smith and Farrald 1988; 59) Summanwar and Marathe 1982; 60) Suzuki and Akazawa 1978; 61) Swirski et al.1991; 62) Theuri et al. 1987; 63) Trumble et al. 1983; 64) Vyanjane and Mali 1981; 65) Wadnerkar and Deshpande 1977; 66) Webb and Argauer 1974; 67) Zhang and Zhong 1990.

some type of human intervention. This organization emphasizes the diversity of the host range and the impact this aphid has on human activities.

In addition to records of hosts from which this aphid has been recovered, the host switching ability of this aphid is also documented. One study reported *A. gossypii* surviving at least 15 days following transfer from plants in the Scrophulariaceae, Brassicaceae, Asteraceae, Lamiaceae, Rosaceae, and Malvaceae to plants in the Asteraceae, Solanaceae, Cucurbitaceae, Liliaceae, Portulacaceae, Commelinaceae, and Araceae (Inaizumi 1980). Another study reported colonies on plants in the Cucurbitaceae surviving at least 3 months following transfer to plants in the Begoniaceae, and Onagraceae (Batchelder 1927). In addition, colonies from plants in the Cucurbitaceae survived over 2 years following transfer to hosts in the Poaceae (Ebert 1994). However, it is also clear that there are many biotypes of this aphid (Guldemond et al. 1994), where biotype is defined as the ability or inability to feed on specific hosts within the host range of the species. Several authors have designated biotypes for this aphid, but they are only of local interest because a lack of standardization precludes comparisons of biotypes.

Host Utilization. Reproduction in *A. gossypii* is mostly asexual with either alate or apterous females. In warmer environments, this aphid exhibits an anholocyclic life cycle, while in cooler areas the aphid exhibits either a heteroecious or autoecious holocyclic life cycle (Slosser et al. 1989, Zhang and Zhong 1990). The heteroecious cycle involves a migration from a primary host to a secondary host in the spring and a return to a primary host in the fall for laying eggs. The primary host for the aphid is the original host for the aphid, but there are problems with using this definition if the aphid has multiple primary hosts. In Japan, this aphid lays eggs on *Citrus* (Rutaceae), *Hibiscus syriacus* L. (Malvaceae), *Rhamnus dahuricust* Pall. (Rhamnaceae), *Celastrus orbiculatus* Thunb. (Celastraceae), and *Rubia cordifolia* L. (Rubiaceae) (Inaizumi 1980, Komazaki et al. 1979). In the USA, this aphid lays eggs on *H. syriacus* and *Catalpa bignonioides* Walter (Bignoniaceae) (Kring 1959). In the Peoples Republic of China, this aphid lays eggs on *Zanthoxylum simulans* Hance (Rutaceae), *Rhamnus* sp. (Rhamnaceae), and *Punica granatum* L. (Lythraceae) (Zhang and Zhong 1990).

It has been suggested that *Z. simulans* was the original host of this aphid (Zhang and Zhong 1990). The rationale was that this host was the more primitive host where sexuals overwinter and that the aphid life cycle is better synchronized with *Z. simulans* relative to *P. granatum*, and *Rhamnus sp.* In Japan, *A. gossypii* on *H. syriacus* will move onto secondary hosts. Of the primary hosts, the aphids did best in the transfer from *H. syriacus* relative to the other primary hosts examined (*Z. simulans* was not examined) (Inaizumi 1980). The argument against *H. syriacus* as the primary host was that *A. gossypii* is almost completely autoecious on this host (in China), and this host is more advanced than either *P. granatum*, or *Rhamnus sp* (Zhang and Zhong 1990).

This conflict highlights fundamental problems in using plant biology or plant phylogeny for determining the original host for a polyphagous species. First, until the geographical point of origin for the aphid is known, the degree of synchronization will not help identify the original host of the aphid. This is because authors from different countries will find different plants best synchronized with the aphid based on local host availability and climate. Second, considering its polyphagous nature, the aphid may secondarily adopt a new primary host that is more primitive than the "true" original host. In conclusion, the original host plant for this aphid will remain unknown - at least until a great deal more is known about the aphid's biology on a global scale.

Population Growth. Reproductive rates in *A. gossypii* are reported in two ways; net reproductive rate (R_o), which is an interaction between birth rate and survival rate (Wilson and Bossert 1971), and birth rate as measured in nymphs produced per day per aphid. The additional information required to estimate R_o is more difficult to obtain than just the birth rate. As a result, authors estimating R_o and other life table parameters use a single host plant at several different temperatures. Authors using birth rate examine differences in aphids feeding on different host plants.

R_o has been estimated for this aphid on many hosts: squash (*Cucurbita pepo* L.) (Aldyhim and Khalil 1993), citrus (*Citrus unshiu* Marc.) (Komazaki 1982), pumpkin (may be squash, no scientific name provided) (Liu and Perng 1987), *Veronica persica* Poir (Nozato 1987a), taro (*Colocasia esculenta* † (L.) Schott. (Setokuchi 1981), and cucumber (*Cucumis sativus* L.) (Kocourek et al. 1994, Owusu et al. 1994b, van Steenis and El-Khawass 1995, Wyatt and Brown 1977). The interaction between host plant and temperature is apparent when examining R_o estimated by several different authors on several different hosts: on squash the maximum R_o (79.7) was at 25°C with a 15% decrease at 30°C (Aldyhim and Khalil 1993); in citrus the maximum R_o (58.68) was at 19.8°C with a 6% decrease at 29.7°C (Komazaki 1982); in pumpkin the maximum R_o (109.14) was at 21°C with a 44% decline at 30°C (Liu and Perng 1987). Comparing the results from research on cucumber gives some idea of the variability induced by differences in biotype augmented by differences in experimental procedure. The differences include 1) location (United Kingdom, Japan, Czech Republic, Netherlands); 2) confinement (leaf discs, whole leaf cage?, leaf clip cages); 3) colony age (unknown, field collected, 64 generations); and 4) range of abiotic variables examined. At a day length of 16.5 hours, 4000 versus 5000 lux, and temperature of 17.5°C, R_o was reported as 109.0 versus 41.9. At the same day length but 24.5°C, R_o was reported as 80.5 (Wyatt and Brown 1977) versus 51.6 (Kocourek et al. 1994). Owusu et al. (1994b) did not report light intensity, but at about 25°C and 16 hours daylight, R_o ranged from 32.9 to 49.8 for collections tested at various times during the year. Also with an unknown light intensity, with a 14-hour day at 25°C, R_o was reported as 53.0 (van Steenis and El-Khawass 1995). Equivalent estimates of R_o under a range of light intensities at 16.5 hours daylight varied from 5.8 to 103.2 (Wyatt and Brown 1977).

Birth rate as a method for comparing aphid populations further emphasizes host plant effects on *A. gossypii* biology. Significant differences were found in birth rates of this aphid reared on cotton (*Gossypium hirsutum* L.), watermelon (*Citrullus lanatus* (Thunb.) Matsum. & Nakai), and groundnut (*Arachis hypogaea* L.) (Ekukole 1990). Significant differences were also found in the reproductive potential of this aphid on cotton, watermelon, sesame (*Sesamum indicum* L.), and eggplant (Moursi et al. 1985). Another study reported that development time was shortest on cotton, longest on melon, with development rate on watermelon (*Citrullus lanatus* †) lying somewhere between (Ghovanlou 1976). In contrast, one paper reported no significant differences in birth rate for this aphid reared on okra (*Abelmoschus esculentus* (L.) Moench.), eggplant (*Solanum melongena* L.), and chili (*Capsicum annuum* var. annuum L.) (Kandoria and Jamwal 1988).

It is difficult to draw conclusions from these studies. Problems include differences in aphid biotype (it is unknown, and may be different on different host plants within a study), and the effects of different methods of handling the insects (mainly cage effects). However, there are two papers which examine the reproductive rate for this aphid on cotton: one from Arizona in 1993 (Akey and Butler 1993), the other from Arkansas in 1946 (Isely 1946). The development time was shortest at 27.5°C and 28°C respectively. At these temperatures, the aphids took 5.0 and 5.18 days to reach maturity. The optimal temperature for fecundity as measured in nymphs/adult/day was different: 25°C versus 20°C, which resulted in 2.85 versus 2.69 nymphs/adult/day respectively.

Stage Structure. One paper reported on the stage structure of field collected aphids. By classifying nymphs as small, medium, and large, the stage structure of this aphid on cotton in the USA was about 53% small nymphs, 25% medium nymphs, 15% large nymphs, 5% apterous adults, and 2% winged adults (Satoh et al. 1995).

Alate Production. There are two forces proposed as triggers for alate production in *A. gossypii*: nutritional factors, and crowding. However, research to date has not conclusively identified the relative importance of nutrition versus crowding in alate formation in this aphid. The problem with the nutritional studies is that they show increased alate production along with increased total number of aphids. Studies on crowding eliminate nutritional stress effects, but they do not eliminate other nutritional factors. The problem is further complicated by insufficient knowledge of the aphid's biology. There is no clear definition of "stress" for this aphid or

how to measure it. Frequently it is measured as a change in aphid fecundity, but this is an average measure of stress rather than an instantaneous measure. A similar problem occurs with defining crowding. There is no clear understanding of what constitutes "crowded" from the aphid's perspective. Papers which demonstrate crowding as the force behind alate development also suffer from a lack of demonstrated (rather than theoretical) mechanism. Aphid-aphid contact has been proposed, but it is equally likely that the aphid has a chemical means of identifying the number of aphids on a leaf or plant. Furthermore, it is not known if the trigger for alate production is continuous, discrete, or a combination of the two. From nutritional studies it would appear that those authors have assumed that they are looking at a continuous process where change in plant nutrition brings about corresponding change in alate production. However, from papers on crowding, it would appear that alate production is partly a discrete process where crowding gets to some threshold level (three-four aphids/plant?) and suddenly alates are produced. From the following examples, it would appear that crowding is the driving force for alate production while other factors (e.g. nutrition, parentage, temperature) all modify the magnitude of the response.

Nutritional stress has been examined by removing aphids from their host for some period of time and then returning them to their host. This treatment was applied to adults and nymphs on cotton for 6- to 8-hour periods each day. Starved nymphs from apterous parents resulted in 13% alates versus 0.4% from unstarved nymphs. However, starvation of nymphs from alate parents resulted in no increase in alate formation. A similar result was also reported for starved parents, where starved apterous parents produced more alate progeny than well fed apterous parents (23% versus 2%, respectively), and there was no increase in alate formation by starved alate parents. The effect of starvation also was detectable in progeny from aphids starved as nymphs but fed normally as adults (Reinhard 1927). However, this might be a crowding response if the aphid is interpreting physical contact with the brush (used to remove the aphid from the plant) as contact with other aphids.

Nutritional factors from other sources also play a role. One of the more interesting hypotheses is the possible role of aphid borne plant viruses. The survival of the virus is dependent on having an efficient aphid vector, and the most efficient vector is alate. Therefore one might expect that a virus would promote conditions favoring alate production in the aphid. Alate production in the melon aphid-zucchini-zucchini yellow mosaic virus system appeared to increase on infected plants. Unfortunately, virus infected plants also had more aphids. The authors discuss this and decide that nutritional factors are the cause for increased alate production (Blua 1991, Blua and Perring 1992a). To further support the authors' argument that the cause might be nutritional, it has been shown that aphid infestations on *Solanum integrifolium* change peroxidase, esterase, and protein content of the plant in proportion to the level of infestation (Owusu et al. 1994a). This shows a change in plant nutrient content associated with aphid density which might form a chemical link between nutrition and alate production. Obviously, more work in this area is required.

Crowding effects have been conclusively demonstrated for this aphid on cotton. One study examined crowding using leaf disks with a single apterous aphid which was removed following reproduction. The resulting colonies contained from one to seven nymphs. From 52 colonies with one or two nymphs, no alates were produced. From 41 colonies with three or four nymphs, less than 10% of the colony became alate. However, of 29 colonies with five to seven nymphs, over 30% of the total number of aphids became alate (Graham 1968). This experiment was repeated as part of the control for another experiment by the same author. Two possibly significant differences were apparent: alate production only occurred in colonies with four or more nymphs, and colonies with five to seven nymphs only produced 12% alates (Graham 1968). The observation that solitary individuals never develop alates was reported earlier for the melon aphid (Reinhard 1927).

Crowding parents also influenced alate development in the progeny. Regardless if the parents are crowded or not, colonies with fewer than three aphids did not develop alate individuals. In colonies with more than three aphids, the progeny from crowded parents developed more alates than did progeny from uncrowded parents (Graham 1968).

Parentage also influenced alate production. Apterous aphids can be reared through 59 generations on cotton with nearly no alate production (1066 aphids reared, with only 17 alates). The author attributed the few alates produced to accidental crowding (Reinhard 1927). Uncrowded conditions were maintained as one aphid per plant. This author tried rearing a continuous line of alate aphids, where alate parents produce alate progeny to provide the alate parents of the next generation. The progeny from a single alate for five generations (59 nymphs) did not produce a single alate.

Abiotic factors could also influence alate production; temperature and light being the two obvious signals used as indicators of seasonal change. However, to date no one has effectively studied these factors. The closest is the paper by Reinhard (1927), but his research in this area was inconclusive. There was also a note which reported sexual morph (winged) development due to short-day conditions (10.5 light: 13.5 dark at about 20°C) (Guldemond et al. 1994).

Color Variation. Yellow and a "green that is almost black" form the extremes of a continuous gradation in color. The yellow form occurs during warmer summer conditions and is smaller. The green form is larger and occurs during cooler spring and fall temperatures, and uncrowded conditions. Color is not a host race trait because color morphs are able to produce progeny of the other color morph (Setokuchi 1981, Wall 1933). Host plant also influences aphid color (Regupathy and Jayaraj 1973). Wall (1933) reported green morphs produced more alate offspring than yellow morphs. However, his observation could be the result of crowding, as the green form also produced more total offspring. Since stresses like crowding, higher temperatures and host plant stress result in a greater proportion of the yellow morph, it was thought that the yellow morph was a stress induced response. However, some insecticides can reverse this response. LC_{10} doses of sulprofos (a stress) produced more dark individuals than either the control or LC_{10} doses of cypermethrin or dicrotophos (Kerns and Gaylor 1992).

Abiotic Environment. One of the most important abiotic factors affecting the life cycle of this aphid is temperature. A lower developmental threshold for this aphid was estimated at 7.34°C on squash in Taiwan (Liu and Perng 1987). Development thresholds have also been estimated for the aphid on cucumber as 5.8°C from birth to age of first reproduction, but the development threshold for the nymphal stages was 6.9°C (Kocourek et al. 1994). A study of this aphid on *Veronica persica* estimated a developmental threshold of 10.47°C for the teneral preflight period (Nozato 1989b). An upper limit to survival of 35°C was reported on squash in Saudi Arabia, but the authors pointed out that the aphid survives in okra fields where the daytime temperature exceeds 45°C (Aldyhim and Khalil 1993). Temperature is also thought to be responsible for some strains of *A. gossypii* being holocyclic while others anholocyclic. A hypothesis was that eggs will be produced in locations where the average temperature during November does not exceed 13°C (Inaizumi 1980).

Light intensity and day length are also important abiotic factors in the reproductive capacity of this aphid. Increasing day length from 6 to 12 to 18 hours significantly increased the intrinsic rate of increase, decreased population doubling time, and decreased generation time. However, longevity, and R_o were maximized at a 12-hour day (Aldyhim and Khalil 1993). Another paper reported *A. gossypii* subjected to longer days (8 versus 16 hours) and increasing light intensities (800, versus 4000, versus 8000 lux) had increased reproduction on cucumber at 18°C with the intrinsic rate of natural increase doubling from 0.22 to 0.44 (Wyatt and Brown 1977). Auclair's (1967a) results regarding light intensity conflict with the above results. Auclair reports that high intensity light (550 lux or brighter) inhibits feeding and colonization, and aphids feeding on diets exposed to 550 lux would move to diets exposed to 54 lux. There was no obvious reconciliation between these two studies other than to suggest that different clones may respond differently to light intensity, or the difference was due to experimental conditions.

The effect of light intensity may be strong enough to result in detectable differences under field conditions. In experimental plots in Texas (USA), cotton was mulched with wheat straw, or left bare ground. In most cases the mulched plots had fewer aphids. The mulch was reflective, so

the undersides of leaves received significantly more light in the 340-1067nm range (Rummel et al. 1995). However, more experiments should be done in this area before assuming a cause and effect relationship.

BEHAVIOR

Flight. Flight is the beginning of the dispersal or sexual reproductive phase in this aphid's life cycle. It begins with the preflight period (from molt to flight) which lasts from 1 to 31 hours with most activity between 10 and 24 hours after molt from colonies reared on Veronica *persica* (Nozato 1987b). The teneral preflight period increased from 10 to over 70 hours with decreasing temperatures from 28° to 12°C (Nozato 1989b). Adults flew from about sunrise to early afternoon, but a few individuals continued to fly after dark. With first light at 0600 hours, and last light at 1930 hours, no aphid flight was detected from 2300 hours to 0700 hours. Considering that the time of molting is independent of time of day, the most common duration of the teneral preflight period is explained by adult inactivity after dark (Nozato 1987b, Nozato 1989b).

In laboratory colonies, the flight period lasted from 1 to 4 days (Nozato 1990). Older colonies produced fewer alates that flew for one day and more that flew for two days. Aphids flew from one to several (about five) times each day, with the first flight always longer than the others. Alates larviposited after flight, and flew again when the number of embryos with pigmented eyes per ovariole decreased. Alates that flew longer had a shorter reproductive period and produced fewer total progeny. Forewing length, teneral period, and first flight duration did not influence the flight period (Nozato 1987b). Two authors reported that alates will not produce offspring on leaves with existing colonies (Nozato 1989a, Reinhard 1927).

Migration. In cotton fields in the Ivory Coast, dispersal from savanna to cotton fields was examined for *A. gossypii* from data collected using pan traps (Duviard et al. 1976). These data showed that most aphids settled at field margins, although there was some settlement in the field. They also reported that pan traps at ground level caught more *A. gossypii* than traps further from the ground. From their graphs, it appears that most aphids were caught no more than 1 meter from the soil surface, and that the closer to the surface, the more aphids were caught.

Light. This aphid is sensitive to different wavelengths and intensities of light, but the nature of the effect is not clearly understood. Aphids were attracted to Auclair's diet illuminated at 570-595 nm while diets illuminated at 420-485 nm were repellent (Auclair 1967a). This contradicts other findings where newly molted alate adult individuals preferred shorter wavelengths down to 357 nm. Adults of mixed age also preferred this short wavelength, and their preference declined with increasing wavelength. However, there was an increase in preference beginning at 547 nm, peaking at 562 nm, and rapidly declining after the peak (Pospisil 1971). This peak is approximately where Auclair (1967a) did his studies, and could explain the different results. Furthermore, there is a stage dependent response to light, adults being more sensitive to different wavelengths than nymphs (Auclair 1967a). In keeping with these results, Rummel et al. (1995) reported fewer aphids in cotton plantings with wheat straw mulch, and light in the 420 to 485 nm wavelength was significantly higher in the mulched plots.

Host Plant. Orientation to host plants was significant at 6 hours after wing development, but was highly significant after 24 hours . Alates were able to distinguish between different plants; *Cucurbita pepo* and *Thunbergia laurifolia* were attractive, and were common hosts for this aphid in Cuba. The occasional host *Hibiscus rosa-sinensis* L. was neither attractive nor repellent, and the non-host plant *Lantana camara* L. was repellent (Pospisil 1972).

In addition to the type of host present in a field, the arrangement of hosts within the field is also important. An experiment was done using soybean, dwarf sorghum and tall sorghum planted in monoculture or interplanted. At the sorghum canopy level, landing rates were highest on monocultures of dwarf sorghum, then monocultures of tall sorghum, and lowest in mixed plantings. This difference was attributed to a lower percentage

ground cover in monocultures. In mixed fields, landing rates were equal in dwarf sorghum and soybean, but in tall sorghum interplanted with soybean the aphid preferred to land on soybean (Bottenberg and Irwin 1992).

Feeding: In one set of experiments, alates were placed on *Cucurbita pepo* plants and watched under a microscope. Given that probing occurs when aphids pressed their labium to the surface, and placed their antennae flush with the body, the aphid took 6 to 9 minutes following contact with the plant before probing (Yuan and Ullman 1996). However, this is the time it took newly emerged alates, and may not be characteristic of alates after migration.

Egg Laying. Egg laying on *H. syriacus* occurred mostly between the leaf scar and the twig near where the buds would emerge in spring. Some eggs were also laid at the branching point of twigs. However, from the wandering behavior of the oviparous females, it appears that females searched for protected places to lay eggs rather than for specific parts of the plant (Inaizumi and Takahashi 1989b).

Virus Interaction. Two papers deal with the effect of viral infection of host on the aphid. The first reported that aphids on Yellow-Vein Mosaic Virus infected okra did not reproduce as fast as aphids from healthy okra (Regupathy and Jayaraj 1972). A possible mechanism for the observation was provided by another author using Zucchini Yellow Mosaic Virus infected zucchini (*Cucurbita pepo*) plants (Blua and Perring 1992b). Aphids on infected plants spent more time probing and less time feeding than aphids on healthy plants. Furthermore, prior to feeding, aphids on infected plants spent more time in forming the salivary sheath.

Within Plant Distribution. On cotton grown in the former Soviet Socialist Republic, *A. gossypii* migrated from the stem apex to the upper leaves and then to the lower leaves in the morning (Tshernyshev et al. 1981). During the day, aphids were mostly on the underside of leaves, and they migrated back to the stem apex at night. The table in Tshernyshev et al. (1981) indicated that many individuals did not conform to this pattern. In eggplant from India, the aphid settled on older mature leaves. It moved to younger tissues only when population pressure forced it to so aphid populations were always greatest on older leaves (Banerjee and Raychaudhuri 1985). In cantaloupe (*Cucumis melo* L.) from the USA, the aphid was most abundant on the basal portion of vines (Edelson 1986). In cotton in the USA, the aphid was most abundant in the middle canopy, followed by the upper canopy. However, this pattern may be a result of high aphid mortality from a fungal pathogen in the lower canopy rather than a result of aphid behavior (O'Brien et al. 1993). Other authors in the USA have related aphid density to leaf age and mostly report larger populations on older leaves (Hardee et al. 1994, Slosser et al., in press). However, year to year variability in location of the highest aphid population has been reported (Slosser et al., in press) and the aphid's preference may change through the season. In Greece, aphids infested the top of the cotton plant early (May-June) and late in the season (October), but preferred the lower parts during the middle of the season (July-August) (Kapatos et al. 1996).

BIOCHEMISTRY

Genetic Characters. Khuda-Bukhsh and Datta (1978) reported that in *A. gossypii* 2n=8 (found on *Ageratum conyzoides* L. (Asteraceae) in India). The chromosomes in cells during metaphase measured 2.3, 3.4, 3.8, and 5.0 µm in length. Using aphids from a different location Khuda-Bukhsh and Pal (1985) reexamined the karyomorphology of this aphid and reported that chromosome lengths were 3.65 ± 0.54, 5.42 ± 0.44, 6.24 ± 0.48, and 7.64 ± 0.85 µm (collected from *Erobtorys japonica* (Rosaceae) in India). These authors provided further discussion of the processes occurring during cell division. Khuda-Bukhsh and Kar (1989) reported differences in chromosomal length within each pair of chromosomes. They reported chromosome lengths of 2.00, 2.25, 3.55, 3.80, 4.00, 4.50, 6.45, and 6.80 µm. The chromosomes are believed to be holokinetic which could simplify structural rearrangement of chromosomes, and permit more rapid adaptation to adverse conditions, including new host plants. This mechanism for adaptation has been shown in *Myzus persicae*. Chromosomal rearrangement in *M. persicae* is one source of pesticide resistance (Blackman et al. 1978). The effect appeared to be due

to a translocation that was correlated with an increase in carboxylesterase (est-4) activity.

Another method for evaluating DNA, random amplified polymorphic DNA polymerase chain reaction (RAPD-PCR), can detect differences in *A. gossypii* colonies (Cenis et al. 1993, Ebert 1994) and between the melon aphid and other aphid species (Cenis et al. 1993). Differences in *A. gossypii* DNA occurs between aphids feeding on different host plants (Ebert 1994, Khuda-Bukhsh and Kar 1989), but neither reported conclusive evidence that the differences were due to mutation rather than selection of adaptable strains within a parent population.

Host Adaptation. A. gossypii requires time to adapt when switching hosts, or feeding on artificial diet. Knowing the duration of this adaptation period is critical in biological and nutritional studies because during this period the response of the aphid is a combination of the effect of the new host and a stress response. Field collected aphids from various cucurbits could adapt to a susceptible muskmelon line in 6 months, as measured by an increase in the reproductive rate (Kishaba and Coudriet 1985). Another author reported that the aphid would not adapt to a new host in 3 months in experiments transferring clones between plants in the Cucurbitaceae and Solanaceae (Saito 1991). Aphids transferred between cucumber and chrysanthemum began to adapt to the new host (as measured by adult weight and development time), and if transferred back to their original host, they did not return to original conditions within three generations (Guldemond et al. 1994). Aphids have also been transferred from plants in the Cucurbitaceae to wheat. While the transfer was successful, the reproductive rate on wheat never achieved that on the cucurbits even after 2 years (Ebert 1994). However, the aphids had probably adapted to wheat as much as their genotype would permit without mutation.

Since different host plants contain different secondary compounds, one might expect that aphids adapted to different hosts would show different enzyme levels. Furthermore, these levels should change when the aphid was switched to a new host. However, one study failed to show this phenomenon with aphids adapted to eggplant, cucumber, taro, or watermelon. The enzymes examined were malic enzyme, phosphoglucomutase, glucosephosphate isomerase, 6-phosphogluconate dehydrogenase, isocitrate dehydrogenase, malate dehydrogenase, esterase, and carboxylesterase. The only difference in the colonies was reported for aphids on taro for malate dehydrogenase (Owusu et al. 1996). This lack of observable differences may be due to using only insecticide resistant aphid clones from each colony. This was done to try and remove effects of past field exposure to insecticides from the results of the experiment. However, this may have also removed much of the natural enzyme variability in the aphid populations.

Insecticide Response. Aphids reared on different plants show different levels of susceptibility to insecticides (see McKenzie and Cartwright 1994). Several mechanisms for resistance have been demonstrated in this aphid: enzymatic differences, target insensitivity, cuticular modifications, and life history modifications at the population level. However, studies in this area suffer from an inability to differentiate between phenotypic plasticity versus differences in aphid genotype as selected by host plant. Confounding the entire issue is the effect of host plant response to insecticides, either through metabolism of the insecticide or insecticide induced changes in plant nutrient levels.

In some cases, pesticide resistance in this aphid was correlated with different aliesterase levels in aphids on different host plants. Aphids reared on melon or cucumber showed elevated aliesterase levels relative to aphids from eggplant or potato (Saito 1991). Aphids with high aliesterase activity maintained original levels of aliesterase activity even when moved to solanaceous crops, and aphids with low aliesterase activity maintained low levels when moved to cucurbitaceous crops. This represents a difference between two strains of this aphid correlated with a difference in host plant, and is clearly not host induced. Other authors also have reported significant differences in esterase patterns and esterase quantity, and have correlated these differences with insecticide resistance (Furk et al. 1980, Hama and Hosoda 1988, O'Brien 1992, O'Brien et al. 1992, Saito et al. 1995, Takada and Murakami 1988) and with host plant preference (Furk et al. 1980). While mixed function oxidases (MFOs) may play a role in detoxification reactions, the esterases and carboxylesterases showed more conspicuous

differences between susceptible and resistant aphid strains (Sun et al. 1987).

Feeding is one method for insecticide entry into the aphid. Peroxidase levels in salivary glands, sheath material, and salivary excretions of *A. gossypii* might play some role in detoxifying systemic insecticides. For this reason, the role of salivary gland enzymes in detoxification has been examined as a possible defense. While the peroxidases were effective in detoxifying hordenine and gossypol (secondary plant compounds in cotton), the role of these enzymes in the natural habitat of the aphid is not clear (Miles and Peng 1989).

This aphid also acquires resistance through target site insensitivity and through increased production of the affected enzymes. Acetylcholinesterase (AChE) insensitivity was shown in aphids resistant to organophosphate and carbamate insecticides (Moores et al. 1988). Furthermore, activity level of acetylcholinesterase has been correlated with pirimicarb resistance (Gubran et al. 1992, Silver et al. 1995, Suzuki and Hama 1994). For two clones, this resistance was shown to be specific to pirimicarb (Silver et al. 1995). These clones were 800 fold more resistant than the susceptible clone, but no more than 22 times more resistant to six organophosphates, six carbamates, and two pyrethroids. The mechanism was linked to AChE with higher catalytic activity and lower affinity to pirimicarb in the resistant clones. Cuticular differences as well as acetylcholinesterase insensitivity played a role in insecticide resistance in parathion and paraoxon (Sun et al. 1987).

In studying the cause for a control failure in cotton (USA) the biological cost of resistance for an organophosphate resistant aphid feeding on cotton was estimated. Over the first few days resistant alate aphids produced more progeny, but over the life span of the adult there was no significant difference between the resistant and susceptible aphids. However, as these authors point out, reproductive rate during the first few reproductive events are the most influential in determining population growth rate (O'Brien 1992, O'Brien and Graves 1992). These authors proposed that the organophosphate insecticide had selected for individuals with higher initial reproduction, as opposed to stimulating reproduction (O'Brien and Graves 1992).

Life stage is another factor affecting the susceptibility of this aphid to insecticides. Alate adults on cotton were more resistant than apterous adults to oxydemeton-methyl, chlorpyrifos, dicrotophos, biphenate, and endosulfan (Grafton-Cardwell 1991).

Pest resurgence is where a pesticide treatment causes some mortality, but ultimately results in much higher pest populations. Pest resurgence can be due to the action of pesticide on the insect, the action of pesticide on the plant, the action of pesticide on natural enemies, or some combination of the three. Classical thought attributes most or all of pesticide resurgence to a reduction in natural enemies. However, under field conditions it is difficult to control for other factors, and they are often ignored. However, a few authors have examined these other factors. Sulprofos treated cotton fields had elevated numbers of *A. gossypii*, but the cotton plants in these fields had significantly elevated levels of threonine and "essential" amino acids (Kerns and Gaylor 1993). Cotton plants treated with the systemic insecticides disulfoton, phorate, dimethoate, and lindane had larger aphids, but treated plants had lower sugar content, lower nitrogen content, lower carbohydrate to nitrogen ratio, and higher amino acid content (Sithanantham et al. 1973). Okra treated with phorate had more aphids which were also physically larger, but the phorate treatment also resulted in elevated levels of NH_4 nitrogen, potassium, and a decrease in carbohydrates, magnesium, and calcium (Regupathy and Jayaraj 1974b). Although altered plant physiology may account for aphid resurgence in some cases, it is not the only cause. Direct applications to the aphid of deltamethrin, methyl parathion and carbaryl stimulated the reproductive rate of *A. gossypii*. Some of this effect was due to elevated feeding levels at LC_{10} doses of deltamethrin and methyl parathion (Gajendran et al. 1986). Other authors also reported elevated aphid populations following pesticide applications, but did not examine causes (Patel et al. 1986, Surulivelu and Sundaramurthy 1986, Thimmaiah and Kadapa 1986).

Host plant mediated susceptibility to DDT, lindane, endrin, endosulfan, malathion, ethyl parathion, methyl parathion, dimethoate, phosphamidon, and

carbaryl was reported for aphids reared on six cucurbitaceous hosts (Juneja and Sharma 1973). Aphids on cucumber were consistently less susceptible to all insecticides relative to aphids from the original culture on bottle gourd (*Lagenaria siceraria* (Molina) Standl.). Other than this, there was no consistency in the level of resistance and host plant. Based on relative toxicity within each host plant, phosphamidon was most toxic followed by methyl parathion, while p,p' DDT was least toxic. The toxicity rank of the other pesticides changed depending on host plant (Juneja and Sharma 1973). Aphid colonies feeding on watermelon versus cotton showed differences in pesticide susceptibility between bifenthrin, chlorpyrifos, dicrotophos, dimethoate, endosulfan, methomyl, and oxydemeton-methyl (McKenzie and Cartwright 1994). An interaction between host plant resistance and pesticide resistance in this aphid can also occur. *A. gossypii* reared on aphid resistant chrysanthemums showed increased susceptibility to parathion or nicotine relative to aphids on a susceptible cultivar (Selander et al. 1972). However, plants producing more detoxifying enzymes (esterases and transferases) in response to insecticides may help aphids by metabolizing insecticides. While a cause and effect relationship could not be proven, there was good correlation between plant enzyme levels and reduced aphid mortality from dichlorvos on okra, cucumber, and eggplant (Owusu et al. 1995).

Aphid Nutrition. Aphid nutrition has been studied by stressing the host plant or using holidic diets. Cotton plants in Arkansas were grown in the greenhouse in sand fertilized with solutions containing 10% of the nitrogen or 5% of the potassium of the full fertilizer (Isely 1946). *Aphis gossypii* took longer to mature, and had a lower larviposition rate on nutrient stressed plants, but only nitrogen stressed plants produced a significant reduction in total offspring and total duration of reproductive period. The effect of nitrogen fertilization was also examined on cucumber using hydroponically grown plants (Petitt et al. 1994). As expected, these authors showed differences in aphid population growth correlated with nitrogen level in the solution. The importance of fertilizers, especially nitrogen, was also recorded for this aphid feeding on eggplant in West Bengal (Banerjee and Raychaudhuri 1987), potato in Egypt (El-Saadany et al. 1976), cotton in USA (Beckham 1970, Slosser et al., in press), and cotton in Egypt (Rasmy and Hassib 1974). However, it is not always possible to detect the effect of fertilizer on aphid populations in the field (Slosser et al., in press).

Another way to change nutrient quality of the host is to limit the availability of water. On cotton, alate aphids did better on leaves with sap densities under 11%, and such leaves occur on the lower part of the plant during flowering (Weismann et al. 1970). Another study on cotton reported that total leaf water content was positively correlated with higher aphid numbers, and lower leaves on cotton have a higher water content (Slosser et al. 1992). It is unclear if water content is the cause for greater aphid numbers on lower leaves, or if cotton aphids prefer lower leaves for other reasons.

While water content may play a role in aphid fecundity, other changes in the plant associated with water balance also influence aphid populations. Aphid population density increased on potted cotton plants as the frequency of watering decreased from once every 3 days to once every 6 days to once every 9 days. The cause was attributed to an observed increased plant nitrogen levels and decreased carbohydrate levels in the foliage associated with decreased watering (Hassib and Rasmy 1974). A different study examined levels of carbohydrate, nitrogen, fat, sterol, and inorganic salts in eggplant, and reported that only nitrogen levels were significantly correlated with aphid populations (Banerjee and Raychaudhuri 1987). Thus, some of the effect of plant drought on aphids may be due to altered nutritional status, but sap density and microclimate changes associated with altered canopy structure are confounding factors (e.g., wilting) (Weismann et al. 1971).

A popular method of studying aphid nutrition is to use artificial diets. So far, no diet has been reported which will indefinitely support *A. gossypii* growth and reproduction. At most, only two generations survive, which is insufficient time for the aphid to adapt to the diet. Therefore, the following reports are subject to alternate interpretation based on stresses from as yet unidentified sources. The authors of the papers

recognize this problem. For this discussion, I treat the nutritional requirements of the aphid as the aphid plus its symbionts.

An artificial diet for the pea aphid was developed by Auclair in 1965 which has been used for *A. gossypii* (Auclair 1965). Aphids transferred to the artificial diet survived, and produced progeny, but the progeny produced by these aphids did not survive (Auclair 1967b). On this diet, the growth and birth rate of *A. gossypii* was highest on diet adjusted to pH 7.4-7.8 (Auclair 1967b). The importance of sugars, both nutritionally and as phagostimulants, are well known. The optimal sucrose concentration for settling was 40%, but the optimal sucrose concentration for growth and reproduction was 20 to 30% (Auclair 1967a). Diets with some or all sucrose replaced with one of the following, raffinose, sorbose, melezitose, glactose, lactose, ribose, or cellobiose, were less suitable than diets with only sucrose (Auclair 1967a). The diet consisting of 20% sucrose and 10% maltose yielded progeny with significantly higher biomass, and more total progeny in the second generation. However, by all other measures this diet was either equal to or inferior to the other diets. These measures include mean adult weight, lifespan, progeny of the first generation, progeny produced per day, and settling of larvae and adults on the diet (Auclair 1967a).

The nutritional requirements of *A. gossypii* were examined by Turner (1971, 1977) using the diet developed by Auclair. Diet was evaluated using a growth index that was a function of the total aphids produced in 6 days and the weight of those aphids. Better diets were identified by having a higher growth index. Both cysteine and methionine are required for maximum growth. Concentrations of either amino acid above 30 mg/ml are toxic. Inorganic sulfur, as sodium sulfate or ammonium sulfate, does not substitute for these amino acids when present in equivalent molar concentrations to the amino acids. While the concentration of methionine is sufficient in Auclair's diet, Turner recommends 700 mg more cysteine be added. Turner also altered concentrations of tyrosine, phenylalanine, and tryptophan from Auclair's diet. He shows that aphids continue to reproduce on diets lacking all three amino acids, but the aphid does better with them present. Phenylalanine concentrations from 0 to 8 mg/ml were examined, and the aphids did best at 2 mg/ml. Tryptophan concentrations from 0 to 8 mg/ml were examined, and the aphids did best at 4 mg/ml. Tyrosine concentrations from 0 to 0.40 mg/ml were examined and the aphids did best at 0.4 mg/ml. All of these represent a recommended increase in the concentrations from the original diet.

PLANT DEFENSES

Among the factors determining host range of phytophagous insects are plant defenses. These can be mechanical or chemical, and the insect must overcome these defenses in order to survive and reproduce. The main characteristic implicated as a physical mechanism of defense is leaf trichome density. Glabrous cotton supported fewer aphids than more pubescent cotton (0.52, 2.34, 4.48, and 6.09 hairs/mm^2) (Dunnam and Clark 1938, Rummel et al. 1995, Weathersbee III et al. 1994). However, pubescence has the opposite effect on this aphid feeding on muskmelon (Kennedy et al. 1978). Leaf color may also have some effect. The red and yellow isolines of TM-1 cotton (USA) had fewer aphids relative to the TM-1 isoline with green leaves (Rummel et al. 1995).

Many crops have some level of physiological resistance to this aphid that can be classified into one of three categories: tolerance, antixenosis, or antibiosis. The causes for resistance were examined in muskmelon and cucumber. Resistance has also been documented in the following crops: okra (Gunathilagaraj et al. 1977, Uthamasamy et al. 1976): *Gossypium hirsutum* and *Gossypium arboreum* (Chakravarthy and Sidhu 1986); *Antigastra catalunalis* (Muralidharan et al. 1977); *Citrullus lanatus* (MacCarter and Habeck 1973); *Solanum melongena* (Sambandam and Chelliah 1970); and *Colocasia esculenta.* (Palaniswami et al. 1980).

Muskmelon. All three mechanisms of plant resistance (tolerance, antibiosis, antixenosis) have been documented for muskmelon. In some muskmelon, several genes confer tolerance by reducing the curling response of leaves subjected to heavy feeding. There appeared to be a single gene controlling the curling response of the plant to aphid attack (Bohn et al.

1973). Resistance in *Cucumis melo* can also be due to antixenosis (Kennedy and Kishaba 1977). The effect remained in excised leaves for at least 4 days. It was not translocatable across a graft union. In some muskmelon, antixenosis was caused by a single dominant gene with a few other genes playing a minor role (Kishaba et al. 1976). Antibiosis has also been reported as a source of resistance in muskmelon. Electronic recording of aphid feeding showed that aphids feeding on resistant plants made fewer contacts with the phloem, and the duration of each feeding episode is two to three times shorter on resistant plants. However, aphids on the resistant plants made more contacts with the phloem. Physical examination of the stylet sheaths showed an increase in the proportion of branched stylet sheaths, and an increase in the number of branches per stylet sheath. However, contrary to the electronic recording, histological evidence suggested that the aphids contacted the phloem less in the resistant plant. Furthermore, while there was no difference in the number of stylet sheath branches ending in xylem, palisade, bundle sheath or epidermis, there were significantly more which ended in the mesophyll. The discrepancy between the electronic recording of aphid feeding and the histological evidence was attributed to the histological evidence looking at a fixed instant in time while the electronic recording includes a time component (Kennedy et al. 1978).

Cucumber. The *Bi* gene permits cucurbitacin production in cucumbers. Aphids on non-bitter *Cucumis sativus* had a higher average daily reproductive rate, and achieved much higher densities than aphids on bitter plants. However, aphids on bitter plants had a shorter development time (Haynes and Jones 1975).

Tissue age plays a significant part in host plant resistance. In cucumber, aphids on older leaves had 82% nymphal mortality versus less than 25% on middle aged and young leaves (van Steenis and El-Khawass 1995). Aphids on older leaves also had reduced fecundity - 45.9 nymphs/female on lower leaves versus 70 nymphs/female on middle and young leaves. In chrysanthemum, the effect of leaf position was determined by cultivar (Storer and van Emden 1995). Survival was unaffected by position in two of the three cultivars (Hero and Purple Anne) and highest on lower leaves in cultivar Surfine. Development time, fecundity, and the intrinsic rate of increase (r_m) were more favorable (when significant) for rapid population growth on lower leaves (Storer and van Emden 1995). The effect of tissue age was reported for cotton in field trials where planting date interacted with season to significantly alter aphid populations (Slosser et al., in press, Slosser et al. 1992).

VIRAL INTERACTIONS

The most important impact that *A. gossypii* has on world agriculture is through its ability to transmit plant viruses. Table 2 lists plant viruses transmitted by this aphid. The list does not contain older references because of problems in proper identification of the aphid and the viruses; see Kennedy et al. (1962) for older references.

The type of transmission is classified as persistent, semipersistent, and nonpersistent using a system first proposed by Watson and Roberts (1939) and later modified by Sylvester (1956). Pirone and Harris (1977) recommend the use of stylet-borne and circulative to categorize aphid borne viruses. However, we have retained the old system because most of the literature uses the old system, and in many cases it is not known if the virus is stylet-borne or not.

This review proposes that the plant-aphid-virus system be thought of as a series of simple effects (e.g., aphid population growth, plant growth, virus replication) plus a series of interactions (e.g., "plant x aphid", "plant x virus", "aphid x virus", and possibly the three way interaction). Breeding plants resistant to virus modifies the "plant x virus" interaction. The "plant x virus" interaction could occur by a modification of leaf cuticle hydrocarbons. This could have the same effect as spraying the crop with oil which has been shown to decrease transmission of non-persistent and semi-persistent viruses (Singh 1981, Vanderveken 1977). Modifying other interactions will also reduce the incidence of disease. Documenting which

effect is responsible for any observed reduction in disease incidence is important for properly evaluating the results. For example, the "aphid x virus" interaction can occur if the virus coat protein changes (Gera et al. 1979). One would also expect that this could occur if the binding sights on the aphid were to change, but this has not yet been demonstrated. The "plant x aphid" interaction is exemplified by plants which are repellent to insects, or lack cues which the insect uses to distinguish host plants from non-host plants (e.g., some melons are resistant to virus transmission, but the cause appears to be due to antixenosis [Pitrat and Lecoq 1980]).

Short literature reviews of citrus tristeza virus, cucumber mosaic virus, and potyviruses highlight the complex nature of the interactions possible in plant-aphid-virus systems.

Citrus Tristeza. Citrus tristeza, a member of the closterovirus group, is a phloem-limited virus attacking plants in the Rutaceae almost exclusively. It is a filamentous particle 11x2000 nm long. The genome is a single strand of RNA. *Aphis gossypii* transmits this virus semi-persistently, remaining infectious for over 24 hours (Bar-Joseph et al. 1989). The system was not sensitive to the culture host of the aphid but was sensitive to temperature (Bar-Joseph and Loebenstein 1973). Different strains of *A. gossypii* did not differ in their ability to transmit citrus tristeza, but different strains of the virus did differ in their transmission rates by this aphid (Raccah et al. 1980). The aphid acquires the virus more easily from some citrus cultivars than from others. The acquisition period can be 5 minutes, but was more efficient at periods of 30 minutes to 24 hours. Infectivity was lost within 48 hours of acquisition, but feeding on alternate host plants does not reduce infectivity (Bar-Joseph and Loebenstein 1973). Aphids reared on cucumber were able to acquire the virus when fed on infected citrus as easily as aphids reared on citrus (Bar-Joseph and Loebenstein 1973). This was also true of aphids reared on muskmelon (*Cucumis melo* L.), and kenaf (*Hibiscus cannabinus* L.) (Norman and Sutton 1969, Roistacher et al. 1984). Transmission rates were significantly lower when plants were held at 31°C relative to those at 22°C (Bar-Joseph and Loebenstein 1973). When the plants were cooled (31° to 22°C), it took about 6 days for an increase in transmission rate. When plants were warmed (22° to 31°C), it took 12 to 20 days for transmission rates to decline. The apparent reason for this effect was different virus titers in trees at the two temperatures. The inoculation period should be 4 to 6 hours (Bar-Joseph and Loebenstein 1973). *Aphis gossypii* was able to transmit the virus to certain cultivars more efficiently than to others (Roistacher and Bar-Joseph 1984).

Cucumber Mosaic Virus. Cucumber Mosaic Virus (CMV) is the type member of the cucumovirus group. The virus is a set of three isometric particles 29 nm in diameter each consisting of a protein coat built from 180 identical subunits, and encapsulating four main single stranded RNA molecules, several minor strands, and a variable number of satellite RNA molecules (molecules requiring the virus for replication and encapsidation, but unnecessary for virus function). In order of decreasing size, the major RNA strands are designated RNA 1, 2, 3, and 4. The minor strands are designated RNA 4a, 5 and 6. The active virus is a set of three distinct particles all of which must be transmitted for infection; one particle has RNA 1, one particle has RNA 2, and the third has RNAs 3 and 4. The remaining RNA molecules may or may not be present (Palukaitis et al. 1992). RNA 1 is necessary for infection and replication. It also influences symptom severity and rapidity of expression of the symptoms. RNA 1 also plays a role in aphid transmission (Francki et al. 1985, Zitter and Gonsalves 1991). RNA 2 is also required for infection and replication. RNA 3 codes for coat protein, but requires RNA 4 to express the trait. RNA 3 is also necessary for aphid transmission. In some cases RNA 3 determines the host plant reaction while in others it is RNA 2, or both (Francki et al. 1985). RNA 4 is generated from RNA 3. RNA 4 is necessary for coat protein synthesis, but not for infectivity.

CMV has the widest host range of any virus, attacking plants from 85 plant families (Palukaitis et al. 1992). It was transmitted non-persistently on the stylets of the aphid vector. Unlike citrus tristeza, previous host changes the ability of the aphid to acquire CMV (Jacquemond 1982), and different aphid clones differ in their ability to transmit CMV (Simons and Eastop 1970). Acquisition time can be short (under 1 minute), but transmission rate increases with longer feeding times up to at least 15 minutes (Camino-Lavin 1970). Changes in the virus coat protein can change

TABLE 2: Virusus Vectored By Aphis Gossypii. Virus type is based on Francki et al 1985. The question mark after the virus type indicates a tentative placement in that group. Viruses of unknown afinity may be new viruses that have not been placed, or may be variants of a virus already listed.

Type	Virus	Host Plant	Country	Source
unknown Afinity	Calotropis Mosaic Virus	*Calotropis procera*	India	20
	Carnation Mottle Virus	*Dianthus caryophyllus*	India	32
	Citrus Woody Gall Virus	Citrus	Peru	41
	Greengram Mosaic Virus	*Vigna mungo* & other hosts	India	23
	Infectious Chlorosis	Banana	India	37
	Leaf Crinkle of Sunflower	Sunflower	Kenya	39
	Mosaic of Bean	*Vigna mungo*	Philippines	6
	Mosaic of Garlic	*Allium sativum* L.	India	1
	Muskmelon Yellow Stunt Virus	*Cucumis melo* & *Cucurbita pepo*	France?	26
	Solanum torvum Mosaic Virus	*Solanum torvum*	India	33,35
	Yellow Blotch of Sunflower	*Helianthus annuus*	Kenya	39
	Yellow Vein Mosaic Virus	*Abelmoschus esculentus*	India	24
Alfalfa Mosaic Virus	Alfalfa Mosaic Virus	*Trifolium alexandrinum*	India	19
		Eggplant	India	40
Carlavirus?	Chinese Yam Necrotic Mosaic Virus	*Dioscorea batatas*	Japan	12
Carlavirus	Lily Symptomless Virus			36
Caulimo-virus	Cauliflower Mosaic Virus			36
Clostero-virus 1	Citrus Tristeza Virus	Citrus	USA	27,43
Cucumo-virus	Cucumber Mosaic Virus	*Zinnia elegans*	India	28
		Turnip	Japan	11
		Banana	India	18
		Cucumber	Japan	42
			Netherlands	7
		Capsicum spp.	India	13,31
		Cucumis melo & *Cucurbita pepo*	USA	5
		Nicotiana tabacum & other hosts	India	29
Luteovirus	Potato Leafroll Virus	Potato	India	34
Potyvirus	Bean Common Mosaic Virus			36
	Cowpea Aphid-Borne Mosaic Virus	*Vigna unguiculata*	Nigeria	4
	Onion Yellow Dwarf Virus			36
	Papaya Ringspot Virus			36
	Pepper Veinal Mottle Virus	Pepper (Capsicum sp.)	Nigeria	3
	Potato Virus Y	*Nicotiana tabacum*	Japan,USA	14,38
		Capsicum annuum & other hosts	India	16
		Potato	India	34

	Sri Lankan Passion Fruit Mottle Virus	*Passiflora edulis* f. flavacarpa	Sri Lanka	9
	Sugarcane Mosaic	Sugarcane	India	17
		Corn	USA	30
		Musa textilis	Philippines	25
	Turnip Mosaic Virus	Turnip	Japan	10,11
		radish	Japan	10
	Watermelon Mosaic Virus 1	Cucumber	Japan	42
		Cucurbita maxima & other hosts	Japan	44
		Cucumis sativus, & other hosts	Mexico	8
	Watermelon Mosaic Virus 2	*Cucurbita* spp.	Israel	2
	Yam Mosaic Virus			36
Potyvirus ?	Commelina Mosaic Virus	*Commelina diffusa*	USA	21
	Sweet Potato Feathery Mottle Virus	*Ipomoea nil*	USA	15
	Zucchini Yellow Mosaic Virus	Pumpkin	Japan	22
		Cucurbits	Israel	2

1) Ahlawat 1974; 2) Antignus et al. 1989; 3) Atiri & Dele, 1985; 4) Atiri et al. 1986; 5) Banik & Zitter, 1990; 6) Benigno 1979; 7) Brouwer & Dorst 1975; 8) Camino-Lavin, et al. 1974.; 9) Dassanayake & Hicks 1992; 10) Fujisawa & Iizuka 1985; 11) Fujisawa 1985; 12) Fukumoto & Tochihara 1978; 13) Gahukar & Nariani 1982; 14) Gooding & Kennedy, 1985; 15) Kennedy & Moyer 1982; 16) Khatri & Sekhon 1974; 17) Khurana & Singh 1972; 18) Mali & Rajegore 1979; 19) Mishra et al. 1980; 20) Mohan & Sharma 1987; 21) Morales & Zettler 1977; 22) Ohtsu et al. 1985; 23) Ramakrishnan, et al. 1973; 24) Regupathy & Jayaraj 1972; 25) Retuerma 1982; 26) Risser et al. 1981; 27) Roistacher et al. 1984; 28) Sastry et al. 1973; 29) Seth & Raychaudhuri 1977; 30) Shaunak & Pitre 1973; 31) Singh & Singh 1977; 32) Singh & Singh 1989; 33) Singh et al. 1975b; 34) Singh et al. 1984; 35) Singh, et al. 1975a; 36) Smith 1972; 37) Summanwar & Marathe 1982; 38) Suzuki & Akazawa 1978; 39) Theuri et al. 1987; 40) Vyanjane & Mali 1981; 41) Wallace & Drake 1969; 42) Yamamoto & Ishii 1983; 43) Yokomi & Damsteegt 1991; 44) Yonaha et al. 1977;

the effectiveness of aphids in transmitting the virus (Gera et al. 1979). Between an aphid transmissible strain (Fny-CMV) and a poorly transmissible strain (M-CMV), there were eight amino acid changes in the coat protein. Amino acids 129, 162, and 168 of the coat protein mediate the aphid-virus interaction, but amino acid 168 plays a relatively minor role relative to the other two amino acid positions (Perry et al. 1994). Aphids lose their ability to transmit following probing or after fasting for about four hours (Simons and Eastop 1970). Transmission rate is dependent on the concentration of virus in the host. A virulent isolate reproduced faster than a less virulent isolate in muskmelon with a corresponding increase in transmission of the more virulent isolate (Banik and Zitter 1990).

An enzyme linked immunosorbant assay (ELISA) has been used to detect CMV from individual aphids (Gera et al. 1978). The aphid transmissible strain carried 0.01-0.1 ng of virus per aphid. The non-transmissible strain was not detectable on the aphid.

Potyviridae. Many members of the potyviridae are transmitted by the melon aphid. Among the better studied members are: potato virus Y (PVY), watermelon mosaic virus I and II (WMVI, WMV2), zucchini yellow mosaic virus (ZYMV), and papaya ringspot virus (PRV). These viruses consist of a flexuous rod 680-900 nm long and ±12 nm in diameter. The genome is a single molecule of single stranded RNA (Francki et al. 1985). Unfortunately, no single virus in this group has been studied extensively in its association with this aphid. As a result, the following examples are pieced together from articles each dealing with a different virus in this group. Different aphid life stages had different vectoring potential of PVY with the adult alate stage having the lowest transmission efficiency (Singh et al. 1983). Differences in virus composition in ZYMV changed the ability of the aphid to transmit the virus (Lecoq et al. 1991). Different clones of the aphid differ in their ability to transmit PRV (Lupoli et al. 1992). Acquisition and transmission times for both ZYMV and WMV2 can be as short as 15 seconds (Perring et al. 1992). The host plant phenotype might also be important (Gooding and Kennedy 1985, Simons 1959).

Several conclusions can be drawn about the nature of non-persistent viruses. From the short acquisition time, it is likely that the source of the virus is in the epidermis of the host plant (Pirone and Harris 1977); therefore, the aphid could acquire the virus with only a brief probe. This hypothesis would be consistent with aphid feeding patterns where many short probes occur prior to a much longer sustained feeding probe. If aphids are to acquire the virus in only a few seconds of probing, the virus needs to be available in the tissues invaded by short probes. This is also consistent with the observation that starvation increases virus acquisition because short probes become more frequent following starvation (Powell 1993). It is also likely that the virus is not just a physical contaminant on the aphid stylet, but involves a chemical reaction to specific binding cites on some part of the stylet. If the interaction was just physical, one should not observe differences in transmission rates from different aphid clones, there should be no difference in transmission rate between aphid species, and specific sites in the virus genome should not alter transmission rates.

OTHER BIOLOGICAL INTERACTIONS

This section deals with other organisms which influence aphid survival. This is not intended to be a review of these other organisms other than as they affect *A. gossypii*. In general, these studies have concentrated on the biology of the other organism. This trait is reflected in the following text on the ecological relationship other organisms have with the aphid, but the emphasis of some papers was changed from that provided by the original author.

Ants. A beneficial effect to the aphid of the presence of ants *Camponotus japonicus* Mayr was reported from research in Japan (Nozato and Nagano 1988). Aphid populations tended by ants increased in spite of the presence of the coccinellid predator *Coccinella septempunctata bruckii* L.; however, the level of protection afforded by the ant was highly variable. A positive correlation between *A. gossypii*, its coccinellid predator *Menochilus sexmaculatus* (Fabrecius), and the ant *Camponotus compressus* Fabrecius was reported from a guava (*Psidium* sp.) orchard in India. However,

there was a negative correlation between ant abundance and coccinellid abundance. The cause for this effect was not investigated, and it was unclear what effect this had on aphid densities (Verghese and Tandon 1987). Laboratory studies on cotton in the USA examined the effect of *Solenopsis invicta* Buren on the predators *Hippodamnia convergens* Guerin-Meneville, *Chrysopa carnea* Stephens, *Scymnus louisianae* Chapin, and *Syrphus* sp. feeding on *A. gossypii* (Vinson and Scarborough 1989). With ants present, all predators except *Syrphus* were unable to control aphid densities. Without ants all predators were able to control aphid densities.

Predators. The effectiveness of predators is highly variable depending on availability of alternate prey, host plant, and environmental factors. The effect of alternate prey was reported for *Chrysoperla rufilabris* (Burmeister) which preferred *Helicoverpa* (=*Heliothis*) *virescens* (Fabrecius) larvae to aphids, but preferred aphids to *H. virescens* eggs (Nordlund and Morrison 1990). Presence of *A. gossypii* was shown to decrease predation on *H. virescens* eggs by the following predators: *Hippodamnia convergens, Chrysopa carnea,* and *Orius insidiosus* (Say) (Ables et al. 1978).

Syrphid flies have shown potential in controlling aphid populations under greenhouse conditions (Adashkevich and Karelin 1988, Babayan and Hovhannisian 1984, Chambers 1986). However, colonization by the syrphid was decreased on older plants, and older larvae would not transfer from young plants to more mature plants (Adashkevich and Karelin 1988). The suggested cause for the latter effect was leaf pubescence.

From the many studies on aphid predators, it is obvious that these organisms can regulate aphid populations under the right circumstances. However, while most reports deal with a single predator, there are a complex of predators in the field. One paper reported on interactions of several predators of this aphid (Rosenheim et al. 1993). The lacewing *Chrysoperla carnea* (Stephens) was able to cause an overall reduction in aphid abundance when caged on field grown cotton in California. Added to this system were several hemipteran generalist predators (*Geocoris* spp., *Nabis* spp., and *Zelus* spp.) which feed on the aphid, lacewing, and each other. As expected, all of these predators reduced aphid populations, though none were as effective as *C. carnea*. However, all of the hemipterans also reduced lacewing survival which resulted in an increased number of aphids. Furthermore, the reduction in the ability of lacewings to control aphid populations was increased with increasing size of the other predators.

Parasitic Hymenoptera. Changes in parasitism based on age structure of *A. gossypii* populations feeding on cotton were reported for the parasites *Trioxys* spp. and *Aphelinus* sp.. These parasites rarely parasitised first- and second-instar aphids. Thus, the percentage parasitism increased as the proportion of older aphids increased (Luo and Gan 1986). This has survival value for both parasite and aphid because aphids parasitized as older nymphs or as adults have a chance to reproduce. Aphids parasitized by *Aphidius colemani* Viereck had a fecundity of 0.5-1.3 nymphs/female when parasitized in the fourth instar, and 10.5-13.3 when parasitized as adults (van Steenis and El-Khawass 1995). Aphids which survived an attack had lower fecundity but equal longevity relative to unattacked aphids (van Steenis and El-Khawass 1995).

Hyperparasitization by *Alloxysta pleuralis* (Cameron) of the primary parasites *Lipolexis scutellaris* Mackauer, and *Trioxys indicus* Subba Rao & Sharma parasitizing *A. gossypii* was reported from research conducted in India. There was significant decline in rates of hyperparasitization of *T. indicus* parasitizing *A. gossypii* feeding on solanaceous crops (*Capsicum frutescens* L., and *Solanum melongena*) versus crops in the Fabaceae (*Cajanus* sp., *Dolichos* sp.), and Cucurbitaceae (*Lagenaria* sp., and *Luffa* sp.). There was also a significant host aphid effect in *T. indicus* where wasps parasitizing *A. gossypii* had higher parasitism rates compared to *Aphis craccivora* Koch and *Myzus persicae* (Sulzer) (Singh and Srivastava 1990).

Fungi. The two best studied pathogens are *Neozygites fresenii* (Nowakowski), and *Cephalosporium* (=*Verticillium*) *lecanii* (Zimm.). Several other fungal pathogens have also been reported: *Arthrobotrys* sp., *Entomophthora aphidis* Hoffm., and *Entomophthora delphacis* Hori (Sanchez-Peña 1993, Shimazu 1977).

Neozygites fresenii (Zygomycetes: Neozygitaceae) takes 3, 4, 5-6, and 6-8 days to develop at temperatures of 30, 25, 20, and 15°C, respectively. Furthermore, at 35°C the fungus did not kill aphids (Steinkraus et al.

1993). *Neozygites fresenii* was able to produce up to 9,835 conidia from a single aphid. The number of conidia was correlated with aphid size, but the authors suggested that handling or storage properties of larger aphids could explain their observation (Steinkraus et al. 1993). Temperatures above 35°C and humidity below 85% inhibit conidial discharge from *A. gossypii* cadavers (Steinkraus and Slaymaker 1994). Infection starts with primary conidia germinating and forming capilliconidia. This process is so sensitive to humidity that at 95% RH and 25°C, 90% of the primary conidia germinate, while only 19% germinate at 89% RH (Steinkraus and Slaymaker 1994). This fungus can be a major cause of aphid mortality in cotton grown in the Texas/Arkansas area of the USA (Steinkraus et al. 1993, Steinkraus et al. 1991). The fungus has a distinct diel periodicity in spore discharge, with greatest activity occurring between the hours of 0100-0500 hours with almost no activity between 0900-2400 hours (Steinkraus et al. 1996). This fungus has not been reared on artificial media, but Steinkraus et al. (1993) reported on propagation in an aphid colony and longevity of the fungus in cold storage. A primary route for infection of new aphid colonies may be through infected alates (Steinkraus et al. 1995). Detection of infected alates may also provide an early indication of impending epizootics (Steinkraus et al. 1995).

Cephalosporium lecanii is an important source of mortality for aphids under greenhouse conditions, but there are no reports of its impact on *A. gossypii* under field conditions. The effectiveness of the fungus is emphasized by its use as an aphicide in commercial greenhouses in the UK (Hall 1985, Sopp et al. 1990). Its success in this capacity is partly due to the ability of the fungus to grow in artificial media. As one might expect, different strains of the fungus show different growth rates and different levels of pathogenicity (Hall 1982, Kitazawa et al. 1984, Yokomi and Gottwald 1988).

Other Biocontrol Agents. One group of organisms is conspicuously missing from the literature on the biological control of this aphid: viral pathogens. Another group (the mites) have just started to show up in the literature with the study of *Allothrombium pulvinum* (Acari: Trombidiidae). However, these papers deal mainly with mite distribution in the field (Zhang et al. 1993, Zhang and Chen 1993).

Miscellaneous. Potts and Gunadi (1991) reported a decrease in *A. gossypii* populations in potato that is intercropped with *Allium cepa* L. or *Allium sativum* L. To get the reduction, the onions had to be planted within 0.75 meters of potato plants. However, intercropping poses a problem when the minor crop harbors a disease of the primary crop. Such a system has been documented in Taiwan where banana was interplanted with cucumber (an alternate host for banana mosaic virus) (Tsai et al. 1986). A similar effect also occurs when alternate hosts (of aphid and virus) are in neighboring fields (Tsai et al. 1986).

Competition is another form of species interaction. Regupathy and Jayaraj (1974a) reported a negative relationship between *A. gossypii* and *Amrasca devastans* (a leafhopper) on okra with an r^2 of 0.6. The relationship was significant only for aphid and leafhopper nymphs, not leafhopper adults. Presumably this effect is a result of crowding and host quality reduction at high aphid densities. The effect of host quality decline due to feeding by *A. gossypii* is a problem during the commercial production of *Kerria lacca* (Kerr) (Lac insects) on *Flemingia macrophylla* † O. KZE. ex Prain (Sen et al. 1987). Aphid feeding causes premature leaf drop, wilting, and desiccation of the plant. The role, if any, crowding may have played in reducing Lac insect densities was not examined.

FUTURE DIRECTIONS

There are four research areas that should be particularly fruitful.

1) The causes for alate production are still not understood. We think that all of the significant elements have been identified, but their order of importance and their interactions have not been examined. However, in order to fully explore the role of nutrition, a suitable artificial diet, one where 30 or more generations can survive, needs to be developed.

Understanding the mechanism for alate formation would be useful in understanding the spread of this insect through the environment and may improve understanding of the role of this aphid as a virus vector.

2) Our understanding of the ecological interactions of *A. gossypii* are improving, but there are still large gaps. The possibility that a virus will alter host physiology to promote alate formation in this aphid should receive more attention. Research on the impact of natural enemies as it affects the aphid's biology would also be useful. Especially important is work which examines the interactions between different mortality factors and the rest of the aphid's biotic and abiotic environment.

3) The relationship between soil fertility and aphid population growth should be examined further. It is clear that effects like soil fertility, soil salinity, and soil moisture all influence plant nutrient levels. It is also clear that this influences aphid abundance. However, when specific elements are examined (like amino acid composition in the phloem) there is no clear correlation.

4) What is the biochemical mechanism for host adaptation in this aphid. One knows that it involves a physiological change, that there is a limit to the degree that the aphid can change during maturation, and that the parent passes some of this change on to the nymphs. The latter effect may sound Lamarckian; however, it may be that nymphs still inside the adult aphid are the most physiologically flexible. Hence, parental stress will produce changes in nymphs such that they appear to adapt to a new host with each passing generation.

As a final note, there are three things we would change about many of the articles used in this paper. First: although, research is easier using leaf cages or excised leaves, these procedures modify aphid biology by modifying the environment in which the aphid lives. Sometimes a cage is necessary. Under such constraints, the cage should enter the analysis as a treatment effect, and the experiment should be planned accordingly. Second: auxiliary information such as light source, light intensity, temperature, relative humidity, and aphid size should be included as routine measurements. It is also important to report the conditions under which the aphid was reared, and for how long. Finally, some record should be kept of the field host upon which the aphid was found. Such information will greatly facilitate comparing one set of research results with the results from other work.

ACKNOWLEDGMENT

I would like to thank Xiong Deng for interpreting articles published in Chinese. We would also like to thank the people in the Interlibrary Loan department at Oklahoma State University. This paper was a part of the senior author's doctoral dissertation, and funded by the Department of Entomology at Oklahoma State University. This work was also supported by Hatch Project OKLO-2040, CSRS-SRIPM grant #91-34103-5844, CSR - special grants #92-34146-6995, and CSRS special grant # 93-34146-8408.

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OVERWINTERING SURVIVAL OF SEVEN IMPORTED COCCINELLIDS IN THE TEXAS HIGH PLAINS

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ABSTRACT

In a four-year study (1990-1993), seven species of exotic coccinellids [*Cycloneda ancoralis* (Germar), *Eriopis connexa* Germar, *Hippodamia tredecimpunctata* Say, *H. variegata* (Goeze), *Oenopia conglobata* (L.), *Propylea 14-punctata* (L.), and *Sycmnus frontalis* F.], imported for potential control of the Russian wheat aphid, *Diuraphis noxia* (Kurdjumov), successfully overwintered in the Texas High Plains when caged on native range grasses. Survival ranged from 58.6% for *H. tredecimpunctata* to a low of 2.4% for *S. frontalis*. Differences in percentage survival over years were due to climatic conditions. Higher percentage recoveries were made in colder years with snow cover. An absence of snow cover in conjunction with cold temperatures was more detrimental to overwintering survival than warmer temperatures.

INTRODUCTION

Russian wheat aphid, *Diuraphis noxia* (Kurdjumov) (Homoptera: Aphididae), is a pest of wheat in many parts of the world (Hughes 1988), and invaded Texas and other western states in 1986 (Stoetzel 1987). After this invasion, parasitoids and predators of the Russian wheat aphid were imported from Africa, Asia, Europe, the Middle East, and South America in a nationwide program directed at biological control of this devastating pest. This program was a joint effort involving the United States Department of Agriculture Animal and Plant Health Inspection Service (USDA-APHIS), USDA Agricultural Research Service (USDA-ARS), and state agricultural experiment stations, including the Texas Agricultural Experiment Station (TAES).

Working in conjunction with the USDA-APHIS National Biological Control Laboratory at Niles, MI, we evaluated various parameters of imported predaceous coccinellids over the course of the importation and release phases of this program. It was recognized that the ability of an imported coccinellid species to overwinter in the Texas High Plains was crucial to its successful establishment, and a three-year experiment was established to determine how well various species would survive through the winter, and if there were differences in the ability of a particular species to overwinter depending on its origin. This paper reports the results of the overwintering survival research.

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MATERIALS AND METHODS

The research was conducted from January 1990 to June 1993 at the Texas Agricultural Experiment Station Research and Extension Center Laboratory at Bushland, TX. Coccinellid species were supplied by personnel of the USDA-APHIS National Biological Control Laboratory at Niles, MI. The species examined by year, their origins, and the initial release densities are given in Table 1. Adult beetles from colonies at the Niles laboratory were sent by overnight express service to the Bushland laboratory. These beetles were 2- to 3-wk old adults, a mixture of F_1, F_2 or F_3 generation beetles from original importations. The beetles were fed greenbugs, *Schizaphis graminum* (Rondani), and a pork liver diet prior to shipment. They had been induced to enter diapause by simulating fall climatic conditions (10:14 h light:dark photoperiod and 5-15°C fluctuating temperatures). The beetles were released into overwintering cages within 24 h of their arrival at Bushland. The overwintering cage (Fig. 1) consisted of a conical Lumite®

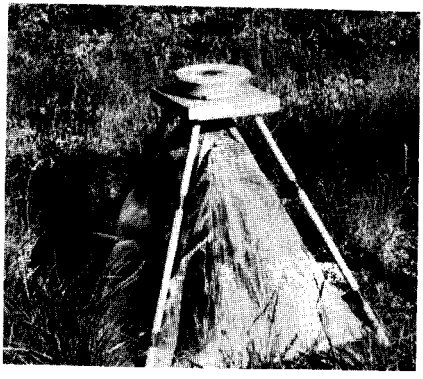

FIG. 1 Exclusion cage

screen enclosure attached to four aluminum legs by plastic reinforced fabric straps. The screened enclosure opened at the top into a metal pan that had a removable plastic lid on top. The cage took advantage of the natural negative geotropic behavior of coccinellids when they encounter a vertical surface (i. e., beetles would climb up the screen wall of the cage and enter the metal pan). The interior surface of the metal pan was coated with Necco Fluon 80-1 (Northern Products, Woonsocket, RI), an anti-friction agent which did not allow the beetles to climb back into the cage. The cages were provided by the USDA-APHIS National Biological Control Laboratory at Niles, MI, and had performed well in overwintering research on coccinellids at that location (Flanders unpublished data).

The cages were placed in a field of native range grasses (composed of *Agropyron* sp., *Bouteloua* sp., *Elymus* sp. and *Poa* sp.) approximately 2 wk prior to release of the beetles. This site was chosen because of the tendency for native coccinellid species to overwinter in the crowns of grasses (Michels pers. obs.).

The cages were arranged in a randomized complete block design with three replications (1 cage = 1 replication) for each species and strain in most years. Initial release densities were originally to be 250 beetles per cage; however, this was modified somewhat as the availability of coccinellids from the USDA-APHIS National Biological Control Laboratory at Niles, MI changed over the four years. See Table 1 for variations in replications and initial release densities for a given year.

TABLE 1. Species Names, Origin, Years Entered and Release Densities for Overwintering Survival of Seven Imported Coccinellids.

Species	Origin Country (Locale[a])	Years entered and number of individuals per replication											
		1990			1991			1992			1993		
		Replication			Replication			Replication			Replication		
		I	II	III	I	II	III	I	II	III	I	II	III
Cycloneda ancoralis (Germar)	Argentina (Santa Fe)							250	250	250	180	170	176
Eriopis connexa Germar	Argentina (Santa Fe)							250	250	250	101	99	98
Hippodamia tredecimpunctata Say	U.S.S.R. (Kirghizia)	250	250	250									
	U.S.S.R. (Moldavia)	250	250	250									
	U.S.S.R. (Ukraine)							164	164				
Hippodamia variegata (Goeze)	Canada (Quebec)	250	250	250	250	250	250	119	119	119			
	U.S.S.R. (Kirghizia)				250	250	250	250	250	250	208	196	165
	U.S.S.R. (Moldavia)	250	250	250	250	250	250	250	250	250	195	276	231
	Morocco (Meknes)				250	250	250	250	250	250	258	45	208
Oenopia conglobata (L.)	U.S.S.R. (Uzbekistan)							250	250	250	315	269	258
Propylea 14-punctata (L.)	Canada (Quebec)	250	250	250				358	150				
	U.S.S.R. (Moldavia)	250	250	250									
	U.S.S.R. (Ukraine)				250	250	250	250	250	250			
Scymnus frontalis Fabr.	Turkey (Beypazari)	250	250	250	250	250	250	250	250	250			

[a]Locale is a province (U.S.S.R. and Canadian entries) or a city (Argentina, Morocco and Turkey).

After beetles were released, the cages were examined weekly for beetles that had entered the collecting pan at the top of the cage. All beetles found in the pan were counted and removed. Sampling continued until no beetles were found after two consecutive sampling dates. Percentage survival was determined as total beetles recovered/total beetles released into a cage *100.

Climatic data were recorded by a USDA-ARS weather station at Bushland, located less than 1 km from the study site. Heating degree days were based on a threshold of 18.3°C (McClure 1987).

Data from the experiments were analyzed using a general linear model (SAS 1989) after undergoing an arcsin transformation, and significant means for percentage survival were separated using the Student-Newman-Keuls test at P=0.05.

RESULTS AND DISCUSSION

Results of the experiments are found in Tables 2 and 3, and Fig. 2. Every species released in a given year survived, although the percentage of the initial population recovered varied from a high of 58.6% for *Hippodamia tredecimpunctata* Say to a low of 2.4% for *Sycmnus frontalis* F. both in 1990. No significant differences in percentage survival were found among groups of the same species from different origins. Therefore these data were pooled and analyzed for differences in survival among species within a year and within a species over years.

In 1990, *H. tredecimpunctata* had a significantly higher percentage survival (58.6%) than *H. variegata* (Goeze) (29.4%), *Propylea 14-punctata* (L.) (33.9%) or *Scymnus frontalis* (2.4%). Significantly fewer *S. frontalis* were recovered than *H. variegata* or *P. 14-punctata*. *H. variegata* and *P. 14-punctata* did not differ significantly in percentage survival. In 1991, *H. tredecimpunctata* was not included in the experiment because of a shortage of specimens that could be used outside the designated release program at the Niles laboratory. Percentage survival for the three species included in the 1991 experiment were similar to that found in 1990. *H. variegata* and *P. 14-punctata* did not differ significantly in percentage survival (20.4% and 25.6%, respectively), and both had a significantly higher percentage survival than *S. frontalis* (5.1%).

In 1992, three new species; *Cycloneda ancoralis* (Germar), *Eriopis connexa* Germar and *Oenopia conglobata* (L.), were added to the experiment and *H. tredecimpunctata* was used again. Percentage survival was greatest with *E. connexa* (31.1%) and *H. tredecimpunctata* (27.4%), although these recoveries were only significantly higher than *S. frontalis* (3.9%). Of those species in the experiment for more than two years, *H. variegata* and *P. 14-punctata* had significantly lower percentage survival in 1992 than in 1990, but not significantly lower than in 1991. No significant difference in percentage survival was observed for *S. frontalis* recovery from 1990 to 1992.

Four species were included in the experiment in 1993. The reduction of species in the experiment was because of the elimination of some older cultures at the USDA-APHIS National Biological Control Laboratory at Niles, MI, or the higher priority to use the available specimens in a continuing, multi-state release effort. The highest percentage survival was found with *C. ancoralis* and *O. conglobata* (7.8% and 10.9%, respectively). These results were significantly higher than those for *E. connexa* (2.7%) which were significantly higher than the percentage survival of *H. variegata* (4.3%). *E. connexa* and *H. variegata* percentage survival in 1993 was significantly lower than in 1992.

TABLE 2. Percentage Survival of Overwintering Coccinellid Adults.

Species	Percentage survival by year											
	1990			1991			1992			1993		
	%	±SE		%	±SE		%	±SE		%	±SE	
Cycloneda ancoralis	--			--			12.9	1.1 ab[a]	A	7.8	4.0 ab	A
Eriopis connexa	--			--			31.1	1.1 a	A	2.7	0.9 b	B
Hippodamia tredecimpunctata	58.6	6.2 a	A	--			27.4	10.4 a	A	--		
Hippodamia variegata	29.4	29.4 b	A	20.4	2.8 a	B	17.9	3.0 ab	B	4.3	1.2 b	C
Oenopia conglobata	--			--			14.1	4.9 ab	A	10.9	4.6 a	A
Propylea 14-punctata	33.9	2.8 b	A	25.6	5.6 a	AB	16.5	1.6 ab	B	--		
Scymnus frontalis	2.4	0.8 c	A	5.1	1.6 b	A	3.9	0.6 b	A	--		

[a]Means followed by the same lower-case letter in a column or upper-case letter in a row are not significantly different ($P<0.05$, Student-Newman-Keuls test, arcsin transformation).

TABLE 3. Temporal and Climatological Parameters Associated with Overwintering Coccinellid Adult Survival, Bushland, TX.

Item	Year			
	1990	1991	1992	1993
Date initiated	1/4/90	11/15/90	11/13/91	1/6/93
Date terminated	5/31/90	4/18/91	6/2/92	6/4/93
Total days	147	154	202	149
Days below 0°C	132	133	121	145
Percent of days below 0°C	90	86	60	97
Date of last freeze	5/1/90	5/1/91	4/3/92	4/21/93
Temp. at last freeze (°C)	0	-3	0	-2
Avg. max. temp. (°C)	15.29	16.44	13.51	18.13
Departure from 20 yr. avg.[a]	-1.01	0.14	-2.79	1.83
Avg. min. temp. (°C)	0.24	-0.81	2.07	2.23
Departure from 20 yr. avg.[a]	0.04	-1.01	1.87	2.03
Absolute min. temp. (°C)	-21.67	-15.56	-15.00	-15.56
Precipitation (cm)	11.30	11.15	18.14	11.63
Departure from 57 yr. avg.[a]	-2.79	-2.95	4.04	-2.46
Snow (cm)	45.72	35.56	57.15	17.78

[a]20-yr. avg., 1976-1995; 57-yr. avg., 1939-1995.

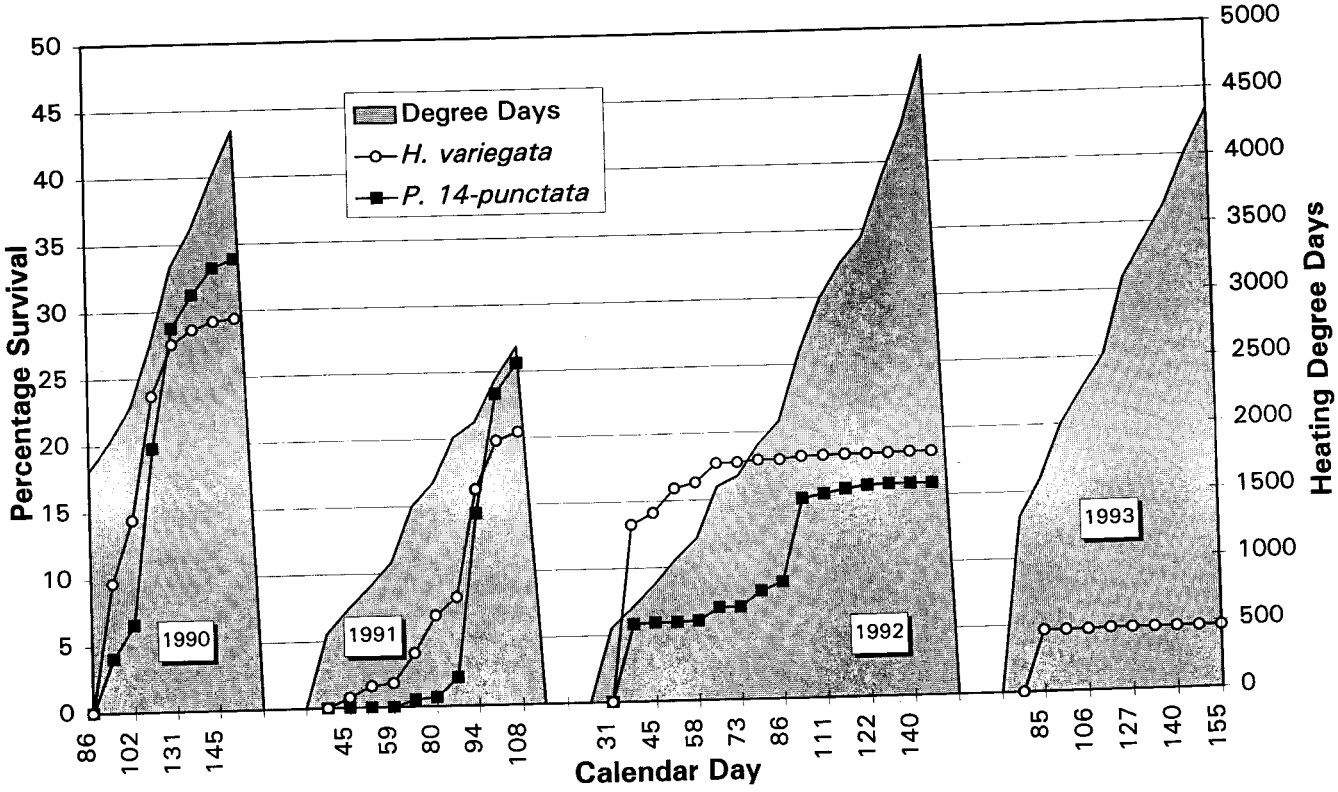

FIG 2. A comparison of heating degree days and percentage survival for two imported coccinellids in the Texas High Plains.

During the four years of our study, a general reduction in recovery occurred. Clues to the differences in survival may be found in the climatic data recorded during the four years. No significant differences were noted in percentage survival between 1990 and 1991, and the climatic data (Table 3) are very similar. From 1991 to 1992, when significant differences in percentage survival were observed for *H. variegata* and *P. 14-punctata*, there were fewer days below freezing in 1992 than 1990 and 1991, a lower percentage of days below freezing in 1992 than 1990 and 1991, the date of last freeze was almost a month earlier in 1992 than 1990 or 1991, the average minimum temperature was higher in 1992 than 1990 and 1991, cumulative heating degree days (Fig. 2) were greater in 1992 than in 1990 and 1991, and total precipitation and snow was the highest observed in the four years. Compared to the winters of 1990, 1991 and 1993, the winter of 1992 was warmer and wetter and the beetles could have been more active.

Significant reductions in percentage survival occurred from 1992 to 1993 for *E. connexa*, and *H. variegata*. Noticeable climatic factors during these two winters (Table 2) include the percentage of days below freezing (97%, or 145 out of 149 days in 1993), and the lack of snow cover (only 17.78 cm total snowfall in 1993). These data indicated a cool, dry winter in 1993 with little insulation from snow cover compared to the warmer and wetter conditions of 1992 which had over three times the snow cover. Surprisingly, percentage survival for *C. ancoralis* and *O. conglobata* did not differ significantly between 1992 and 1993. Although their percentage survival was low in comparison to other species over the four years (7.8% and 10.9% respectively), their apparent ability to survive with a lack of snow cover is noteworthy.

The results of the experiments clearly demonstrate that all species could overwinter in the Texas High Plains. The percentage survival of adult beetles fell within the limits reported by others. Harper and Lilly (1982) reported a range of 30 to 80% survival for *H. quinquesignata* Kirby in Southern Alberta. McClure (1987) noted winter survival of 10% of *Harmonia axyridis* Pallas in Connecticut, and Ali and El-Saeady (1982) found winter survival rates of a phytophagous species, *Epilachna chrysomelina* (F.), in Egypt ranging from 41.8 to 73.1%.

Coleomegilla maculata lengi Timberlake survival declined during winter hibernation in an artificially controlled environment with increasing temperatures due to increased activity of the insects (Jean et al. 1990). Constant temperatures above 0°C did not provide the necessary conditions for *C. maculata* survival during hibernation. Similar results were reported for *Coccinella septempunctata* L. and *Adalia bipunctata* (L.) hibernating at 4°C (Hamalainen 1977). McClure (1987) reported less than 10% of overwintering *H. axyridis* adults survived in overwintering cages in Connecticut. They reasoned that the overwintering cages may have been too restrictive to the beetles, not allowing them to reach protective overwintering sites (e.g., cliffs, caves and rock outcroppings) *H. axyridis* naturally uses in Asia. Jean et al. (1990) also noted that daily lipid catabolism increased exponentially between 0 and 20°C, and lipid content per insect decreased more rapidly over time as temperatures increased from -10 to 10°C. Because 1992 was warmer than the other three years, the reduction in percentage recovery in our research could have been caused by increased temperature and an exhaustion of lipid reserves in the beetles if they remained in a state of hibernation. However, because of the variable climate in the Texas High Plains, where cold periods can be quickly followed by relatively warm periods (ranges of 0 to 22°C in 24 hours are not uncommon), native *Hippodamia* species are found foraging for prey every month of the year (Michels, unpublished data). It may be that the beetles did not enter a true state of hibernation for the entire time the experiment was conducted in 1992, but cycled between periods of

activity and quiescence based on temperature. We were unable to determine if the cage or cage size had a direct effect on survival, but active beetles would likely disperse from a specific site to search for food. In these experiments, the beetles were unable to feed during active periods, and starvation of active adults probably resulted in the reduction in percentage survival.

Harper and Lilly (1982) suggested that higher winter mortality of *H. quinquesignata* may have been caused by reduced snow cover in a given year. This mortality varied among aggregation sites in the Rocky Mountains in Alberta, Canada. Based on supercooling points for adult beetles in hibernation, Harper and Lilly (1982) also noted that lady beetles were more cold hardy during mid-winter, and lost this cold hardiness in the spring. They reasoned that cold periods in April and May could result in high mortality, especially with little or no snow cover. Our recovery data for 1993 suggested a similar pattern. Cold temperatures, or a large number of days below freezing, with little or no snow cover was probably responsible for the low percentage recovery.

One concern in this kind of experiment is confining beetles to cages. Although we did not examine cage effects, it should be noted that the beetles may have dispersed to other sites during the year if they were free to do so. The beetles we recovered during the course of the experiment may or may not have tried to disperse to other sites. Even though the cages prevented dispersal, the objective of the experiment, to determine whether the beetles could survive winters in the Texas High Plains, was accomplished. Unconfined coccinellid adults would probably have higher overwintering survival rates if they were allowed to select their own hibernation sites. In this regard, the experiments we conducted should be considered more stringent tests of the ability these beetles have to overwinter in the High Plains.

Coupled with the overwintering experiments reported in this paper, all species included in this study except *H. tredecimpunctata* were part of a release program to establish these beetles in the Texas High Plains. Between 1987 and 1993, 130,634 predaceous coccinellids from seven species were released in the Texas High Plains (Michels et al. 1994). The one species released for colonization but not included in the overwintering experiments was *Semiadalia undecimpunctata* Schneider. As of this date, no recoveries of any species have been made. From the research reported here, it does not seem that the lack of recovery is caused by overwintering survival.

ACKNOWLEDGMENTS

The authors thank R. L. Romero (presently with the Department of the Army, Headquarters, Ft. Carson, Attn: AFZC-TMD (Romero), DECAM Bldg. 302, Ft. Carson, CO 80913) for her technical assistance. We also thank T. L. Archer, M. D. Lazar, and D. A. Fritts of the Texas Agricultural Experiment Station and C. D. Patrick of the Texas Agricultural Extension Service for their reviews of this manuscript. This research was partially funded by a cooperative agreement between the Texas Agricultural Experiment Station and the United States Department of Agriculture - Animal and Plant Health Inspection Service (CA 12-34-81-0137)

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HIGH CLEARANCE SPRAYER METHODS FOR APPLICATION OF CORN ROOTWORM (COLEOPTERA: CHRYSOMELIDAE) SEMIOCHEMICAL-BASED BAITS

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ABSTRACT

Application of a semiochemical-based bait to silking corn (*Zea mays* L.) with a high clearance sprayer effectively reduced the numbers of northern, *Diabrotica barberi* Smith & Lawrence, and western, *D. virgifera virgifera* LeConte, corn rootworm adults during studies conducted in 1994 and 1995. Bait was applied using water volumes of 19 and 37 L/ha above canopy nozzles vs nozzles on drop lines, and nozzles on drop lines on every row vs nozzles on drop lines on alternating rows. There were no significant differences among the high clearance sprayer nozzle configurations in reducing corn rootworm beetle populations; however, nozzles on booms above the canopy were more likely to deliver discrete droplets and were less likely to break due to entanglement of the drop lines with foliage. Semiochemical insecticide-baits had minimal effects on predaceous adults of the family Coccinellidae. Seven coccinellid species were observed in the plots in 1994 and 4 species in 1995.

INTRODUCTION

Western (WCR) and northern corn rootworms (NCR), *Diabrotica virgifera virgifera* LeConte and *D. barberi* Smith & Lawrence, are primary insect pests of corn, *Zea mays* L., in the United States. Use of soil insecticides or crop rotation remains the major management options for control of these pests in corn production systems. However, soil insecticides are generally used in a prophylactic manner without knowledge of their actual need (Stamm et al. 1985). Of even more concern is the fact that soil insecticides applied at planting-time often only protect basal roots from damage and do not manage or reduce corn rootworm (CRW) populations (Sutter et al. 1991, Gray et al. 1992). Within the last seven years, semiochemical-based baits targeted at adult WCR and NCR have been evaluated for use as a new management tool (Lance and Sutter 1990, 1991, 1992; Sutter and Lance 1991; Sutter et al. 1996; and Weissling and Meinke 1991a,b). These baits, currently composed of dried, powdered root of buffalo gourd, *Cucurbita foetidissima* H.B.K., a toxicant (carbaryl), and

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a carrier, are effective for managing CRW adults in individual fields and in large area units, and are reportedly not toxic to non-target and beneficial insects (Sutter et al. 1996).

The current sprayable formulations of semiochemical-based bait can be applied using aerial application technology (Hoffman et al. 1996). With this technology, the bait is easy to use, is equal to or less than the price of applying a soil insecticide at planting, and fits well into the systems of large-scale corn growers. However, there are situations where baits cannot be applied by aircraft due to either public concerns/perceptions, lack of aerial applicators, and difficulty in getting to fields that are irregular in shape, are near dwellings, or are near sensitive environmental areas. Therefore, there is a need to develop alternative application technology to apply baits. This study evaluated 1) high clearance sprayer nozzle configurations and water volumes for bait applications, 2) the efficacy of high clearance sprayer application of baits in reducing populations of NCR and WCR beetles, and 3) the effects of semiochemical-based baits applied with high clearance sprayers on adults of the family Coccinellidae, important beneficial insects in corn ecosystems.

MATERIALS AND METHODS

Corn was planted on 5 May 1994 (Pioneer 3737) and 18 May 1995 (Pioneer 3769) in a 4-ha field at the Eastern South Dakota Soil and Water Farm in Brookings Co., SD. The field was planted to soybeans in 1993. Each year the field was divided into 24 plots, each 26 rows (76 cm row width) wide and 60 m long. Ten m-wide buffer strips were established between plots. Five high clearance sprayer application treatments and an untreated control were then arranged in a randomized block design with four replications. Treatments were as follows:

1. 19 L/ha spray volume; 1 nozzle/row above canopy;
2. 37 L/ha spray volume; 1 nozzle/row above canopy;
3. 19 L/ha spray volume; 2 nozzles on single drop; alternate rows; (2 nozzles/2 rows)
4. 37 L/ha spray volume; 2 nozzles on single drop; alternate rows; (2 nozzles/2 rows)
5. 37 L/ha spray volume; 2 nozzles on single drop; every row;
6. Untreated control.

A semiochemical-based bait (SLAM®, BASF Corp., Research Triangle Park, NC and MicroFlo Co., Lakeland, FL) was applied twice (day 217 and day 227) in 1994 and once (day 221) in 1995 to silking corn using the above sprayer configurations. The bait was applied at 561 gms/ha of formulated product in the listed spray volumes. Spray volumes were selected based on the sprayer capabilities and configurations of nozzles and droplines. The 19 L/ha volume is approximately twice that normally recommended for aerial application of SLAM. The bait is formulated into microspheres which, when added to water, hydrate and lose some integrity as they go into suspension. The suspended microspheres can then be easily sprayed with conventional aerial or ground-based equipment. However, it is imperative that screens be removed from the system to avoid problems with clogging. All applications were made with a Modern Flow® high clearance sprayer equipped with a 12 row (76 cm row width) boom and TX-4 hollow cone nozzles. Applications were made at 0.9 kg/cm^2 pressure and at variable speeds to accommodate the differing spray volumes and nozzle configurations. Droplines were extended downward from the spray boom 91 cm (below the plant canopy) and nozzles were pointed upward at 45° angles. We assumed that these sprayer configurations would result in application of discrete droplets of bait to the foliage, particularly to the underside of leaves which would increase resistance to washoff by rain and irrigation.

The effectiveness of the applications was determined by three methods. First, we determined densities of CRW beetles by species from 25 (1994) and 50 (1995) randomly selected plants down either of the center two rows in each plot. Population estimates were

made just before the bait applications and at predetermined intervals afterwards. The number of adult coccinellids by species per plant was also determined. Second, two yellow sticky traps (Pherocon AM®) were placed in each plot approximately one week before bait applications to capture CRW and coccinellid beetles. Each trap was placed 20 m from each end of the plot. Traps were changed every seven days and returned to the laboratory to determine capture of CRW by species and sex; coccinellids were enumerated only by species. Finally, wood framed screen trays (73 cm x 95 cm) were placed under the plant canopy (3/plot) at approximately 15-m intervals to collect dead CRW and coccinellid adults following bait application. Insects were collected periodically from the trays, placed in vials and brought back to the laboratory for counting. Counts were conducted as with the yellow sticky traps.

Means and standard errors were calculated for all data. An analysis of variance procedure with mean separation (P=0.05) by Tukeys HSD test (SAS 1985) was used to evaluate effectiveness of all application treatments. Sexes were combined for CRW species to conduct analyses of the number caught on yellow sticky traps and the numbers of dead individuals collected from the screen trays following application. Additionally, coccinellid numbers per species were combined for analysis. Orthogonal comparisons (contrasts) (Steele and Torrie 1960), with significance noted at P=0.05, were conducted to evaluate 1) spray volume effectiveness - 19 vs 37 L/ha, 2) nozzles on droplines vs. nozzles above the canopy, and 3) use of droplines on every row vs. droplines on alternating rows. Henderson and Tilton's (1955) modification of Abbott's formula was used to determine percent control based on adult plant counts.

RESULTS AND DISCUSSION

Corn Rootworm. Numbers of NCR in the 1994 test plots were light to moderate. The maximum average number of adults per plant in the untreated plots was 0.89 on day 220 (Table 1). Few WCR adults were noted during the study. Highest average number of WCR adults per plant in the untreated control was 0.14 on days 230 and 237. An initial application (day 217) of semiochemical-based bait significantly reduced the number of NCR within 3 days in all high clearance sprayer plots (Table 1). Percent control ranged from 22.6% (one nozzle/row above the canopy at 19 L/ha) to 74.8% (two nozzles/dropline on alternate rows at 19 L/ha). No significant differences in beetle numbers per plant were observed between above canopy and dropline applications, 19 and 37 L/ha applications, or use of droplines on every row vs. alternating rows. NCR numbers per plant were significantly lower 6 days after spraying in the one nozzle/row above canopy at 37 L/ha and in the two treatments using droplines on alternate rows than in the untreated control plots (Table 1). A second application of insecticide-bait on day 227 continued to suppress beetle populations. NCR adults per plant were significantly lower 3 days after application in the above canopy treatments compared to the untreated control plots (Table 1). Numbers of WCR adults were significantly lower 3 days after the second application in all but the two nozzles/row on dropline treatment (Table 1). Percent reduction ranged from 35.7% (two nozzles/dropline on alternating rows at 19 L/ha) to 91.1% (2 nozzles/dropline on alternating rows at 37 L/ha). No differences among the tested contrast groups were observed. NCR males (69.8 to 96.0% per collection date) were more numerous than females on the yellow sticky traps through the entire study. WCR males were more numerous on traps from day 216 through day 230 (78.4 to 81.8% per collection date). Thereafter, females were more numerous (60.4 and 72.9% per collection date) until termination of this study. There were no significant differences among treatments in the number of NCR beetles caught on yellow sticky traps 6 days after initial treatment (Table 2). However, WCR numbers were significantly higher on traps from the

TABLE 1. Numbers of Northern and Western Corn Rootworm Adults per Plant Following Application of Semiochemical Insecticide-Bait, 1994[a].

Nozzle Configuration	L/Ha Spray Volume	Canopy Placement	$\bar{x}$ ± SE No. Adults/Plant[b]				
			Day 216	Day 220	Day 223	Day 230	Day 237
					Northern Corn Rootworm		
1/Row	19	Above	0.32 ± 0.06	0.45 ± 0.07 B	0.49 ± 0.07 AB	0.16 ± 0.04 B	0.25 ± 0.06
1/Row	37	Above	0.39 ± 0.06	0.23 ± 0.05 B	0.38 ± 0.07 BC	0.28 ± 0.06 B	0.44 ± 0.07
2/2 Rows	19	Drops	0.35 ± 0.06	0.16 ± 0.04 B	0.20 ± 0.05 C	0.30 ± 0.06 AB	0.40 ± 0.08
2/2 Rows	37	Drops	0.39 ± 0.08	0.31 ± 0.07 B	0.32 ± 0.06 BC	0.29 ± 0.06 AB	0.30 ± 0.05
2/Row	37	Drops	0.33 ± 0.06	0.36 ± 0.06 B	0.49 ± 0.08 AB	0.37 ± 0.06 AB	0.28 ± 0.05
Control	--	--	0.49 ± 0.08	0.89 ± 0.13 A	0.74 ± 0.09 A	0.53 ± 0.08 A	0.29 ± 0.06
			N.S.				N.S.
					Western Corn Rootworm		
1/Row	19	Above	0.02 ± 0.01	0.02 ± 0.01	0.05 ± 0.03	0.02 ± 0.01 B	0.13 ± 0.04
1/Row	37	Above	0.03 ± 0.02	0.02 ± 0.01	0.03 ± 0.02	0.03 ± 0.02 B	0.22 ± 0.05
2/2 Rows	19	Drops	0.05 ± 0.02	0.01 ± 0.01	0.02 ± 0.01	0.03 ± 0.02 B	0.09 ± 0.03
2/2 Rows	37	Drops	0.08 ± 0.03	0.01 ± 0.01	0.04 ± 0.02	0.02 ± 0.01 B	0.12 ± 0.04
2/Row	37	Drops	0.05 ± 0.02	0.00	0.01 ± 0.01	0.05 ± 0.02 AB	0.11 ± 0.03
Control	--	--	0.05 ± 0.02	0.03 ± 0.02	0.05 ± 0.02	0.14 ± 0.04 A	0.14 ± 0.03
			N.S.	N.S.	N.S.		N.S.

[a] Plots sprayed on Day 217 and Day 227

[b] Means followed by the same letter in a column are not significantly different (Tukeys HSD Test, $P \leq 0.05$). N.S. indicates no significant differences among means in a column.

TABLE 2. Number of Northern Corn Rootworm and Western Corn Rootworm Adults per Trap (Pherocon AM) Following Application of Semiochemical Insecticide-Bait, 1994[a].

Nozzle Configuration	L/HA Spray Volume	Canopy Placement	$\bar{x}$ ± SE No. Adults/Trap[b] Day 216	Day 223	Day 230	Day 237	Day 244
					Northern Corn Rootworm		
1/ Row	19	Above	4.1 ± 1.3	2.0 ± 0.6	1.8 ± 0.7 AB	3.5 ± 0.6 B	14.1 ± 1.7
1/ Row	37	Above	4.6 ± 1.6	1.8 ± 0.5	1.0 ± 0.4 AB	5.0 ± 0.3 AB	10.5 ± 2.8
2/2 Rows	19	Drops	3.5 ± 0.8	2.3 ± 0.8	3.4 ± 1.3 A	6.0 ± 1.3 AB	12.1 ± 2.4
2/2 Rows	37	Drops	5.9 ± 1.1	1.5 ± 0.6	0.9 ± 0.3 AB	3.5 ± 0.7 B	12.4 ± 2.4
2/ Row	37	Drops	3.0 ± 0.3	1.6 ± 0.7	0.1 ± 0.1 B	3.3 ± 0.6 B	10.9 ± 1.3
Control	--	--	6.4 ± 1.7	5.0 ± 1.6	3.1 ± 1.1 AB	7.4 ± 1.0 A	14.5 ± 2.6
			N.S.	N.S.			N.S.
					Western Corn Rootworm		
1/ Row	19	Above	1.0 ± 0.4	0.5 ± 0.5 AB	2.4 ± 0.9	4.3 ± 1.4	5.8 ± 0.7
1/ Row	37	Above	1.3 ± 0.6	0.4 ± 0.3 AB	1.5 ± 0.5	2.1 ± 1.0	3.8 ± 1.0
2/2 Rows	19	Drops	1.1 ± 0.3	0.0 B	0.8 ± 0.4	3.4 ± 0.7	5.0 ± 1.1
2/2 Rows	37	Drops	1.4 ± 0.5	0.5 ± 0.4 AB	0.8 ± 0.3	4.0 ± 1.3	5.4 ± 0.6
2/ Row	37	Drops	1.1 ± 0.7	0.4 ± 0.2 AB	1.1 ± 0.2	2.1 ± 0.7	3.9 ± 1.1
Control	--	--	1.8 ± 0.5	1.9 ± 0.7 A	1.9 ± 0.5	3.9 ± 1.1	4.5 ± 0.9
			N.S.		N.S.	N.S.	N.S.

[a]Plots sprayed on Day 217 and Day 227.
[b]Means followed by the same letter in a column are not significantly different (Tukey's HSD Test, $P \leq 0.05$). N.S. indicates no significant differences among means in a column.

untreated control plots compared with numbers on traps from the two nozzles/dropline on alternating rows at 19 L/ha plots (Table 2). NCR adults were significantly less numerous on traps collected 10 days (day 237) after the second application in all but the one nozzle/row above canopy at 37 L/ha and two nozzles/dropline on alternating rows at 19 L/ha treatments compared to the untreated control (Table 2). WCR numbers per trap were not significantly different among any treatment following the second application. Contrasts using mean numbers of either NCR or WCR adults per trap were not significant for any comparisons following the second application.

Numerous NCR and WCR adults were found dead in the screen collection trays 24 hours after each application in 1994. There were no significant differences in the number of dead WCR beetles collected in trays during any period of the test, with numbers of beetles ranging from 0 to 1.1/tray. Female WCR beetles were more numerous than males (75.6% vs 24.4%, respectively). However, significantly greater numbers of dead NCR were observed in the two nozzles/dropline on alternating rows at 37 L/ha treatment (day 220) compared to the untreated control. The majority of these dead NCR beetles were male (68.3% for male vs 31.7% for female). Collection trays in the bait treatments collected numerically more dead beetles than trays in the untreated control. Some movement of CRW adults from treated plots into control plots may have accounted for the observance of dead beetles in the untreated control plots. A similar pattern of dead CRW beetles was observed 3 days after the second application. Significantly more dead NCR adults were collected from trays in the one nozzle/row above the canopy at 37 L/ha treatment than in the untreated control (Table 3).

Populations of NCR and WCR per plant were somewhat higher in 1995 compared to 1994. However, populations per plot were not uniformly distributed at the initiation of the test resulting in unequal beetle numbers among treatments (Table 4). A single application of bait resulted in a 59.6% (one nozzle/row above the canopy at 37 L/ha) to 71.2 % (one nozzle/row above the canopy at 19 L/ha) reduction in NCR numbers. A 81.1% (one nozzle/row above the canopy at 37 L/ha) to 97.2% (two nozzles/dropline on alternating rows at 37 L/ha) reduction in WCR adults was observed 24 hours after application. Percent reductions ranged from 26.3% (one nozzle/row above the canopy at 37 L/ha) to 76.7% (two nozzles/dropline on every row at 37 L/ha) and 20.4% (one nozzle/row above the canopy at 37 L/ha) to 53.5% (two nozzles/dropline on every row at 37 L/ha) for WCR and NCR adults, respectively, 14 days after application. All application methods significantly reduced WCR numbers per plant 24 hours after treatment compared to the untreated control (Table 4). Numbers of NCR were significantly higher 24 hours after treatment in the untreated control compared to the one nozzle/row above the canopy at 19 L/ha and both 37 L/ha nozzles on dropline treatments (Table 4). No differences between the number of NCR or WCR adults on plants in untreated controls and treated plots were observed after the 24 hour evaluation date. Significant contrasts were observed for the dropline on every row vs. alternating rows ($F = 4.53$, $df = 1$, $Pr > F = 0.0336$) and the 19 vs 37 L/ha ($F = 6.62$, $df = 1$, $Pr > F = 0.0102$) comparisons for NCR 6 days after treatment. More adults were observed on plants in the alternating row and in the 37 L/ha treatments. No other differences were noted with contrast groups during the test period. The small size of the plots and interplot movement of CRW adults may have contributed to the lack of differences in adult numbers among treatments throughout much of the remainder of study (after 24 hours). The number of beetles caught on yellow sticky traps was not affected by the semiochemical-based bait treatment nor the application method. Trap catches ranged from 17 to 26 and 8 to 15 adults per trap, for NCR and WCR, respectively, 24 hours before applications were made. Six days after application trap catches ranged from 18 to 23 and 3 to 8 for NCR and WCR, respectively. Additionally, no differences among contrast groups in the number of adults per trap were observed. Male

TABLE 3. Number of Dead Northern Corn Rootworm Adults per Metal Collection Tray Following Application of Semiochemical Insecticide-Bait, 1994[a].

Nozzle Configuration	L/Ha Spray Volume	Canopy Placement	$\bar{x} \pm$ SE No. of Dead Adults/Tray[b]				
			Day 216	Day 220	Day 223	Day 230	Day 237
					Northern Corn Rootworm		
1/ Row	19	Above	0	6.9 ± 1.9 AB	1.2 ± 0.5	1.1 ± 0.4 AB	0.5 ± 0.3 AB
1/ Row	37	Above	0	5.2 ± 1.8 AB	0.2 ± 0.2	4.7 ± 2.0 A	0.9 ± 0.4 AB
2/2 Rows	19	Drops	0	2.6 ± 1.3 AB	0.7 ± 0.3	0.8 ± 0.3 AB	0.4 ± 0.2 AB
2/2 Rows	37	Drops	0	8.1 ± 1.8 A	0.9 ± 0.7	1.2 ± 0.6 AB	0.6 ± 0.3 AB
2/ Row	37	Drops	0	3.9 ± 0.7 AB	0.4 ± 0.2	2.8 ± 0.7 AB	1.5 ± 0.4 A
Control	--	--	0	1.8 ± 0.8 B	0.1 ± 0.1	0.4 ± 0.3 B	0.2 ± 0.2 B
					N.S.		

[a]Plots sprayed on Day 217 and Day 227.
[b]Means followed by the same letter in a column are not significantly different (Tukey's HSD Test, $P \leq 0.05$). N.S. indicates no significant differences among means in a column.

TABLE 4. Number of Northern and Western Corn Rootworm Adults per Plant Following Application of Semiochemical Insecticide-Bait, 1995[a].

Nozzle Configuration	L/HA Spray Volume	Canopy Placement	$\bar{x}$ ± SE No. Adults/Plant[b] Day 220	Day 222	Day 227	Day 235
				Northern Corn Rootworm		
1/Row	19	Above	1.01 ± 0.09 A	0.24 ± 0.04 B	0.21 ± 0.04 B	0.24 ± 0.04
1/Row	37	Above	0.87 ± 0.08 ABC	0.29 ± 0.05 AB	0.38 ± 0.06 AB	0.34 ± 0.05
2/2 Rows	19	Drops	0.89 ± 0.09 AB	0.29 ± 0.05 AB	0.33 ± 0.05 AB	0.26 ± 0.05
2/2 Rows	37	Drops	0.92 ± 0.08 A	0.23 ± 0.04 B	0.42 ± 0.07 A	0.21 ± 0.04
2/ Row	37	Drops	0.59 ± 0.06 BC	0.21 ± 0.04 B	0.27 ± 0.05 AB	0.22 ± 0.04
Control	--	--	0.57 ± 0.07 C	0.47 ± 0.07 A	0.30 ± 0.05 AB	0.28 ± 0.04
						N.S.
				Western Corn Rootworm		
1/ Row	19	Above	0.30 ± 0.05 AB	0.02 ± 0.01 B	0.05 ± 0.02	0.06 ± 0.02
1/ Row	37	Above	0.19 ± 0.03 BC	0.03 ± 0.01 B	0.06 ± 0.02	0.07 ± 0.02
2/2 Rows	19	Drops	0.31 ± 0.05 AB	0.01 ± 0.01 B	0.05 ± 0.02	0.06 ± 0.02
2/2 Rows	37	Drops	0.28 ± 0.04 ABC	0.03 ± 0.01 B	0.09 ± 0.02	0.08 ± 0.02
2/ Row	37	Drops	0.43 ± 0.06 A	0.01 ± 0.01 B	0.06 ± 0.02	0.05 ± 0.01
Control	--	--	0.12 ± 0.02 C	0.10 ± 0.02 A	0.10 ± 0.03	0.06 ± 0.02
					N.S.	N.S.

[a] Plots sprayed on Day 221.

[b] Means followed by the same letter in a column are not significantly different (Tukey's HSD Test, $P \leq 0.05$). N.S. indicates no significant differences among means in a column.

WCR collected on traps were more numerous (64.3% to 81.3% per collection date) than females from day 216 through day 234. On day 241, 50.5% of the WCR beetles captured were female. NCR males were more numerous (54.9% to 95.8% per collection date) on traps through all of the study.

As in 1994, dead NCR and WCR beetles were readily collected from the screen trays 48 hours after application of bait (Table 5). Male NCR were found more frequently than females (69.5% vs 30.5%, respectively), while dead female WCR were again more numerous than males (58.0% vs 42.0%, respectively). Greater numbers of dead NCR beetles were collected from the one nozzle/row above the canopy at 19 L/ha, the two nozzles/dropline on alternating rows at 19 L/ha, and the two nozzles/dropline on every row treatments 48 hours after application compared to the untreated control plots (Table 5). Dead NCR continued to be collected from trays 14 days after application. Dead WCR beetles were collected in significantly greater numbers from the two nozzles/drop on every row treatment than from the untreated control plots (Table 5).

Coccinellids. Adults in the family Coccinellidae were abundant during both years of the study, but populations were more numerous and diverse in species composition in 1994. A total of 1108 individual coccinellids were collected on yellow sticky traps during the 1994 study. Species collected and percentage of total abundance in 1994 were: *Hippodamia tredecimpunctata tibialis* (Say), 48.5%; *Coleomegilla maculata* DeGeer, 46.9%; *Hippodamia convergens* Guerin-Meneville, 2.7%; *Coccinella septempunctata* L., 0.8%; *Cycloneda munda* (Say), 0.8%; *Adalia bipunctata* (L.), 0.2%; and *Hippodamia parenthesis* (Say), 0.1%. Only four species were observed on plants (379 individuals) in 1994. These were: *C. maculata,* 62.4% of total; *H. t. tibialis,* 27.2%; *H. convergens,* 5.4%; and *C. septempunctata,* 5.0%. A total of 458 coccinellid adults of three species were caught on yellow sticky traps in 1995. These were: *C. maculata,* 97.6% of total; *H. t. tibialis,* 2.2%; and *C. munda*, 0.2%. The only coccinellid observed while doing our visual surveys was *C. maculata*; 89 individuals were counted during the study.

Coccinellid numbers per plot were numerically reduced three days after the initial semiochemical-bases bait application (day 217) in 1994. However, no significant differences in beetle numbers were observed among any treatment. Beetle numbers ranged from 2 to 5 per plot before application and 0.3 to 2 per plot after application. Similar patterns were observed on yellow sticky traps with 6 to 9 and 1 to 4 beetles per trap observed before and after treatment, respectively. Average number of dead coccinellids observed per screen tray 3 days after the initial bait application in 1994 ranged from 0.1 to 0.8. No significant differences in dead coccinellid numbers among treatments were observed. Coccinellid numbers increased in all plots by day 223 (6 days after application). A second bait application on day 227 significantly reduced the number of coccinellids observed per plot 3 days after treatment in the two nozzles/dropline on every row treatment (0.5 ± 0.3 SE beetles/plot) compared to the untreated control (6.3 ± 0.6 beetles/plot). No differences in coccinellid numbers were observed on yellow sticky traps following the applications with numbers ranging from 4 to 6 per trap. Greater numbers of dead coccinellids were observed on the screen trays in the one nozzle/row above the canopy at 37 L/ha treatment (1.8 ± 0.6/tray) than in the untreated control (0/tray) 3 days after the second bait application. Coccinellid numbers increased in all plots within 7 days after the second application.

A single application of bait in 1995 (day 221) did not result in significant differences among treatments in the number of coccinellids observed on plants 48 hours after treatment. However, coccinellids were fewer in number in all treatments than before the application (0.3 to 2 and 0 to 0.5/plot before and after treatment, respectively). Numbers increased 14 days after application in all treatments. The number of coccinellids caught on yellow sticky traps was minimally affected by bait applications. Significantly greater numbers of coccinellids

TABLE 5. Number of Dead Northern and Western Corn Rootworm Adults per Metal Collection Tray Following Application of Semiochemical Insecticide-Bait, 1995[a].

Nozzle Configuration	L/Ha Spray Volume	Canopy Placement	$\bar{x}$ ± SE No. of Dead Adults/Tray[b]			
			Day 220	Day 223	Day 227	Day 235
					Northern Corn Rootworm	
1/ Row	19	Above	0	14.3 ± 3.5 A	4.0 ± 1.3	0.1 ± 0.1 B
1/ Row	37	Above	0	7.4 ± 1.2 ABC	5.2 ± 1.6	0.1 ± 0.1 B
2/2 Rows	19	Drops	0	11.0 ± 2.1 AB	5.2 ± 1.8	1.2 ± 0.5 AB
2/2 Rows	37	Drops	0	2.6 ± 1.3 BC	2.3 ± 0.9	0.2 ± 0.2 B
2/ Row	37	Drops	0	12.2 ± 3.8 AB	6.1 ± 1.8	1.8 ± 0.7 A
Control	--	--	0	0.0 C	1.1 ± 0.6	0.0 B
					N.S.	
					Western Corn Rootworm	
1/ Row	19	Above	0	2.1 ± 0.8 AB	0.7 ± 0.4	0.0
1/ Row	37	Above	0	0.7 ± 0.4 B	0.1 ± 0.1	0.0
2/2 Rows	19	Drops	0	1.2 ± 0.3 AB	0.4 ± 0.2	0.1 ± 0.1
2/2 Rows	37	Drops	0	0.5 ± 0.3 B	0.2 ± 0.2	0.0
2/ Row	37	Drops	0	5.0 ± 2.1 A	2.0 ± 1.0	0.1 ± 0.1
Control	--	--	0	0.0 B	0.6 ± 0.4	0.0
					N.S.	N.S.

[a]Plots sprayed on Day 221.

[b]Means followed by the same letter in a column are not significantly different (Tukey's HSD Test, $P \leq 0.05$). Only 2 dead coccinellids found during the duration of the test -- both in the 1 nozzle/row, 6.2 l/Ha above the canopy application. N.S. indicates no significant differences among means in a column.

were caught in the untreated control plots (2.0 ± 0.7) than in the one nozzle/row above the canopy at 37 L/ha treatment (0.5 ± 0.3) and in the two nozzles/dropline on alternating rows at 37 L/ha 20 days after application (0.3 ± 0.2). This difference could be related to natural population fluctuations. Only 2 dead coccinellid adults were collected in the screen trays during the 1995 study. Both were *C. maculata* which were collected 48 hours after application in the one nozzle/row above the canopy at 37 L/ha plots. Numbers of coccinellid adults on traps remained stable following the insecticide-bait application until study termination.

Conclusion. Results from the 1994 and 1995 studies clearly demonstrated the effectiveness of high clearance sprayer application of semiochemical-based bait in reducing NCR and WCR adult numbers. Initial CRW adult population reduction with baits applied with high clearance sprayers compared favorably with results obtained following aerial application of SLAM (L. Chandler, personal observation). This test was primarily designed to compare application parameters using high clearance equipment. The ability of CRW adults to move between plots probably influenced the results of this small plot test which was not designed to "manage" populations. Additional tests of high clearance sprayer methods should be conducted in commercial fields for evaluation of population management potential.

None of the high clearance sprayer application methods provided a consistent advantage over any other in reducing beetle populations. This is somewhat surprising but results in a more flexible set of parameters for growers to consider when setting up sprayers for their use. Decreased water volume in combination with the use of droplines did not appear to be important factors in improved CRW beetle kill. There may be several reasons for this. Nozzles on droplines can be directed to spray at any angle and direction within the canopy. However, corn plant architecture during silking/tasseling may prevent droplines from being more effective in targeting preferred CRW adult feeding habitats (corn ears and silks). As droplines move down a row, they can become entangled with ears, stalks, and leaves. If the field being treated has poor row configuration due to weather related events or insect feeding resulting in goosenecked or lodged plants, droplines can catch on the plant parts. Entangling droplines on plant parts can result in poor distribution of spray droplets, and, at times, broken droplines and/or nozzle housings. Additionally, sprays from dropline nozzles impact leaves or stems at close proximity resulting in bait traveling as streams on the plants (L. Chandler, personal observation). This phenomena, though effective in killing CRW adults (especially with greater water volumes), could have reduced the chances for obtaining a higher percentage of control since discrete droplets are preferred for insecticide-bait deposition. Studies by Hoffman et al. (1996) indicated that 600 micron volume median diameter (VMD) droplets of SLAM applied with an airplane reduced CRW populations up to 85% compared to 60% with 900 micron VMD droplets and 80% with 300 micron VMD droplets. It would appear that spraying above the canopy minimizes sprayer problems and would be the preferred method for high clearance sprayer use.

Application of semiochemical-based baits appeared to have minimal effects on coccinellid populations within the study site. Although dead coccinellid adults were observed in the screen trays following spray applications, the numbers were small (only two collected in 1995) and appeared to rebound quickly as observed on traps and with plant counts. With few individuals noted in the trays it is difficult to know for certain the effects insecticide-baits have on coccinellid populations. The mechanics of spraying could have disturbed individual beetles and forced them to move out of the plots. Thus, they may have avoided much of the initial insecticide-bait application. One could also speculate that the cucurbitacin-based bait may have repelled these insects from the study site thus contributing to lower numbers of insects observed and collected on traps following spray applications.

ACKNOWLEDGMENT

The author wishes to thank Microflo Co. and BASF Corp. for supplying the semiochemical insecticide-bait. Denise Hovland is acknowledged for data management. Deb Hartman, Lynn Fleming, Kurt Dagel, Gene Dempster, Jesse Jenson, Kyle Groos, Bill Clausen, Jason Nieber, and Shannon Larkin are acknowledged for data collection. Bart Larson applied the semiochemical insecticide-bait and set up the spray nozzle configurations for the high clearance sprayer.

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THE PRESENCE OF AN INTERCALATED DISK-LIKE STRUCTURE IN THE HINDGUT MUSCLES OF THE COCKROACH

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ABSTRACT

An interfibrillar junction with many ultrastructural features of an intercalated disk was found in the circular muscle of the anterior rectum of the cockroach, *Leucophaea maderae* (Fabricius). This junction consisted of a central region with a large amount of electron-dense material associated with the I band of the myofibrils. A zona occludens was present in the plasma membrane that extended from the region of the myofilaments to the periphery of the cells.

Intercalated disks have been frequently reported in insect cardiac muscle, but this is the first recorded instance of its presence in insect non-cardiac muscle. Such interdigitated junctions appear to provide a means of adhesion between individual cells to prevent any separation during contraction. Also, gap junctions in the plasma membrane may provide an avenue for intercellular communication by ion exchange. Finally, the frequent and uniform rhythmic contractions of the muscles in the rectal valve suggest a heart-like action.

INTRODUCTION

A unique cytological structure called the intercalated disk has been observed in cardiac muscle. Although such muscle fibers have cross striations or Z lines like skeletal muscles, darkly staining bands wider than Z lines are evident under the light microscope. These wider bands are inserted between the Z lines and have been termed intercalated disks. The significance of these regions, however, was not appreciated until the electron microscope revealed that such disks actually represent the boundaries between individual cells in cardiac muscle (Ham and Cormack 1979). This discovery corrected the earlier impression that cardiac muscle is a multi-nucleated network of cytoplasm called a syncytium. We now know that the cells in this muscle tissue are joined end to end by these special interfibrillar junctions to form a network of muscle fibers.

Intercalated disks have been identified in the cardiac tissues of several insect species (Sanger and McCann 1968, Cook and Meola 1983, Cook and Meola 1988, Meola and Loeb 1995). The fine structure described in these examples is similar to that found in vertebrate heart tissue (Ham and Cormack 1979). In the present study, the ultrastructural features of an interfibrillar junction in non-cardiac muscle of the cockroach *Leucophaea maderae* (Fabricius) are described. These junctions were detected in the circular muscles of the rectal valve and had all the characteristics reported for intercalated disks found in insect hearts. The significance of this discovery may be related to the pace-maker action of the muscles in the region of the rectal valve of the cockroach.

MATERIALS AND METHODS

The *L. maderae* cockroaches used in the present study were taken from stock colonies maintained at 27°C and 40% R.H. The insects were fed dry dog food and water *ad libitum*. All dissected organs were perfused with saline solution that had the following composition (in mM): Na 154, K 2.7, Ca 1.8, Cl 160, glucose 22. The pH was adjusted to 6.8 with sodium hydroxide. Adult cockroaches of either sex were decapitated, and the legs and wings were removed. Then a dorsal incision at the midline was made just anterior to the last abdominal sclerite and continued through the pronotum. After opening the dorsal surface, the Malpighian tubules and tracheae were cut from the surface of the hindgut. Once freed from these restraints, the hindgut was severed just anterior to the evagination of the Malpighian tubules and placed to one side. The posterior end of the hindgut was then carefully released from its attachments to the terminal sclerites by cutting suspending muscles and the thin cuticular membrane around the anal aperture. The isolated hindgut was transferred to a paraffin-filled petri dish for further dissection.

Preparation of Tissue for Microscopic Examination. Samples of the strap muscles (SM) on the surface of the anterior rectum (Fig. 1) were carefully removed from the whole hindgut preparation. These single muscle bundles were excised with a small amount of rectal tissue attached to both ends.

For observation under transmission electron microscopy, the muscle bundles were fixed in 1% acrolein and 2.5% glutaraldehyde in a 0.1 *M* sodium cacodylate buffer (pH 7.4) for 1 h at room temperature or overnight in the cold (4°C). The specimens were then rinsed in the buffer three times (10 min each) in the cold (4°C) and postfixed in a 1% OsO_4 solution buffered with 0.1 *M* sodium cacodylate for 2 h in the cold (4°C). The tissues were dehydrated in a series of acetones, 30, 50, 70, 95, and 100% (three times in 100%), before embedding in Spurr's resin as modified by Mollenhauer (1986). Tissues were sectioned with a microtome (Reichert Ultracut S, Leica, Deerfield, IL) either at 1 μm and stained with tolluidine blue (1%) or thin sectioned and stained with uranyl acetate (1%) and lead citrate. Appropriate specimens were viewed under a light microscope or under an electron microscope (Hitachi H 7000, Nissei Sangyo, Gaithersburg, MD) at 75 kV.

For observation under the scanning electron microscope, samples of the anterior rectum were fixed as described and dried with liquid CO_2 in a Denton critical point drier. The dried specimens were then mounted with silver conducting paints on SEM stubs, coated with gold-palladium, and observed with a Cambridge Stereoscan S-4 SEM at 10 kV (Cook and Meola, 1988).

Preparation of Separate Regions of the Hindgut for Myographic Recording. Two types of isolated preparations were used to study spontaneous contractile activity in the hindgut. The first preparation included the entire rectum with suspending threads attached to the anal aperture and a point just posterior to the rectal valve as shown in Fig. 12A. The second preparation consisted of the posterior colon and the rectal valve. The suspending threads for this preparation were tied as shown in Fig. 12B.

These isolated regions of the hindgut were suspended in a 5 ml muscle chamber between a metal hook and a balsa-wood beam with cotton thread. This beam was inserted into a plastic hub that served as a fulcrum to register changes in torque on the Brush isotonic muscle transducer, model No. 33-03-981. The balsa-wood beam was then sufficiently counter-weighted to produce a tension of approximately 180 mg on the hindgut. The muscle chamber was continuously aerated by a hypodermic needle inserted into a rubber stopper at the bottom of the chamber. Myographs were recorded by connecting the transducer to an oscillographic recorder (Gould Brush 220, Cleveland, OH).

RESULTS AND DISCUSSION

The hindgut of *L. maderae* is divided into four distinct parts (Cook and Reinecke 1973): the ileum, a short, narrow section of the gut extending from the evagination of the Malpighian tubules to the abrupt expansion of the intestinal wall, which marks the anterior limit of the colon; the colon, which is separated into anterior and posterior limbs by a median sphincter; and the rectum.

The muscular networks were distinctive for each region of the hindgut (Fig. 1). The rectum was readily identified by the symmetrically arranged longitudinal muscles which appeared in six distinct straps (SM). In general, the circular muscles (CM) did not encompass

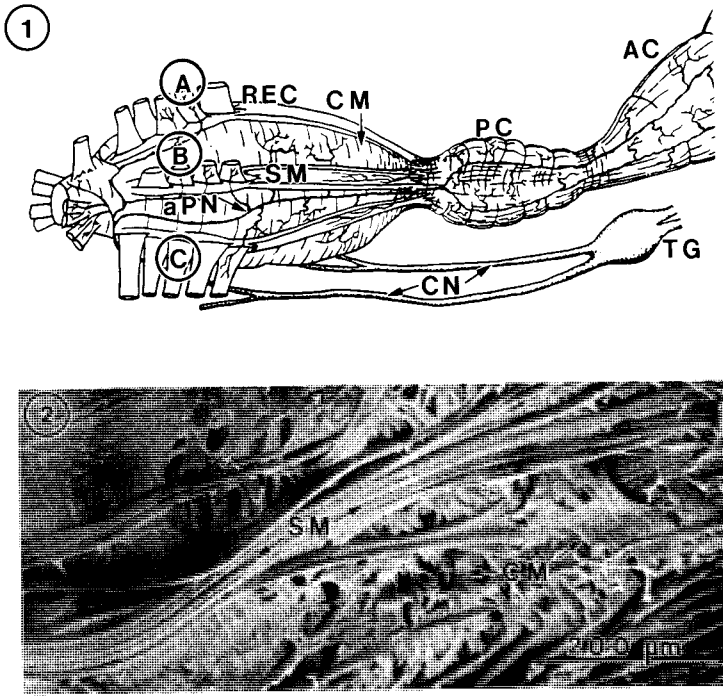


FIG. 1. Dorsolateral view of the principal subdivisions AC = anterior colon; PC = posterior colon and Rec = rectum and innervation pattern of the hindgut of *Leucophaea maderae*. The rectum is suspended from the exoskeleton by dorsal (A), lateral (B) and ventral (C) groups of strap muscle (SM). Five muscle groups (*) suspend and support the anal aperture. The hindgut is innervated bilaterally from proctodeal nerves (aPN = anterior branch of proctodeal nerve) that arise from cercal nerves (CN) which proceed from the terminal ganglion (TG). SM = strap muscle and CM = circular muscle.

FIG. 2. Scanning electron micrograph of the topography of muscle networks close to the rectal valve of the hindgut. SM = strap muscle and CM = circular muscles.

the entire rectum but simply extended from one longitudinal strap muscle to the next. These circular muscles appeared as a uniform layer over the rectal pads, which tended to protrude between the longitudinal muscles. The strap muscles in the anterior portion of the rectum consisted of superior and inferior bundles and both terminated in the sphincter between the posterior colon and the rectum. Three pairs of rectal dilator muscles arose from the posterior terminations of the superior bundles of the longitudinal strap muscles. The dorsal pair (A) was inserted on the terminal abdominal tergite just above the rectum. The lateral dilators (B) were attached to the same tergite but at a more lateral position, and the ventral pair (C) was attached to the lateral borders of a ventral genital sclerite in the male. A second group of suspensory muscles was associated with the anal aperture (*). A single dorsal depressor was attached to the supraanal plate, and a pair of extensors and depressors was attached to the perianal plates on either side of the anal aperture. The muscles of the posterior colon were arranged in an irregular lattice with the longitudinal fibers superimposed on the circular ones. In the central portion, the circular fibers were more sparsely located than they were at either end; also, there was an evident weave in the lattice. In the anterior colon, the same irregular lattice between circular and longitudinal fibers persisted, but the weaving between the layers was more pronounced and resembled woven cloth.

The hindgut of the *L. maderae* cockroach was innervated bilaterally from a pair of dorsally directed branches of the cercal nerves (CN). Shortly after these proctodeal nerves reached the posterior lateral surface of the rectum, they bifurcated and gave rise to anterior and posterior branches (Fig. 1). The posterior branch innervated the dorsal and lateral dilator muscles of the rectum, the suspensory muscles of the anal aperture, and the circular muscles of the posterior rectum. The anterior branch (aPN) innervated the circular and longitudinal muscles of the anterior rectum. At the constriction between the rectum and posterior colon (rectal valve), these branches bifurcated to give rise to two nerves on either side that innervated the surface of the colon as they proceeded toward the anterior limit of the organ.

The scanning electron micrograph in Fig. 2 shows the anterior ends of a longitudinal strap muscle entering a field of circular muscles on the surface of the rectal valve. It was in such a region that the intercalated disk-like structures were first detected in circular muscle (CM) fibers. A transection of this region is shown in Fig. 3 as revealed by Nomarski optics. In this section, both the strap muscle and some of the circular muscle fibers have been pulled away from the surface of the rectum. At a higher magnification (Fig. 4), the darker intercalated disk-like junction (between the opposed arrow heads) was positioned between the lighter striations of the muscle fiber. Additional details of these cellular junctions and their arrangement from sarcomere to sarcomere within the muscle bundle are shown in Fig. 5. In this electron micrograph, a characteristic step-like arrangement of the junctions was evident. Such a feature is often reported to occur in vertebrate cardiac muscle (Ham and Cormack 1979). Fig. 6 shows another series of interfibrillar junctions that had a more linear arrangement across the sarcomeres. At higher magnifications (Fig. 7) these intercalated disks in circular muscles of the rectal valve showed a characteristic series of interdigitating elements bounded by electron dense (*) desmosome-like regions. In addition to these features of the central junctional region between opposing sarcomeres, a zona occludens (ZO) was observed in the plasma membrane leading to the periphery of the cell.

These same ultrastructural details were evident in the intercalated disks of the heart muscle of the stable fly larva as shown in Fig. 8. Other reports on disk junctions in the cardiac tissues of insects support the same conclusion (Sanger and McCann 1968, Cook and Meola 1983, Meola and Loeb 1995).

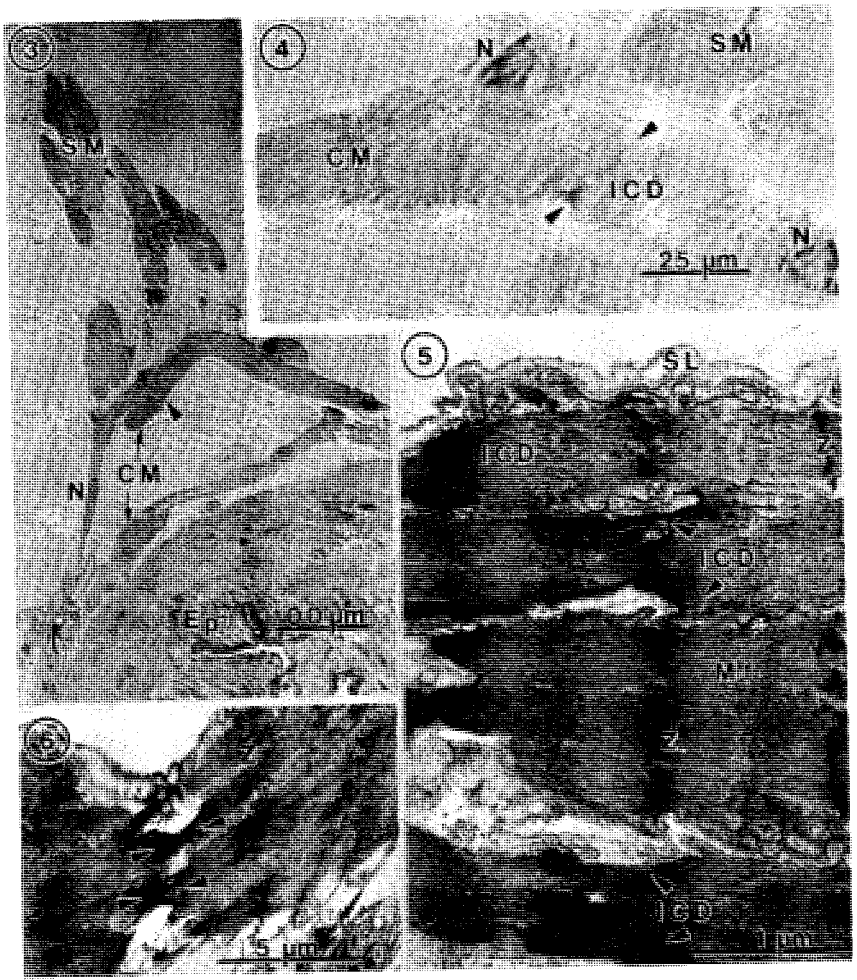
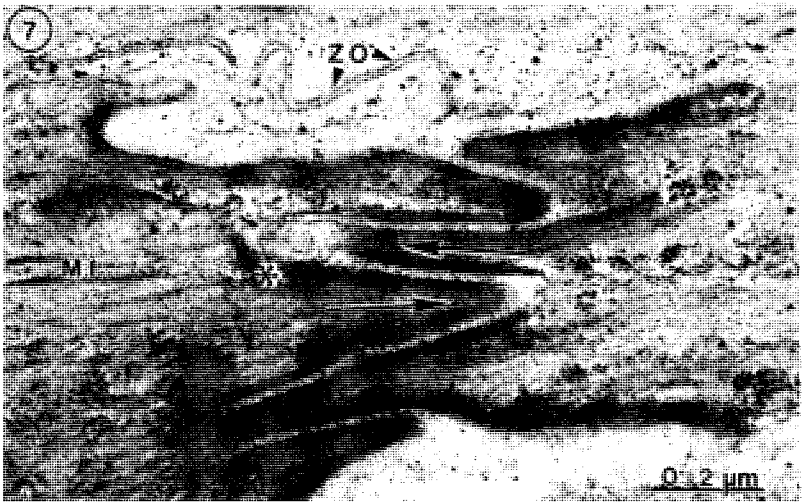


FIG. 3. A differential interference contrast (DIC) micrograph that shows the close relationship between circular muscles (CM) and strap muscles (SM) in transection near the rectal valve. Several circular muscle fibers have been pulled away from the surface of the rectum. Both nuclei (N) and striations (arrow head) are evident in these muscle cells. An epithelial layer (Ep) is shown next to the lumen (*) of the rectum.

FIG. 4. Higher magnification of a circular muscle fiber (CM) with an intercalated disk (ICD) positioned between striations. SM = strap muscle and N = nuclei.

FIG. 5. Electron micrograph that illustrates the ultrastructural features of a circular muscle fiber which contains a series of intercalated disks (ICD). Mf = myofilaments; SL = sarcolemma and Z = Z bands.

FIG. 6. Another circular muscle fiber that contains several intercalated disks between arrow heads. Z = Z band.



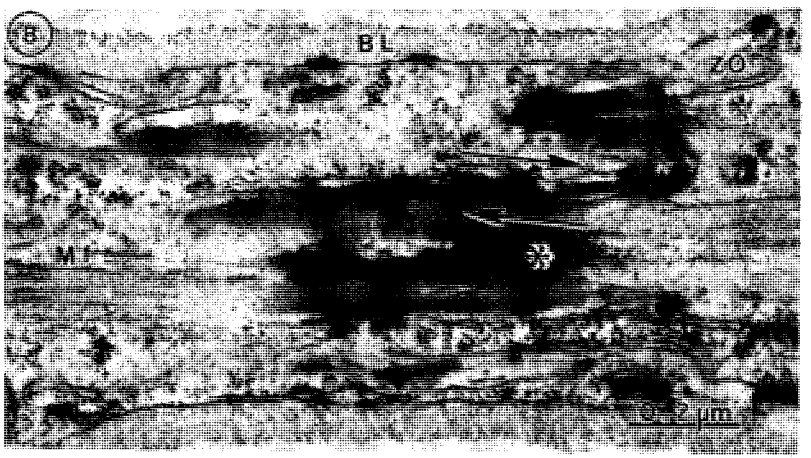


FIG. 7. Higher magnification of an intercalated disk in a circular muscle that shows the fine structure of the interdigitating elements (opposing arrows) of the disk with its numerous electron dense (*) regions and the zona occludens (ZO) which leads from the central myofilament (Mf) region to the periphery of the cell.

FIG. 8. Electron micrograph of a comparable magnification to Figure 7 shows the interdigitating elements (opposing arrows) of an intercalated disk in the heart muscle of the stable fly larva. ZO = zona occludens; BL = basal lamina; Mf = myofilaments and * = electron dense region.

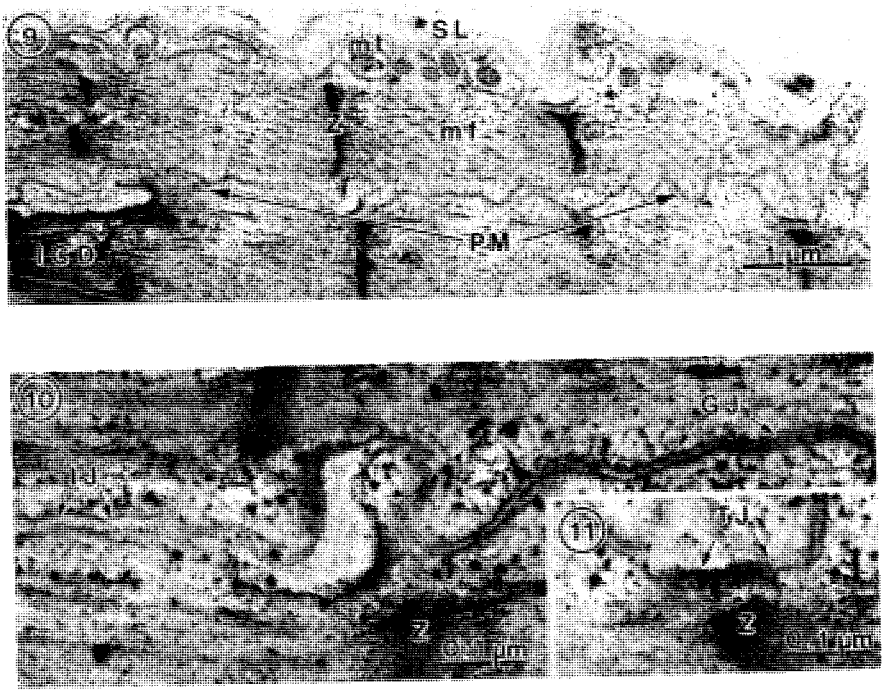


FIG. 9. Electron micrograph of several circular muscle fibers in the rectum which illustrates a long section of the plasma membrane (PM) that arises near the electron dense region of the intercalated disk (ICD) and extends toward the periphery of the cell. Z = Z band; mt = mitochondria; mf = myofilaments; SL = sarcolemma.

FIG. 10. Higher magnification of the transection in Figure 9 that shows examples of an intermediate junction (IJ) and a gap junction (GJ) along the course of the plasma membrane. Z = Z band.

FIG. 11. Shows a tight junction (TJ) along the zona occludens near the periphery of the cell. Z= Z band.

A long section of plasma membrane (PM) between two circular muscle cells of the rectum is shown in Fig. 9. This membrane arose near the electron dense desmosome-like region of the intercalated disk and extended toward the periphery of the cell. Regions of this membrane at higher magnification showed three types of intercellular junctions: an intermediate junction (IJ), a gap junction (GJ) in Fig. 10 and a tight junction (TJ) in Fig. 11. Such a variety of junctions suggest a multifunctional nature for this membrane.

In an effort to correlate the unusual structural features present in these muscles of the rectal valve with a function, a comparison was made between the myographic activity of the isolated rectum which excluded the rectal valve and the posterior colon which included the rectal valve (Fig. 12). Contractions recorded from the rectum had a frequency range of 1 to

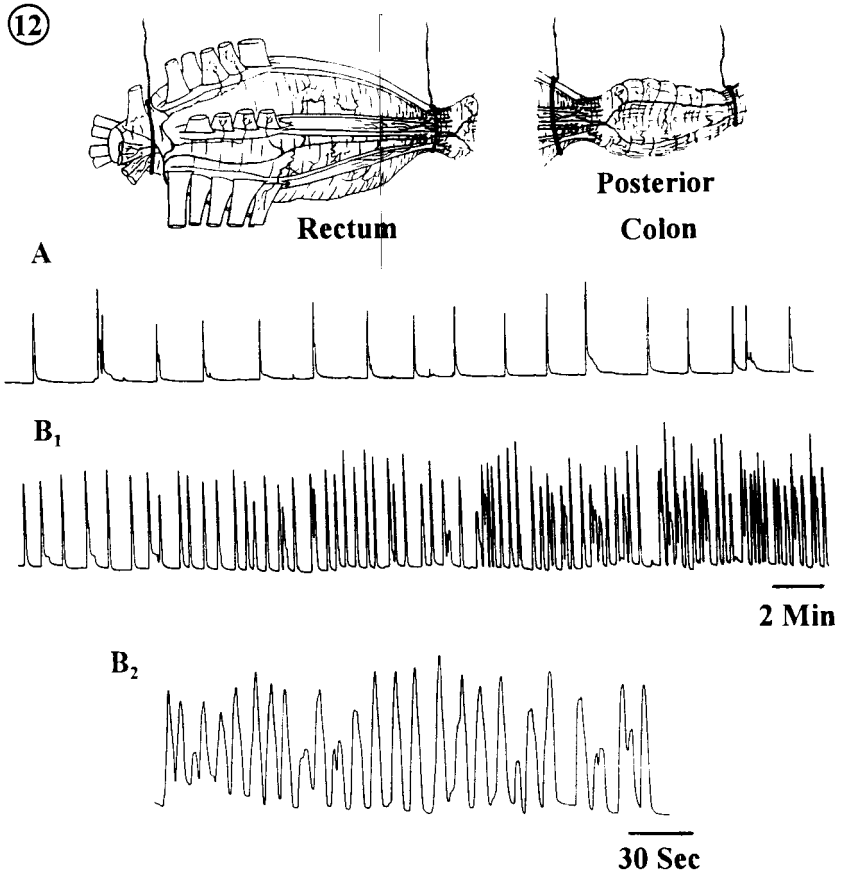


FIG. 12. Drawings of the isolated preparations used to compare the separate actions of the rectum and posterior colon of the cockroach hindgut. A) Myographic record of the rectum without the rectal valve. The time mark for this record is the same as that shown in B_1. B_1) Myographic record of the posterior colon which included the rectal valve. B_2) Record of the preparation shown in B_1 at a faster scan speed.

2 every two minutes (Fig. 12A) while those in the posterior colon had a range in frequency from 3 to 10 every two minutes (Fig. $12B_1$). Contraction frequencies reached a rate of one every 10 seconds (Fig. $12B_2$).

A comparison of the myogenic characteristics of these two separate regions revealed quite distinctive patterns of activity. The frequency of contractions in the rectum was low and often tended to be irregular in amplitude while those in the posterior colon had a higher frequency rate and a more uniform amplitude. Indeed, comparative observations from many such preparations (unpublished data) suggested that the rectal valve may be the site of a pace-maker which sets the frequency rate and amplitude for contractions in the hindgut. The discovery of intercalated disk-like structures in the muscles of the rectal valve offer an interesting correlative to these observations. However, further research will be required to establish the full significance of this relationship between structure and function.

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A CONVERSION FACTOR FOR CORRECTING NUMBERS OF ADULT TARNISHED PLANT BUGS (HETEROPTERA: MIRIDAE) CAPTURED WITH A SWEEP NET IN COTTON

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ABSTRACT

Adult tarnished plant bugs, *Lygus lineolaris* (Palisot de Beauvois), were sampled with a sweep net and an absolute visual sampling method during the first five weeks of fruit production in cotton at Stoneville, Mississippi during 1993 and 1994. The absolute visual sampling method captured significantly higher mean numbers of adults than were found with sweep net sampling in all sample weeks of both years. A significant regression of mean numbers of adults from absolute visual samples on mean numbers of adults in sweep net samples was obtained. This regression showed that sweep net samples underestimated numbers of adults by a correction factor of three. This is the only correction factor currently available for converting numbers of adult tarnished plant bugs captured in the sweep net to the more accurate numbers captured with an absolute sampling method.

INTRODUCTION

The tarnished plant bug, *Lygus lineolaris* (Palisot de Beauvois), is an important pest of cotton in the southeastern United States. Treatment thresholds for plant bugs in cotton vary from state to state and are usually based on combined numbers of adults and nymphs. Numbers of plant bugs are estimated by producers or crop consultants using relative sampling methods, most commonly by visual examination of plant terminals or by use of a drop cloth or sweep net. Producers or crop consultants will not use most absolute sampling methods because they require more time and are more expensive than samples obtained by relative sampling methods. Comparison between relative and absolute sampling methods is done by regressing insect densities obtained with both types of sampling methods from the same field. Factors can then be calculated for converting insect densities obtained with the relative sampling method to the more accurate densities obtained with the absolute sampling method.

The drop cloth was found in two studies to capture significantly higher numbers of tarnished plant bug nymphs in cotton than were captured with the sweep net (Young and Tugwell 1975, Snodgrass 1993). Snodgrass (1993) also developed a regression equation for correcting counts of nymphs taken with a drop cloth in cotton based on a study of the efficiency of the drop cloth for nymphs using a nymphal population of known density at different plant heights. Accurate sampling of adult tarnished plant bugs has proven to be a difficult task. Young and Tugwell (1975) compared several sampling methods including the drop cloth, D-Vac, sweep net, and visual examination of terminals. They found the sweep net to be the

most rapid method which also produced the most bugs per minute of effort. Visual inspection of terminals was the least efficient of the methods compared. Fleischer et al. (1985) compared numbers of tarnished plant bugs captured with a drop cloth and sweep net to two absolute sampling methods [a whole plant bag sampling (WPBS) method and a visual method where plants were cut up and examined piece by piece in the field]. They obtained a significant regression for adults in comparisons between drop cloth and absolute visual estimates of plant bug densities. The equation had a reasonable level of predictability ($R^2 = 0.61$) with a conversion factor of 4.4, but the visual sampling method did not detect populations on several occasions which suggested the need for additional research. Byerly et al. (1978) compared densities of plant bugs from sweep net samples to those obtained in absolute WPBS samples for *L. hesperus* Knight. Regression analysis showed that the sweep net underestimated adult plant bug densites by a conversion factor of 3.65 ($R^2 = 0.64$).

No usable conversion factors are available for adults of the tarnished plant bug with any relative sampling method. This study was conducted to evaluate the relationship between densities of tarnished plant bugs determined using sweep net sampling of a cotton field compared to plant bug densities obtained by absolute sampling of the same cotton field.

MATERIALS AND METHODS

The test was conducted in a cotton field (0.8 ha) located on the Delta Branch Experiment Station, Stoneville, MS, during 1993 and 1994. Cotton 'DES119' was grown in the field in both years, and plant stand densities averaged 14 per m of row in both years. The test began each year during the first week of square production (28 June and 17 June in 1993 and 1994, respectively). Average plant height was determined each sample week in each year by measurement of 100 plants chosen at random. Measurement of each plant was from the soil surface to the tip of the mainstem terminal.

The relative sampling method tested in both years was the sweep net. A standard (38-cm diam.) sweep net was used and the author (G.L.S.) took all sweep net samples in both years. Sweeps were made by moving the net opening through the cotton foliage upward through the mainstem terminals, alternately from and toward the sampler, as the sampler walked down a row. Adult plant bugs captured were counted and recorded.

Two absolute sampling methods were used in 1993. One was the WPBS method adapted from Byerly et al. (1978). In this method a cylindrical cloth bag, with a draw string at each end for closure, was placed over plants on 38-cm of row. The bottom drawstring was then pulled to snug the bag up to the plant bases. The bag was then collapsed to the ground. When a sample was taken, two people quickly pulled the bag up over the plants and closed the bag opening with the drawstring. Plants in the bag were then cut off at the soil line and entirely enclosed in the bag which was closed at the bottom with the drawstring. The bag was taken to the laboratory where the number of adult plant bugs captured was determined by visual examination of the bag and plants. Bags were placed in the field at least 1 week prior to being used. The second absolute method was a visual method in which four observers examined plants on 38-cm of row for adult plant bugs. The 38-cm section of row and plants to be examined were marked with colored tape around the bases of the plants at the beginning and end of the sample row section at least 1 week prior to taking the sample. Observers approached the section of row to be sampled quietly with two observers on each side of the row. On each side one observer examined the plants from the top down, while the other observer worked from the bottom plant branches upward. Adults nearly always flew when found and the four observers communicated findings to avoid counting the same bug twice.

Experimental design in both years was a randomized complete block with ten replications. Each block was a row in the field picked at random except that adjacent rows were not used since it was impossible to sample a row without disturbing plant bugs on the adjacent rows. Each sweep net sample in 1993 was made up of five sweeps, and the three treatments were assigned at random three times in each row. The WPBS absolute sampling method was not used in 1994, and sweep net and absolute visual samples were the treatments tested. Each sweep net sample was ten sweeps and both treatments were assigned at random to each row four times. Data were analyzed by analysis of variance (SAS Institute 1989), and means were separated using least significant difference (LSD) ($P = 0.05$). Means for both absolute sampling methods were for adults found on plants on 38 cm of row. For comparison, adults captured with the sweep net were converted to the mean number found per sweep of the 38 cm diam. net. Absolute visual and sweep net estimates of adult plant bug densities were compared by regression of absolute visual counts on sweep net counts (SAS Institute 1989). To determine if plant height or sample year affected this relationship, analysis of covariance was performed with year as a class variable and sweep net counts and plant height as continuous variables (SAS Institute 1989).

RESULTS AND DISCUSSION

Both absolute sampling methods found significantly higher numbers of adult plant bugs than were found with the sweep net in every sample week of 1993 (except for the WPBS method on 25 July) (Table 1). Mean numbers of adults found with the visual method were higher than those found with the WPBS method in all weeks, and mean numbers of adults were significantly higher than those found with the WPBS method in three of the five weeks. Significantly higher mean numbers of adult plant bugs were found with the absolute visual sampling method compared to mean numbers found with the sweep net in every sample week of 1994. These results agree with those of Garcia et al. (1982) who found that absolute visual samples found significantly higher numbers of *L. hesperus* adults in cotton than were found with WPBS samples. Fleischer et al. (1985) could not obtain significant regressions of adult counts taken with a drop cloth or sweep net on counts taken with the WPBS method. The WPBS method was not used in the present study in 1994 because of the 1993 results which showed absolute visual sampling to be better than WPBS sampling.

Garcia et al. (1982) and Fleischer et al. (1985) both found that absolute visual sampling (where plants were cut into sections and visually examined in the field) was the best method for estimating densities of *Lygus* nymphs. However, only the study by Fleischer et al. (1985) included the sweep net in the sampling methods tested. They did not obtain a significant regression of adult counts with the sweep net on adult counts with the visual sampling method. Adult plant bugs are easily disturbed, and when disturbed, they usually fly. Adults flying from plants being cut up in the field would be easy to miss. Plants in the present study were examined in place, and four observers were used to increase accuracy.

Plant height did not have a significant effect ($F = 2.17$, $df = 95$, $P > F = 0.14$) on numbers of adult bugs captured per sweep, and year did not have a significant effect on the intercept ($F = 0.34$, $df = 95$, $P > F = 0.56$) or the slope ($F = 0.92$, $df = 95$, $P > F = 0.34$). Therefore, data for average plant height was not used and data from both years for sweep net and absolute visual sampling of adults were combined for regression analysis. The regression equation from the combined data was : y (absolute visual) = $0.034 + 0.33x$ (x = mean number of adults per sweep). The regression was significant ($F = 130.9$, $df = 98$, $P > F = 0.001$) with an R^2 of 0.57. Thus, the conversion factor for sweep net samples was 3.0 (if the intercept is ignored the relationship is $y = 0.33x$). The number of adults captured in sweep net samples can be

converted to the more accurate counts taken with the absolute visual samples by multiplying the mean number of adults captured per sweep by three. This converts the counts to the mean number per 0.38 m of row which can then be converted to number per ha or acre. The R^2 value of 0.57 and 3X conversion factor were both fairly close to the R^2 of 0.64 and conversion factor of 3.65 obtained by Byerly et al. (1978), despite their use of a different absolute sampling method and a different plant bug species. The conversion factor obtained in the present study is the only one available for sweep net sampling of adult tarnished plant bugs in cotton. It needs to be tested by other researchers in other locations for comparison and possible use in sampling adult tarnished plant bugs.

Table 1. Results from Sampling Adult Tarnished Plant Bugs in Cotton with Absolute and Relative Sampling Methods at Stoneville, Mississippi.

	Mean[a] no. adults per 0.38 m of row				
Date	Absolute visual[b]	Absolute bag	Sweep net	F	P > F
		1993			
28 Jun	1.13a	0.63b	0.28c	16.35	0.0001
07 Jul	0.70a	0.60a	0.18b	4.81	0.0200
14 Jul	1.70a	1.13b	0.45c	14.93	0.0002
20 Jul	5.27a	4.30a	1.07b	43.48	0.0001
25 Jul	1.40a	0.67b	0.55b	6.24	0.0090
		1994			
17 Jun	0.38a		0.09b	6.85	0.0300
27 Jun	0.55a		0.10b	19.37	0.0020
01 Jul	0.88a		0.18b	15.81	0.0030
08 Jul	2.25a		0.38b	33.70	0.0003
19 Jul	1.60a		0.47b	19.13	0.0020

[a] Means in a row followed by the same letter are not significantly different ($P < 0.05$); least significant difference test (SAS Institute 1989).

[b] See text for a description of the three sampling methods.

The use of the conversion factor to correct the number of adult plant bugs captured with a sweep net would create a problem in using recommended treatment thresholds. These thresholds were not established using the correction factor, and they would be lowered if the correction factor was used (since the number of adults captured would be multiplied by three). However, the more accurate number of adults obtained by using the correction factor could improve research on thresholds since it could improve correlation of numbers of bugs with plant damage and yield loss.

ACKNOWLEDGMENTS

The authors would like to express their appreciation to R. Drake for his technical assistance in the preparation of this manuscript.

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MEXICAN RICE BORER (LEPIDOPTERA: PYRALIDAE) REPRODUCTIVE RESPONSES TO DELAYED MATING

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ABSTRACT

Adult longevity, fecundity, and fertility of Mexican rice borer, *Eoreuma loftini* (Dyar), females mated at 0, 1, 3, and 5 d after eclosion were examined under reversed photoperiodic and thermoperiodic conditions. Mating delays of 1 to 5 d did not significantly affect longevity. One- to 5-d delays in mating reduced fecundity primarily by shortening the oviposition period; however, females did not oviposit their entire egg complement regardless of mating age. The proportion of fertile eggs was not significantly reduced by mating delays of <5 d. Fertility was reduced by mating delays of 3 or 5 d, but these reductions were $>50\%$ only when mating was delayed for 5 d. Substantial reductions in fertility of *E. loftini* populations in response to synthetic pheromone applications would probably require that mating of most females be disrupted or delayed for >5 d.

INTRODUCTION

The Mexican rice borer, *Eoreuma loftini* (Dyar), is the key pest of sugarcane, *Saccharum* spp., in the Lower Rio Grande Valley of Texas (Johnson 1984, Meagher et al. 1994). Conventional chemical control methods for *E. loftini* populations in commercial sugarcane are impractical because multiple applications of insecticide necessary for effective control (Meagher et al. 1994) are prohibitively expensive and reductions in sugarcane yield and quality occur despite control efforts (Ring et al. 1991). Shaver and Brown (1993) concluded from small plot studies that mating disruption techniques using synthetic pheromone could be useful for controlling field populations of *E. loftini*. Subsequent studies conducted over larger areas did not demonstrate substantial population impacts from pheromone applications, but increased captures of virgin females and reductions in mating on mating tables suggested that some delay in mating was associated with those applications (Spurgeon et al. 1997).

Both sexes of *E. loftini* are capable of mating on the night of adult eclosion, but either sex will mate until at least 5 d of age (Spurgeon et al. 1995a). The reproductive impacts of delayed mating on *E. loftini* have not been examined. Our objective in the present study was to quantifiy the effects of delayed mating on adult female longevity, fecundity, and fertility.

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MATERIALS AND METHODS

E. loftini larvae obtained from a laboratory colony maintained for three to four generations at the USDA-ARS laboratory in Weslaco, TX, were reared singly in 29.5-ml plastic cups on artificial diet (Shaver and Raulston 1971) that had been finely shredded. Larvae were observed daily for pupation. Pupae were sexed and weighed to the nearest 0.01 mg on the day after pupation, and held individually in rearing cups containing approximately 10 ml of moistened vermiculite until adult eclosion. All stages of *E. loftini* were held under reversed photoperiod (14:10 [L:D]) and thermoperiod conditions (ranging from approximately 22 to 33°C during the scotophase and photophase, respectively) as described by Spurgeon et al. (1994).

Pupae were observed daily for adult eclosion. At the time of eclosion, each female was assigned a treatment (mating age) and identifying number. Except during mating, each female was confined in a separate 473-ml cardboard carton lined with oviposition substrate (a pleated, unbleached coffee filter). The periphery of the filter was folded over the carton rim, covered with a sheet of clear plastic wrap, and held in place by the rim of the carton lid. Females were supplied fresh drinking water daily in a saturated cotton ball contained in a plastic rearing cup.

Twenty-five each of 0-, 1-, 3-, and 5-d-old females were mated. On the day of mating, about 2.5-4.5 h after the beginning of the scotophase, each female was introduced into a small mating chamber containing a male (1- to 3-d-old) and mating was monitored using a video camcorder equipped with a night vision lens as described by Spurgeon et al. (1995a). Following mating, females were returned to their respective cartons and any eggs deposited in the mating chambers were recovered for fertility determination.

The oviposition substrate of each female was replaced daily at the beginning of the photophase, both before and after mating. Eggs found on the filters were counted and the filters were held in individual plastic bags with a moistened paper towel to determine egg fertility. Eggs were classified as fertile if head capsules or larval stripes were visible after 5 d.

Because both size of the egg complement and realized fecundity are related to pupal weight (Spurgeon et al. 1995b), pupal weights among mating ages were compared by analysis of variance using the SAS procedure PROC ANOVA (SAS Institute 1988). Longevity, fecundity (total of oviposited eggs), fertility (number of fertile eggs), and the proportion of fertile eggs were also compared among mating ages using analysis of variance. Means were separated using the Ryan-Einot-Gabriel-Welsch multiple-range test (REGWQ option of PROC ANOVA). Regression analyses (PROC REG) (SAS Institute 1988) using pupal weight as the independent variable and fecundity as the dependent variable were also conducted for each mating age for comparison with previously reported data.

RESULTS AND DISCUSSION

Mean pupal weights were similar among mating ages ($F=0.72$; df=3, 96; $P=0.545$), ranging from 44.42 mg (mated 5 d after eclosion) to 48.43 mg (mated on the night of eclosion) and averaging 46.68 mg. Therefore, differences in pupal weights did not contribute greatly to differences detected in other parameters.

Mean longevity of all females was 9.02 d and ranged from 8.52 d (mated on the night of eclosion) to 9.92 d (mated 5 d after eclosion). Although mean longevity appeared to increase with increased delay in mating, significant impacts of delayed mating were not demonstrated ($F=2.61$; df=3, 96; $P=0.056$).

Fecundity was significantly decreased with increased delay in mating (Fig. 1) ($F=9.40$; df=3, 96; $P<0.01$). Predictions from regressions relating pupal weight and

fecundity for different mating ages also indicated decreasing fecundity with increasing age at mating. In addition, these relationships yielded smaller estimates of fecundity than the regression relating pupal weight to total egg complement reported by Spurgeon et al. (1995b) (Table 1). Thus, females did not oviposit the entire egg complement before dying regardless of age at mating.

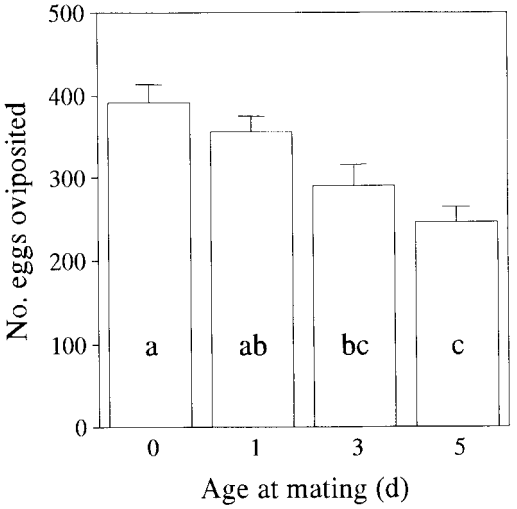

FIG. 1. Mean fecundity (±SEM) of *E. loftini* females mated at 0, 1, 3, and 5 d after eclosion. Bars labelled with the same letter are not significantly different (Ryan-Einot-Gabriel-Welsch multiple-range test; $\alpha=0.05$).

TABLE 1. Parameter Estimates of Regression Models Relating *E. loftini* Pupal Weights to Fecundity for Females Mated at Different Ages.

Age at mating (d)	*n*	Slope (SEM)	Intercept (SEM)	*F*	df	*P*	r^2
0	25	7.63(1.22)	22.42(60.67)	39.0	1, 23	<0.01	0.629
1	25	6.01(1.73)	74.16(82.43)	12.1	1, 23	<0.01	0.344
3	25	10.84(2.35)	-220.91(112.17)	21.3	1, 23	<0.01	0.481
5	25	4.19(1.58)	59.79(72.27)	7.0	1, 23	0.015	0.233
0[a]	50	9.92(0.68)	54.37(32.74)	211.2	1, 48	<0.01	0.815

[a]Regression relating total egg complement to pupal weight reported by Spurgeon et al. (1995b).

Observed decreases in fecundity were accompanied by changes in temporal patterns of oviposition (Fig. 2). Oviposition tended to be greatest on the second night after mating for females mated on the night of eclosion (29.7% of total oviposition) or 1 d after eclosion (33.2% of total oviposition). These observations are consistent with reports by Spurgeon et al. (1995b). However, peak oviposition of moths mated 3 or 5 d after eclosion tended to occur on the night after mating (43.3 and 53.1% of total oviposition, respectively).

Increased delays in mating also shortened the period over which most oviposition occurred. Moths mated at 0, 1, 3, and 5 d after eclosion required 6, 5, 4, and 3 d, respectively, for >90% of total oviposition to occur, although oviposition typically continued at a low level for several days afterwards.

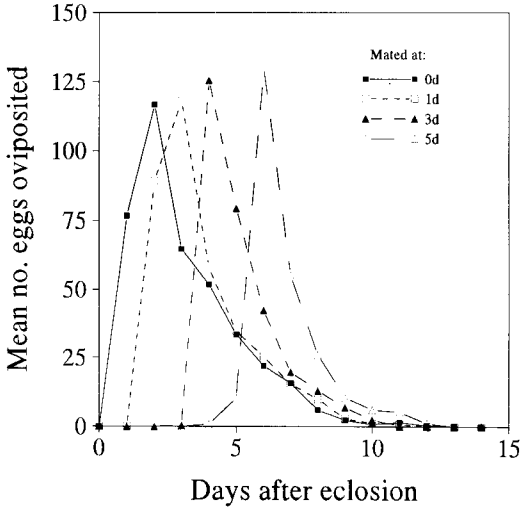

FIG. 2. Temporal patterns of oviposition of *E. loftini* females mated at 0, 1, 3, and 5 d after eclosion.

Females mated 3 and 5 d after eclosion oviposited a portion of their eggs before mating (0.2 and 4.8%, respectively). Females contain a full complement of oocytes at eclosion (Spurgeon et al. 1995b) and oocyte development occurs without mating (D.W.S., unpublished data). Oocyte maturation results in distention of the ovarioles and is probably responsible for eventual oviposition of unfertilized eggs. These observations, combined with estimates of longevity, suggest reductions in fecundity were a consequence of reduced vigor or other factors associated with aging rather than a simple shortening of the period available for oviposition.

Mean percentage fertility of eggs was reduced by delayed mating ($F=31.26$; df=3,96; $P<0.01$) (Fig. 3). No differences in egg fertility were demonstrated among moths mated at 0 to 3 d after eclosion, although a slight trend of reduction in fertility with increasing age at mating was observed. However, fertility of eggs oviposited by females mated at 5 d was substantially reduced.

Impacts of delayed mating on fertility (oviposition of fertile eggs) were products of the respective impacts delayed mating on fecundity and the proportion of fertile eggs. We observed significant reductions in fertility only when mating was delayed for 3 or 5 d after eclosion ($F=18.42$; df=3, 96; $P<0.01$) (Fig. 4). Fertility of moths mated 3 d after eclosion was about 69% of that for moths mated on the night of eclosion. Reductions in fertility of >50% were observed only after a 5-d delay in mating, but these females still produced an average of 178 fertile eggs. Thus, effective population suppression through mating disruption would probably require delays in mating of >5 d. Levels of mating disruption previously observed in large-area studies (Spurgeon et al. 1997) were unlikely to have represented delays in mating of more than 1 or 2 d. The lack of impact of short delays

in mating may explain the absence of measurable population impacts resulting from these pheromone applications.

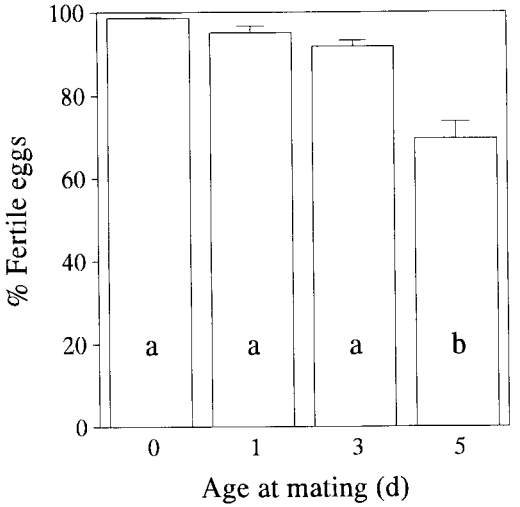

FIG. 3. Mean percentage fertile eggs (±SEM) oviposited by *E. loftini* females mated at 0, 1, 3, and 5 d after eclosion. Bars labelled with the same letter are not significantly different (Ryan-Einot-Gabriel-Welsch multiple-range test; $\alpha=0.05$).

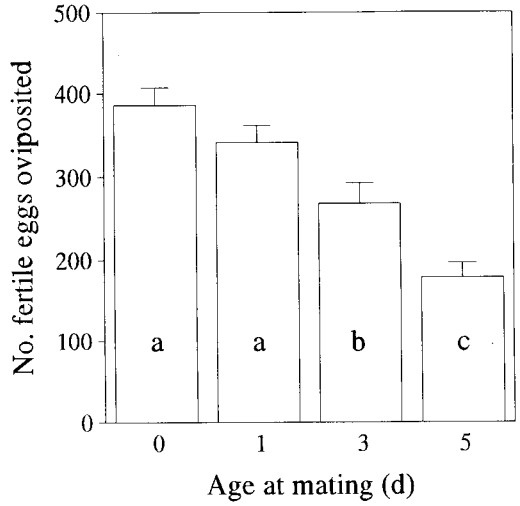

FIG. 4. Mean fecundity (±SEM) of *E. loftini* females mated at 0, 1, 3, and 5 d after eclosion. Bars labelled with the same letter are not significantly different (Ryan-Einot-Gabriel-Welsch multiple-range test; $\alpha=0.05$).

ACKNOWLEDGMENT

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.

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A TECHNIQUE FOR THE COLLECTION AND REARING OF LATE-STAGE 3RD-INSTARS OF *HYPODERMA LINEATUM* (DIPTERA: OESTRIDAE)

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ABSTRACT

An inexpensive and efficient technique for collection and rearing of late-stage, third-instar larvae of the common cattle grub, *Hypoderma lineatum* (Villers), is described. Yearling calves were maintained in individual stalls with expanded metal floors. Larvae exited the back tissues naturally and fell through the expanded metal floors and were collected on screens located beneath the floor. In two seasons (1994-1996), 633 late-stage, third-instar larvae were collected from a total of 29 infested calves. A total of 500 adult flies, with a male:female sex ratio of 249:247, were produced from 597 larvae placed in an incubator for adult fly production.

INTRODUCTION

Hypoderma lineatum (Villers), the common cattle grub, has a 1-yr life cycle, approximately 10 mo of which are passed within the host tissues (Scholl 1993). Larvae digest breathing holes, molt twice, and develop to late-stage, third-instars in the back tissues over a period of approximately 60 days (Pruett and Kunz 1996). Given the time of adult fly activity, larval appearance in the back can be accurately predicted for a specific geographic region (Scholl et al. 1992). The seasonal availability of adult flies for use in research projects in a given geographic region is only 2 to 3 mo, limiting experimental study of the biology of this parasite and hindering use of the sterile insect technique as an effective control option (Kunz et al. 1990).

Collection of larvae for laboratory studies is difficult and unpredictable (Minar and Breev 1982, Kunz et al. 1990). Many techniques have been developed to facilitate collection. These include collection from hides at the abbatoir (Chamberlain and Scholl 1991), gowns designed for collection as larvae exit the back (Bishopp et al. 1926, Gregson and Holland 1944, Barrett 1981), collection from concrete floors (Weintraub et al. 1961), and collection from tethering infested cattle over woven wire flooring (Minar and Breev 1982). In this study we report a modification of the method of Minar and Breev (1982) as an inexpensive and labor saving method for collection of third-instar larvae.

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

MATERIALS AND METHODS

Hypoderma lineatum infested yearling calves (n=27), determined by back palpation, were purchased from a livestock auction in Fredericksburg, TX, during November through January 1994-1996. Hair on the backs of the calves was clipped and warbles counted. Calves were then moved into larval collection stalls (10) as previously described (Pruett and Kunz 1996). An individual stall (2.3 m x 1.4 m x 1.3 m) has an expanded metal floor (2.54 cm, raised, 0.64 cm thick) located 66 cm above the concrete floor. A metal tray (2.3 m x 1.4 m, 1.9 cm flat, 9 gauge) covered with a fine nylon mesh screen is situated 45.72 cm below the expanded metal floor and 25.40 cm above the concrete floor. As larvae exited the back of the host, they fell through the metal floor onto the screen below. Screens were checked daily, at midday, for larvae. Larvae were collected, recorded, and placed into individual cells of an emergence tray containing heat treated hardwood laboratory bedding (Beta Chips, Northeastern Products, Warrensburg, NY) and placed in an incubator (IR-23 Sherer Evironmental Incubator/Refrigerator, Rheem Manufacturing Co., Asheville, NC) maintained at 10:14 h (L:D) and 26:16°C.

In 1995-1996, three laboratory infested calves were included in the collection group. They were Y251, previously infested (natural) and naive calves Y161 and Y163. These calves were stanchioned and infested by allowing tethered, gravid female flies to oviposit on their backs (Gingrich and Barrett 1975).

Development time, from pupation to eclosion, was compared for male and female flies by analysis of the data using the Mann-Whitney Rank Sum Test (Sigmastat Software, Jandel Scientific, San Rafael, CA).

RESULTS AND DISCUSSION

Third-instar larval collections from infested calves held in collection stalls for the 8 wks during early December through mid-February for the collection years 1994-1995 and 1995-1996 are presented in Fig. 1. Collections declined rapidly after the middle of February. Production results for each year are presented in Table 1 (1994-1995) and Table 2 (1995-1996). In 1994-1995, 14 infested calves had a total of 583 warbles and yielded 421 larvae, a recovery of 72.2 %. Of larvae collected and used for fly production (385), 88.6% developed successfully, yielding 341 adult flies (158 males and 179 females). In 1995-1996, 15 infested calves had a total of 395 warbles and yielded 212 larvae, a recovery of 53.7 %. Of larvae collected (212), 75% developed successfully, yielding 159 adult flies (91 males and 68 females). The 2-yr total of eclosed adult flies yielded a male:female sex ratio of 249:247.

Flies eclosed from pupae maintained in incubators within 16-17 d (Table 3). Male flies eclosed approximately 1 d before females. In 1994-1995, male flies eclosed in 16.9 d while females required 17.4 d (Mann-Whitney, T=7025, n_s=95, n_b=94, P<0.001). In 1995-1996, male and female flies eclosed in 15.9 d and 16.4 d, respectively (Mann-Whitney, T=4901, n_s=80, n_b=60, P=0.002). In 1994-1995, some pupae were maintained up to 6 d in a laboratory room because of an incubator problem. Males and females eclosed from these pupae within an average of 18.0 and 18.4 d, respectively (Table 3). Development time was extended by holding these pupae for up to 6 d at room temperature. The longest development time observed was 21 d. Eclosion rate was not affected by this treatment as 88.8% (151/170) of pupae held up to 6 d completed development; 88.4% (190/215) of pupae maintained for the duration of development in the incubator completed development.

This study demonstrates that *H. lineatum* adult flies can be produced for 7 wks from the collection of 3rd-instar larvae from locally purchased, naturally infested yearling calves using a modification of the technique of Minar and Breev (1982). Minar and Breev (1982)

FIG. 1. Total weekly collections of mature 3rd-instar *Hypoderma lineatum* that naturally exited the backs of infested calves. Larval collections for 1994-1995 began on December 3, 1994, and ended on February 21, 1995. Larval collections for 1995-1996 began on December 12, 1995 and ended on February 19, 1995.

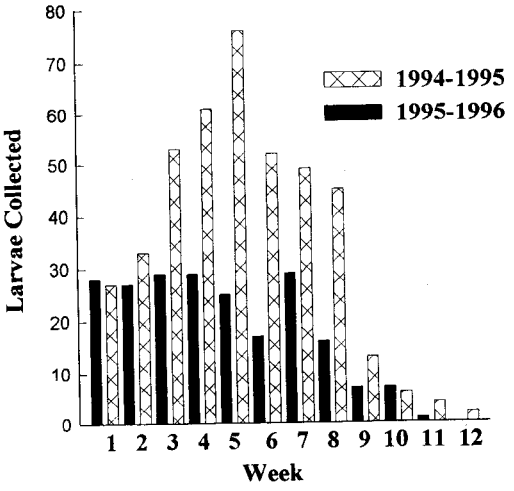

TABLE 1. *Hypoderma lineatum* Production for 1994-1995.

Calf	Larvae in Back	Total Pupae	% Yield	Pupae for Flies[b]	Flies	% Eclosed	Male	Female
Y249	64	36	56.3	30	24	80.0	10	14
Y250	64	52	81.3	50	46	92.0	19	26
Y251	197	169	85.8	151	145	96.0	68	74
Y258	21	11	52.4	9	9	100.0	5	4
Y259	61	37	60.7	36	31	86.1	19	12
Y260	25	16	64.0	15	12	80.0	8	4
Y261	44	34	77.3	34	29	85.3	11	18
Y262	22	11	50.0	5	2	40.0	0	2
Y263	19	15	78.9	15	13	86.7	6	7
Y264	16	9	56.3	9	6	66.7	2	4
Y265	25	17	68.0	17	15	88.2	8	7
Y267	7	5	71.4	5	2	40.0	1	1
Y268	11	7	63.6	7	6	85.7	1	5
Y270	7	2	28.6	2	1	50.0	0	1
Total	583	421		385	341		158	179
Mean	41.6	30.1	63.9			76.9		
Std.	47.4	41.1	14.5			19.2		

[a] Percentage of larvae in the back that were collected after exit from the back.
[b] Pupae from calf that were used in fly production, remaining pupae used in other experiments.

TABLE 2. *Hypoderma lineatum* Production for 1995-1996.

Calf	Larvae in Back	Total Pupae	% Yield[a]	Flies	% Eclosed	Male	Female
Y161[b]	34	15	44.1	12	80.0	6	6
Y163	48	13	27.1	6	46.2	5	1
Y251[c]	53	20	37.1	14	70.0	7	7
Y382[d]	14	6	42.9	4	66.7	1	3
Y383	21	16	76.2	8	50.0	4	4
Y384	41	29	70.7	22	75.9	13	9
Y385	27	22	81.5	16	72.7	8	8
Y386	16	8	50.0	5	62.5	2	3
Y387	24	18	75.0	16	88.9	11	5
Y388	13	7	53.8	6	85.7	3	3
Y389	9	7	77.8	4	57.1	2	2
Y405	9	5	55.6	5	100.0	2	3
Y406	62	38	61.3	33	86.8	20	13
Y408	11	5	45.5	5	100.0	4	1
Y409	13	3	23.1	3	100.0	3	0
Total	395	212		159		91	68
Mean	26.3	14.1	54.8		76.2		
Std.	16.8	9.7	18.0		17.0		

[a] Percentage of larvae in the back that were collected after exit from the back.
[b] Y161 and Y163, naive calves that were laboratory infested.
[c] Y251 had 1 previous natural infestation and was laboratory infested in 1995-1996.
[d] All remaining calves had natural infestations. Y382 (2 larvae), Y385(1 larva), Y389(1 larva), and Y405(1 larva) had larvae remaining in their backs when sold.

TABLE 3. Days to Eclosion for *Hypoderma lineatum* Pupae Held in an Incubator at 10:14 h (L:D) and 26:16°C.

	1994-1995	1994-1995	1995-1996	1995-1996	1994-1995	1994-1995
	Males	Females	Males	Females	Males[a]	Females[a]
N[b]	95	94	80	60	62	84
Days[c]	16.9 ± 0.7	17.4 ± 0.7	15.9 ± 0.9	16.4 ± 0.8	18.0 ± 0.7	18.4± 0.8

[a] Pupae were affected by incubator problems and were maintained in a room environment (10:14 h, 26°C:16°C) for 6 d.
[b] Number of pupae.
[c] Mean days of incubation ± standard deviation.

reported a 62.7% mean eclosion rate for the northern species, *Hypoderma bovis*, over a series of different experiments. The eclosion rate reported in the current study for *H. lineatum* was 88.6% for 1994-1995 and 75% for 1995-1996. The larval collection technique reported in this study provided, from a limited number of calves, a sufficient number of adult flies to infest 20 calves per week for 7 wks. This represents a significant improvement over previous, logistically demanding, larval collection techniques.

Larval collection from hides at the abbatoir (Scholl et al. 1989) is by chance; pupation can be expected of only the most developmentally advanced larvae (Chamberlain and Scholl 1991). Continued development of less developed larvae requires considerable laboratory manipulation (Chamberlain and Scholl 1991). In the current study, larvae were allowed to exit the host naturally and were placed in the incubator as either fully pupated larvae or black P3 motile larva (Scholl et al. 1989) that pupated within a few hours.

Considerable problems are also associated with gowning of cattle with fabric for larval collection. A technical improvement that lessened gown maintenance was the technique of Barrett (1981) where the gown material was glued to the host. However, this technique requires frequent checks, as larvae can be crushed by the host, and gown seams can come unglued. In addition, the effect of the gown on the microenvironment of the warble and the continued development of early phase 2nd- and 3rd-instar larvae has never been adequately evaluated. The technique of gowning is labor intensive, with production efficiency (Weintraub and Scholl 1984) less than the results reported in the current study.

Utilizing the stall technique for larval collection allows us to predict that > 50% of all warbles counted in the backs of yearling calves will yield viable 3rd-instar larvae that will successfully pupate. We can reliably estimate that the eclosion rate will be greater than 75%. The cost of producing reproductive adult flies with the stall technique is much less than previous collection techniques as labor was reduced to about 1 h per day for feeding, cleaning, and larval collection by one to two technicians. Feed cost was limited to 20 to 60 d per calf. The principal expenditure was for the purchase of infested calves; however, these funds were recovered at resale. This technique should find utility for research applications involving the requirement for 3rd-instar larvae or adult flies.

ACKNOWLEDGMENT

The authors gratefully acknowledge the technical assistance of Melinda May, Keith Shelley, Fidel Tijerina, and Leonard Pawelek.

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INFLUENCE OF PLANT MATURITY AND APPLICATION TIMING ON EFFICACY OF DICROTOPHOS FOR CONTROL OF COTTON APHID[1]

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ABSTRACT

Efficacy of dicrotophos for controlling the cotton aphid, *Aphis gossypii* Glover, was investigated at the Texas Agricultural Experiment Station at Chillicothe in 1992-1994. Cotton was planted in late April, late May, and late June each year, and dicrotophos, at 0.5 lb AI/acre, was applied as aphid numbers began increasing during August. A single application was made at different aphid densities within each planting date. A treatment threshold of 35-50 aphids per leaf can be used for all three planting dates. A single application prevented development of high numbers for extended periods in the late April and late May planting dates; in some years populations of 50 aphids per leaf did not develop in these two plantings. Multiple applications will probably be necessary to control aphids in late June cotton.

INTRODUCTION

The cotton aphid, *Aphis gossypii* Glover, has become a serious pest in cotton in West Texas, with problems intensifying since the mid-1970's. Based mostly on experience and observations, the Texas A&M University Cotton Aphid Task Force (1994) recommended a treatment threshold of 50 aphids per leaf. Fuchs and Minzenmayer (1995) reported lint and seed yield reductions when cotton aphid populations exceeded 50 aphids per leaf for 3 weeks or 100 aphids per leaf for 2 weeks in irrigated cotton.

There are many factors that influence efficacy of insecticides for controlling cotton aphids. The most serious factor is the development of resistance to organophosphates, organochlorines, and pyrethroids (O'Brien et al. 1990, Gubran et al. 1993). Additionally, insecticide resistance varies within and between years. Kerns and Gaylor (1992) reported variable resistance ratios among years, within insecticide classes, in populations collected from the same geographical areas. Grafton-Cardwell (1991) found that resistance to some organophosphate and organochlorine insecticides varied among populations within the same year and that resistance declined in some populations as the season progressed. She suggested

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that these changes might be related to aphid life stage, nutrition effects, and insecticide selection pressure. However, O'Brien and Graves (1992) reported that organophosphate resistance remained stable in the absence of selection pressure.

Insecticides also alter the reproductive potential of cotton aphids. For example, O'Brien and Graves (1992) reported reproduction of organophosphate resistant aphids was greater than in susceptible aphids. Brown and Reed (1992) reported increased population levels in plots treated with azinphosmethyl, cypermethrin, and endosulfan compared to untreated plots.

Agronomic factors influence insecticide efficacy also. McKenzie et al. (1995) found increased levels of leaf nitrogen, as influenced by amount of nitrogen fertilizer applied at planting, were associated with decreased susceptibility to bifenthrin and methomyl. Fuson et al. (1995) attributed better control to increased percentage of leaf coverage in a mid-May planting, compared to spray coverage in late March and late April plantings.

O'Brien (1994) obtained best control of cotton aphids with chlorpyrifos with droplets in the 200-400 µm range for ground application and in the 175-225 µm range for aerial application. Adjuvants did not affect efficacy of chlorpyrifos (French et al. 1992).

This overview shows that many factors influence insecticide efficacy in cotton, and any one factor or a combination of factors may be responsible for perceived control failures or successes. The purpose of this report is to document the relationship between planting date (i.e., plant age) and efficacy of dicrotophos for control of the cotton aphid in dryland cotton.

MATERIALS AND METHODS

Experimental Design. This study was conducted at The Texas Agricultural Experiment Station at Chillicothe in 1992, 1993, and 1994. 'Paymaster 145' cotton was planted each year in east-west row directions on 40" row spacings. Cotton was grown dryland each year, and fertilizer, 32-0-0 (N-P-K) lbs/acre, was applied at planting. A split-plot experimental design, with three replications, was used. Whole plots were three planting dates: 27 April, 22 May, and 25 June 1992; 26 April, 28 May, and 24 June, 1993; and 6 May, 31 May, and 24 June 1994. In subsequent discussions, these are referred to as late April, late May, and late June plantings. Seeds were planted at 6.7, 4.2, and 4.2/row ft in the late April, late May, and late June plantings, respectively. Subplots were treatments of dicrotophos (Bidrin 8E®), at 0.5 lb AI/acre. Subplots were 8 rows wide by 75' long.

A single application was made at different aphid densities within each planting date. Insecticide application dates were 11, 14, and 18 August 1992; 16, 20, and 23 August 1993; and 10 August 1994. Insecticide was applied using a tractor-mounted sprayer with drops to obtain three nozzles per row. The middle six rows of each eight-row plot were treated. Total solution applied was 11.5 gal/acre in 1992, 10.5 gal/acre in 1993, and 10.0 gal/acre in 1994.

Aphid Sampling. Average numbers of aphids per leaf were estimated once each week beginning in early August. Sample size was 20 upper leaves (top-half of plant) and 20 lower leaves (bottom-half of plant) in each plot, but as aphid numbers increased in late August, sample size was reduced to ten top and ten bottom leaves. Aphids were individually counted until numbers reached about 100/leaf, after which numbers were estimated by counting groups containing five or ten aphids per group. A leaf was picked from the plant, and examined immediately, every two or three steps along a row. All upper leaves were taken from the same

row, and the sampler then moved to another row to examine lower leaves.

Data Analysis. Aphid counts were averaged over the three consecutive dates with highest numbers posttreatment in 1992 and 1993. Only two posttreatment dates were sampled in 1994 because aphid numbers declined rapidly after the second sample. Data for each year were analyzed by analysis of variance for a split-plot experiment. Analyses were performed using the FACTOR and RANGE programs of MSTAT-C (MSTAT Development Team 1988), and means were separated using protected least significant difference (LSD) ($\alpha = 0.05$). When only two means were compared, LSD was used.

Yield data were not analyzed because boll weevil, *Anthonomus grandis* Boheman, damage to squares and bolls was high in all plots. These infestations made it impossible to determine the influence of cotton aphid infestation levels on yield.

RESULTS AND DISCUSSION

1992 Results. Aphid populations began increasing in early August, and peak densities were reached on 3 September, after which numbers declined rapidly. In the untreated plots, peak population levels were 173, 142, and 393 aphids per leaf in the late April, late May and late June planting dates, respectively. Dicrotophos was applied on 11, 14, and 18 August in each planting date. Pretreatment aphid numbers per leaf ranged from 8 to 28 in the late April planting, from 9 to 22 in the late May planting, and from 12 to 50 in the late June planting (Table 1).

In both the late April and late May plantings, a single application maintained average posttreatment densities near 50 aphids per leaf. Even though the season high density on 3 September exceeded 50 aphids per leaf in all timing schedules in these two planting dates, numbers did not exceed 50/leaf or 100/leaf for more than 1 week.

In the late June planting, average posttreatment numbers ranged from 75 to 145 aphids per leaf, and the season high density exceeded 183 aphids per leaf in all three timing schedules. However, numbers exceeded 50/leaf and 100/leaf for 2 weeks in the 11 and 14 August schedules, but for 1 week only when treatments were applied on 18 August.

1993 Results. Populations began increasing in mid-August and peaked on 9 September, after which populations declined rapidly. In the untreated plots, peak population levels were 299, 77, and 841 aphids per leaf in the late April, late May, and late June planting dates, respectively. Dicrotophos was applied 16, 20, and 23 August in each planting date. Pretreatment aphid numbers per leaf were 21 to 41 in the late April planting, 14 in the late May planting, and 28 to 117 in the late June planting (Table 2).

In the late April planting, the dicrotophos applications maintained average posttreatment aphid densities near 50/leaf. However, numbers exceeded 50/leaf for 2 weeks in the 16 August application, but for only 1 week in the 20 and 23 August applications. The season high densities on 9 September ranged from 69 to 118 per leaf. Numbers exceeded 100/leaf for only 1 week in the 16 and 20 August treatments, but numbers did not exceed 100/leaf in the 23 August treatment.

In the late May planting, treatments were applied on 16 August only. Average posttreatment aphid numbers were <50/leaf in the treated and untreated plots. The season high was 72 aphids per leaf in the treated plot, and numbers exceeded 50/leaf for 1 week. Dicrotophos applications did not effectively control aphids in the late June planting. Average posttreatment numbers in treated plots ranged from 199

TABLE 1. Cotton Aphid Population Response to Timing of Dicrotophos Applications in Three Planting Dates. Chillicothe, TX. 1992.

Planting Date	Date of Treatment[a]	Average Number of Aphids per Leaf			No. Weeks Populations Exceeded:	
			Posttreatment			
		Pretreatment[b]	20 Aug-3 Sep[c]	3 Sep[c]	50/Leaf	100/Leaf
27 Apr	Untreated	8.1	109.6 a	172.7 a	2	2
	11 Aug	7.9	27.8 c	57.0 c	1	0
	14 Aug	23.0	67.9 b	140.1 ab	1	1
	18 Aug	28.4	32.5 c	76.1 bc	1	0
22 May	Untreated	10.2	105.0 a	141.7 a	2	2
	11 Aug	9.4	58.2 b	123.6 a	1	1
	14 Aug	19.6	60.0 b	125.5 a	1	1
	18 Aug	21.5	39.0 b	95.0 a	1	0
25 Jun	Untreated	13.9	226.7 a	393.4 a	4	2
	11 Aug	12.3	144.6 b	266.6 b	2	2
	14 Aug	31.4	129.6 b	255.2 b	2	2
	18 Aug	50.3	75.2 c	183.7 c	1	1

[a]Dicrotophos applied once on indicated dates at 0.5 lb AI/acre.
[b]Pretreatment counts taken on 10 Aug for untreated and 11 Aug applications, and on 13 and 17 Aug for the 14 and 18 Aug applications, respectively.
[c]Posttreatment counts are averages of samples taken 20 and 26 Aug and 3 Sep, while the season high count represents only the data collected 3 Sep.

TABLE 2. Cotton Aphid Population Response to Timing of Dicrotophos Applications in Three Planting Dates. Chillicothe, TX. 1993.

Planting Date	Date of Treatment[a]	Average Number of Aphids per Leaf			No. Weeks Populations Exceeded:	
			Posttreatment			
		Pretreatment[b]	26 Aug-9 Sep[c]	9 Sep[c]	50/Leaf	100/Leaf
26 Apr	Untreated	19.0	224.5 a	298.7 a	4	3
	16 Aug	24.9	76.2 b	109.5 b	2	1
	20 Aug	21.0	60.3 b	117.9 b	1	1
	23 Aug	40.6	44.3 b	69.3 b	1	0
28 May	Untreated	13.5	46.0 a	77.1 a	1	0
	16 Aug	13.8	37.7 a	72.2 a	1	0
	20 Aug	7.8	-	-	-	-
	23 Aug	-	-	-	-	-
24 Jun	Untreated	20.7	520.9 a	840.5 a	4	3
	16 Aug	27.5	326.6 ab	644.9 ab	3	2
	20 Aug	30.1	285.1 b	532.0 b	4	2
	23 Aug	116.5	198.7 c	387.6 b	3	3

[a]Dicrotophos applied once on indicated dates at 0.5 lb AI/acre.
[b]Pretreatment counts taken on 16 Aug for untreated and 16 Aug application, and on 19 and 23 Aug for the 20 and 23 Aug applications, respectively.
[c]Posttreatment counts are averages of samples taken 26 Aug and 2 and 9 Sep, while the season high count represents only the data collected on 9 Sep.

to 327 aphids per leaf, and season high numbers on 9 September exceeded 387/leaf in all treatments. Numbers exceeded 100/leaf for 2 weeks in all treatments.

1994 Results. Aphid populations began increasing in early August, and peak densities were reached on 19 August, and numbers per leaf rapidly declined thereafter. In the untreated plots, peak population levels were 19, 37, and 339 aphids per leaf in the 6 May, late May, and late June planting dates, respectively (Table 3). Dicrotophos was applied once on 10 August in each planting date. Pretreatment aphid numbers were 7/leaf in the late April planting, 16/leaf in the late May planting, and 140/leaf in the late June planting.

Populations did not reach high levels in the late April or late May planting dates, and aphid numbers remained below 50/leaf in both treated and untreated plots. In the late June planting date, average posttreatment densities were 60 aphids per leaf. The season high density was 105 aphids per leaf in treated plots, and numbers exceeded 100/leaf for 2 weeks.

Discussion. Aphid densities declined rapidly in early September 1992. There were many dead aphids on the leaves, with mortality apparently caused by the entomopathogenic fungus *Neozygites fresenii* (Nowakowski). High numbers of lady beetles, *Hippodamia convergens* Guerin-Meneville, entered the plots in mid- to late August in 1993 and 1994, and aphid numbers declined. In all 3 years, aphid numbers became quite variable soon after peak densities were reached; plots in one replication would have few aphids while plots in another replication would have high numbers. As a result, accurate determination of insecticide efficacy was not possible after numbers began to decline.

In late April cotton in 1992 and 1993, lowest posttreatment populations occurred when dicrotophos was applied to pretreatment populations of 28 and 41 aphids per leaf, respectively. Additionally, populations exceeded 50/leaf for only 1 week, which is below the damage level of 50/leaf for 3 weeks (Fuchs and Minzenmayer 1995). In 1994, the season high infestation in untreated plots did not exceed 19/leaf, so an insecticide application was not necessary. In late April cotton, best control was obtained when the dicrotophos application was delayed until aphids reached densities of 28-41/leaf.

In cotton planted in late May, average infestations in untreated plots did not exceed economic levels in 1993 or 1994. In 1992, there were no significant differences between average posttreatment infestations when treatments were initiated over the pretreatment range of 9 to 22 aphids per leaf. In all 3 years, a single application at pretreatment levels ranging from 9 to 22 aphids per leaf prevented populations from exceeding 100/leaf for more than 1 week. Since aphid numbers may remain below 50/leaf, dicrotophos should not be applied in late May cotton until aphid numbers exceed at least 37/leaf, which was the highest level attained in 1994.

In cotton planted in late June, populations exceeded 50/leaf for 1-3 weeks when a single application was made at pretreatment levels ranging from 12 to 140 aphids per leaf. Populations exceeded 100/leaf for 2 weeks in all timing schedules except the 18 August 1992 treatment; in this treatment numbers exceeded 100/leaf for 1 week only. These results indicate that multiple applications will generally be necessary to prevent populations from exceeding 50/leaf for several weeks in late-June planted cotton. Lowest posttreatment densities were attained when the application was delayed until populations were ≥50 aphids per leaf.

Plant maturity in August, as governed by planting date, significantly affects insecticide efficacy. Without the benefit of an untreated check, treatments applied at ≤16 aphids per leaf appeared to provide season-long control in the late April and

TABLE 3. Cotton Aphid Population Response to Timing of Dicrotophos Treatments in Three Planting Dates. Chillicothe, TX. 1994.

Planting Date	Date of Treatment[a]	Average Number of Aphids per Leaf			No. Weeks Populations Exceeded:	
		Pretreatment[b]	Posttreatment 15-19 Aug[c]	Posttreatment 19 Aug[c]	50/Leaf	100/Leaf
6 May	Untreated	9.5	15.1 a	19.2 a	0	0
	10 Aug	7.3	7.0 a	10.8 a	0	0
31 May	Untreated	15.5	32.0 a	37.3 a	0	0
	10 Aug	16.1	12.4 a	18.6 a	0	0
24 Jun	Untreated	124.4	254.7 a	339.2 a	3	3
	10 Aug	139.5	59.6 b	105.4 b	2	2

[a]Dicrotophos applied once on indicated dates at 0.5 lb AI/acre.
[b]Pretreatment counts taken on 9 Aug.
[c]Posttreatment counts are averages of samples taken 15 Aug and 19 Aug, while the season high count represents only the data collected on 19 Aug.

late May planting dates in 1994 and in the late May planting in 1993. However, an application at ≤21 aphids per leaf in the late June planting date in the 1992 and 1993 tests did not provide effective control, because populations exceeded 50/leaf for 2-3 weeks after the application. The influence of plant maturity probably explains why some growers and consultants like to treat at ≤10 aphids per leaf (Spray 1992), because treatments at such low levels can appear to be very effective.

Maturity of the cotton plant at the time of aphid population increase is as important a regulating factor as an application of dicrotophos. For example, cotton planted in late April or late May is setting bolls by mid-August, and cotton aphid populations often fail to reach economic levels as happened in 1993 and 1994. Cotton planted in late June is just beginning to bloom in mid-August, and aphid numbers consistently increase during August in this planting date (Slosser et al. 1997). Nutritional quality of cotton during August, as influenced by plant maturity, apparently regulates reproductive potential of aphid populations. Maturing plants (setting bolls) hinder population growth, making dicrotophos more effective, while immature plants (beginning to bloom) enhance population growth, making dicrotophos less effective.

The results of these tests also suggest that resistance to dicrotophos, and perhaps other insecticides, could be implicated in field tests, when in fact resistance may not be involved. For example, if a test in the first year was conducted in maturing cotton, dicrotophos could appear to be very effective. Then, in tests in subsequent years in young cotton, dicrotophos would appear to be ineffective. In this scenario, high aphid reproductive potential in young cotton would make dicrotophos seem ineffective, and resistance would appear to explain loss of efficacy. Thus, efficacy of insecticide applications in young cotton at time of population increase must be interpreted with caution.

The results of these tests indicate that dicrotophos treatments should be delayed until populations exceed 35-50/leaf. In some years in the northern Rolling Plains, this level may not be reached in cotton planted in late April or late May, and a single application should maintain average posttreatment infestations near 50/leaf. Multiple applications may be necessary in cotton planted in late June, but dicrotophos should not be applied until populations are near 50/leaf.

ACKNOWLEDGMENT

This research was supported in part by grants from The Cotton Foundation (Project #III.5) and Cotton Incorporated, Texas State Support Committee (Cooperative Agreement No. 93-893TX). Additional support was provided by Texas Agricultural Experiment Station projects H-8136 and H-6819 (Vernon) and H-6035 (Lubbock). We appreciate the assistance of Roxanne Montandon.

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ADULT CONTROL OF STRIPED CUCUMBER BEETLE[1] FOR MANAGEMENT OF LARVAL INJURY TO CANTALOUPE

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ABSTRACT

Applications of chemical treatments directed at adult stages of the striped cucumber beetle, *Acalymma vittatum* (F.) were made during the period when eggs were being laid that were anticipated to produce late instar larvae at the time of harvest, approximately 20 to 30 days prior to harvest. In the first study (1995), methamidophos (Monitor 2WM), two formulations of carbaryl (Sevimol, Adios) and esfenvalerate (Asana XL) all provided acceptable control when applied sequentially on three dates, beginning 35 days prior to harvest. Systemic insecticide treatments (carbofuran, imidacloprid), applied as a band over the furrow after planting, and seed treatments with imidacloprid provided poor or unacceptable control. In the subsequent study (1996), the effect of different treatment timings was evaluated. Treatments applied 29 days before harvest provided significantly better control of later larval damage than did later treatments, 19 and 9 days before harvest. Somewhat greater control was observed with carbaryl (Sevin XLR-Plus, Adios) than with esfenvalerate. The use of cucurbitacin-based feeding stimulant baits (Adios) does provide an effective treatment that is generally compatible with protection of pollinators present in fields at this time.

INTRODUCTION

The striped cucumber beetle, *Acalymma vittatum* (F), is the most important insect pest of cantaloupe in the Arkansas Valley. Damage can be caused by both the adult and larval stages (Chittenden 1919, Houser and Balduf 1925). Adults chew on leaves, sometimes seriously injuring seedlings. The striped cucumber beetle is also the primary vector of bacterial wilt of cucurbits (Gould 1944), although this disease is extremely uncommon in this growing area due to the arid climate.

However, the greatest injury to the Colorado cantaloupe crop is done by the larvae that develop below ground. During development striped cucumber beetle larvae primarily feed on roots and substantial root injuries are well documented for this insect (Chittenden 1919, Houser and Balduf 1925, Isely 1927). However, far less commonly recognized is the problem of migration by late-instar larvae into fruit resting on soil. This damage to cantaloupe rinds can cause serious scarring, and wounds allow entry of rotting organisms. Periodically, whole fields of cantaloupe in the Arkansas Valley of Colorado can be so badly

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damaged by larval fruit injuries that the crop is discarded prior to harvest. Wet weather conditions and irrigation that produce moist soils prior to harvest appear to contribute to larval injury to fruit, as has been observed by Isley (1927). In the Arkansas Valley, injury is primarily associated with the second generation of striped cucumber beetle, which produces full-grown larvae in mid summer.

Larval control is difficult as they occur below ground beneath a dense canopy. Since larval injuries to fruit occur at harvest, planting time treatments may provide poor control due to the extended period required for persistence of control. Therefore, studies were conducted in 1995 and 1996 to develop better information on managing larval injuries to cantaloupe. Attention was directed at control of adult stages, particularly those present during periods when egg laying would most likely later result in larval injuries near harvest. Studies on the biology of striped cucumber beetle (Houser and Balduf 1925, Sweetman 1925, Isely 1927, Gould 1944) indicate that it takes approximately three to four weeks from the time of egg deposition to completion of the larval period, and treatments focused on this period.

Since flowering typically begins five to six weeks prior to harvest, and continues through harvest, protection of pollinating insects is also an important concern. Therefore a secondary objective was to evaluate treatments of reduced risk to pollinators. One of these involves formulating carbaryl into a cucurbitacin-based feeding stimulant bait (Adios) that substantially reduces risk to bees (Mayer et al. 1996).

METHODS AND MATERIALS

In the first year studies (1995), evaluations were made to identify effective insecticides, particularly those that would produce less injury to pollinators of the crop. Determination of the best time to apply treatments was the focus of the 1996 trial. All studies were conducted at the Arkansas Valley Research Center in Rocky Ford.

1995 Studies. The experimental area was planted 14 June 1995 using the cultivar "PMR-45". Plots consisted of 4, 60-inch beds, each 20 ft in length. The soil systemic treatments carbofuran (Furadan 4F) and imidacloprid (Admire 2F) were applied as 8-inch bands on 16 June, and were immediately followed by an irrigation to move the application to the root zone. Foliar treatments were applied 8, 16 and 23 August using a hand operated compressed air sprayer delivering 27 gal/A. First treatments were made to coincide with the initial presence of small fruit.

Fields were flood irrigated a week prior to evaluations to increase larval activity, and subsequent fruit injury near the soil surface. Evaluations were made 12 September by examining ten fruit per plot and rating the amount of larval tunneling injury to the fruit rind. For this evaluation, a four point system was used (0 = no injury, 1 = slight injury, 2 = moderate injury, 3 = severe injury). Pest pressure from striped cucumber beetle larvae was moderate to heavy throughout the trial except along the southeastern corner where the drier soils, not reached with flooding, limited damage. As a result, five outlier plots in this area were eliminated from the analysis.

1996 Trials. The subsequent trial was established in an approximately similar manner. Cantaloupe 'PMR-45' was seeded 31 May to 60-inch rows and individual plots were expanded slightly to consist of five, 27-ft rows. Insecticides were applied on three dates (29 July, 8 August, and 18 August), with individual plots receiving single or combinations of multiple applications. Again, the experiment was initiated (29 July) to coincide with the first presence of small fruit. Applications were made using a hand-operated compressed air sprayer delivering 8 gal/A. Evaluations were conducted 27 August by examining ten fruit per plot and rating the amount of larval tunneling injury to the rind, using a 0 (no injury) to 3 (severe rind injury) scale, similar to that used the previous season.

RESULTS AND DISCUSSION

All of the foliar treatments in 1995 provided significant control compared to the untreated check (Table 1). Control from planting time treatments of Admire 2F, but not Furadan 4F, were significantly better than the untreated check, but were less effective than most foliar treatments. Seed treatment with imidacloprid (Gaucho) was also not effective at limiting larval damage to fruit. The cucurbitacin bait formulation of carbaryl, Adios, provided control comparable to standard carbaryl foliar treatment (Sevimol). Foliar applications of carbaryl, methamidophos (Monitor 2WM), and esfenvalerate (Asana XL) all produced acceptable control of larval injury, while dimethoate (Cygon) provided only marginal control.

TABLE 1. Control of Striped Cucumber Beetle Larval Injury to Cantaloupe Fruit by Foliar and Soil Treatment Directed at Adult Stages, Rocky Ford, CO 1995

Treatment and rate[a]	Application	Percent undamaged fruit[b]	Average fruit damage rating[b,c]
Monitor 2WM 1.5 pt/A	Foliar treatment	85.0 a	0.18 a
Sevimol 4 1 qt/A	Foliar treatment	77.5 ab	0.28 a
Asana XL 9.6 fl oz/A	Foliar treatment	72.5 abc	0.35 a
Adios 0.75 lb/A	Foliar treatment	72.5 abc	0.43 a
Admire 2F 1.9 fl oz/1000 row-ft	Band over row	53.6 bcd	0.69 a
Cygon 4E 1 qt	Foliar treatment	47.9 cd	0.68 a
Furadan 4F 2.4 fl oz/1000 row-ft	Band over row	27.9 de	1.32 b
Untreated Check I		30.0 de	1.28 b
Gaucho 21 gr ai/kg seed	Seed treatment	20.2 e	1.36 b
Untreated Check II		16.7 e	1.46 b

[a] Foliar treatments applied 8, 13, and 23 August 1995. Soil systemic treatments banded over row 16 June.
[b] Numbers followed by the same letter are not significantly different (P = 0.05) by SNK test. Analysis involved removing outlier plots in southeastern corner that did not receive flooding irrigation and replacing with least squares mean estimates.
[c] Harvest date 12 September. Average amount of injury to 40 fruit (4 replications). Individual fruit damage ratings are based on a 4 point system (0 = no damage; 3 = severe injury to rind).

In 1996, all of the treatments again were able to reduce fruit damage by striped cucumber beetle larvae (Table 2). There was a trend to slightly greater control with carbaryl (Sevin XLR-Plus, Adios) compared to esfenvalerate (Asana XL). Based on these data, it is suggested that Adios be considered as an effective striped cucumber beetle treatment that is most compatible with applications during bloom (Mayer et al. 1996) when pollinators are present.

Differences in control existed among the different treatment timings. Overall, plots sustained least fruit damage from larvae when treated 29 July, approximately one month prior to the harvest evaluation. This is somewhat inconsistent with what one would have expected from the report by Isely (1927) that the period from egg laying to completion of

the larval stage in Arkansas averaged 24 days; treatment 29 days before harvest might have been expected to be a bit premature. However, others have reported slightly longer developmental periods for the immature stages of striped cucumber beetle (Chittenden 1919) and the insecticides used likely would have persisted for several days after application.

TABLE 2. Evaluations of Treatment Timing of Sprays Directed at Adult Striped Cucumber Beetles to Control Subsequent Damage by Larval Stages, Rocky Ford, CO 1996.

Insecticide and rate	Treatment Timing[a]	Average Fruit Damage Rating[b]	Percent fruit marketable[c]
Sevin XLR-Plus 1 qt/A	1	0.43 a	92.5
Sevin XLR-Plus 1 qt/A	2	0.78 b	85.0
Sevin XLR-Plus 1 qt/A	3	0.83 b	72.5
Sevin XLR-Plus 1 qt/A	1, 2	0.18 a	97.5
Sevin XLR-Plus 1 qt/A	2, 3	0.68 ab	77.5
Sevin XLR-Plus 1 qt/A	1, 3	0.45 a	97.5
Sevin XLR-Plus 1 qt/A	1, 2, 3	0.30 a	92.5
Adios 0.75 lb/A	1, 2	0.30 a	97.5
Adios 0.75 lb/A	2, 3	1.13 c	60.0
Adios 0.75 lb/A	1, 3	0.53 ab	90.0
Asana XL 5.6 fl oz/A	1, 2	0.75 b	82.5
Asana XL 5.6 fl oz/A	2, 3	0.95 bc	67.5
Asana XL 5.6 fl oz/A	1, 3	0.63 ab	90.0
Untreated check		1.55 d	42.5

[a] Treatment dates were July 29 (1), August 8 (2) and August 18 (3).
[b] Harvest date 27 August. Fruit injury scale: 0 - no injury to fruit rind, 1 - slight injury, 2 - moderate injury, 3 - severe injury. Numbers followed by the same letter are not significantly different (P = 0.05) by SNK.
[c] Plants receiving injury ratings of 2 or 3 were considered not marketable.

A single application of Sevin XLR-Plus applied 8 August, 19 days prior to harvest, was not effective as an earlier treatment. However, applied as a second follow-up treatment, control was improved compared to a single earlier application.

Treatments made 9 days prior to harvest (19 August) did not provide control of larval injury to fruit at harvest. This is consistent with the described biology of the striped cucumber beetle (Sweetman 1925, Isely 1927) as eggs laid on 19 August would only have recently hatched and only small larvae would be present at a 27 August harvest.

These studies demonstrate that striped cucumber beetle can be controlled by insecticide applications directed at adult stages. Most critical is the timing of these treatments, which should be applied when adults are laying eggs that will develop into late-instar larvae at time of harvest. Although this application also coincides with bloom, the use of selective cucurbitacin-based feeding stimulant bait formulations should be compatible with pollinator protection.

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SPECIES COMPOSITION OF HELIOTHINAE CAPTURED IN CONE TRAPS BAITED WITH SYNTHETIC BOLLWORM[1] OR TOBACCO BUDWORM[2] PHEROMONES

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ABSTRACT

The species composition of the Heliothinae captured in cone traps baited with synthetic pheromones of the bollworm, *Helicoverpa zea* (Boddie), or tobacco budworm, *Heliothis virescens* (F.), was determined in Louisiana in 1992. Only tobacco budworms comprised the 49,729 specimens from traps baited with tobacco budworm lure. The 25,819 specimens collected in traps baited with bollworm lure included one *Heliothis phloxiphaga* Grote and Robinson, 10 specimens that may be hybrids between the bollworm and *H. phloxiphaga*, and 15 tobacco budworms. These small numbers of other Heliothinae, however, would not affect the interpretation of pheromone trap catches of these species or insecticide resistance monitoring data.

INTRODUCTION

Traps baited with pheromone are often used to capture adult male bollworms, *Helicoverpa zea* (Boddie), and tobacco budworms, *Heliothis virescens* (F.), for predictive purposes in IPM programs (Slosser et al. 1987, Goodenough et al. 1988, Schneider et al. 1989) and for insecticide resistance monitoring (Graves et al. 1988, 1991; Plapp et al. 1990). Related species also may be attracted to these traps but may not be recognized because of their similar appearance or because the wings are often void of the scales needed for identification. Kaae et al. (1973) reported a moderate degree of cross attraction between males of the bollworm and *Heliothis phloxiphaga* Grote and Robinson to traps baited with virgin females of the opposite species. Later, Raina et al. (1986) demonstrated a low level (< 5%) of cross-attractiveness of bollworm males by *H. phloxiphaga* females. In California, *H. phloxiphaga* was attracted to traps baited with the bollworm pheromone, and the potential for misinterpretation of trap catch data was greatest in May, June, and July when both species were present (Hoffmann et al. 1991).

Heliothis phloxiphaga is very similar in appearance to the bollworm, but the darker markings of the fore wings are usually more distinct than in the bollworm (Lange and Michelbacher

[1]*Helicoverpa zea* (Boddie) (LEPIDOPTERA: NOCTUIDAE)
[2]*Heliothis virescens* (F.) (LEPIDOPTERA: NOCTUIDAE)

1937), and the discal spot on the hind wing is large and black while in the bollworm it varies from a faint vein line to a distinct lunule which is narrower than in *H. phloxiphaga* (Forbes 1954). The genitalia were illustrated by Matthews (1991). In the U.S., it occurs from Washington to California, east to Texas in the south and Massachusetts in the north (Forbes 1954, Crumb 1956). This species also has been collected in Louisiana (V. A. Brou, personal communication) and in Washington County, MS. (Stoneville Exp. Stn., 1-18-June-1986, R. E. Furr, Jr [MEM]). It was recorded from hosts in 12 plant families by Matthews (1991), but there were no records from Malvaceae.

Two species of *Heliocheilus*, *H. paradoxus* Grote and *H. turbata* (Walker), occur in Louisiana (V. A. Brou, personal communication). These species were placed in *Raghuva* (Forbes 1954) and *Heliothis* (Kimball 1965, Hardwick 1970) before Matthews (1987) removed *Heliocheilus* from synonymy with *Heliothis*. Forbes (1954) gave a brief description of the adults, and Matthews (1991) reported that known host records indicate that larvae of this genus feed on grasses (Poaceae).

The adult of *H. paradoxus* was illustrated by Holland (1920), Kimball (1965), and Matthews (1991); the genitalia were illustrated by Hardwick (1970) and Matthews (1991). Forbes (1954) reported the distribution as New Jersey to Florida, west to Minnesota, Colorado and Arizona. Specimens of *H. paradoxus* were also collected in southern Texas (E. G. Riley, personal communication). Matthews (1991) listed its host as common bermudagrass, *Cynodon dactylon* (L.) Pers.

Heliocheilus turbata and *H. lupata* were treated as separate species (Forbes 1912, 1954; Kimball 1965; Hardwick 1970) until Poole (1989) placed the latter name as a synonym of the former one. The adult of *H. turbatus* was illustrated by Kimball (1965, as *lupatus*) and Covell (1984, as *Heliothis phloxiphagus*). The species was reported from North Carolina and Georgia by Forbes (1954) and from Florida by Kimball (1965). Specimens were collected in Green County, AR (Crowley's Ridge St. Pk., 24 July 1990, R. L. Brown [MEM]), Oktibbeha County, MS (6 mi. SW Starkville, 21 Aug. 1986, 26 Aug. 1984, and 27 Aug. 1987, R. L. & B. B. Brown [MEM]), and in College Station, TX (29 Sep. 1956, Tex. Agric. Exp. Stn. light trap, det. E. L. Todd [TAMU]). The distribution and host records given by Covell (1984) apparently refer to *H. phloxiphaga*. Forbes (1954) listed the host as *Triodia fusca* Hitchcock.

Heliothis virescens and *H. subflexa* have alternate bands of olive green and white scales on the front wings. Because of their similarity, the latter was included in a discussion of *Heliothis* spp. as cotton pests in Louisiana and Arkansas by Brazzel et al. (1953). Poole et al. (1993) reviewed the differences between the two species and reported that the tobacco budworm has a wide host range, while *H. subflexa* is usually associated with ground cherry, *Physalis* spp. The purpose of our study was to determine if collections of bollworm and tobacco budworm moths in cone traps baited with synthetic pheromones included any of the four related species which occur in Louisiana.

MATERIALS AND METHODS

Wire cone traps (Hartstack et al. 1979) were used to capture male tobacco budworm and bollworm moths during 1992. Traps were placed on the perimeter of cotton fields at 24 locations in 12 parishes (counties). Each trap was mounted on a steel reinforcement rod (1.82 m long, 0.64 cm diameter) pushed into the soil with the bottom of the cone approximately 1-1.5 m above the soil surface. Traps for each species were placed at least 50 m apart at each site to reduce interference between lures. Prevailing wind direction was not considered in trap placement.

The bollworm pheromone, zealure, was obtained from Hercon Environmental, Emigsville, Pa. The tobacco budworm pheromone, virelure, was obtained from Scentry (now Ecogen), Langhorne, Pa. Lures were placed in the center of the trap at the bottom of the large cone on a 10 cm length of wire attached to the cross brace. Lures were changed every 2 wk as suggested by Lopez et al. (1987).

Moths were collected from traps at weekly intervals in all sites but the Macon Ridge (Winnsboro, Franklin Parish) and Red River (Bossier City, Bossier Parish) Research Stations, where moths were collected daily, Monday through Friday. Catches were refrigerated or frozen until they could be examined. Species other than Heliothinae were discarded.

Bollworm trap catches were examined for the presence of *Heliothis phloxiphaga*, *Heliochilus paradoxa*, and *H. turbata*. Tobacco budworm trap catches were examined for the presence of *Heliothis subflexa*.

In *Heliochilus*, the male forewing is modified with the costa bowed and swollen at about the middle of the wing, a character that is lacking in the other two genera (Matthews 1991). *Helicoverpa zea* males were identified by the cluster of coarse specialized scales on the underside of the prothoracic femur that are lacking in *Heliochilus* (Hardwick 1965, Matthews 1991) and *Heliothis phloxiphaga*. Similar scales are present in *H. virescens* and *H. subflexa* (Poole et al. 1993), but they are not as distinct and, usually, enough of the wing pattern remained to make separation possible without reference to the legs or genitalia. If specimens lacked the prothoracic legs or the scales on the fore femur, the front wings and/or the male genitalia were examined. In the bollworm, the valve is broad, the uncus is straight at the tip, and the aedeagus has well-developed thorn-like cornuti. In *H. phloxiphaga*, the valve is narrow, the uncus is curved at the tip, and the aedeagus is about 1/3 shorter with a scrobinate bar at the distal end and without the barbs illustrated by Lange and Michelbacher (1937).

Specimens of *H. virescens* were identified by removing and examining the claspers. To make examination easier, moths were drenched with alcohol and then placed in a sieve under hot running water to remove the scales and soften the abdomen. In *H. virescens*, the saccus is broadly rounded, and the base of the valve has a large hair pencil on the outer face. In *H. subflexa*, the saccus is pointed, the base of the valve lacks a large hair pencil, and the hairs are reduced to a fine line.

RESULTS AND DISCUSSION

Trap locations and number of specimens examined are given in Table 1. Only males of *H. virescens* comprised the 49,729

specimens from traps baited with tobacco budworm lure. According to Chapin and Callahan (1967), *H. subflexa* has been collected in the vicinity of Baton Rouge from May to November. Specimens in the Louisiana State Arthropod Museum were collected on 17 April and 23 November in Edgard by V. A. Brou. There is, however, no information on how common this species is in the state.

TABLE 1: Trap Locations and Numbers of Heliothinae Examined.

Trap location			Number of specimens examined	
Parish (county)	Nearest town	Collection dates	Bollworm traps	Tobacco budworm traps
Bossier	Bossier City	02/24-11/10	17,574	26,890
Caddo	Belcher	07/01-09/15	557	---
		07/01-09/29	---	2,354
	Dixie	05/29-09/15	1,090	---
		05/29-09/29	---	3,170
	Gilliam	06/23-09/15	409	---
		06/12-09/29	---	7,025
Caldwell	Bosco	06/16	8	5
Catahoula	Peck	04/22-10/22	167	377
Concordia	Ferriday	05/06-10/12	186	263
Franklin	Crowville	06/16-10/08	110	243
	Jigger	07/01-09/02	119	312
	Winnsboro	03/11-10/28	2,702	---
		04/10-10/28	---	2,617
Iberville	St. Gabriel	04/07-09/28	170	484
Morehouse	Collinston	06/16-08/11	212	300
	Mer Rouge	06/23	6	3
	Oak Ridge	06/16-07/21	85	---
		06/16-08/18	---	392
Natchito-ches	Allen	04/08-10/06	258	1,113
	Derry	04/08-09/10	266	1,135
Rapides	Alexandria	04/08-09/11	207	66
	Cheneyville	05/15-09/22	673	---
		05/22-09/22	---	345
	Lecompte	10/06	2	---
		06/03-10/06	---	183
Richland	Archibald	07/21-10/08	52	---
		06/02-10/08	---	550
	Hebert	08/31-10/29	---	243
Tensas	Newellton	06/16-07/27	25	---
		04/22-08/11	---	270
	St. Joseph	05/06-08/11	91	---
		04/22-08/18	---	125
	Waterproof	04/09-10/12	202	544
		04/09-09/28	268	---
		04/16-10/12	---	532
		04/16-09/28	380	---
		04/22-10/12	---	188
		Totals	25,819	49,729

The 25,819 Heliothinae collected in the traps baited with synthetic bollworm lure included 25,784 male and 9 female bollworms, one *H. phloxiphaga*, 10 odd "*zea*", and 15 tobacco budworms (1, sex undetermined; 7 males; 7 females).

The specimen of *H. phloxiphaga* was collected at the Red River Research Station near Bossier City on 11 May, and the 10 odd "*zea*" at the following locations: Dixie, 9 September 1992 (1), and at the Red River Research Station on 22 July (1), 2 September (4), 8 September (2), 25 September (1), and 15 October (1). The odd moths were in poor condition and the forelegs were missing. Dr. R. W. Poole (personal communication) examined two of these specimens and reported that while they looked most like the bollworm, they also had certain characteristics, in particular the light spots on the outer margins of the hindwing, of *H. phloxiphaga*. The male genitalia were malformed, and the aedeagi were poorly developed and clearly non-functional. He suggested the possibility that these were hybrids between the bollworm and *H. phloxiphaga*, a speculation that would need to be verified by attempting to produce hybrids in the laboratory.

The small number of Heliothinae, other than bollworms and tobacco budworms, that were collected would not affect the interpretation of moth captures in pheromone traps of these species or insecticide resistance monitoring data. We do not, however, know the distribution and abundance of *H. phloxiphaga* in Louisiana, and our results may not be applicable to other areas. Furthermore, our study was done over a one-year period, and populations of *H. phloxiphaga* obviously vary among years. Since there is some cross-attraction between the bollworm and *H. phloxiphaga* (Kaae et al. 1973, Raina et al. 1986, Hoffman et al. 1991), captures of moths in traps, including those made using automatic moth detection systems that are baited with zealure (Hendricks 1993), may need to be manually checked occasionally in areas where *H. phloxiphaga* is more common. Also, commercially formulated zealure may not be adequately specific for bollworm moths.

The seasonal captures of bollworm and tobacco budworm moths in traps located on the Macon Ridge and Red River Research Stations are depicted in Fig. 1. More bollworms (approximately 2.6x/trap) and tobacco budworms (approximately 4.1x/trap) were captured on the Red River Research Station compared to the Macon Ridge Research Station. Bollworm moths were captured earlier than tobacco budworm moths at both locations. Thus, subsequent generational peaks of abundance were not in synchrony. The highest populations of both species occurred in September as has been previously reported (Leonard et al. 1989). Also, 59% of all moths captured at these two sites and 66% of all moths captured at all sites during 1992 were tobacco budworms. Thus the shift toward increased abundance of tobacco budworms relative to bollworms noted by Leonard et al. (1989) has continued. In the early 1980's, bollworm moths were more abundant than tobacco budworm moths and comprised from 67 to 95% of all moths captured in pheromone-baited traps (Leonard et al. 1989). In 1986 and 1987, bollworm moths constituted only 64 and 53%, respectively, of moths captured. Significant levels of resistance to carbamate, organochlorine, organophosphorus and pyrethroid insecticides in tobacco budworms (Sparks et al. 1993) has no

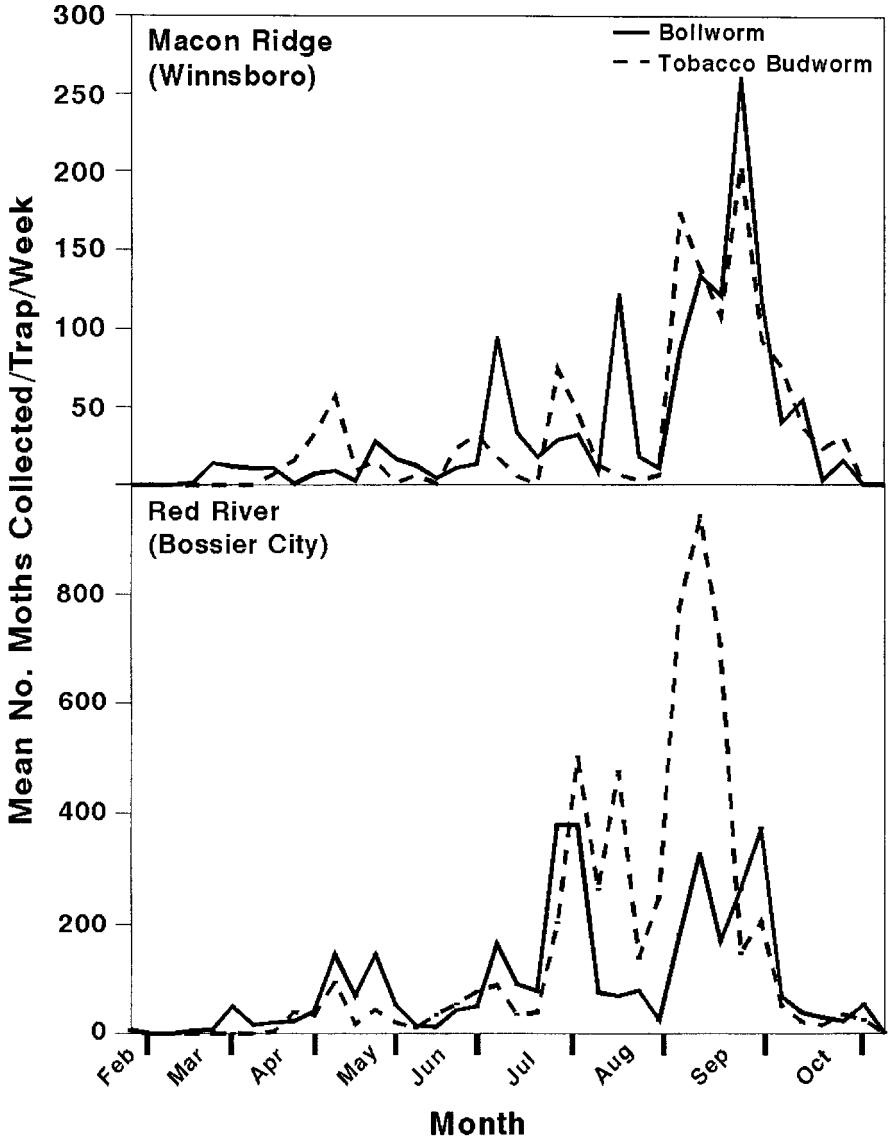

FIG. 1. Seasonal captures of bollworm and tobacco budworm moths in pheromone-baited wire cone traps at the Macon Ridge (Winnsboro) and Red River (Bossier City) Research Stations during 1992.

doubt contributed to this shift in relative abundance of these two species.

ACKNOWLEDGMENT

The authors wish to thank Vernon Brou, 74320 Jack Loyd Rd., Abita Springs, LA, for the identification of *Heliocheilus paradoxus* and for information about the occurrence of *Heliocheilus* spp. and *Heliothis phloxiphaga* in Louisiana; Robert W. Poole, USDA Systematic Entomology Laboratory, Washington, D.C., for identifying the possible hybrids between *Helicoverpa zea* and *Heliothis phloxiphaga*; and Richard L. Brown, Mississippi Entomological Museum (MEM), Starkville, MS, and Edward G. Riley, Texas A&M University (TAMU), College Station, TX, for permission to use the data from specimens in their collection. This manuscript is approved for publication by the Director of the Louisiana Agricultural Experiment Station as manuscript number 96-17-0308.

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SUSCEPTIBILIDAD DEL PARASITOIDE *CEPHALONOMIA STEPHANODERIS* [1] A DIFERENTES CEPAS DE *BEAUVERIA BASSIANA* Y *METARHIZIUM ANISOPLIAE* [2]

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ABSTRACT

Laboratory bioassays at 27 ± 2 ^{0}C, 90 ± 5% RH and 12:12 h L:D were used to determine the susceptibility of *Cephalonomia stephanoderis* Betrem (Hymenoptera: Bethylidae), a parasitoid of the coffee berry borer *Hypothenemus hampei*, to 9 strains of the entomopathogenic fungus *Beauveria bassiana* and 5 strains of *Metarhizium anisopliae* (Metsch.) Sorokin. The most virulent strains of *B. bassiana* were from Ecuador (code named Bb-4) and two from Mexico (Bb-26 and Bb-17) with CL_{50}'s of $5.3x10^6$, $21.9x10^6$ and $29.3\ x10^6$ conidia/ml respectively. Three aggressive strains of *M. anisopliae* were identified (code named Ma5, Ma23 and Ma4) with CL_{50} values of $5.4x10^7$, $7.6x10^7$ and $10.6x10^7$ conidia/ml respectively. Lethal time (LT_{50}) tests with *B. bassiana* showed a range of values between 3.8 and 6.8 days with a mean of 5.3 days. LT_{50} values *in M. anisopliae* ranged from 4.4 to 7.2 days with a mean of 5.8 days. The highest incidence of sporulation of *B. bassiana* on parasitoid cadavers was observed in strains Bb-4 (Ecuador), Bb-17 and Bb26 (Mexico) all with values of 90%. In the case of *M. anisopliae*, the highest incidence of sporulation on insects was observed in strains Ma5 (84.3%), Ma4 (71.6%) and Ma23 (62.7%).

RESUMEN

Mediante bioensayos en laboratorio, bajo condiciones controladas a 27 ± 2 ^{0}C, 90 ± 5% de HR y 12:12 horas L:O, se determinó la susceptibilidad del parasitoide *Cephalonomia stephanoderis* Betrem (Hymenoptera: Bethylidae), enemigo natural de la broca del café *Hypothenemus hampei* (Ferrari) (Coleoptera: Scolytidae), a nueve cepas del hongo entomopatógeno *Beauveria bassiana* (Bals.) Vuill., y cinco cepas de *Metarhizium anisopliae* (Metsch.) Sorokin. Los esultados obtenidos demostraron que las cepas más virulentas de *B.bassiana* fueron la Bb-4 (Ecuador), Bb-26 (México) y Bb-17 (México) con concentraciones letales medias (CL_{50}) de $5.3x10^6$, $21.9x10^6$ y $29.3\ x\ 10^6$ conidios/ml de suspensión. Para *M.anisopliae* las cepas más agresivas fueron la Ma5, Ma23 y Ma4 con CL_{50} de $5.4x10^7$, $7.6x10^7$ y $10.6\ x\ 10^7$ conidios/ml respectivamente. El tiempo letal medio (TL_{50}) se ubicó entre 3.8 a 6.8 días con un promedio de 5.3 días para *B. bassiana* y 4.4 a

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[2] Deutoromycotina: Hyphomycetes

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7.2 días con un promedio de 5.8 dìas para *M. anisopliae*. Los máximos porcentajes de insectos esporulados con *B. bassiana* sobre los cadáveres del parasitoide lo obtuvieron las cepas Bb-4 (Ecuador), Bb-17 (México) y Bb-26 (México) todas con un 90%. Para *M. anisopliae* los máximos porcentajes los obtuvieron las cepas Ma5 (84.3%), Ma4 (71.6%) y Ma23 (62.7%).

INTRODUCCION

La cafeticultura en México es una de las principales actividades agrícolas distribuídas en 760, 786 has cultivadas e importantes en la captación de divisas para el país (285.4 millones de dólares en 1993). A esta actividad se dedican poco más de dos millones de mexicanos, lo que representa el 10% de la población rural (Santoyo *et al.*, 1994). En México se reportan 16 plagas de importancia económica y 17 enfermedades para el cultivo de café, siendo la Broca del Café *Hypothenemus hampei* (Ferr.) (Coleoptera: Scolytidae) la principal plaga (Regalado, 1993). *H. hampei* fue introducida a México en 1978 (Baker, 1984), y los daños que ocasiona varían de un 21 a 50% cuando no se hace ninguna medida de control (Benavides *et al.*, 1990). El método de control más eficaz contra esta plaga ha sido el químico, utilizando el insecticida endosulfán (Ingram, 1965), sin embargo, una de las posibles limitaciones de este producto es el desarrollo de resistencia de la plaga, tal como sucedió en Nueva Caledonia, Oceanía (Brun *et al.*, 1989). El control biológico de *H. hampei* en México se inició en 1988 con la importación desde Africa del parasitoide *Cephalonomia stephanoderis* Betrem (Hymenoptera: Bethylidae), constituyendo este hecho un gran logro para el control biológico de la broca del café, ya que se ha tenido una eficacia del 22 al 56% de control (Barrera *et al.*, 1989, Barrera, 1994). Murphy y Moore (1990), mencionan a los hongos entomopatógenos *Beauveria bassiana* (Bals.) Vuill. y *Metarhizium anisopliae* (Metschnikoff) Sorokin (Deuteromycotina) como agentes potenciales en el control biológico de *H. hampei*. Tanto los hongos como el parasitoide brindan nuevas posibilidades en el manejo integrado de la plaga (Alonzo, 1985; Barrera *et al.*, 1990). Sin embargo, no existe información sobre la actividad biológica de *B. bassiana* y *M. anisopliae* sobre *C. stephanoderis*. Por tal razón el objetivo de la presente investigación fue determinar las susceptibilidad del parasitoide a diferentes cepas de ambas especies de entomopatógenos.

MATERIALES Y METODOS

Origen de las cepas. Se utilizaron nueve cepas del hongo *B. bassiana* y cinco de *M. anisopliae* obtenidas de Brasil, Ecuador y México (Tabla 1). A cada cepa se le asignó una clave de acuerdo con la fecha de recepción del material biológico en el cepario del laboratorio de patología de insectos de El Colegio de la Frontera Sur (ECOSUR) ubicado en Tapachula, Chiapas, México.

Producción de conidios. Con la finalidad de uniformizar el sustrato de crecimiento y evitar mutaciones de los hongos, todas las cepas fueron sembradas en el medio de cultivo SDA (40 g de dextrosa, 10 g de peptona, 15 g de agar, 0.2% extracto de levadura, 40 mg del antibiótico tetraciclina para 1000 ml de agua destilada). El medio de cultivo se distribuyó en cajas de Petri estériles de 90 x 15 mm y en tubos de ensayos (22 x 175 mm) y el sembrado de conidios se llevó a cabo dentro de una campana de flujo de aire. El medio inoculado con los hongos se mantuvo a 27 ± 2 °C y humedad relativa (HR) de 80 ± 5% y 12:12 horas L:O, para inducir el crecimiento y esporulación. Después de 12 a 15 días los conidios fueron cosechados raspando el contenido de cada caja de Petri o del tubo de ensayo con una asa bacteriológica estéril y se almacenaron a 4 °C en recipientes de plástico.

TABLA 1. Origen de las cepas de *B. bassiana* (Bb) y *M. anisopliae* (Ma) utilizadas en los bioensayos sobre el parasitoide *C. stephanoderis*.

Pais	Clave de la cepa	Hospedero original	Orden: Familia
"	Bb-14	*Chalcodermus aeneus*	Coleoptera: Curculionidae
Brasil	Bb-16	*Chalcodermus aeneus*	Coleoptera: Curculionidae
	Bb-4	*Hypothenemus hampei*	Coleoptera: Scolytidae
Ecuador	Bb-8	*Leptinotarsa* sp.	Coleoptera: Chrysomelidae
México	Bb-17	No identificado	Lepidoptera
	Bb-18	No identificado	Lepidoptera
	Bb-24	*H. hampei*	Coleoptera: Scolytidae
	Bb-25	*H. hampei*	Coleoptera: Scolytidae
	Bb-26	*H. hampei*	Coleoptera: Scolytidae
	Ma3	Cepa comercial (USA)	Homoptera: Cercopidae
	Ma4	*Diatraea saccharalis*	Lepidoptera: Pyralidae
	Ma5	*Spodoptera frugiperda*	Lepidoptera: Noctuidae
	Ma10	*Geraeus senilis*	Coleoptera: Curculionidae
	Ma23	*Macrodactylus murinus*	Coleoptera: Scarabaeidae

Viabilidad de los conidios. La viabilidad de los conidios se verificó antes de llevar a cabo los bioensayos de patogenicidad, usando el método de microcultivos (Jímenez, 1989). Los microcultivos se prepararon poniendo una capa de SDA sobre la superficie de portaobjetos, que posteriormente fueron inoculados con los hongos. El inóculo se preparó suspendiendo una cantidad pequeña de conidios (0.5 mg) en 5 ml de agua destilada estéril; después esta suspensión se mezcló con 0.025% del dispersante-adherente Tritón X-100 en un tubo de vidrio con tapón de rosca (16 x 125 mm) y se homogenizó en un vortéx a alta velocidad durante 45 segundos. De la suspensión resultante se tomaron alicuotas de 0.3 ml (10^6 esporas/ml) para inocular las placas de SDA. Los portaobjetos así preparados se colocaron en cajas de Petri sobre papel filtro humedecido y se incubaron durante 36 horas en las condiciones ambientales arriba citadas. Los microcultivos se observaron usando un microscopio óptico compuesto y la viabilidad de las esporas se determinó en base a la presencia del tubo germinativo en 100 a 200 conidios por portaobjeto, considerando una cepa viable aquellas que registraron más del 90% de esporas germinadas.

Cría del parasitoide. En los bioensayos se utilizaron hembras adultas de *C. stephanoderis* obtenidas de una cría de los laboratorios de ECOSUR a partir de frutos de café infestadas naturalmente por la broca del café, técnica establecida por Barrera *et al* (1989). Los parasitoides fueron colectados del laboratorio un día antes de llevar a cabo los bioensayos.

Ensayo preliminar de patogenicidad. Con la finalidad de seleccionar las cepas más virulentas, se utilizó un diseño experimental completamente al azar, utilizando grupos de 96 parasitoides adultos (8 repeticiones de 12 insectos cada una), se sumergieron durante 30 segundos en 0.5 ml de suspensión de cada cepa, a concentraciones de 1 x10^8 con/ml para *B. bassiana* y *M. anisopliae*. Un grupo de parasitoides se dejó como testigo (sin conidios) tratándolos únicamente con agua destilada estéril y 0.025% de Tritón X-100. Los insectos tratados se colocaron en una caja de Petri que contenía papel filtro estéril para eliminar el exceso de humedad y posteriormente se traspasaron a tubos de vidrio de fondo plano (1.7 x 8 cm) cerrados con malla fina. En cada tubo se colocaron 12 insectos, alimentándolos

diariamente con miel de abeja diluida en agua (relación 2:1; Barrera *et al.*, 1993), los tubos fueron mantenidos en una cámara húmeda a 27 ± 2 °C, 90 ± 5 % de HR y 12:12 horas (luz:obscuridad). Las observaciones de mortalidad del parasitoide se realizaron diariamente, llevando un registro por cada cepa evaluada; los parasitoides muertos se colocaron en cámaras húmedas para propiciar la emergencia del micelio de los hongos y comprobar que la muerte de los insectos se debió al efecto de los entomopatógenos. Los porcentajes de mortalidad aparente fueron corregidos utilizando la formula de Abbot (1925) para obtener el porcentaje de la mortalidad real.

Determinación del tiempo letal medio (TL_{50}). Basándose en el ensayo preliminar, todas las cepas se compararon simultáneamente en un bioensayo con el fin de estimar el tiempo letal para matar al 50% de los insectos tratados. Se utilizaron grupos de 96 insectos por cepa con ocho repeticiones de 12 insectos cada una, sumergiéndolos durante 30 segundos en suspensiones de 10^8 conidios/ml. Los insectos se colocaron en tubos de vidrio y se introdujeron a una cámara húmeda a condiciones ambientales controladas antes mencionadas. El número de insectos muertos se registró diariamente durante 10 días.

Determinación de la concentración letal media (CL_{50}). Se evaluaron todas las cepas utilizando las concentraciones siguientes 10^5, 10^6, 10^7 y 10^8 conidios/ml determinadas con un hemocitómetro (Neubauer). La metodología para esta prueba fue similar a la descrita anteriormente en el ensayo de patogenicidad. Los resultados fueron analizados usando el programa PcProbit (Camacho, 1990) del Centro de Estadística y Cálculo (CEC) del Colegio de Posgraduados de Montecillo, México.

RESULTADOS Y DISCUSION

Bioensayo preliminar de patogenicidad. En la prueba preliminar de patogenicidad todas las cepas de *B. bassiana* y *M. anisopliae* resultaron ser patógenas al parasitoide *C. stephanoderis* (Tabla 2). Los porcentajes corregidos de mortalidad aplicando la fórmula de Abbot (1925) variaron del 78.9 al 100% para *B. bassiana* y de 72.4 al 100% para *M. anisopliae*. Las cepas Bb14 y Bb24 fueron estadísticamente menos patógenas que las demás cepas evaluadas de acuerdo a la prueba de Tukey ($P< 0.05$). Las siete cepas más patógenas al enemigo natural causaron mortalidades entre 84 y 100%. En el caso de *M. anisopliae*, las cepas Ma4 y Ma23 causaron estadísticamente los mayores porcentajes de mortalidad (100 y 93.8% respectivamente). Aunque no existe ningún reporte del efecto de estos hongos sobre *C. stephanoderis*, sí existe en otros enemigos naturales como lo mencionan Gusev *et al* (1977); Donegan y Lighthart (1989), al reportar que *B. bassiana* es patógeno a *Crysoperla* spp (Neuroptera: Chrysopidae). Balfour-Browne (1960), establece que *Campsomermis quadrifasciata* (Hymenoptera: Scoliidae) es afectado por *M. anisopliae*. Este ensayo muestra una variabilidad en la patogenicidad de las cepas de *B. bassiana* y *M. anisopliae* sobre *C. stephanoderis*. Esta variabilidad puede ser atribuída, según Roberts (1989), a la diversidad bioquímica de los hongos con respecto a su hospedero, a su distribución geográfica y a la concentración de las enzimas presentes como proteasas, lipasas y/o quitinasas.

Tiempo Letal Medio (TL_{50}). El TL_{50} para las nueve cepas del hongo *B. bassiana* varió de 3.8 a 6.8 días. Según la prueba de Tukey ($P<0.05$), no se presentaron diferencias significativas entre las cepas con respecto al TL_{50} (Tabla 2). Se detectaron diferencias numéricas en los tiempos letales, sin embargo el valor entre el TL_{50} mínimo y el máximo fue de 3 días. En el caso de *M. anisopliae*, el TL_{50} para las cincos cepas estudiadas varió de 4.4 a 7.2 días; tampoco se presentaron diferencias estadísticas entre las cepas (Tabla 2). La diferencia del TL_{50} entre el valor máximo y mínimo fue de 2.8 días. Generalmente los aislamientos de *B. bassiana* tienen una alta virulencia para el hospedero de donde son

aislados o para especies que pertenecen al mismo orden de insectos; sin embargo, en algunos casos el hongo puede ser más agresivo hacia otro tipo de insectos, no importando el hospedero de origen (Xu, 1988).

TABLA 2. Porcentajes de mortalidad y Tiempo Letal Medio (TL_{50}) de hembras adultas del parasitoide *C. stephanoderis* causadas por *B. bassiana* y *M. anisopliae*.

Cepas	Mortalidad (%)	Mortalidad corregida (%) Abbot (1925)	TL_{50} ± D. E.
Bb4	98.0	97.8 a *	4.7 ± 1.3 a*
Bb8	85.0	84.2 a	4.6 ± 1.1 a
Bb14	80.0	78.9 b	6.8 ± 1.6 a
Bb16	90.0	89.4 a	5.2 ± 0.7 a
Bb17	100.0	100.0 a	4.3 ± 1.2 a
Bb18	95.0	94.7 a	5.6 ± 1.5 a
Bb24	82.0	81.0 b	3.8 ± 1.6 a
Bb25	97.0	96.8 a	4.8 ± 1.1 a
Bb26	97.0	96.8 a	4.3 ± 1.4 a
Control	5.0	0.0	
Ma3	76.0	72.4 b	5.6 ± 0.8 a
Ma4	100.0	100.0 a	4.9 ± 1.2 a
Ma5	76.0	72.4 b	7.2 ± 0.6 a
Ma10	76.0	72.4 b	4.4 ± 1.4 a
Ma23	94.0	93.8 a	5.8 ± 1.3 a
Control	2.0	0.0	

* Los valores seguidos por la misma letra no son diferentes estadísticamente de acuerdo a la prueba de Tukey ($P < 0.05$).

Concentración Letal Media (CL_{50}). En la Tabla 3 se presentan los resultados de la virulencia correspondientes al análisis Probit (Finney, 1971) para las nueve cepas de *B. bassiana* y las cinco cepas de *M. anisopliae* sobre *C. stephanoderis*. Las tres cepas más virulentas de *B. bassiana* fueron la Bb-4 (Ecuador), Bb-26 (México) y Bb-17 (México), registrando los siguientes valores CL_{50}: (límites fiduciales al 95%): 0.007 (0.005-008), 0.022 (0.017-0.028) y 0.029 (0.023-0.035), correspondiendo a 5.3, 21.9 y 29.3 x 10^6 conidios/ml de suspensión.

De acuerdo con la pendiente de las ecuaciones la respuesta más homogénea la obtuvieron la Bb-18 (México) con un valor de 1.80 ± 0.1 y la Ma10 con 1.14 ± 0.7. Las respuestas más heterogéneas fueron manifestados por la Bb-24 (México) con un valor de la pendiente de 1.06 ± 0.3 y la Ma4 con 0.5 ± 1.6. La variación en la virulencia de las cepas pudo estar relacionada con la actividad y producción de enzimas durante el curso de penetración de la cutícula del hospedero, la especie del hongo, la cepa (patotipo) y/o las pruebas de combinación de hospederos (Hall, 1982; Ignoffo *et al.*, 1982, Morrow *et al.*, 1989; Roberts, 1989; Bidochka y Khachatorians, 1990; Hayden *et al.*, 1992; Ignoffo y Boucias, 1992).

TABLA 3. Resultados del bioensayo de concentración letal (CL_{50}) de varias cepas del hongo *B. bassiana* y *M. anisopliae* sobre adultos hembras del parasitoide *C. stephanoderis*.

Cepas	CL_{50} y límites fiduciales al 95%	Concentración Conidios /ml	CL_{95}	Pendiente ± Error estándar
Bb-4	0.007 (0.005 - 0.008) a*	5.3×10^6	0.096	1.53 ± 0.2
Bb-26	0.022 (0.017 - 0.028) ab	21.9×10^6	0.287	1.19 ± 0.3
Bb-17	0.029 (0.023 - 0.035) b	29.3×10^6	0.366	1.50 ± 0.2
Bb-18	0.030 (0.024 - 0.042) bc	30.0×10^6	0.239	1.80 ± 0.1
Bb-16	0.034 (0.028 - 0.042) c	36.3×10^6	0.404	1.54 ± 0.6
Bb-25	0.037 (0.030 - 0.045) cd	44.5×10^6	0.287	1.49 ± 0.4
Bb-8	0.064 (0.052 - 0.079) d	45.2×10^6	0.967	1.40 ± 0.4
Bb-14	0.072 (0.058 - 0.088) d	58.1×10^6	0.913	1.49 ± 0.2
Bb-24	0.081 (0.063 - 0.105) d	79.4×10^6	2.938	1.06 ± 0.3
Ma5	0.033 (0.027 - 0.040) a	5.4×10^7	1.83	0.94 ± 1.2
Ma23	0.050 (0.036 - 0.068) ab	7.6×10^7	21.76	0.62 ± 1.4
Ma4	0.059 (0.042 - 0.080) b	10.0×10^7	113.34	0.50 ± 1.6
Ma3	0.079 (0.065 - 0.951) bc	10.6×10^7	3.06	1.03 ± 0.4
Ma10	0.084 (0.070 - 1.495) c	24.6×10^7	1.11	1.14 ± 0.7

* Los valores seguidos por la misma letra no son diferentes estadísticamente de acuerdo a los límites fiduciales al 95%.

Emergencia del micelio de B. bassiana y M. anisopliae sobre C. stephanoderis. Después de 24 horas, el micelio de *B. bassiana* emerge de las partes intersegmentales del cuerpo de *C. stephanoderis*; a las 48 horas el micelio cubre totalmente el cuerpo dejando libre solamente la cabeza, la parte superior del tórax y el abdomen; a las 72 horas se presenta una esporulación completa y el hongo cubre totalmente al insecto, dando el aspecto de una pelotita de color blanco-yeso de apariencia algodonosa. Estas características de emergencia del micelio de *B. bassiana* han sido reportadas para otros insectos por Roberts (1989). En *M. anisopliae* se observa que a las 24 horas emerge un micelio de color blanco por la región bucal, la región pigidial, las uniones de la cabeza-tórax, y tórax-abdomen y la base de las alas; a las 48 horas el micelio toma un color verde olivo; y a las 72 horas, el micelio se observa de un color verde obscuro cubriendo parcialmente el cuerpo del parasitoide y alcanzando una esporulación completa; para entonces, el micelio presenta una forma de empalizada, característica muy común de este patógeno (Alves, 1986; Butt *et al.*, 1992).

Esporulación de B. bassiana y M. anisopliae sobre C. stephanoderis. El porcentaje de insectos esporulados fue calculado en base al número de cadáveres de *C. stephanoderis* que mostraban los signos de la infección de los hongos. Estos datos fueron corregidos por transformación angular (Little y Jackson, 1989). Algunos de los parasitoides muertos durante los bioensayos no mostraron la evidencia del hongo esporulado, aún cuando los insectos fueron colocados en cámaras húmedas (HR: 90 ± 5%) para estimular su emergencia, tal como lo mencionan Franz (1971); Moore (1973) y Ramoska (1984). Un análisis de regresión lineal aplicado a los datos de cada una de las especies de entomopatógenos mostró una relación positiva entre la dosis (con/ml) utilizada de inóculo (x) y el porcentaje de insectos esporulados (Y), *B. bassiana* ($r^2 = 0.89$; $Y = 5.80 + 0.75X$) y *M. anisopliae* ($r^2 = 0.92$; $Y = 5.48 + 0.52X$) (Fig. 1 y 2). Los máximos porcentajes de

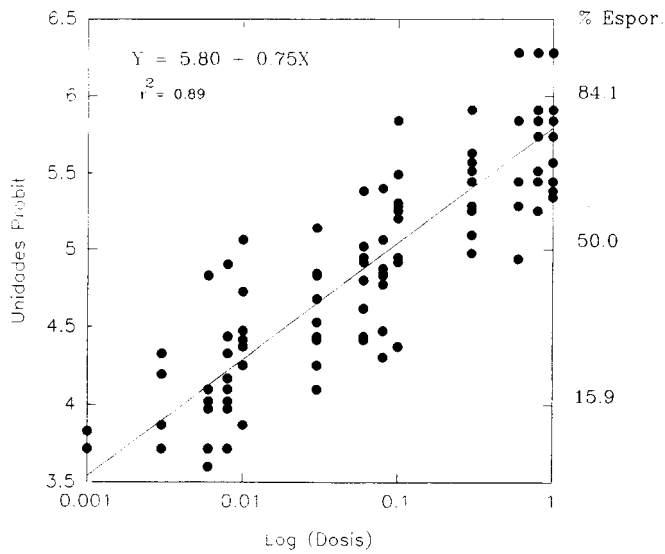

FIG. 1. Relación entre la dosis y el porcentaje de esporulación de nueve cepas de *B. bassiana* sobre el parasitoide *C. stephanoderis*.

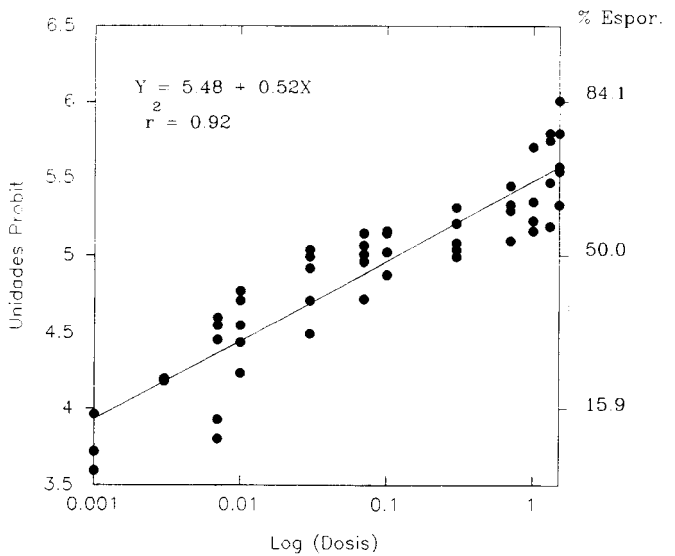

FIG. 2. Relación entre la dosis y el porcentaje de esporulación de cinco cepas de *M. anisopliae* sobre el parasitoide *C. stephanoderis*.

esporulación de *B. bassiana* los obtuvieron las cepas Bb-4, Bb-17 y Bb-26, todas con un 90%; el menor porcentaje de esporulación se presentó en la Bb-14 (67%). Las cepas de *M. anisopliae* que obtuvieron los porcentajes más altos de esporulación fueron la Ma5 (84.3%), la Ma4 (71.6%) y la Ma23 (62.7%). Los resultados de este estudio en condiciones de laboratorio indican que los hongos *B. bassiana* y *M. anisopliae* son patógenos al parasitoide *C. stephanoderis*, sin embargo no se conoce la interacción que puedan tener ambos enemigos naturales (hongos-parasitoide) en condiciones de campo para integrarlo en el control biológico de la broca del café.

AGRADECIMIENTOS

Esta Investigación se llevó a cabo gracias al apoyo financiero brindado por El Consejo Nacional de Ciencia y Tecnología (CONACyT) de México, proyecto 2010-N9301. Se agradece el apoyo del personal Técnico del Proyecto Broca del café de ECOSUR. Así mismo queremos agradecer a J. F. Barrera por la revisión del manuscrito.

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PRESENCIA DE CUCARACHAS EN VIVIENDAS DE LA PAZ, BAJA CALIFORNIA SUR, MEXICO

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Hasta ahora se conocen de 3,500 a 4,000 especies de cucarachas en el mundo; de éllas solo el 1% están asociadas al hombre y son consideradas como sinantrópicas o plagas domésticas de importancia sanitaria (Harwood y James 1987).

En Norte América existen 69 especies de cucarachas que se agrupan en 32 géneros del Canadá, Estados Unidos y el Norte de México (Atkinson et al. 1991). De ellas *Blattella germanica* (Linneo), *Blatta orientalis* (Linneo) y *Periplaneta americana* (Linneo) están ampliamente distribuídas en México (Morón y Terrón 1988).

Debido a la importancia sanitaria de las cucarachas (Cloarec et al. 1992) y a los escasos estudios que sobre ellas se han publicado en México, en este trabajo se dan a conocer las especies de blátidos de la ciudad de La Paz, Baja California Sur, su incidencia y sus posibles enemigos naturales.

El estudio se realizó de Enero a Noviembre de 1993, en esta localidad, ubicada a los 24° 09' N y 110° 20' O. La vegetación que rodea a la ciudad es del tipo matorral sarco-crasicaule (Wiggins 1980, Domínguez 1992). El clima es muy seco y árido, la humedad relativa media anual es de 62 % y la temperatura media anual es de 22 a 24° C (Domínguez 1992).

Se llevaron a cabo once recolecciones mensuales en el interior y exterior de 30 viviendas de la ciudad de La Paz seleccionadas al azar. La captura se realizó manualmente conservando a los insectos en alcohol al 70%. En seis viviendas, se realizaron observaciones matutinas durante una hora y media, para conocer depredadores de las cucarachas; se colectaron 15 ootecas, que se mantuvieron en frascos de vidrio para la obtención de sus parasitoides.

Se obtuvo un total de 1,267 ejemplares pertenecientes a cuatro familias, ocho géneros y nueve especies de cucarachas: *Neostylopyga rhombifolia, Periplaneta americana,* (Blattidae); *Arenivaga sp.1, Arenivaga sp.2,* (Polyphagidae); *Blattella germanica, Supella longipalpa, Symploce pallens,* (Blattelidae); *Nauphoeta cinerea y Pycnocelus surinamensis* (Blaberidae); las tres últimas se registran en este trabajo, por primera vez para Baja California Sur.

Es probable que *Symploce pallens, Nauphoeata cinerea* y *Pycnocelus surinamensis*, consideradas como plagas domésticas, hayan sido recientemente introducidas en la ciudad de La Paz por vía marítima, por cargamentos de frutas, tal y como han sido transportadas otras especies no domiciliarias desde América a otras partes del mundo (Roth y Willis 1960). Estas especies sólo han sido citadas para los estados de la República Mexicana que limitan el Golfo de México (Atkinson et al. 1991).

Las familias con mayor riqueza específica fueron Blattellidae (47%) y Blattidae (42%) y las especies más abundantes fueron *P. americana* (77%), *S. pallens* (42%), y *S. longipalpa* (28%) y *B. germanica* (24%).

Periplaneta americana fué la especie más numerosa (38%) tanto en el exterior (38%) como en el interior (40%) de las viviendas (Fig. 1a,b). Se registró durante todo el año con

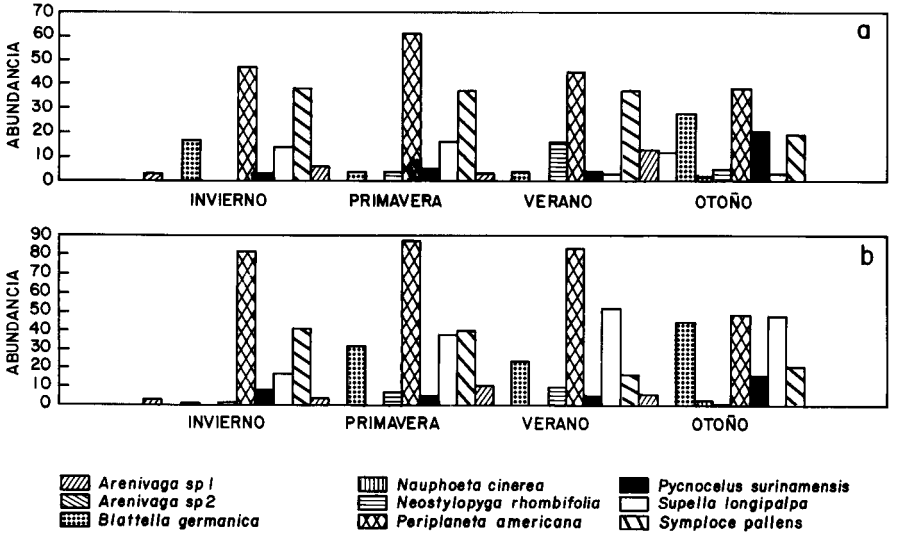

Fig. 1 Incidencia de organismos del orden Dictyoptera colectados en el exterior (a) e interior (b) en casas-habitación a través del período invierno-otoño de 1993.

mayor incidencia durante la primavera. Harwood y James (1987), mencionan que esta especie entra en diapausa en el otoño e invierno en zonas templadas, sin embargo para esta región desértica, sólo fue menos frecuente en estas estaciones. En las viviendas localizadas en la periferia de la ciudad de La Paz, donde el mal manejo de los desechos de basura proporcionan alimento y refugio, hubo una mayor abundancia de esta especie; es por ello que la alta incidencia de *P. americana*, probablemente se debió a que estos insectos migraron de estos lugares a las viviendas.

Symploce pallens especie extradomiciliaria (16%) fue más abundante durante el invierno y el verano (Fig. 1a,b); se le encontró en los árboles frutales de: limón (*Citrus aurantifolia*), mango (*Mangifera indica*), guayaba (*Psidium guajava*), tamarindo (*Tamarindus indica*) y en benjaminas (*Ficus benjamina*), eucaliptos(*Eucalyptus sp.*) y palmas (*Puenis dactilyfera* y *Coconut palm*) alimentándose de los frutos maduros, por lo que su incidencia en el interior de las viviendas fue menor.

S. longipalpa tuvo mayor incidencia dentro de los domicilios durante el verano (21%), en recámaras y cuartos de enseres; mientras que *B. germanica* fue más abundante en el otoño (13%), en baños y cocinas (Fig. 1a,b). Esta última especie no es territorial (Rivault 1990) y es probable que la migración de las hembras adultas se lleve a cabo durante estas estaciones, tal y como sucede en Alabama y Texas (Akers y Robinson 1981, Appel y Tucker 1986). *B. germanica*, *S. longipalpa* también se les

encontró en muebles y aparatos electrodomésticos de viviendas con malas condiciones sanitarias. Ramírez (1989), Akers y Robinson (1981) indican que por medio de esta vía, estas especies invaden ciudades enteras.

P. surinamensis, *N. rhombifolia*, *Arenivaga* sp 1, *Arenivaga* sp 2 y *N. cinera*, fueron menos numerosas en los domicilios, teniendo en verano y otoño su máxima incidencia (Fig. 1a,b). Estas cucarachas fueron colectadas en los jardínes particulares (*P. surinamensis*), en las viviendas cerca de la Bahía de La Paz (*N. cinerea*) y en las ubicadas en la periferia (*Arenivaga sp. 1 y 2 y N. rhombilofia*). Esta distribución se debe a que estas especies son de hábitos silvestres en climas cálido-húmedos o cálido-secos; pero llegan a invadir las zonas suburbanas y urbanas, para establecerse principalmente en áreas verdes (Hawke y Farley 1972, Roth y Willis 1960). Las especies del género *Arenivaga* son comensales en nidos y cuevas de reptiles, aves, roedores, hormigas, avispas y escarabajos, localizados en matorrales desérticos, donde se alimentan de hojas y raices (Roth y Willis 1954, Atkinson *et al.* 1991) es por ello que, al igual que *P. surinamensis, N. rhombifolia y N. cinerea* fueron capturadas únicamente al ser atraidas por la luz artificial de las viviendas.

Las hormigas (*Pogonomyrmex* sp), arañas (*Heteropoda venatoria* y *Latrodectus mactans*), lagartijas (*Urosaurus nigricaudus* y *Uta stanburiana*), roedores (*Mus musculus*) y gatos (*Felis catus*) se alimentaron de *P. americana*. De nueve ootocas de *P. americana* y cinco de *N. rhombifolia*, se obtuvieron dos especies de parasitoides, *Prosevania sp.* (Evanidae) y una microavispa de la familia Eulophidae. Roth y Willis (1960) mencionan que las hormigas, arañas y avispas son eficientes depredadores de cucarachas y Boraiko (1981) cita a algunos roedores que consumen a las de gran talla; sin embargo, hasta ahora la araña *L. mactans*, los gatos y lagartijas no habían sido registrados como sus enemigos naturales.

AGRADECIMIENTOS

Se agradece el apoyo brindado al personal del C.I.B., al Biol. Armando Tejas, al Médico José Luis Jeman y personal del Hospital Militar, por la ayuda en la colecta de cucarachas en las viviendas de La Paz. Este trabajo fue realizado dentro del Proyecto "Impacto Social por Insectos, Arachnidos y Aves en áreas urbanas en La Cuenca del Golfo de California" (Clave 94/SA-03), apoyado por el Sistema de Investigación del Mar de Cortéz (SIMAC).

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SOUTHWESTERN ENTOMOLOGICAL SOCIETY

PERSPECTIVE

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

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EARLY BOLL WEEVIL FIGHTERS

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ABSTRACT

Biographical sketches are presented for eight federal and state entomologists who conducted research on the boll weevil during approximately the first twenty-five years of its occurrence in the United States. The contributions of each of them to the knowledge of the species are reviewed in the context of the scientific, political, economic, and social conditions prevailing at that time. Their work in other areas of entomology is also briefly summarized. Review of the lives and work of these scientists reveals that they were born and educated outside of the Cotton Belt of the United States, all were broadly trained in the biological sciences, and to a remarkable degree each became a leader in some aspect of entomology.

INTRODUCTION

During approximately a century of study of the boll weevil, *Anthonomus grandis* Boheman, many entomologists have dedicated a significant part of their professional careers to investigating the life processes and control of this species. When the boll weevil first appeared in Texas in 1892, there was little information available on its life history and habits and nothing was known about its control. When it became obvious that the species presented a serious threat to the cotton industry of the United States, there was an urgent need for basic information on the species with the main objective being to develop a quick control strategy. Subjects such as when the boll weevil first appeared in the United States, its subsequent dispersal, the considerable damage which it caused to cotton, and the attendant economic and social disruptions are well documented (Hunter and Pierce 1912, Loftin 1946, Helms 1977, Wagner 1980, Burke et al. 1986). What is lacking, however, is an appreciation for the work of the pioneer entomologists who were suddenly charged with the difficult task of marshalling forces, accumulating information, and quickly finding a way to stop the advance of this formidable pest. It is obvious a hundred years and many millions of dollars later what a difficult task they had before them. Certainly, they could not have anticipated just how expensive and time-consuming the job would be. Except for brief obituaries and occasional biographical sketches, little has been written about these early researchers. Where did they come from, how were they trained, how did they face the daunting research problems posed by a new exotic and voracious pest, what contributions did they make to accumulation of knowledge of the pest, and what were their overall contributions to the field of entomology as a whole? These and many other questions may be asked about the early boll weevil "fighters," many of whom had never seen a live cotton plant, and certainly not a boll weevil, before becoming involved with research on this insect.

In the late 1800's and early 1900's the southern states did not have adequate academic programs in colleges and universities to train entomologists capable of dealing with a newly introduced pest of such devastating potential as the boll weevil. Consequently, early in the study of the boll weevil entomologists had to be recruited from the northeastern and midwestern universities of the United States where entomology was being taught. Most of the early workers were recruited immediately following graduation

with baccalaureate degrees, although at least one, W. E. Hinds, had just recently completed the Ph.D. degree when he came to Texas to work with W. D. Hunter. These young entomologists were broadly trained in insect biology and ecology and were capable of working on a wide range of insects and insect problems. The scientists recruited in the early days to work on the boll weevil were excellent examples of the products of entomological education of the time. Although they were not adverse to using chemicals for insect control whenever such materials were available, of necessity their main thrust in pest management was through adaptation and use of the ecological and cultural principles and methods of the time. In keeping with their broad biological training and interests, the early students of the boll weevil also made significant contributions to entomology in areas outside of their primary area of research. Several had considerable interest in the systematics of various groups of insects. C. H. T. Townsend, for example, is best known for his work on the systematics of Diptera. In addition to their boll weevil studies, W. D. Hunter and W. D. Pierce contributed to the field of insect systematics, as well as to the advancement of medical and veterinary entomology. Most of the early entomologists who began their careers working on the boll weevil quickly became leaders in the field of entomology.

The objective here is to review the lives and contributions of some of the more prominent entomologists who worked on the boll weevil from the time of its first discovery in the United States in 1892 through approximately the first two decades of the 20th century. By the end of this period, the weevil had spread throughout most of the cotton growing area of the southern United States and the battle had expanded, both geographically and technically, to the point that additional entomologists were needed to combat it. Although some of the pioneer workers were still involved in study of the boll weevil past the end of the first decade of the 1900's, their influence on research on the species was generally reduced through assignment to other entomological projects and diluted by the increased numbers of entomologists being employed to work on the problem. The first era of research on the boll weevil had ended by this time and a new one with a greatly expanded knowledge base and new participants was beginning. The following discussions of selected individuals are designed to provide biographical information as well as to point out the contributions that each made to the knowledge of the boll weevil and to a broader entomological field.

CHARLES HENRY TYLER TOWNSEND (1863-1944) (Fig. 1)

The first entomologist to conduct scientific study of the boll weevil was C. H. T. Townsend (sometimes cited as Charles H. T. or C. H. Tyler Townsend). Townsend was born in Oberlin, Ohio, December 5, 1863. He developed an early interest in natural history, having at the age of 10 assembled a collection of insects at Lawrence, Kansas, under the guidance of Dr. G. H. Gaumer. He graduated from high school at Constantine, Michigan, in 1882. During the 1800's those who developed interests in natural history early in their lives often pursued degrees in medicine. Townsend followed this pattern and during the period 1887-1891 enrolled in a medical course at Columbian College in South Carolina. Apparently he did not receive a medical degree as a result of this study because such an accomplishment was not included in a later list of his academic achievements (Townsend 1943). The B.S. and Ph.D. degrees were obtained in 1908 and 1914, respectively, from George Washington University. His first entomological job started in 1888 as an office assistant to C.V. Riley in the Division of Entomology of the U.S. Department of Agriculture (USDA) in Washington. It was here that he began his lifelong interest of the taxonomy of Diptera, the field of entomology for which he is best known. It was also during his tenure in this position that he and his work came to the attention of the later Chief of the Division, Leland O. Howard. The two became friends and whenever Howard needed someone to take on a special, short-term job he turned to Townsend. Howard was aware of Townsend's restless nature and knew that he could count on him to be interested in projects where travel, especially in the tropics, was involved. Without benefit of an academic degree at the time, Townsend managed to obtain a teaching position on the faculty of New Mexico College of Agriculture and Mechanical Arts (now New Mexico State University) at Las Cruces and remained there from 1891 to 1893. In 1893 he exchanged positions with T. D. A. Cockerell, a friend and naturalist/entomologist, and for one year served as curator of the Public Museum in Kingston, Jamaica (Townsend 1943).

Increased concern about the destructiveness of the newly introduced boll weevil and its potential threat to the southern cotton growing areas of the United States prompted Howard to call upon Townsend to obtain preliminary information on the life history, behavior, and ecology of the weevil. Townsend accepted the brief temporary assignment offered by Howard and travelled to Eagle Pass in South Texas in mid-November 1894 (Helms 1977, Wagner 1980) to begin his investigations. From there he immediately departed for the state of Coahuila, Mexico, where he made observations on the weevil and talked to farmers and others about their experiences with this pest. He was on the road in northern Mexico and southern Texas for about a month in his quest for information on the species. On the basis of the data accumulated during this short period he published the first scientific paper on the boll weevil and its habits. This paper, written during Townsend's travels in the area in 1894, entitled "Report on the Cotton Boll Weevil in Texas," was published in the periodical *Insect Life* in 1895 (Townsend 1895). Considering the short time he spent in the area, this paper, based on personal observations and anecdotal information, contains a remarkable amount of pertinent data about the weevil. Brief descriptions of the developmental stages were presented and observations were made on adult and larval behavior, seasonality, overwintering, and parasites. He discussed ways which the weevil could be spread on trains, horse-drawn stages, and other conveyances. Flight was not considered as a possible means of long-range dispersal. Townsend speculated on the original home of the weevil, concluding that it was native to the Coahuila, Mexico, area where there were many unsubstantiated reports that it had been for a long time and had caused considerable damage to cotton there. It was stated that the weevil had even forced discontinuance of cotton production in some cases. No host plant of the weevil other than cultivated cotton was found during his brief study. This question was a persistent one and many years elapsed before other hosts were found.

FIG. 1. C. H. T. Townsend, ca. 1893. Courtesy National Archives 7-H-323.

Townsend considered Brownsville, Texas, to be the site of entry of the weevil into the United States. He warned of the danger this insect posed to cotton and described the very heavy damage that the species caused in both Mexico and Texas. It was estimated that about one-fifth of the cotton grown in Texas at that time was infested and damage was frequently as high as 90%. He generally painted a bleak future for cotton production if something was not done to stop the spread of the boll weevil.

The first extensive recommendations for control of the boll weevil were made by Townsend on the basis of the information he gathered during his short visit to the weevil-infested areas in Texas and Mexico in 1894. His recommendations included burning infested plants during the winter, flooding fields, rotating crops, picking and burning bolls (fruit), destroying volunteer cotton, grazing by hogs and cattle, quarantine, and use of the chemicals Paris Green and London Purple mixed with molasses. Some of these proposed remedies were generally not feasible but he nevertheless offered a broad menu of options. Townsend was convinced that parasites would be an important factor in control of the pest and he did not pass up any opportunity to espouse his views on this subject. It is of interest to note that the use of parasites for weevil control is being actively investigated today. He also emphasized the need to establish a no-cotton zone (50 miles wide) to halt the advance

of the weevil but expressed a lack of confidence that the Texas Legislature would enact such a law, much less enforce it. He strongly recommended that the infested area in Texas be quarantined. The USDA adopted most of Townsend's recommendations and embraced still others, such as hand picking of infested squares. There was considerable disagreement among entomologists for many years concerning which of these measures was most effective and some of the early USDA recommendations were dropped after further study proved them to be of no use for weevil control. However, several of the general cultural methods recommended by Townsend became the basis of control procedures for many years to come (Helms 1977, Townsend 1895).

Townsend's brief trip to southern Texas in the fall of 1894 apparently convinced him that this would be an excellent place to live and to pursue investigations on the boll weevil as well as on other insects of interest to him. The Lower Rio Grande Valley of Texas, with its semitropical biota and climate, was at that time highly attractive to insect collectors from the northern states. It was here that many collectors first became acquainted with semitropical and tropical insects and this experience prepared them for cautious advances into the wilds of Mexico and other truly tropical areas. The potential of new discoveries to be made on the Lower Rio Grande insect fauna was surely not lost on Townsend. Regardless of his motives, he requested that L.O. Howard make him a permanent employee of the USDA and to station him in Brownsville in 1895 where he could conduct experiments and study the weevil more thoroughly. This request fell on receptive ears because of the gravity of the boll weevil situation and the lack of response on the part of the State of Texas in trying to solve the problem. There was also both an urgent need for USDA presence in the area and a need for more information on the weevil. In response to these pressures, Townsend was appointed as a Special Agent with his base of operation being Brownsville. He moved his family from New Mexico in February 1895, set up a laboratory, obtained use of land for experimental plots, and made other arrangements to study the boll weevil that season. Although he actively sought this assignment, all did not go well, and he complained profusely to Howard about the hot weather, storms, mosquitoes, fleas, ticks, chiggers and the loud singing emanating from the churches near his laboratory and home (Wagner 1980). Eugene A. Schwarz, a USDA entomologist from Washington, D. C., who visited Brownsville in 1895 while on a trip to southern Texas to make observations on the boll weevil, remarked about the nice little house and laboratory Townsend had but said that Townsend was not a field man because he did not know how to collect insects in the brushy country in the area (Sherman 1929). Schwarz was an inveterate collector and the latter qualification was probably his standard for measuring the worth of an entomologist. In spite of the many personal and scientific problems Townsend encountered, he persevered and sent in reports on his observations and experiments, as well as investigated all kinds of concoctions and strange machines people were touting for control of the boll weevil. Neither Townsend nor L. O. Howard was entirely satisfied with the results of the work that summer and Townsend wrote to the latter apologizing for the lack of accomplishment (Wagner 1980). It has often been the case in the past that the efforts of those working in the field and facing the vagaries of nature and lack of cooperation by the organisms they studied have failed to satisfy their remote, office- or laboratory-bound supervisors. Townsend's summer work added to the core of badly needed information on the boll weevil even if it did not answer as many questions as both he and Howard had wished. The boll weevil continued its devastation along the Rio Grande and many farmers there and elsewhere decided not to plant cotton the following year (Helms 1977).

After Townsend and his family returned to New Mexico at the end of the less than successful 1895 season, he requested that L. O. Howard send him to southern Mexico, specifically Yucatan, to survey for parasites of the boll weevil. Howard did not immediately grant the request fearing that the results of such a survey would be minimal and not of practical value. But in 1896 he changed his mind and Townsend was allowed to travel in an official capacity to Mexico with instructions to search for parasites and host plants of the boll weevil (Helms 1977). Townsend (1897a), in a paper on the biogeography of Mexico and the southwestern United States, described his travels to Vera Cruz, Puebla, Tabasco, Campeche and Yucatan, a trip that took place from February to approximately mid-July of 1896. His work was a failure as far as the boll weevil was concerned since he did not find either parasites or wild hosts of the weevil (in fact, he found only one boll weevil specimen). However, Townsend surely did not consider this a wasted effort overall

as he collected other insects extensively and wrote about the biogeography of the country and the animals and plants observed. He later described a large number of species of insects from Mexico and visited the country many times on collecting and hunting trips. He never passed up an opportunity to travel in Mexico and eventually covered most of the country.

Even though Townsend's work with the boll weevil during the past season had not been as productive as hoped, Howard apparently did not lose faith in his ability to accomplish research. Consequently, Townsend was sent to Texas in August 1897 to study fall migration of the weevil. His last tour of duty on the boll weevil for the USDA in Texas was in 1898 when he conducted experiments on the effectiveness of the poisons London purple and Paris green (Helms 1977). Despite various setbacks, some of which were beyond his control, Townsend made good use of his time in the Brownsville area. He accumulated a considerable amount of experimental and observational data on the boll weevil as well as collected and described other insects occurring in this biologically interesting area. He published several papers on the insects of the Lower Rio Grande Valley and the biogeography of the area (Townsend 1897b,1903).

After completion of his 1898 work in Texas, Townsend was again employed by the New Mexico Agricultural Experiment Station for about a year during 1898 and 1899. Always the restless one, he soon moved to El Paso, Texas, and became involved in other endeavors, serving as natural history editor of the El Paso Herald in 1900, employee of U.S. Customs during 1900-1901, and head of the Townsend-Barber Zoological Company during the period 1901-1903. While residing in El Paso he collected reptiles in Mexico and acted as a guide for parties hunting large game in the Mexican Sierra Madre (Mallis 1971, Townsend 1943). As was usual with Townsend, this work did not last long and he next moved to the Philippines where he spent two years teaching in the Provincial School at Batangas. Pierce (1974) attributed Townsend's wandering ways to being unable to "remain out of the tropics very long at a time." It is interesting to note that in a list of activities that he compiled, Townsend considered travelling "around [the] world without a passport" as one of his notable accomplishments (Townsend 1943). The few references made here to Townsend's activities demonstrate the broad range of his interests and the diverse occupations held. Stone (1980) summarized his wanderlust and interests best: "his career is probably one of the more varied of any entomologist, ranging all the way from economic entomologist for the U. S. Department of Agriculture and foreign governments to customs collector and leader of big game hunting parties in Texas [should read "Mexico"], and geographically from Washington, D. C. to Jamaica, the Philippines, Peru and Brazil. His interests ranged from entomology to ornithology to anthropology and physics. He also claimed to have explained gravity; recorded exact atomic weights; determined the exact velocity of light; defined cosmic units of length, time and mass; explained the moon's origin and the earth's axial inclinations; set Pleistocene duration and man in America at two million years."

Although Townsend did not conduct further field work on the boll weevil after 1898, he did contribute much to the field of entomology in general during the following years. He was employed as Entomologist and Director of the Experiment Station in Peru from 1909 to 1914. He investigated many entomological problems while in Peru but the work for which he is best known there is his discovery in 1913 of the transmission of a virulent bacterial disease of man known as Carrión's disease (verruga or oroyo fever) by a sand fly. He wrote extensively on this subject during 1913 and 1914 (Arnaud 1958, Pierce 1975).

From 1914 until 1919 Townsend was back in the United States. Mallis (1971) states that he worked at the U. S. Gypsy Moth Parasite Laboratory in Massachusetts after returning from Peru but Townsend himself listed his employment there as being during the period 1907-1909, preceding the Peruvian work. In any event, Townsend was associated with the USDA again as systematic entomologist at the U. S. National Museum from 1914 to 1919. He worked there on the muscoid Diptera. This was a very productive period for him and many papers were written describing new species and genera of flies.

After returning to South America in 1919, Townsend conducted work in Peru and Brazil on various agricultural, including some cotton, and medical pests. His *magnum opus* was the 12 volume, 3,760 page *Manual of Myiology*, a treatise on flies, published in Brazil during the period 1934-1942 (Townsend 1934-1942). All told, he published about 1,000 papers on insects and other subjects and described 1,491 genera and 1,555 species-level

taxa of flies (Arnaud 1958, James 1945). Townsend never failed to philosophize whenever he got the chance and some of his writings were highly controversial. For example, by a series of complicated calculations, he determined that a bot fly of the genus *Cephenomyia* was able to fly between 500 and 700 miles per hour (Townsend 1943), and by observation one supposedly achieved a speed of 820 miles per hour, at least 13 times the speed of the fastest known insect today. One should peruse the 12th part of the *Manual of Myiology* to get a true feeling for the breadth of the man's thinking. Townsend shared with many of the early researchers on the boll weevil a long and active life, dying in a suburb of São Paulo, Brazil, on March 17, 1944 at the age of 81.

EUGENE AMANDUS SCHWARZ (1844-1928)

Eugene A. Schwarz was not a major contributor to the early knowledge of the boll weevil, but he conducted some field work during it's initial dispersal in the United States and provided information in his reports that was useful for understanding the life history and habits of this little known species. Schwarz was born in Liegnitz, Silesia (then in Prussia, now in Poland), April 21, 1844. Very little is known of his early life in Europe but it is thought that he probably left home because of a disagreement with his parents about the direction of his career, he being more interested in zoology than becoming a high school teacher of literature and culture as was their plan for him. Fortunately, however, we have excellent accounts of Schwarz's life and activities in the United States through obituaries prepared by Howard (1929) and Howard et al. (1928), Schwarz's letters edited by Sherman (1929), and papers on Schwarz and F. H. Chittenden by Blake (1951,1952).

Schwarz came to the United States in 1872 and worked first at the Museum of Comparative Zoology at Harvard University where he organized the H. A. Hagen collection of insects. He began working for the USDA in 1878, followed C. V. Riley to the U. S. Entomological Commission in 1879, and then returned to the USDA in 1881. In 1895 Schwarz travelled to South Texas to make observations on the boll weevil as part of the USDA efforts to assemble as much information on the species as quickly as possible. Much interesting information about Schwarz may be gleaned from the 211 page publication of his letters as a special supplement of the Journal of the New York Entomological Society (Sherman 1929). Some of these letters pertain to his early South Texas travels. For example, on May 19, 1895, he wrote from San Diego, Texas: "I am sent here into this miserable Mesquite and Cactus brush to investigate *Anthonomus grandis* which at present is so rare that it takes three men a whole day to find a single specimen. I wonder how I can investigate it under the circumstances except by waiting until it becomes more numerous." He obviously was not inactive due to the rarity of boll weevils, however, since as an inveterate collector he found the brush country of South Texas to contain many other interesting species of insects and he took full advantage of the opportunity to collect them, especially the beetles. He travelled by stage to Brownsville where he visited Townsend at the request of L. O. Howard. There seems to have been no purpose of this trip except to see "how Townsend is situated there" and no mention is made in his edited letters of the boll weevil work being carried out by the latter. Perhaps an official report was made to Howard about this trip.

Schwarz published little on the boll weevil himself but he made reports to L. O. Howard, the contents of which were often used in the various bulletins and pamphlets produced on the species by the USDA. He travelled in Cuba in 1903 and 1904 for the purpose of determining if the boll weevil had hosts other than cultivated cotton. In a series of letters (mostly to L. O. Howard) dated from February 16, 1903 to June 4, 1904, Schwarz described the situation in Cuba concerning cotton and boll weevil infestations (Sherman 1929). Since the first appearance of the boll weevil in Texas in 1892 there had been speculation about whether cultivated cotton (or at least species of *Gossypium*) was the only host of the species. Howard was anxious to investigate this question since it was obviously important to the development of a control strategy. In the only paper that he published on the boll weevil, Schwarz summarized his observations on the hosts plants and parasitism of the species in Cuba (Schwarz 1904). The weevil was found on both cultivated cotton and "kidney cotton" (a "wild" form of *Gossypium barbadense*) and there was no evidence that the species was being parasitized there. In his letters to Howard, he stated: "Up to the time of my visit to Cuba, I had shared in the opinion held by many entomologists connected with the Boll-weevil investigation, that the original food-plant of

the weevil would prove to be some Malvaceous plant generically distinct from Gossypium." After Schwarz had investigated other malvaceous plants in Cuba without finding weevils he said: "I do not hesitate to assert, after my experience in Cuba, that *Anthonomus grandis*, wherever it occurs, has no other food plants than the various species or varieties of the genus Gossypium." The question on additional hosts of the weevil remained unanswered for approximately the next 50 years.

As noted above, Schwarz was not a major contributor to the knowledge of the boll weevil but he was certainly a loyal "foot soldier" when called upon to help. Another example of his unheralded work was his identification of the innumerable insect fragments taken from stomachs of birds shot by Bureau of Entomology personnel during 1903 and 1904 to determine the extent to which birds controlled the boll weevil.

Eugene A. Schwarz was looked upon as a "walking encyclopedia" about insects and entomological literature. He traveled widely in the United States, Cuba, Mexico, and Central America collecting insects. Even though most of his 395 publications were brief scientific notes, his productivity was remarkable, especially when the breadth of entomological subjects covered is considered. Schwarz's favorite insects were beetles which he collected extensively with Henry Hubbard, a close friend who shared his fondness for these insects. The two collected extensively in Florida, Arizona, and Colorado. Schwarz was a generalist in entomology, the likes of which will not be seen again. Blake (1951-1952) presents a personal and touching account of her association with him during his last years of activity as a coleopterist in Washington. Although he was approximately 80 years of age at the time of which Blake speaks, he was still enthusiastic about his work and yearned to visit his old collecting grounds. This dedication to his profession was typical of that of many of the early boll weevil fighters. Schwarz died October 15, 1928 at the age of 84 years.

FREDERICK WILLIAM MALLY (1868-1939) (Fig. 2)

Although attempts were being made by the USDA during the earliest days of boll weevil study to learn more about the species and to devise effective control measures, there was widespread dissatisfaction among farmers, legislators, and the general public about the lack of a quick solution to the boll weevil problem. Because of other demands on its resources, the USDA was unable or reluctant to commit additional funds and personnel to speed up the process. Under these circumstances, political pressure developed in Texas for the state to make a commitment to the control of the pest. As a result, an entomology position was established at Texas A&M College and Frederick W. Mally was employed with the primary responsibility of destroying the boll weevil.

Frederick Mally was born November 30, 1868, near Des Moines, Iowa. He entered Iowa State College in February 1884 and was awarded the B.A. degree in 1887 and the M.S. degree in 1889. It was during his first year of agricultural study at Iowa State that Mally came under the tutelage of Seaman A. Knapp, then President of the College but who later was involved in developing the use of farm demonstrations to educate farmers about modern agricultural practices. This type of work eventually led to establishment of the agricultural extension service. Mally carried the demonstration idea forward in his later work with the boll weevil, making good use of the methods and principles taught by Knapp. The following account of Mally's work on the boll weevil in Texas is derived mostly from the publications of Little (1960), Osborn (1946), and Wagner (1980). A manuscript copy of Mally's presidential address to the Texas Entomological Society, February 9-11, 1939, has been of considerable assistance in recreating accounts of some of his activities.[1]

For a brief period after graduation from Iowa State in 1889, Mally was employed to assist Dr. Stephen A. Forbes of the Illinois State Laboratory of Natural History. His next position began in 1890 with the Division of Entomology, USDA, where he assisted C. V. Riley. Here he became acquainted with L. O. Howard and other USDA entomologists who had a large influence upon his subsequent philosophy about and approach to research. Mally was sent to Texas in 1891 on a special assignment to study the cotton bollworm. It was during this project that he demonstrated the ability to work with farmers and others in assessing the problem and gathering useful information for future control programs. The results of his brief study of the bollworm were summarized in a USDA bulletin entitled

"Report on the Boll Worm of Cotton" published in 1893, one of the important early publications on this pest (Mally 1893). Later in 1893 Mally resigned from the USDA to become the manager of the Galveston Nursery and Orchard Co. near Dickinson, Texas. He was subsequently Director of the Texas Commission on Insect Pests and Fungus Diseases and in this position was involved in getting passed the first orchard and nursery inspection law in Texas. It was in this capacity as spokesman for nurserymen and orchardists that Mally demonstrated his exceptional ability to interact with the press, act as a mediator, educate the public, and generally serve as an organizer for the industry in Texas.

FIG. 2. **F. W. Mally.** Reprinted from Little (1960).

The Department of Entomology at Texas A&M College was established by a legislative act in 1899 with the decree to employ "an expert entomologist, whose duty it shall be to devise means, if possible, of destroying the Mexican boll weevil, bollworm, caterpillar, sharpshooter, chinch bug, peach bug, fly and worm and other insect pests, and to perform the duties of professor of entomology of the Agricultural and Mechanical College of Texas." Frederick W. Mally became that "expert entomologist" in April 1899, shortly after the bill became law. Mally's attempt to satisfy the difficult task he undertook is well documented in his presidential address to the Texas Entomological Society in 1939. Part of this address was published by Osborn (1946), but if a fuller account of his activities is desired a copy of the original manuscript should be consulted. In his address, Mally commented at some length on the lack of understanding among farmers about the need to have basic information about pests and to conduct proper experiments before recommending control measures. Even Mally with his excellent ability to communicate with farmers and his frequent use of the press to disseminate information, was never able to get this message across. There was still much to learn about the life history and habits of the boll weevil at the time but farmers and the general public continued to expect immediate relief from its ravages. Mally's task was made even more difficult because he was the only entomologist employed by the state of Texas and had to deal with insect problems other than that of the boll weevil as well as to satisfy his teaching and administrative responsibilities. Furthermore, he initially had an annual appropriation of only $1,000 to cover travel, experiments, and the many other costs associated with research on the boll weevil and other insects. Little (1960) speculated that if Mally had known the problems which would confront him he probably would not have taken the job. Nevertheless, Mally planned and carried out ambitious research and public relations programs. He built a field laboratory on the Colorado River, near Eagle Lake, to study the boll weevil, the first such laboratory established in Texas if the one Townsend had in a residence in Brownsville in 1895 is excepted. In addition to the many other problems Mally faced, the weather did not cooperate. Exceptionally heavy rains occurred early in the spring of both 1899 and 1900, inundating crop land and delaying planting. The flood of 1899 even washed his newly built field station down the Colorado River.

In spite of the many difficulties of conducting research on the boll weevil and dealing with unreasonable expectations, Mally persevered, fighting both the weevil and political pressures to obtain quick results. He traveled extensively about the areas of the state that were infested with the boll weevil talking to farmers and setting up demonstration plots, essentially what we have come to identify as extension work. In this respect he preceded his old mentor, Seaman Knapp, who in 1903 established what is now known to be the first farm demonstration, an activity which evolved into the extension service. In the aforementioned presidential address, Mally stated that to accomplish what he was hired to do he had "to put on four educational campaigns in fifty counties in which the boll weevil had appeared during the second and third years of the investigations made by the State of Texas. Most of my time for these two years was devoted to one campaign each spring during the planting season for cotton and the spring migration of the boll weevil, the other, a fall campaign each year, during the fall grazing season and plow-out periods ahead of

frost. The first year of my administration was almost peremptorily taken for research and life history work, some field tests, and remedial measures, and cultural methods." All of this was in addition to his teaching load, albeit relatively light as only two entomology courses were offered at Texas A&M at that time, and administrative tasks. Mally doggedly pursued his goals of developing suitable means to control the weevil and to stop its spread. He recommended using arsenate of lead or London purple and Paris green mixed with molasses. He also recommended spraying over dusting and emphasized the value of such methods as grazing and planting trap crops. The USDA entomologists at the time recommended several cultural practices and also urged use of dusts rather than sprays. However, experiments by USDA entomologists indicated that Paris green, which Mally recommended, was ineffective and the chemical was subsequently dropped from their recommendations. It was well known that Mally and W. D. Hunter, who came to Texas in May of 1901 to head the USDA effort to halt the spread of the boll weevil, had differences of opinion about how to control the weevil. Their disagreements received considerable public attention, but both were reasonable and tactful men and, aside from the competition to be expected between these two strong-willed individuals, it is not entirely evident what their problems were. Hunter obviously had more financial and human resources to conduct research than did Mally. It was probably inevitable that "turf wars" would break out between state and federal entomologists in the highly charged political and emotional atmosphere which posed a threat to a way of life for many in the infested zone. It is possible that the strain on Mally to develop an immediate control measure for the weevil with the inadequate resources he had available took its toll on his patience and endurance. According to Little (1960): "He had his problems, but not within the college [Texas A&M College]." For whatever reason, Mally resigned from Texas A&M in October 1902, having served about 3 1/2 years in the position. There is some evidence that he was forced to resign because he ran afoul of Texas politics (Wagner 1980); however, other factors probably also played a part. During the short period of Mally's intense work on the boll weevil he published two significant bulletins on its life history and control in addition to many popular articles on the subject (Mally 1901,1902). He also made hundreds of talks on the subject and conducted many farm demonstrations on control. A handbill printed by the *Bryan Eagle*, June 1, 1901, reproduces a talk Mally presented to farmers and businessmen at Bryan and exhorts farmers to save their cotton crops by following Mally's method. "Prof. Mally says if you cannot get the Arsenate of Lead don't wait, but use the other poisons; use as follows: FOUR OUNCES London Purple or Paris Green; TWO GALLONS of Sorghum or Molasses; ADD ENOUGH water to make Fifty Gallons." At the same time Mally recommended grazing in the fall before the first frost, trap crops and handpicking weevils and squares. Mally's formula for control of the weevil was a mixture consisting of "1 ounce of arsenic and boil in one gallon of water until dissolved, 8 ounces arsenate of lead and rub up in one gallon and a half of cold water until dissolved, 1 gallon of molasses diluted with one gallon of water, mix well and add sufficient water to make 25 gallons."[2] Wagner (1980) cites many excerpts from newspapers concerning Mally and the recommendations he presented to various citizen groups in the infested areas on how to control the weevil.

Dr. V. A. Little, a longtime teacher of entomology at Texas A&M and a chronicler of historical events of Texas entomology, credited Mally with many major accomplishments during his brief work on the weevil, of which the following five appear most important: (1) first to work out the life cycle of the boll weevil; (2) had a more complete understanding of the flight activities and overwintering of the weevil than anyone else; (3) was the first to stress the use of early maturing varieties; (4) was the first entomologist to use lead arsenate (as a spray) for control of cotton insects; and (5) conducted educational campaigns which were the forerunners of present day extension work.[3]

After Mally left Texas A&M in 1902, he pursued private nursery and orchard business interests in Texas. During the period 1910-1915 he was agricultural director for the Cross S Farming Company. In 1915 Mally returned to extension agriculture when he became County Agent in Webb County, Texas, and in 1925 transferred to the same position in Bexar County where he worked until retirement in 1938. Little (1960) considered extension work and day-to-day contact with farmers to be in Mally's blood. In recognition of his contributions to agriculture and his statue as an entomologist, Mally was

elected President of the Texas Entomological Society in 1938 and served a full term which ended February 11, 1939. He was in poor health at the time he presided at the 1939 meeting of the society and died approximately three months later, on May 7, 1939 (Little 1960, Sasscer 1939).

Mally's short tenure as one of the early workers on the boll weevil was marked by major accomplishments as well as failure and disappointment. He arrived on the scene at a point when little basic information was available on the weevil, there were no effective control methods, and farmers and others were demanding an immediate solution to the problem. No one, including the entomologists, understood the adaptability and tenacity of the boll weevil and the difficulty they would face in trying to control it. Mally was probably the man for the times. It was obvious that research alone would not solve the problem. There had to be a mixture of experimental studies to obtain basic life history and ecological information and extension work to educate farmers about the problem and available remedies. If there was to be only one person to do all of these things, as was the case for a short period from about 1899 to 1902, then Mally was surely among the best candidates for the job. For such a short tenure as one of the early boll weevil fighters, he left his mark and any historical account of the times must take his contributions into consideration.

EZRA DWIGHT SANDERSON (1878-1944) (Fig. 3)

Mally's successor at Texas A&M College was E. Dwight Sanderson. The following description of Sanderson's work in Texas with the boll weevil is based mostly on information presented by Little (1960), Phillips (1944), and Wagner (1980). As with the other early workers on the boll weevil in Texas, Sanderson was born and received his education outside of the Cotton Belt. He was born in Clio, Michigan, in 1874. Ezra Dwight Sanderson graduated from Michigan Agricultural College in 1897 and received a second B.S. degree from Cornell University in 1898. Sanderson's next position was Maryland Agricultural College where he was state entomologist. During the summer of 1899, he was temporarily employed by the Division of Entomology of the USDA where, according to Wagner (1980), he received his first experience working with the boll weevil in Texas. From late 1899 until 1902, he was employed by the Delaware Agricultural Experiment Station as an entomologist. During part of this time he also taught zoology at Delaware College.

FIG. 3. **E. D. Sanderson.** With permission of the Entomological Society of America. From Phillips (1944).

E. D. Sanderson was employed on October 1,1902 to succeed Mally at Texas A&M. His position was professor of entomology and state entomologist. Like Mally, he was also the

only entomologist employed by Texas A&M; however, he had more financial support to develop a department than Mally. The present insect collection and entomological library at Texas A&M University dates mostly from the brief tenure of Sanderson. He also seems to have had a better relationship with USDA personnel working on the boll weevil, serving as a special agent with the Bureau of Entomology and cooperating on various projects. However, he immediately criticized Mally's recommendations for boll weevil control, thus causing some friction between himself and Mally who had just left the Texas A&M position. W. D. Hunter, who had been working to improve relations with Mally, saw his efforts being jeopardized. In an attempt to keep relations amiable, Hunter wrote Mally and was successful in getting Sanderson to drop his criticism (Helms 1977). Wagner (1980) claims that Sanderson was instrumental in planting the idea (conceived by others) in the mind of then Governor S. W. T. Lanham that the state of Texas should offer a monetary prize to anyone who "devises or invents a practical way to eradicate the boll weevil." As a result, a bill providing for an reward of $50,000 was introduced in the Texas Legislature and was passed in 1903, becoming law in July of that year. Some legislators and scientists (W. D. Hunter, for example) opposed it on the general grounds that it would be detrimental to the overall adoption of remedies developed through the research of USDA and state entomologists (Helms 1977). It would encourage all sorts of outlandish proposals which would have to be considered and perhaps tested, thus taking up the valuable time of the few scientists working on the problem. Although the reward was never paid, some of the dire predictions did come true and the whole matter was disruptive to boll weevil research and control. If Sanderson played a significant role in the event, this was not one of his more notable accomplishments as a boll weevil fighter. He did, however, make worthwhile contributions to the knowledge of the boll weevil and its control even though he was in Texas for only a short time. Some valuable bulletins were written on the species and the many popular articles and talks produced expanded his influence as a spokesman about the boll weevil. In 1903 he published circulars emphasizing control methods such as planting early, using early maturing varieties, practicing good cultivation, handpicking infested squares (under certain conditions), grazing, and burning stalks in the fall rather in the winter (Sanderson 1903a, b). It is of interest that in the 1903 recommendations published by the Texas Agricultural Experiment Station he does not mention the use of insecticides for control. Wagner (1980) states that Sanderson advocated using Paris green until about the middle of 1904 but decided that it was not effective for control of the weevil. His 1903 recommendations would indicate that Sanderson had abandoned Paris green earlier. Because of the general confusion of other insects with the boll weevil, Sanderson wrote a Texas Agricultural Experiment Station bulletin on this subject. Other insects found around the farm that were likely to be misidentified as boll weevils were described and illustrated (Sanderson 1904). In 1907, several years after Sanderson left Texas, a bulletin entitled "Hibernation and development of the cotton boll weevil" was published by the Bureau of Entomology, USDA (Sanderson 1907). The valuable biological information on the weevil contained in this bulletin was obtained by Sanderson during his research as state entomologist of Texas. Given Sanderson's interest in rural development (an area in which he later gained prominence), it is not surprising that he attempted to more closely evaluate the economic and social effects of the boll weevil on cotton production. Unfortunately, his brief stay in Texas did not allow him time to adequately determine the true costs of the pest.

While highly competent entomologists were attracted to Texas to work on the boll weevil in the days following its invasion, not many remained for more than a few years. Dwight Sanderson was no exception. He left Texas in 1904 and for the next 10 years held a series of positions in New Hampshire and West Virginia. His first employment was as professor of zoology at the University of New Hampshire. After three years in this position, he became Director of the New Hampshire Agricultural Experiment Station. In 1910 he was appointed as Dean of Agriculture at West Virginia University. Two years later he accepted the position of Director of the Agricultural Experiment Station of that state, where he remained until 1915. With his interests in rural life and agricultural development becoming stronger, he resigned the West Virginia position and enrolled in graduate work at the University of Chicago to study rural sociology, receiving the Ph.D. degree from that institution in 1921. While pursuing the degree he accepted a position in 1918 as Head of the Rural Social Organization at Cornell University. He remained at Cornell until his retirement in 1943. During his tenure there he developed an especially strong program in rural sociology.

V.A. Little (1960) said that Sanderson "was the most versatile and probably the most brilliant entomologist who ever worked at the Agricultural and Mechanical College of Texas. He had the unusual distinction of distinguishing himself in three separate fields: entomology, administration and rural sociology." Although a large part of his career was spent outside of entomology, Sanderson contributed a considerable amount of information on the boll weevil and also conducted extensive educational programs for farmers during the two years he spent in Texas. His overall contributions to entomology are also well known through the several books he wrote on various aspects of the subject. Dwight Sanderson died September 27, 1944.

WALTER DAVID HUNTER (1875-1925) (Figs. 4, 5, 6, 7)

No account of the early study of the boll weevil would be complete without reviewing the many contributions W. D. Hunter made to the knowledge of this species. For a period of approximately 25 years he was deeply involved in USDA efforts to control cotton insects, especially the boll weevil and the pink bollworm, *Pectinophora gossypiella* (Saunders).

FIG. 4. **W. D. Hunter.** November 2, 1911. At USDA laboratory, Dallas, Texas. Courtesy of C. R. Parencia.

Hunter was born December 14, 1875, in Lincoln, Nebraska. As was often the case with early entomologists, he developed an interest in the broad field of natural history at a

young age and continued this interest throughout his life. In this regard, Hunter's mother remembered: "There was not a fence corner within eight miles that the children did not know what birds, plants and insects could be found there. Eight was about the limit of our old gray horse. Later on they extended their knowledge on foot many more miles." (Howard 1925a)

After spending several years in the preparatory school of the University of Nebraska, Hunter enrolled as an undergraduate student at the university in 1891 and received an A. B. degree in 1895, at the young age of slightly less than 20 years. He studied ornithology, taxidermy and entomology under Lawrence Bruner but soon decided that he was most interested in insects. During the first two years following graduation, he published four papers on the systematics of syrphid flies. Hunter continued study at the University of Nebraska and received the M.A. degree in 1897 while serving also as an instructor during this period. He took over some of Lawrence Bruner's field work on grasshoppers and continued teaching in the Department of Entomology and Ornithology when Bruner travelled to Argentina in 1897. In a letter dated May 1,1897, Hunter informed Bruner in South America of administrative problems that were occurring at the University of Nebraska at that time "At this date I do not know exactly what was done with this department. All I know I read in the paper where it stated that I was made instructor and placed in charge of the department."[4] After Bruner's return in 1898, Hunter served as his assistant in work on grasshoppers, the fall armyworm, and other insects until 1900. At that time funds were cut at the University of Nebraska and Hunter found himself without a job. He did not stay unemployed long, however, as in the same year he became an assistant to H. E. Summers, entomologist at Iowa State College. Although the salary was only $400 per year, Hunter saw this as a step to something better and he rejected the urgings of relatives to seek employment in some more lucrative area in the commercial world (Howard 1925b). He was correct in assessment of his opportunities as he subsequently advanced quickly in the entomological field.

During his work for Bruner on grasshoppers Hunter's talents caught the attention of entomologists in the Bureau of Entomology in Washington. When the boll weevil problem in Texas became more acute and Congress appropriated special funds for its study, Hunter was appointed March 1901 as a Special Field Agent of the Bureau to direct the work under the supervision of Dr. L. O. Howard. Anticipating that he would be offered the position, Hunter began studying cotton culture and cotton entomology in advance of his appointment. Although he had never seen a growing cotton plant, he prepared himself well for the task ahead. He continued his education on this subject for the short time he was in Washington prior to leaving for Texas. Charles N. Gould, a geologist, wrote about his friendship with Hunter while they were both students at the University of Nebraska. He, Hunter and C. A. Fisher formed a group known as the "Triumvirate." It was agreed that the first one to get a job was to treat the others to a meal. Hunter's employment with the USDA qualified him for the honor of treating Gould and Fisher to a fifty cent dinner (Gould 1959).

W. D. Hunter arrived in Texas at a critical period in the history of the boll weevil. The species was spreading into new areas, none of the available remedies for its control were successful, basic information was still needed on its life history and ecology, and there was considerable economic and social chaos connected with the damage it caused. The state entomologist, F. W. Mally, had been working on the problem since 1899 but he was poorly funded, overworked, and his efforts were hampered by the unreasonable expectation existing among a populace demanding an immediate solution to the problem. The situation had reached a point where an organized effort with adequate research resources was absolutely necessary. The young Hunter was chosen as the person to direct this effort. Hunter lost no time in acquainting himself with the situation after he arrived in Texas. We are fortunate to have as a source of information his field notebook for March 20, 1901-May 29, 1901, the period when he was learning first-hand about cotton and the boll weevil in Texas.[5] During this time he travelled through much of the area of the state infested by the boll weevil, making notes on cotton culture, soils, rainfall patterns, farmer attitudes, economics, and control measures. These notes allow one to follow the path of Hunter's thoughts as he obtains anecdotal and first-hand information on the ravages of the weevil. His broad training and ecological approach to solving insect problems becomes evident in the statements he recorded. Considerable attention is given to the use of trap

crops which were generally considered ineffective because of the abundance of competing volunteer cotton and the large influx of weevils from other areas late in the season. Hunter records the point that early planting was considered to be an effective method of avoiding weevil and boll worm infestations by many farmers and, when successful, resulted in less loss to pests as well as higher prices for the earlier cotton. He writes "Look up this idea of early planting. It saves trapping, the farmers will practice it, it is practical in every way." However, he subsequently points out that climatic and other factors may prevent early planting or affect the survival of early planted seeds. Hunter also presents information on handpicking of weevils and the costs of such a practice. It was obviously also an objective of his to locate sites for experimental work as well as to identify local farmers and others who could be counted upon to cooperate in these studies. He had to travel mostly by horse and buggy and stay in hotels when available and in many of his his daily notes there are entries such as "Small hotel, poor livery, sandy roads." and "Good livery but poor hotel."

FIG. 5. USDA Boll weevil laboratory, Victoria, Texas, ca. 1903. Courtesy of C. R. Parencia.

Schoffelmayer, an agricultural writer for the Dallas Morning News, stated: "Scientists have told me that the young Hunter learned more about cotton in a few months of travel and research than others learn in a lifetime."[6] Hunter's first entry in his field notebook dated March 20, 1901, Austin, Texas, is an example of his attempt to quickly accumulate information on the boll weevil only a few days after arriving in Texas:

> Talked with Jefferson Johnson, who is nominally Commissioner of Agriculture, but who has had no funds for agricultural work, and now simply exercises office of state insurance inspector. He states that he does not believe that the weevil extends over thirty miles north of Austin. There is practically no damage by the boll worm in the uplands about Austin, though some in the lower regions of the river bottoms. Hence delaying planting here would result in no additional damage by the boll worm. He states that winter has been very mild; and that he has seen weevils all winter in most healthy condition. There is practically no volunteer cotton whatever raised here. In Bastrop Co. just to the south of Travis, in which Austin is situated, he states that the cold of the winter of 1897 killed the weevils. At least the spring following there were none to be found and they have since reappeared only very slowly. States that in Bastrop Co. they have found that grazing is a practical and effective means of destroying pests. (Note: This idea of his does not seem to be more than a conjecture.)

> Gives names of the two following: The first is one of his tenants and [is] entirely suitable for carrying out experiments. Mr. _____ Carleton, 7 miles east of Austin on main road, one mile beyond village of Dell Valle, in corner where road leads north. Mr. R. M. Castleman, 4 miles east of Austin on road same as above. Did not find this man, storekeeper at store 3 1/2 miles did not know him (This man is generally to be found at Harold Smith Wilcox store in Austin (Johnson).
>
> The region along valley east of Austin is all cotton land and seems very good for experiment.[5]

After a short introduction to the current boll weevil problem in Texas, Hunter began to plan and conduct studies on the pest. The first USDA boll weevil laboratory was officially established in 1902 at Victoria, Texas after some political pressure had been applied to locate it farther east at Wharton (Howard 1925a). The laboratory consisted of a converted residence (Fig. 4) and field studies were made in the vicinity of Victoria and in other weevil-infested areas of Texas. Hunter was in charge of the laboratory and had the responsibility of staffing it and directing all of the USDA research on the boll weevil in Texas. Federal funds were being provided primarily to prevent the spread of the weevil throughout the Cotton Belt and Hunter recognized that accumulation of basic information on its life history, behavior, and ecology was essential to the success of this effort. He began to assemble a group of scientists at the Victoria laboratory to study the boll weevil and other cotton pests. To the town of Victoria with a population of about 4,000 on the coastal plains of Texas came several young scientists born and educated in the northern and midwestern parts of the United States, most of whom had never been to Texas. W. E. Hinds, W. D. Pierce, W. W. Yothers, A. C. Morgan, A. L. Quaintance, and A. W. Morrill, among others, worked at the Victoria laboratory or in other areas of Texas under the direction of Hunter. Although most of these men got their start in research on the boll weevil at the Victoria laboratory, they went on to individually establish outstanding reputations in other areas of entomology, often entirely outside of cotton entomology. This was an enthusiastic group of entomologists to whom the diverse insect fauna of Texas was new and interesting. According to Howard (1925a, b), Hunter was instructed to discourage employees from collecting insects outside of their work on the boll weevil. However, he recognized that it was impossible to curb the entomological interests of such a dedicated group of young scientists in a new and biologically rich area and it is doubtful that he ever seriously attempted to do so.

The overall accomplishments of the researchers at the Victoria laboratory are difficult to assess because the laboratory was in operation for only about three years and the results of much of the research were published long after discontinuation of work there. However, it is safe to say that a considerable amount of the basic knowledge of the weevil was accumulated from research conducted under the direction of W. D. Hunter during the Victoria days. The first comprehensive report on the boll weevil was published by Hunter and Hinds in 1904 as Bulletin 45 (The Mexican Cotton Boll Weevil) of the USDA Division of Entomology. For many years this was the boll weevil "bible" (Hunter and Hinds 1904). A review of the bulletin reveals that an immense amount of information had been collected on the boll weevil by Townsend, Mally, Sanderson, Schwarz, and Hunter and his group of scientists since study of the species was started in 1894. This publication was revised (as Bulletin 51) in 1905, and in 1912 it was expanded with Hunter and Pierce as authors (Hunter and Hinds 1905, Hunter and Pierce 1912). Other entomologists at the laboratory were involved in specific areas of research and additional publications were forthcoming. The accomplishments accredited specifically to Hinds and Pierce will be covered later under discussion of these individuals.

Hunter was not only involved in conducting and directing research but had to contend with a wide array of political and public relations problems. There were many chemical and mechanical nostrums proposed for control of the boll weevil and Bureau of Entomology personnel were involved in testing these to the extent of their ability (Helms 1977). Much of the task of testing the proposed remedies and dealing with the claims of some persistent individuals who sought Bureau confirmation of their often outrageous suggestions for solutions to the boll weevil problem fell upon Hunter and his staff. The offer of a $50,000 reward by the state of Texas to anyone who could provide a remedy to the boll weevil exacerbated the problem by increasing the number of claims that Hunter and

state entomolgists had to deal with. In addition to the sometimes caustic exchanges between F. W. Mally and USDA entomologists over differences of opinion about control measures and the "turf war" that developed between Hunter and Mally, Hunter found that he and Seaman Knapp had disagreements over some of the same areas. Knapp was able to obtain funds to conduct demonstration farms through the Bureau of Plant Industry (True 1928). Since, in his demonstrations, Knapp used some control recommendations made by the Bureau of Entomology, immediate problems developed between individuals of the two organizations about methods and credit (Helms 1977).

The differences of opinion between F. W. Mally and Hunter over the most effective methods of control of the boll weevil and perhaps other aspects of their work have been touched upon briefly under the discussion of Mally. Minute analysis of the politics of the boll weevil problem and the differences in personalities of boll weevil fighters of the time are outside the objectives of this paper. However, it should be said that considerable heat was generated by the Mally-Hunter dispute and although this must have been distracting to the participants, it did not radically change the direction of events of boll weevil history. The fact of the matter is that neither Mally, Hunter nor Knapp had a complete remedy for the boll weevil and their efforts did little to prevent the continued spread of the species. Whether emphasis was on chemical, cultural, or biological control, neither by itself or in combination with the others, was sufficient to obtain the desired results, that is, to eliminate the pest as an economic factor. Thus, the quest for a magic solution continued.

In 1905 the boll weevil laboratory at Victoria was transferred to Dallas, presumably to be closer to the advancing front of the boll weevil's spread. Political, economic and other scientific reasons were likely also involved in the decision to move the laboratory. In any event, research at the Dallas laboratory was expanded to include veterinary pests, especially

FIG. 6. **W. D. Hunter.** September 14, 1903. In his office in the USDA boll weevil laboratory, Victoria, Texas.

ticks, and other crop pests. Field experiments on the boll weevil continued there as well as in various other places in Texas, including the Victoria area. Hunter had always had a broad interest in entomology and in addition to many scientific papers and popular writings on the boll weevil, by 1905 he had published on a variety of other insect pests, including aphids, grasshoppers, leaf worms, cotton stainers, fall army worms, and many others. He had developed considerable interest in medical and veterinary entomology as well, and after

moving to Dallas was involved in conducting research on ticks. Mrs. A. S. Margoulis, Hunter's daughter, remembered that he and his colleagues worked extensively with ticks at the Dallas Laboratory, feeding them on dogs.[7] In subsequent years he published several important papers on various species of ticks with W. A. Hooker, F. C. Bishopp, and J. D. Mitchell. Work on the boll weevil continued to have priority, however, and several other papers were published on this species between the time Hunter moved to Dallas and when his last paper on the weevil appeared in 1924. A total of 38 papers on the boll weevil was either authored solely by Hunter or coauthored with colleagues. Occasionally, Hunter was accused of pirating the research information of his staff and publishing it as his own, much as was the custom of Charles Valentine Riley, an earlier USDA entomologist and at one time Chief of the Federal Entomological Service, who considered the work of those he supervised as being his own.[10] It is of interest that Hunter was senior author on all coauthored papers and bulletins bearing his name. However, in his defense it should be noted that a few members of his staff, for example W. E. Hinds and W. D. Pierce, published some work done under Hunter's direction without him being included as an author.

In 1909 the USDA laboratory responsible for boll weevil research was again moved, this time eastward to Tallulah, Louisiana. This move was presumably made to place researchers in closer proximity to the advance of the weevil and to provide research more applicable to the humid conditions of the southeastern United States. Some research activities continued at the Dallas laboratory. Hunter remained in Dallas and in 1911 was placed in charge of the Southern Field Crop Insects Investigation section of the Bureau of Entomology, with expanded responsibility for insects of crops other than cotton. He apparently also spent some time in Washington because Gould (1959) mentions finding him in 1910 in a "little cubby hole office in the Department of Agriculture Building on the Mall. In 1912 he and W. D. Pierce officially moved to Washington, D.C. where Hunter remained until 1917.[10] He did not like administrative work and life in Washington and although L. O. Howard wanted him to remain there, he preferred field work and was transferred back to Texas to direct the new program on the pink bollworm.[7] During this period of his life, Hunter's reputation as an entomologist was being recognized and he began to reap the benefits of his exceptional service to science. He was elected President of the American Association of Economic Entomologists in 1912 and President of the Entomological Society of Washington in 1915. As further recognition of his accomplishments, an honorary degree was conferred upon him by Tulane University in 1916 for his contributions to southern agriculture (Howard 1925b).

Hunter entered another phase of his entomological career in 1917 when the pink bollworm was first found in the United States at Hearne, Texas. He was assigned to the Federal Horticultural Board to take charge of the USDA response to the threat of this potentially damaging pest. It was to this project that he dedicated the remaining few years of his life. The task was not an easy one and Hunter had to summon all of his tact and resourcefulness to handle the technical and political aspects of this project. There was considerable resistance in Texas by farmers who by law were forced to discontinue planting of cotton in certain areas. Like the boll weevil previously, the pink bollworm continued to spread in spite of the measures taken to stop it. The history of the invasion of the pink bollworm and the early part Hunter played in the politics of quarantine and control of the pest is a subject deserving of special study. Hunter died in El Paso, Texas, October 14, 1925, at the age of 50 years, while on one of his frequent pink boll worm inspection trips. Ed L. Ayers, who was acquainted with Hunter through pink bollworm work, related that Hunter was relentless in his dedication to the pink bollworm project and would often leave in the middle of the night on a trip from his headquarters in Houston to El Paso or some other distant place. He would be accompanied by Paul Hoidale, his driver and "right hand man."[8] Gould (1959) stated that Hunter died of "pneumonia contracted in the field" and continued to say that he was "a soldier of science who gave his life in the line of duty." According to Howard (1925b), Hunter died suddenly of "apoplexy." Regardless of the cause of death, the quick pace at which he worked and the stress of dealing with the explosive politics of the pink bollworm problem were surely detrimental to his health.

The premature death of W. D. Hunter robbed the entomological world of one of its brightest and most dedicated students. Hunter served entomology at critical points in the introduction of two major pests of cotton, the boll weevil and the pink bollworm. He

assembled staffs of well trained entomologists, sometimes called "Hunter's men," who contributed much to the knowledge of life histories, ecology, and control of these pests. Later in his career he developed a deep interest in veterinary and medical entomology and contributed many papers on these subjects. Howard (1925a) gave special praise to Hunter for his addresses and papers on these subjects. W. D. Pierce, who worked closely with Hunter from 1904 until 1918, accredited Hunter with being a "fine organizer" and a person who was able to establish an excellent rapport with farmers.[10] As an indication of Hunter's excellent administrative abilities, Pierce further stated "Each year Hunter had me make a detailed analysis of each problem before us, and this was set up as the standard for the experiments for the year. He was constantly alert for new problems and so we added cotton insects, rice, sugar cane, and tobacco insects; and then we took on the cattle tick, and built

FIG. 7. **L. O. Howard** (second from left) and **W. D. Hunter** (fourth from left) at Calvert, Texas, June 8, 1903, on a boll weevil inspection tour. Names of other men not known. Courtesy of C. R. Parencia.

this section into insects affecting the health of man and animals." A comprehensive paper on cactus insects which Hunter coauthored with F. C. Pratt and J. D. Mitchell (Hunter et al. 1912) provided knowledge about American cactus insects upon which the Australians later based much their study and successful biological control of cacti in Australia (Mann 1969). Many years after Hunter's death he was still being remembered for his encyclopedic entomological knowledge and personal characteristics. Victor Schoffelmayer wrote in 1942: "During my many years of close acquaintance with many distinguished scientists, dating back to about 1913, when my investigations of agriculture in the Southwest led me from Chicago to Texas, I have never known a more interesting, refreshing, broadly informed and highly cultured personality than the late Dr. Walter D. Hunter."[6] There were surely those associated with Hunter who would not have praised him as highly as did Howard, Pierce, and Schoffelmayer; however, these individuals were mostly silent in their public assessments of him. The criticism that he sometimes used data accumulated by his assistants as his own has been related above. The boll weevil and pink bollworm problems both had potential for causing friction between individuals and groups with different views involving credit for research and effectiveness of various control measures. Whatever faults

Hunter may have had and enemies he made, the fact remains that he was totally dedicated to his work and assembled and supervised a young and enthusiastic group of like-minded scientists who made great strides in accumulating information about the boll weevil and other pests. As an indication of his ability to recognize and attract the best people, it should be noted that practically every one he chose to work with him eventually became a leader in some special area of entomology. Like Mally, Hunter was the man for the times in boll weevil research and one wonders what his additional accomplishments would have been had he not died prematurely. Perhaps the best judge of this was L. O. Howard who knew him well: "Although Hunter's mind was peculiarly fitted to research work and although he had done much excellent work of that character, it is doubted that, had he lived, he would ever have returned to personal research, although he often said that he would like to do so. Undoubtedly, however, his great ability in directing and stimulating research would have had full play and the world would have been a great gainer" (Howard 1925a).

WARREN ELMER HINDS (1876-1936) (Fig. 8)

One of the outstanding young entomologists who W. D. Hunter brought to Texas to work on the boll weevil was W. E. Hinds. Not only did Hinds accomplish excellent work on the boll weevil in those early years in Texas, but he also became a highly respected administrator/researcher in Alabama and Louisiana in his later years.

FIG. 8. **W. E. Hinds.** November 7, 1903. In his laboratory at the USDA boll weevil laboratory, Victoria, Texas.

W. E. Hinds was born September 20, 1876, in Townsend, Massachusetts, where he spent his early years on a farm. He received the B.S. and Ph.D. degrees from the Massachusetts Agricultural College in 1899 and 1902, respectively. It is of interest that he was the first to receive a Ph.D. degree from his alma mater (Thomas 1936). Hind's graduate thesis was on the taxonomy of the Thysanoptera but as is the case with many entomologists, his graduate speciality was not continued into his subsequent professional career. The broad training received in departments of entomology in those days prepared students to study a large diversity of insects and insect problems. Under these circumstances, it was probably not unusual that Hinds spent his professional life in the South working mostly on cotton insects. He was appointed as a Special Field Agent in the

U.S. Department of Agriculture by L. O. Howard in 1902 and moved to the Victoria, Texas boll weevil laboratory to work under the direction of W. D. Hunter. Hinds took a very active role in the boll weevil work and was eventually placed in charge of the laboratory. Within two years of his arrival in Texas he was junior author with W. D. Hunter on USDA Bulletin 45, The Mexican Cotton Boll Weevil, the first major publication on this pest (Hunter and Hinds 1904). A considerable revision of this bulletin was published the following year (Hunter and Hinds 1905), indicating a rapid change in the amount of information being accumulated on the species. This bulletin contained a remarkable amount of basic life history and ecological information on the species as well as information on its natural and chemical control. Dr. Hind's wife, Edith, also worked in the Victoria laboratory although it is not evident that she was an employee of the USDA. Her work was on mosquitoes, a group in which W. D. Hunter also had an interest.

Hinds moved to Dallas in 1905 when laboratory work on the boll weevil was transferred there. He wrote a comprehensive bulletin on the effects of proliferation of tissue of buds and fruit of cotton in the natural control of the boll weevil (Hinds 1906), and another on effects of climate, cultural control, and other factors on the natural control of the species (Hinds 1907). The data used were collected by himself and through the observations of others at the Victoria laboratory. Another bulletin on the hibernation of the boll weevil was written in collaboration with W. W. Yothers (Hinds and Yothers 1909). This bulletin, a summarization of the experiments and observations of several entomologists, included the first comprehensive studies of this aspect of the weevil's biology and was much consulted and quoted for many years to come.

The research and administrative abilities of W. E. Hinds became well known through his studies on the boll weevil for the USDA and in 1907 he became Head of the Department of Zoology and Entomology at Alabama Polytechnic Institute (now Auburn University) and Entomologist with the Alabama Agricultural Experiment Station. There he continued his work on various aspects of cotton entomology, including further studies on the boll weevil. His main entomological interests were in insect control and his research was primarily devoted to this area. Much of his work in Alabama concerned the use of calcium arsenate for control of the weevil. He compared the effectiveness of calcium and lead arsenate, and investigated different methods of application of dusts and various mechanical and cultural means for control of the weevil.

In 1924 Hinds moved to Baton Rouge, Louisiana where he was employed by the Louisiana Agricultural Experiment Station and Extension Service. He continued work there on the boll weevil and other cotton insects. During February and March, 1926, Hinds was invited by cotton growers in the Canete Valley of Peru to visit and study cotton insects there. During the six weeks visit he obtained sufficient information to write articles on the Peruvian cotton square weevil and other major cotton insects in the country (Hinds 1927, 1928). He returned from Peru to continue work on insects of cotton and other crops in Louisiana. In 1933 he was elected President of the American Association of Economic Entomologists and presided over the 46th annual meeting of that organization in 1934. His presidential address, entitled "Achievements in Economic Entomology," demonstrated an intimate knowledge of all aspects of contemporary economic entomology as well as an excellent grasp of entomological history (Hinds 1934).

Dr. Hinds died in Baton Rouge, Louisiana, January 18, 1936, at the age of 59. His accomplishments in entomology were many. He was author or coauthor of over 100 publications on many groups of economically important insects, including those affecting cotton, stored products, sugar cane, and truck crops (Mickel 1937, Thomas 1936). The early studies which he conducted on the biology of the boll weevil while working with W. D. Hunter ranks among the most important basic studies on this species. The Hunter and Hinds bulletin (No. 45) published in 1904 and its enlarged successor (No. 51) in 1905 were the first comprehensive publications on the boll weevil. Hinds' later work on the boll weevil mostly involved control, but he remained a respected authority on all aspects of the species throughout his life. W. E. Hinds was involved in many civic and professional activities and was truly an outstanding representative of the early boll weevil workers. A chime of 10 bells was erected on the campus of the University of Massachusetts, Amherst, and dedicated to Hinds with the inscription "In memory of Warren Elmer Hinds, class of 1899, a distinguished scientist to whom music and the beauty of this valley were ever dear, these bells are presented to Alma Mater by his classmate Bernard H. Smith, A. D.1937" (Bailey 1938).

WILLIAM DWIGHT PIERCE (1881-1967) (Fig. 9)

W. D. Pierce was another scientist who began his entomological career working on the boll weevil with W. D. Hunter at the Victoria laboratory. Pierce contributed substantially to the knowledge of this species as well as to many other areas of entomology. He was born in Champaign, Illinois, November 16, 1881. His entomological interests were developed during his high school days in Omaha, Nebraska when he and a like-minded group of young neighborhood acquaintances formed the Polyphemus Club. They even wrote by hand a monthly report on their experiences in rearing butterflies and moths. To his great fortune, as a youth he was able to attend weekly lectures on entomology by Professor Lawrence Bruner of the University of Nebraska. The die was cast and in 1900 he enrolled at the University of Nebraska to study entomology just as W. D. Hunter, with whom he was later associated in boll weevil research, had done nine years earlier. There he worked his way through college at various tasks, further developed his broad interests in the biological sciences, and had the opportunity to gain experience working with many groups of insects. Like Hunter, he graduated from the University of Nebraska, receiving the A.B. degree in 1904 and the A.M. in 1907. The Ph.D. degree was obtained from George Washington in 1917, long after his initiation to research on the boll weevil in Texas. Since little biographically has been published on Pierce, we are fortunate that late in his career he wrote Arnold Mallis and related considerable information on the early part of his highly diverse entomological career.[10]

FIG. 9. **W. D. Pierce.** Courtesy of B. Brown and the Los Angeles County Museum of Natural History.

Upon completion of work for the A.B. degree in 1904, Pierce obtained a position as Assistant State Entomologist in Mississippi working under the supervision of Glenn W. Herrick. He was there for only a few months when he was appointed as Special Field Agent of the Bureau of Entomology, USDA, and was sent to Victoria, Texas to work on

the boll weevil with Hunter. He arrived in Victoria on the same day as three other entomologists who were to become well known in their profession: W. A. Hooker, A. C. Morgan and W. W. Yothers. Entomologists already at the laboratory were Hunter, W. E. Hinds, A. W. Morrill, and J. C. Crawford. No one in the farming and ranching town of Victoria could have ever predicted that such a remarkable group of young savants would one day be residents there. Surprisingly, published histories of Victoria ignore this event and, furthermore, I have not found any account by the entomologists regarding their views of Victoria and adjustment to life there.

Immediately after Pierce's arrival in Victoria, he became interested in the insects of the area and in connection with his boll weevil work he collected and learned much about the biologies of other species. He was especially interested in all of the species of weevils that serve as alternate hosts of parasites which attack the boll weevil. He collaborated with J. D. Mitchell, a local naturalist and Bureau of Entomology collaborator, to write papers on the weevils and ants of Victoria County (Mitchell and Pierce 1911, 1912). Pierce's thesis for the M. A. degree at the University of Nebraska grew out of his work on the boll weevil (Pierce 1907). In this thesis he included data from field work that he had conducted on the biologies of weevils along with information gleaned from the literature to provide a comprehensive summary of the subject. The resulting publication is still being cited as the most complete, and in many cases the only, available source of information on the biology and host plants of many species of weevils.

In view of the present resurgence of interest in the parasites of the boll weevil and their effectiveness in controlling the species, it is of interest to note that much of Pierce's work on this pest at Victoria and later at the Dallas laboratory dealt with this subject. Parasites were reared from the boll weevil from different areas in the infested zone as well as from many other species of weevils. It was obvious that other species of weevils served as hosts of parasites which attacked the boll weevil and this aspect of the study received considerable attention. The first of his major contributions on this subject were published in 1908 (Pierce 1908a, b, c; Hunter et al.1908). Much basic information was provided on the various biological and climatic conditions that contributed to the effectiveness of parasites in controlling the weevil. A comprehensive report of studies of Pierce and his colleagues on natural enemies of the boll weevil was published in 1912 (Pierce 1912). Of special interest is his figure showing the intricate relationships between plants, weevils, parasites and other natural enemies, and other pest species. It is obvious from this diagram that he viewed the relationships of the weevil, its natural enemies, and plants as constituting a complex ecological network. The work on parasites of the boll weevil was Pierce's most important contribution to the knowledge of the species. The earlier bulletin (Number 51) by Hunter and Hinds (1905) was revised and brought up-to-date by Hunter and Pierce in 1912 (Hunter and Pierce 1912). Another contribution made by Pierce that is of considerable interest to the boll weevil problem was his description of *Anthonomus grandis thurberiae* Pierce (the thurberia weevil) from *Gossypium thurberi* in Arizona as a new variety (later to be considered as a subspecies) (Pierce 1913). The description of this form (or race) of the boll weevil isolated on a wild host in the West has fostered extensive study on its ecology, life history, and relationship to the boll weevil of Texas and the southeastern United States. A summary of the taxonomic status of the thurberia weevil was presented by Burke et al. (1986), wherein it was concluded that it represents a form of *Anthonomus grandis* that does not warrant subspecies status. However, its ecology and relationship to cultivated cotton and the other forms of the boll weevil continues to both interest and confound entomologists. After description of the thurberia weevil, Pierce collaborated with others to study it further (Coad and Pierce 1914; Pierce and Morrill 1914). During the period 1915-1919 Pierce collaborated with Hunter to publish a brief account each year of the previous year's movement of the boll weevil. In 1919 he left the Bureau of Entomology and only occasionally thereafter wrote about the boll weevil.

Early in his career, and probably as an extension of his boll weevil work, Pierce developed an interest in the systematics of the superfamily Curculionoidea, an interest that was to remain with him throughout the remainder of his life. His first papers on weevils appeared in 1907 and the last was published posthumously in 1975, eight years after his death. During this period he published about 30 papers on weevils other than the boll weevil. Some of these papers were fairly substantial in coverage and many new taxa were described and nomenclatural changes made. It is difficult to assess a person's total contributions to a field of taxonomic endeavor and Pierce's work is no exception. Most of

the genus and species-level taxa that he described have withstood the test of time. However, he had a somewhat unorthodox view of the classification of weevils and tended to excessively divide species and higher taxa (in other words, he was a "splitter"). Many of the new tribes that he recognized are no longer considered to be distinct entities. He was prone to use varietal and subspecies categories to the extreme; for example, in his posthumous paper on *Trigonoscuta*, a genus of sand dune weevils (Pierce 1975).

Strepsiptera is another group of insects in which Pierce developed an early interest His first publication on this group was in 1904, followed by another in 1908, and the following year by an extensive monograph on the group (Pierce 1909). This was a remarkable accomplishment for a young entomologist approximately five years removed from graduation with the A.B. degree, especially when his work with the boll weevil during this period must have been very time consuming. A considerable amount of biological information on the twisted-wing parasites was included in the latter monograph. He returned to this group from time to time throughout his career, describing new taxa and discussing his views on the phylogenetic relationships of the group. There has been considerable disagreement among specialists through the years as to where the strepsipterans fit phylogenetically in relation to other insects and Pierce was apparently quite eager to enter the fray. In fact, his last paper on these insects dealt with this subject, concluding that they constitute a distinct order that has characters that ally it more closely related to whiteflies and scale insects on the one hand and Diptera on the other (Pierce 1964). Two families and many genera and species of North American Strepsiptera owe their names to Pierce. Bohart (1941), in the most recent treatment of North American taxa, stated: "The most important contribution in recent years was that of W.D. Pierce who in several papers (1908-1918) brought together most of the information on the group and described and figured many new forms."

As Pierce's work with the boll weevil was being phased out, he became involved with other insects in his capacity as a USDA scientist. As mentioned above, he moved from Dallas to Washington in 1912 and gradually became less involved with work on cotton pests. In 1918 he edited a book on dangerous insects likely to be introduced in the United States (Pierce 1918b). During World War I he was "acting in charge" of both Southern Field Crop Insects and Insects Affecting Man and Animals.[10] The latter responsibility apparently encouraged his interest in medical entomology as he wrote extensively about this subject in 1918 and occasionally at other times throughout his career. One example is an edited book on sanitary entomology (Pierce 1921). Also during this period he was working toward the Ph. D. degree which was awarded by George Washington University in 1919. The research for his degree was a study of the comparative morphology of the Strepsiptera (Pierce 1918a).

In 1919 Pierce left the USDA and entered the field of commercial entomology as a consultant. He was Vice-President of Gage-Pierce Research Laboratories, Inc. (1919-1920), and Managing Director of the Biology Department of the Mineral, Metal and By-Products Co. (1920-1923). In *American Men of Science* (1967, 11th. ed.) he is listed as Consulting Biologist for the period 1919-1927. During this period he wrote on a large array of subjects, including religion, social and economic issues, medical and sanitary entomology, and insect enemies of grape, ornamentals and many other commercially valuable plants. His deep religious beliefs are evident in the many items he wrote on this subject in 1925 and 1926. In 1927 Pierce accepted a position as entomologist with the Victorias Milling Co. in the Philippines. It was not long after arriving there that he started publishing articles on sugarcane insects. Having always been one who emphasized the practical side of his science, he immediately assessed the pest situation on sugarcane in the Philippines and made recommendations for control. Many papers were published on this subject during 1928 and 1930.[9]

In 1931 Pierce was back in the United States serving as Assistant Editor for *Biological Abstracts* in Philadelphia, a position he held until 1936. His entomological output was considerably reduced during this time but he did manage to publish a paper of substantial size on weevils and a few brief ones on other entomological subjects. Pierce became Curator of Paleoentomology at the Los Angeles County Museum in 1937 and remained there until 1952, at which time he became Emeritus Curator. It was during this period that he began to work on sand dune insects and fossil arthropods, publishing numerous papers on these subjects. He was active throughout his life and had two large

manuscripts in various stages of completion at the time of his death on April 29, 1967. These manuscripts, one composed of essays on various aspects of medical entomology (Pierce 1974) and the other on his beloved *Trigonoscuta* weevils (Pierce 1975), were published posthumously as stipulated in his will.

Little in the way of assessment of the nature of W. Dwight Pierce and his accomplishments in science has been written. There was not even a proper obituary written for publication in the scientific literature as is usually the case for prominent scientists. Perhaps this was because of his private nature and no one really felt close enough to him to review his life, or perhaps he simply outlived those who had worked closely with him during his long career and knew him best. We are therefore restricted to the few available published comments about him. Ashley B. Gurney (1978) states: "Dwight Pierce seems to have been a consistently imaginative, studious entomologist, glad to tackle new and difficult problems. Even if not always successful in an outstanding way, he made important and unforgettable contributions. Throughout his career, he was a scholarly naturalist of the old school." Elbert L. Sleeper, a weevil specialist himself who had many opportunities to talk with Pierce in the latter's last years and who also edited the posthumous *Trigonoscuta* weevil paper, remarked that he never ceased to be amazed at Pierce's devotion to so many tasks with such an unbelievable degree of organization. He scheduled his time for maximum efficiency (Pierce 1975b). Sleeper continues "Each day of the week was a particular study day and had a specific task assigned to it as 'stamp collection day'; 'nodule day' [fossils]; 'religious study day'; etc. Tuesday of each week was, without fail, *Trigonoscuta* day." In another context, Sleeper assesses his character thusly: "A more honest, moral, frugal gentleman I have never met." [11] On such a note I end this brief essay on W. Dwight Pierce, another one of "Hunter's men" and an early boll weevil fighter who contributed significantly to useful information about the species.

JOSEPH DANIEL MITCHELL (1848-1922) (Fig. 10)

It has already been emphasized that W. D. Hunter was highly successful in selecting bright and capable young entomologists trained in the North and Midwest to conduct early research on the boll weevil in Texas. With the addition of J. D. Mitchell to his staff, Hunter also proved that he was equally astute in recognizing local talent. When Hunter was in Washington preparing to come to Texas in 1901 to initiate studies on the boll weevil he inquired widely among scientists there about the names of persons in Texas who were knowledgeable about the natural history of the state (Hunter 1922). He was repeatedly referred to J. D. Mitchell of Victoria, Texas. Hunter concluded: "A man whose knowledge has made such a deep impression on the leaders of several departments of science must certainly have been in some degree remarkable." When Hunter arrived in Texas he sought out Mitchell and the two developed a deep friendship, resulting in Mitchell's association in 1904 with the boll weevil research program at Victoria.

J. D. Mitchell was born October 22, 1848 at Mitchell's Point in the Coastal Bend area of Texas. His family had large ranching and farming interests there and it was in this expansive and biologically rich setting that Joseph developed a lifelong interest in natural history. Because of the lack of adequate schools in the area he received little formal education and even that had to be obtained at such places as Galveston and San Antonio, Texas, and Bay St. Louis, Mississippi. Furthermore, the Civil War interrupted his plans to attend college. In spite of the lack of a proper formal education Mitchell became a progressive and highly successful rancher while at the same time nurturing his love of nature. In 1887 he moved to Victoria and began to dispose of his ranch and farm holdings. The financial independence gained from sale of the property gave him the opportunity to pursue his many interests, including that of collecting and observing a wide variety of animals and plants. (Burke 1978)

Mitchell's observations on snakes, mollusks, mammals, and insects and his other natural history pursuits have been discussed by various authors (for example, Bailey 1923, Burke 1978, Neck 1989, and Roell 1990), therefore, I will concentrate on his contributions to the knowledge of the boll weevil. He had an early interest in cotton insects, dating back at least into the 1870's, at which time there was considerable concern about the damage caused by the cotton leafworm, *Alabama argillacea* Hübner, and the bollworm, *Helicoverpa zea* (Boddie). Mitchell apparently participated in research being conducted by

the U.S. Entomological Commission on the use of trap lanterns for control of these species. He was also the first person to bring attention to the damage caused by the cotton fleahopper, *Pseudatomoscelis seriatus* (Reuter). By the late 1890's he was experimenting with a mixture of starch, cresylic ointment, arsenic and molasses for the control of the boll weevil. When the boll weevil laboratory was established at Victoria in 1902, W. D. Hunter as its leader began to assemble a staff of competent young scientists. Some of these entomologists remained with the laboratory until it moved to Dallas in early 1905 while others stayed a shorter period of time or merely made brief visits to the laboratory. This influx of entomologists into his hometown must have been a delight to Mitchell whose previous association with scientists had been mainly via correspondence. In 1904 Mitchell became a collaborator, specifically a Special Field Agent, with the Bureau of Entomology and immediately became involved in work on the boll weevil as well as with ticks and other insects in which Hunter and the laboratory staff were interested. Although Mitchell played the role of an assistant in research, his contributions to the success of various experiments were quite evident. Hunter (1908) provides an indication of how Mitchell was involved in boll weevil research. Credit was given to Mitchell for conducting experiments in 1906 and 1907 involving the destruction of cotton plants as a control measure. The success of the large-scale experiments conducted in Calhoun County was in no small part due to Mitchell's ability to obtain cooperation from farmers with whom he was familiar. Howard (1922) credited Mitchell with being of great assistance to Hunter and other researchers of the U. S. Department of Agriculture and stated: "His knowledge of cotton conditions and his keen powers of observation of everything in nature gave him a unique standing."

When most of the personnel of the Victoria laboratory were transferred to Dallas in early 1905, as noted above, Mitchell remained in Victoria and continued working on field studies of the boll weevil and other insects in cooperation with USDA entomologists. He continued his association with the U. S. Department of Agriculture until 1919 or 1920. In 1918 he became ill and moved from Victoria to San Antonio where he died February 27, 1922.

FIG. 10. **J. D. Mitchell.** Victoria, Texas. Courtesy of A. G., Wheeler, Jr. and Penn State University Entomology Archives.

Insects were only one of Mitchell's many natural history interests. He corresponded with many scientists of different disciplines and freely contributed specimens and information for their studies and collections. Upon his death, several well known scientists wrote obituaries highly complementary of Mitchell's accomplishments in the field of natural history. Hunter (1922) referred to him as a "fountain of accurate information for technical men and was a modest, patient and painstaking imparter of knowledge." Bailey (1923) stated: "His careful observations have helped settle many vexed questions of the economic status of mammals, birds, reptiles and insects and his accuracy and integrity have won the confidence of naturalists and residents of his state." Perhaps the highest praise of all came from L. O. Howard (1922) who related: "He was a rare man, quite the type of his more famous contemporaries, John Muir and John Burroughs." Although Mitchell's name does not appear as author on any publication on the boll weevil, he observed the species from the time of its first appearance in his area and later had the opportunity through the USDA to participate in research on the species. His name is more likely to be found in the body of early papers on the boll weevil where authors quote his observations or acknowledge his assistance in the

research. Although he did not have the academic credentials possessed by his associates in boll weevil work and his contributions were not as evident, he certainly deserves to be counted among them as a pioneer in study of the species.

ACKNOWLEDGMENT

The writing of biographical sketches such as those presented here is invariably dependent upon the assistance of many individuals. Some of those who helped me most are now deceased: Dr. V. A. Little, Mr. Charlie Parencia, and Dr. Ernest R. Tinkham. Dr. Little, whose history of the Department of Entomology at Texas A&M University has been quoted frequently here, was highly knowledgeable about the "oldtime" entomologists and freely shared this information. To him I am indebted for the encouragement he gave me in pursuing the history of entomology. Charlie Parencia had a wealth of information about the early cotton entomologists and worked to preserve this history. I am especially fortunate that he shared some of this with me. A special gift from Charlie was a series of photographs of W. D. Hunter and other entomologists from the Victoria boll weevil laboratory. Dr. Ernest Tinkham and I once entertained the idea of writing a biography of W. D. Pierce. We began collecting biographical information on Pierce and Tinkham sent me copies of the material he obtained in California. Ernest died before we were able to finish the biography and what I have presented here is essentially a summary of our research up to that time. Others have also given generously of their time on my behalf. Dr. Sloan Jones, a longtime USDA scientist and now retired at Kerrville, Texas, has always responded quickly and fully to my inquires about cotton entomologists. Dr. Jones gave me the copy of W.D. Hunter's first field notebook which piqued my interest in Hunter and the contributions he made to the study of the boll weevil and other insects. Dr. Brian Brown, Los Angeles County Museum of Natural History, kindly provided a photograph of W. D. Pierce, and Dr. A. G. Wheeler, Jr., Pennsylvania Department of Agriculture, located a photograph of J. D. Mitchell in the Pennsylvania State University Entomology Archives which he obtained for me. I would also like to acknowledge the help of Dr. Douglas Helms, Soil Conservation Service, Washington, DC, for his assistance in obtaining the photograph of C. H. T. Townsend. Dr. Helms has conducted extensive research on the history of the boll weevil and I am appreciative of his encouragement of my efforts. Appreciation is also due Prof. Charles Spurlin of Victoria College who marshalled local historians in tracking down information on the early Victoria boll weevil laboratory and who provided information on J. D. Mitchell. To all of the above and to others not named who have helped me in my historical efforts, I give my sincere thanks.

NOTES

[1] Fred W. Mally. Presidential Address, Texas Entomological Society-11th. Annual Meeting, Dallas, Texas-February 9-10-11, 1939, 9 pp. A first- hand account of some of the earliest work conducted on the boll weevil and fruit and nursery pests in Texas. A copy of the address is deposited in the Texas A&M University Archives.

[2] Bryan {Texas] Eagle, June 1, 1901. A handbill entitled: Save the Cotton Crop. Kill the Boll Weevil! To the Farmers of Brazos and Adjoining Counties. Includes a list of merchants and citizens supporting the use of arsenate of lead to poison the boll weevil with instruction for preparation of Mally's mixture of poison and molasses. Also reports on a meeting of F. W. Mally with farmers and business men to instruct them on methods of boll weevil control.

[3] V. A. Little. 1970. Some contributions to the knowledge and control of the boll weevil made by Frederick W. Mally, the first entomologist at Texas A&M (1899-1902), typescript, 2 pp.

[4] Typed letter, W. D. Hunter to Lawrence Bruner, May 1, 1897. Department of Entomology, University of Nebraska. Provided by R. E. Hill. In this letter Hunter informs Bruner in Argentina of the firing of faculty, administrative changes, and student unrest at the University of Nebraska. Hunter was in charge of the department while Bruner was studying grasshoppers in South America. A note accompanying the letter indicates that Bruner did not get the unsettling news until August 9, 1897.

[5] W. D. Hunter field notes, March 20,1901-May 29,1901. Original copy in possession of Horace R. Burke. These notes reveal a considerable amount of information about the status of the boll weevil at an early period in its invasion and the attitude of farmers toward the pest and its damage to cotton.

[6] Schoffelmayer, V. 1942. Throne of besieged King Cotton defended by northern expert. The Dallas Morning News, Sec. II, April 11, 1942, p. 16. A lengthy newspaper article about W. D. Hunter written by a longtime observer and writer of the Texas agricultural scene. Schoffelmayer knew Hunter from the early days of the pink bollworm invasion and traveled with him on some of his inspection trips around the state. Especially interesting is the account of their trip to the Big Bend region of Texas in 1921 when roads fit for travel by automobile were practically nonexistent. Hunter's adventurous nature is exemplified in his approach to the problems they encountered along the unusually muddy trails over which they drove. Schoffelmayer's article praises Hunter highly for his leadership on projects involving the boll weevil and pink bollworm, tactfulness, dedication to a purpose, and scholarship.

[7] Telephone conversation between Mrs. A. S. Margoulis, Houston, Texas, and Horace R. Burke, January 3, 1980. Mrs. Margoulis is the daughter of W. D. and Mary P. Hunter. She remembers only vaguely the entomological events at the Dallas laboratory and those later in Washington, D.C. when the family lived there for "six or seven years." She recalls the affection which Hunter had for J. D. Mitchell of Victoria. Mitchell was apparently the person who introduced Hunter to Mary P. Smith of Victoria who Hunter later married. According to Mrs. Margoulis, L. O. Howard wanted Hunter to remain in Washington in an administrative capacity but Hunter declined the offer. Mrs. Hunter liked Washington but W. D. wanted to get into the field and was transferred back to Texas to direct the pink bollworm project.

[8] Conversation between Ed L. Ayers (now deceased) and Horace R. Burke, March 21, 1980, concerning W. D. Hunter.

[9] Publications of William Dwight Pierce. Photocopy of list compiled by Pierce with last entry dated 1965. Two posthumous publications have been added. This list has Pierce's notes about the publications, number of editions of books published and a list of new taxa described and nomenclatural changes proposed. This list is especially valuable as a source of Pierce's activities because the many items he wrote in newspapers, trade journals and other nonscientific publications are included. A copy is in the possession of Horace R. Burke, courtesy of Ernest R. Tinkham and Richard Loomis (both deceased).

[10] Letter from W. Dwight Pierce to Arnold Mallis, April 9, 1962, in response to questions posed by Mallis concerning the entomologists Lawrence Bruner, Walter David Hunter, and Charles L. Marlatt. The original is in the Archives of Entomology, Pennsylvania State University. Copy in possession of Horace R. Burke, courtesy of Charles W. Rutschky. This letter has been helpful in compiling information on Hunter as well as providing a personal account of Pierce's early life, education and boll weevil work.

[11] Letter from Elbert L. Sleeper to Ernest R. Tinkham, April 13, 1982, in response to request for information on the life and work of W. D. Pierce. Copy provided to Horace R. Burke by E. R. Tinkham.

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MINUTES OF THE 1997 ANNUAL MEETING OF THE EXECUTIVE COMMITTEE AND THE ANNUAL MEETING OF THE SOUTHWESTERN ENTOMOLOGICAL SOCIETY

The Executive Committee met at 7:00 a.m., February 19, 1997, at the Radisson Inn in Oklahoma City, Oklahoma during the Annual Meeting of the Southwestern Branch of the Entomological Society of America. President Joe Ellington and Secretary-Treasurer Allen Knutson were present. The financial records of the Society were reviewed and ballots for President-elect were counted. Since the minimum number of three officers necessary for a quorum were not present, the meeting was adjourned.

The 21st Annual Meeting of the Society was called to order by President Joe Ellington at 6:00 p.m. February 19, 1997, at the Radisson Inn in Oklahoma City, Oklahoma, during the Annual Meeting of the Southwestern Branch of the Entomological Society of America. Minutes of the 1996 annual meeting as published in the June issue of the Southwestern Entomologist were distributed. The Secretary-Treasurer's report and Editor's report for 1996 were distributed, reviewed and approved. President Ellington reported on the decision by the Executive Committee to cease publication of manuscripts in Spanish in response to concerns by the Editor that the review process of Spanish manuscripts had become too difficult and time consuming. Problems included the lack of acceptable camera-ready copies from Mexico, delays of up to two years in the revision process, the limited number of reviewers for Spanish manuscripts and the need to ensure only quality papers were published in the Southwestern Entomologist.

Greg Cronholm , Society Archivist, reported he had deposited material relating to the history of the Society with the University Archivist at TAMU. The Society again awarded a one year's membership to the first place winners in the Student Poster and Student Paper Competition. As was later announced, the winners were I. S. Aquino, Oklahoma State University and S. Lingren, Texas A&M University.

President Ellington reported that 108 ballots had been received and that Jerry Michels was the next President-elect. There being no other business, Allen Knutson thanked Joe Ellington for his service and presented him with a plaque from the Society. The meeting was then adjourned.

Respectfully submitted,

Allen Knutson
Secretary-Treasurer

1996 SECRETARY-TREASURER'S REPORT

Balance on hand as of January 31, 1996		$15,804.81
Income: February 1, 1996-January 31, 1997		
Memberships	$ 2,300.00	
Subscriptions	1,760.00	
Page Charges	18,348.62	
Back Issues, Misc.	147.01	
Royalties	43.75	
Total Income		$22,599.38
Expenses:February 1, 1996-January 31, 1997		
Journal:		
Printing	$17,994.26	
Secretary	1,975.00	
Postage & Admail Service	2,056.41	
Editor's Fee	1,800.00	
Society:		
Secretary	900.00	
Supplies	161.43	
Postage, Duplicating	507.32	
President's Plaque	176.94	
Secretary-Treasurer	1,200.00	
Total Expenses		$ 26, 771.36
Balance on hand January 31, 1997		$ 11,632.83

As of January 31, 1997, there were 392 paid members in the Society and 105 subscribers to the Southwestern Entomologist. There were 25 unpaid pages charges, totaling $6,877.50. Most of these unpaid charges were for the December 1996 issue which was delayed at the printers with the result that invoices were not mailed until January. The journal printing costs includes $2,968 for the December 1995 issue.

AUDIT COMMITTEE REPORT:

On February 19, 1997, I examined the 1996 financial records of the Southwestern Entomological Society as prepared by Secretary-Treasurer Allen Knutson and found the records of income and expenses to be in order.

Joe Ellington

EDITOR'S REPORT:

There were 58 manuscripts, totaling 492 pages, published in the four regular issues of the Southwestern Entomologist during 1996 compared to 58 manuscripts and 529 pages in 1995. Two supplements, Numbers 19 and 20, also were published during the year.

I received a total of 72 manuscripts for consideration for publication, compared to 70 in 1995. A number of these are still in the review process; however, 13 of these have already been rejected. This represents a considerable increase over the three manuscripts that were rejected during 1995.

Spanish-language manuscripts are no longer accepted for consideration effective with the notice published in the September issue of the journal. Abstracts printed in Spanish remain optional with the author(s). A number of Spanish-language manuscripts submitted prior to that date remain in the review process and, if accepted, will be published in Spanish.

Editor's Financial Report

Balance Forward 01/01/96	219.09
Receipts	200.00
Expenditures	291.91
	$127.18 Ending Balance

Respectfully submitted,
Darrell E. Bay, Editor

USING REMOTE SENSING TO DETECT AND MONITOR A WESTERN PINE BEETLE INFESTATION IN WEST TEXAS

J. H. Everitt[1], J. V. Richerson[2], J. P. Karges[3], and M. R. Davis[1]

ABSTRACT

We studied the feasibility of using remote sensing techniques to detect and monitor an infestation of western pine beetles, *Dendroctonus brevicomis* LeConte, in a ponderosa pine, *Pinus ponderosa* Dougl. ex. Laud., forest in the Davis Mountains of west Texas. Aerial photography and videography were used in this study. Color-infrared (CIR) and conventional color aerial photography were evaluated and compared on four dates over a 10-week period. Although both films were useful for detecting and monitoring stressed trees, CIR film was better because trees with early stress symptoms could be more clearly delineated from trees showing no symptoms. Trees under moderate to severe stress could also be distinguished better with CIR film. CIR videography was evaluated on a single date and also showed potential for detecting pine beetle infestations.

INTRODUCTION

Bark beetles of the genus *Dendroctonus* are responsible for killing more mature coniferous trees each year than any other group of forest insects in the United States. Although these beetles occur in spruce (*Picea* spp.) and larches (*Larix* spp.), they are predominantly in pines (*Pinus* spp.) (Aldrich et al. 1983). The beetles usually attack the largest and most valuable trees and kill them by sheer force; infestations of several hundred to several thousand beetles may attack one tree. Bark beetles also carry a symbiotic fungus, usually deposited in the beetle galleries, which grows and causes occlusion within the water-conducting tissues of the tree. Girdling by the beetles in conjunction with occlusion of the wood by fungal hyphae causes a rapid decline in tree vigor and eventual death (Aldrich et al. 1983).

The western pine beetle, *Dendroctonus brevicomis* LeConte, is periodically destructive to ponderosa pines, *Pinus ponderosa* Dougl. ex Laud. It occurs in the western and southwestern United States and in British Columbia. Normally this beetle breeds in overmature trees, in windfalls, in root-rotted trees (Cobb et al. 1974), or in trees weakened by drought, stand

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stagnation, or fires. Under epidemic conditions, it becomes aggressive and kills apparently vigorous trees of all ages with bark sufficiently thick to protect the insect in its environment. During the severe drought of the 1920's and 1930's on the Pacific Coast of the United States, 60-90% of the ponderosa pine forest on extensive acres were killed (Furniss and Carolin 1977). Since 1992, drought conditions have prevailed in the Davis Mountains of west Texas and several infestations of western pine beetles in ponderosa pines have been observed in this area. This insect is usually considered less significant in west Texas due to the lack of commercial production value for timber or pulp production in this region. However, ponderosa pines and other pine trees in this region are geographically isolated and have considerable aesthetic value and are a reservoir of genetic diversity; therefore, their preservation is important to land owners, natural resource managers, and the general public.

Remote sensing techniques have proven to be an effective means for detecting and monitoring spread of infestations of several species of bark beetles in forested regions (Caylor and Thorley 1970, Aldrich et al. 1983). Trees attacked by bark beetles show signs of stress by changes in foliage color that facilitates in their detection on aerial photos (Heller et al. 1959, Ciesla 1977).

Although aerial photography has been used to detect western pine beetle infestations in ponderosa pines in the western United States (Caylor and Thorley 1970), this technique has not been used in west Texas. The major objective of this study was to evaluate conventional color and color-infrared (CIR) aerial photographs for detecting and monitoring a western pine beetle infestation in ponderosa pines in the Davis Mountains of west Texas. A secondary objective was to determine the potential of aerial CIR videography for detecting the pine beetle infestation.

MATERIALS AND METHODS

This study was conducted in the Davis Mountains of west Texas. The study site was a stand of ponderosa pine trees located on the north slope of Mount Livermore (Lat. 30° 39' 35", Long. 104° 09' 36"; 6900 feet elevation) about 17 miles west of Fort Davis. On 20 August 1996, an early infestation of western pine beetles was discovered and confirmed in several of the pine trees in the stand. A few of the infested trees exhibited slight chlorosis with some yellow tinged needles, while other trees had evidence of early beetle infestation but showed no change in foliage color. Other trees showed no evidence of beetle infestation.

Aerial photographs and video imagery were obtained of the stand of ponderosa pine trees to evaluate the potential of remote sensing techniques for detecting and monitoring the western pine beetle infestation. Ground observations were made to verify the aerial imagery.

Kodak* Aerochrome conventional color (0.40-0.70μm) type

* Mention of a company name or trademark 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.

2448 and CIR (0.50-0.90μm) type 2443 films were used; photographs were taken with a large format Fairchild camera (23x23cm) and small format twin Hasselblad cameras (5.7x5.7cm). Only conventional color film was used in the large format camera which was equipped with a 305mm lens. The aperture setting was F11 at 1/250 sec. The two small cameras were loaded with CIR film and conventional color film, respectively. The camera containing CIR film was equipped with an orange (minus blue) filter. This camera had an aperture setting of F8 at 1/500 sec. The camera containing conventional color film had an aperture setting of F11 with a shutter speed of 1/500 sec. Both small format cameras were equipped with 80mm lens.

Aerial photography was obtained of the study site at 3-4 week intervals to monitor the western pine beetle infestation. Imagery was taken on 21 August, 9 September, 9 October, and 5 November 1996. On 21 August, conventional color photography was acquired with the large format camera while the CIR photography was taken with one of the small cameras. On 9 September, 9 October, and 5 November 1996, conventional color and CIR photographs were taken simultaneously with the small format twin cameras. Original photographs were obtained at variable scales ranging from 1:2,400 to 1:15,000. All photographs presented here were enlarged to approximately the same scale in order to compare imagery across dates.

Video imagery of the study site was obtained with a three-camera multispectral digital video imaging system (Everitt et al. 1995). The system was comprised of three charge-coupled device analog video cameras and a computer equipped with an image digitizing board. The cameras are visible/near-infrared (NIR) (0.4-1.1μm) light sensitive. Two of the cameras are equipped with visible yellow-green (YG, 0.555-0.565μm) and red (R, 0.623-0.635μm) filters, respectively, while the third camera had a NIR (0.845 to 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 image signals from the cameras are subjected to RGB inputs of the computer digitizing board, thus giving a CIR composite digital video image similar in color rendition to that of CIR film. The hard disk can store 1000 CIR composite images. Video imagery was obtained of the study site on 9 September 1996 at an altitude of approximately 700m above ground level and provided a horizontal ground pixel size of about 0.8m. The video image shown here was photographed off a high resolution monitor.

A GPS (Trimble Transpack II) was integrated with the video system. The navigation system constantly received data from GPS satellites and continuously calculated and displayed the flight direction (bearing), altitude, time, ground speed, and latitude/longitude coordinates. An interphaser (Compix model LP-701) transfered the continuous GPS information on the last two lines of the R-filtered camera, which in turn is also superimposed on the CIR composite image. The latitude/longitude coordinates corresponded to the approximate center of each image and had an accuracy of ±100 m. The altitude data was relative to sea level. All imagery was acquired with photographic and videographic imaging systems mounted vertically in the floor of an Aero Commander fixed-wing aircraft. Imagery was obtained between 1100 and 1500 hours under sunny conditions.

RESULTS AND DISCUSSION

Fig. 1 shows a composite of four CIR (left column) and conventional color (right column) aerial photographs obtained of a stand of ponderosa pine trees infested with western pine beetles near Fort Davis. The photographs were obtained on four dates: 21 August (A and B), 9 September (C and D), 9 October (E and F), and 5 November (G and H) 1996. The arrow on the CIR print points to the pinkish-white hues of trees showing signs of stress by changes in foliage color. These trees visually exhibited a slight yellowing tinge (chlorosis) to the foliage. Other infested trees can be distinguished in the lower center and upper center of the photograph. Pine trees with the typical light green foliage have a magenta tone. Some of the these trees showed evidence of beetle infestation, but had not shown a change in foliage color on 21 August. The small block in the upper right portion of the photograph denotes one such tree. Other hard wood species (primarily oaks, *Quercus* spp.) have dark magenta to red image tones. In the conventional color photograph (B), the infested pine trees with yellow-tinged foliage have a slight greenish-white hue and can not be readily distinguished from the normal light green color of healthy pine trees. A single dead ponderosa pine tree (whitish response) can be seen on the upper top-left margin of both photos (A and B). We surmise that this tree was the source of the current infestation, as it had been infested by western pine beetles the previous year. It appeared to have been initially stressed after having been struck by lightning.

The western pine beetle infestation became more apparent in the photographs acquired on 9 September (Figs. 1C, 1D), than in the 21 August photos. Several of the infested trees whose foliage had not shown the effects of stress in August were exhibiting considerable yellowing of foliage by September. The trees that initially showed slight yellow-tinged foliage in August had a significant increase in discoloration by September, with some leaves turning yellow-tan. The infested trees that had a whitish-pink response on CIR film in August had a more distinct whitish-tan CIR film response in September (Fig. 1C); pine trees showing only very faint or no stress in August had a whitish-pink CIR image tone in September. This change in CIR image response is evident by comparing the tree enclosed in the box in Fig. 1A with the same tree in Fig. 1C. The beetle-infested trees exhibiting stress symptoms were more distinguishable in the conventional color photograph (Fig. 1D) taken in September than in the August photograph (Fig. 1B); stressed trees had a whitish-green to whitish-tan image response in the September photograph.

The image responses of the stressed trees showed generally slight changes in both the CIR (Fig. 1E) and conventional color (Fig. 1F) photographs obtained on 9 October, compared to the 9 September photographs. Ground surveys indicated that the trees had an increase in yellow and yellow-tan foliage over the one-month period, but apparently this did not greatly affect their photographic responses.

Stressed ponderosa pines could be distinguished in both the CIR (Fig. 1G) and conventional color (Fig. 1H) photographs obtained on 5 November. However, the stressed trees could be more clearly delineated in the CIR photograph where they have a conspicuous white image response. In the conventional color

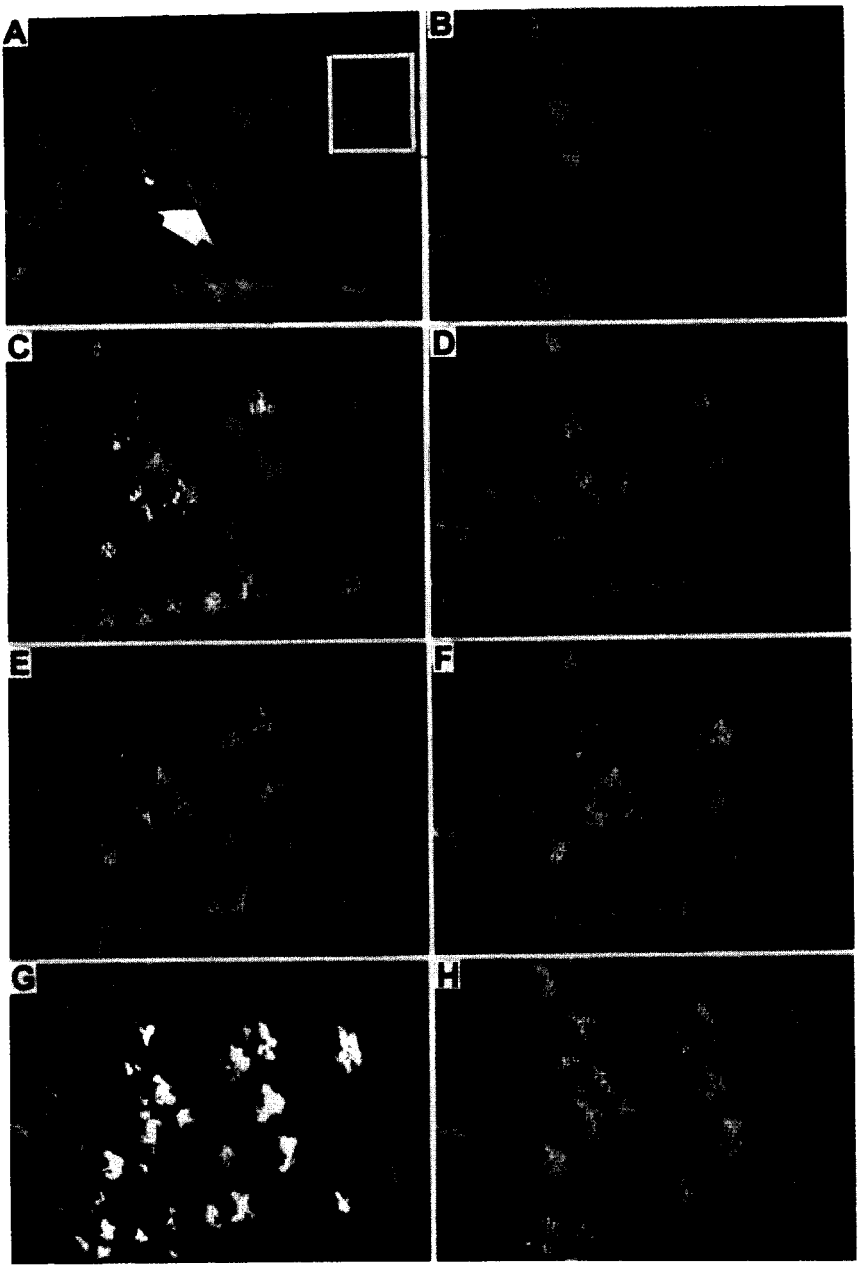

FIG. 1. Color-infrared (left column) and conventional color (right column) aerial photographs of ponderosa pine trees infested with western pine beetles in west Texas. Imagery was acquired on 21 August (A and B), 9 September (C and D), 9 October (E and F), and 5 November (G and H), 1996. The arrow on print A points to a stressed tree, whereas the block denotes a tree showing no stress.

photograph, the stressed trees have a whitish-tan tonal response. Ground surveys of the site showed that the majority of the needles on the stressed trees were a yellow-tan to yellow-red color, indicating that the trees were dying. By comparing the CIR image response of the tree enclosed in the box in Fig. 1A across the four dates, the change in spectral response is conspicuous from that of a tree showing no stress to that of a dying tree in Fig. 1G.

The CIR photographs were better than conventional color photographs for distinguishing western pine beetle infestations in ponderosa pines. This agrees with the findings of Ciesla et al. (1967) who used both types of film to detect southern pine beetle, *Dendroctonus frontalis* Zimmerman, infestations in the southeastern United States.

The photographs shown in Fig. 1 are enlargements (3x-10x) of original photographic transparencies taken at variable scales (1:2,500-1:10,000). Determining the optimum scale to detect individual ponderosa pine trees infested with western pine beetles is dependent on the amount of foliage discoloration in the stressed trees. For detecting individual trees exhibiting early stress (slight yellow-tinged foliage), we found the best scale to be 1:2,500 to 1:5,000. However, more severely stressed trees with a uniform yellow to tan foliage could be detected at scales of 1:8,000 to 1:12,000.

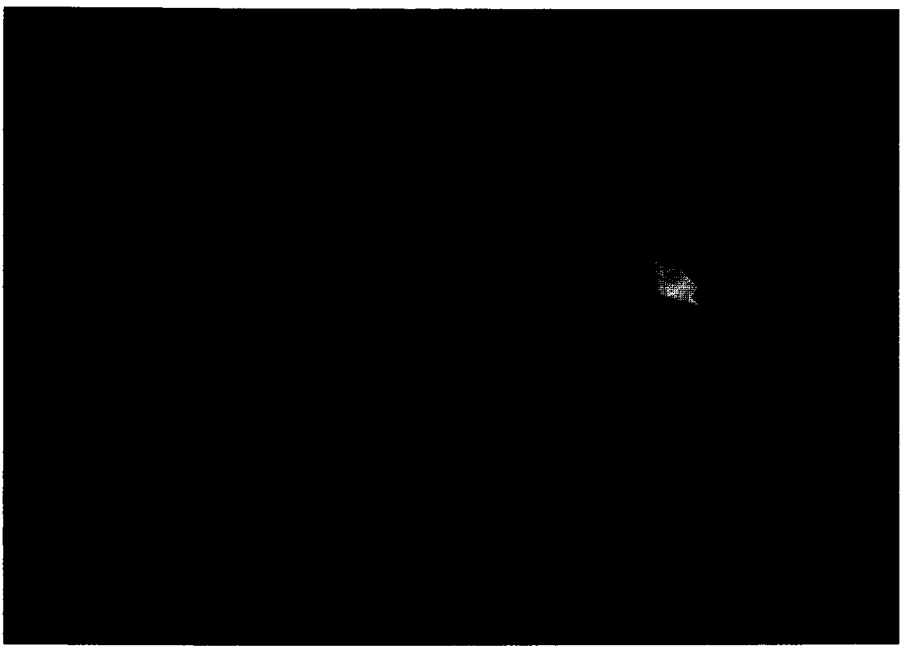

FIG. 2. Color-infrared digital video image (full frame) of a forested area in west Texas with a stand of ponderosa pine trees infested with western pine beetles. The image was obtained on 9 September, 1996. The arrow points to the pinkish-white tonal response of the stressed ponderosa pine trees.

Fig. 2 shows a CIR digital video image of the stand of ponderosa pine trees infested with western pine beetles. The image was obtained on 9 September 1996, approximately 3 weeks after the infestation was discovered. The arrow points to the pinkish-white image tonal response of the stressed trees. Pine trees showing no stress and other deciduous hardwood species have various magenta to red tones, while sparsely vegetated and bare soil areas have a light gray response. Unlike the photographic images shown in Fig. 1 which show enlargements of a small portion of the original photos, the video image shows a full frame of the surrounding area and lacks the detail of the photographs. The stand of stressed trees are oriented differently in the video image than in the photographs (Fig. 1) because the aircraft did not follow the same path in acquisition of the two kinds of imagery. The coarser resolution of the video imagery than that of the photography does not lend it to enlargements. The poorer resolution of the video also limits its use for detecting pine trees showing early stress from the beetle infestation, but it appears adequate for distinguishing stressed trees with a significant amount of yellow-tan foliage (moderate stress).

The GPS data at the bottom of the video image is important to locate the western pine beetle infestations over remote areas with few landmarks. This information can be plotted on maps or integrated with a geographic information system (GIS).

These results demonstrated that aerial remote sensing techniques are useful for detecting and monitoring western pine beetle infestations in ponderosa pine trees in the Davis Mountains of west Texas. Both CIR and conventional color aerial photography were useful for detecting stressed trees. However, CIR film was better because pine trees exhibiting early stress symptoms could be more clearly delineated from trees showing no stress. This would facilitate in monitoring the spread of infestation. Aerial CIR digital videography also has potential for detecting pine trees with moderate stress symptoms.

These findings are part of an on-going study integrating remote sensing with GPS and GIS technologies for mapping the spatial dynamics of western pine beetle infestations in ponderosa pines in this region of west Texas.

ACKNOWLEDGEMENTS

The authors thank Mario Alaniz for preparation of illustrations, David Escobar and Buck Cavazos for image processing, Fred Gomez for assisting with ground surveys and obtaining the aerial photography, and Angie Cardoza for word processing. We also thank Donald D. McIvor, ranch owner, and Randy Glover, ranch foreman, for permission to enter and use the study area. We remind readers that the study area is private property and not accessible without the written consent of the owner.

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DETECTING AND MAPPING WESTERN PINE BEETLE INFESTATIONS WITH AIRBORNE VIDEOGRAPHY, GLOBAL POSITIONING SYSTEM AND GEOGRAPHIC INFORMATION SYSTEM TECHNOLOGIES

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ABSTRACT

Aerial color-infrared digital videography was used with global positioning system (GPS) and geographic information system (GIS) technologies for detecting and mapping western pine beetle, ***Dendroctonus brevicomis*** LeConte, infestations in ponderosa pines, ***Pinus ponderosa*** Dougl. ex Laud., in the Davis Mountains of west Texas. Ponderosa pines infested with western pine beetles could be detected in the CIR video imagery due to their pinkish-white or yellowish-gray image responses. The integration of the GPS with the video imagery permitted latitude-longitude coordinates of pine beetle infestations to be recorded on each image. The GPS latitude-longitude coordinates were entered into a GIS to map western pine beetle infestations in a 8 x 10km study area.

INTRODUCTION

The western pine beetle is a pest that can cause mass destruction of ponderosa pines in the southwestern United States and British Columbia. Infestations often occur when trees are weakened by drought stress (Cobb et al. 1974, Furniss and Carolin 1977). Drought conditions have prevailed in the Davis Mountains of west Texas since 1992, and several western pine beetle infestations have been observed in ponderosa pines in this area.

Aerial photography has long been shown to be a useful tool for detecting infestations of several species of pine beetles (***Dendroctonus*** spp.) in forested regions (Heller et al. 1959, Ciesla et al. 1967, Caylor and Thorley 1970, Ciesla 1977, Aldrich et al. 1983). Everitt et al. (1997) recently demonstrated the potential of aerial photography and videography for detecting a western pine beetle infestation in ponderosa pines in west Texas.

Within 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 for detecting and monitoring insect activity over forested areas (Bobbe and Isikawa 1992,

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Myhre 1992). The latitude-longitude data provided by the GPS were entered into a GIS to georeference forest pest problems (Myhre 1992). Aerial videography has also been integrated with GPS and GIS technologies to detect and map citrus blackfly, *Aleurocanthus* woglumi Ashby, infestations over a large agricultural area (Everitt et al. 1994a) and Chinese tamarisk, *Tamarix chinensis* Lour., infestations in riparian zones (Everitt et al. 1996).

The objectives of this study were: (1) to demonstrate the potential of aerial videography for detecting western pine beetle infestations in ponderosa pines in the Davis Mountains of west Texas, and (2) to demonstrate the merging of videography, GPS, and GIS technologies for mapping the distribution of western pine beetle infestations.

MATERIALS AND METHODS

The study area was located approximately 27km northwest of Fort Davis in the Davis Mountains of west Texas. The specific study site was a 8 x 10km forested area on the north slope of Mount Livermore. This area has a large population of ponderosa pines. Several western pine beetle infestations were observed in ponderosa pines in this area during the fall of 1995.

Airborne video imagery integrated with a GPS was used to detect and map western pine beetle infestations within the study site. Imagery was obtained with a three-camera multispectral digital video imaging system (Everitt et al. 1995). The system was comprised of three charge-coupled device analog video cameras and a computer equipped with a digitizing board. The cameras are visible/near-infrared (NIR) (0.4-1.1μm) light sensitive. Two of the cameras are equipped with visible yellow-green (YG, 0.555-0.565μm) and red (R, 0.623-0.635μm) filters, respectively, while the third camera had a NIR (0.845-0.857μm) 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 image signals from the cameras are subjected to the RGB inputs of the computer digitizing board, thus giving a CIR composite digital video image similar in color rendition to that of CIR film. The hard disk can store 1000 CIR composite images. Video imagery was obtained of the study site on 8 May, 15 May, 21 August, 9 September, and 5 November 1996. Imagery was obtained of the study site at altitudes ranging from 300 to 1200m above ground level and provided horizontal ground pixel sizes of 0.3 to 1.3m, respectively.

A GPS (Trimble[4] Transpak II) was integrated with the video imagery to obtain location coordinates of western pine beetle infestations. The GPS was equipped with a navigation system that constantly received data from GPS satellites and readily calculated and displayed continuously the flight direction (bearing), altitude, time, date, ground speed, and latitude/longitude coordinates of the aircraft above ground. An interphaser (Compix model LP-701) was used with the GPS which

[4] Mention of company name or trademark 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.

permitted the transfer and recording of this continuous information on the last two lines of the video imagery. The latitude/longitude coordinates correspond to the approximate center of each image and have an accuracy of ±100m. The altitude data is relative to sea level.

Personal computer ATLAS-GIS software was used to generate a map of Jeff Davis County and detail maps of the study site based on the 1990 post census TIGER/Line files for the State of Texas. The U.S. Census Bureau developed and trade marked a machine readable referenced map 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 maps provided greater detail of the study area such as major highways, roads, hydrography, and other landmarks. These maps were produced to geographically map the locations of the western pine beetle infestations using the airborne video survey GPS data.

To facilitate locating pine beetle infestations, an aerial photographic mosaic map was made of the study site. Kodak Aerochrome conventional color (0.40-0.70μm) type 2448 film was used to obtain aerial photography of the 8 x 10km study site. Photographs were taken with a large format Fairchild camera (23 x 23cm). The camera was equipped with a 305-mm lens and had an aperture setting of f11 at 1/250 sec. Aerial photographs were obtained in nine flight lines at an altitude above sea level of 3600m on 20 August 1996. The altitude of the study site ranged from 1800 to 2400m. Consequently, photographic film scale ranged from 1:4000 to 1:6000. Photographic prints of this film were used to construct the photo mosaic.

All imagery was acquired with videographic and photographic imaging systems mounted vertically in the floor of an Aero Commander fixed-wing aircraft. Imagery was obtained between 1000 and 1500 hours under sunny conditions. Ground surveys were made on the study site on several dates during the late summer and fall of 1996, and the winter and spring of 1997 to confirm the western pine beetle infestations detected with airborne videographic data. Georeferenced pine beetle infestations detected with the video data were located on the photo mosaic before visiting the sites. The presence of western pine beetles at each site was confirmed by stripping bark from dying and dead trees to locate beetles and their galleries. Other observational data recorded were plant species, density, and cover. Ground photographs were also obtained at several sites.

RESULTS AND DISCUSSION

Fig. 1A shows a stand of dead ponderosa pine trees infested with western pine beetles in the Davis Mountains study site. Mount Livermore can be seen in the upper left portion of the photograph. The conspicuous yellow-orange to orange-brown color of the dead trees can be readily separated from the surrounding green color of the healthy ponderosa pines and other tree species. These trees had a heavy infestation of western pine beetles. The dead trees retain their foliage for several months before eventually defoliating.

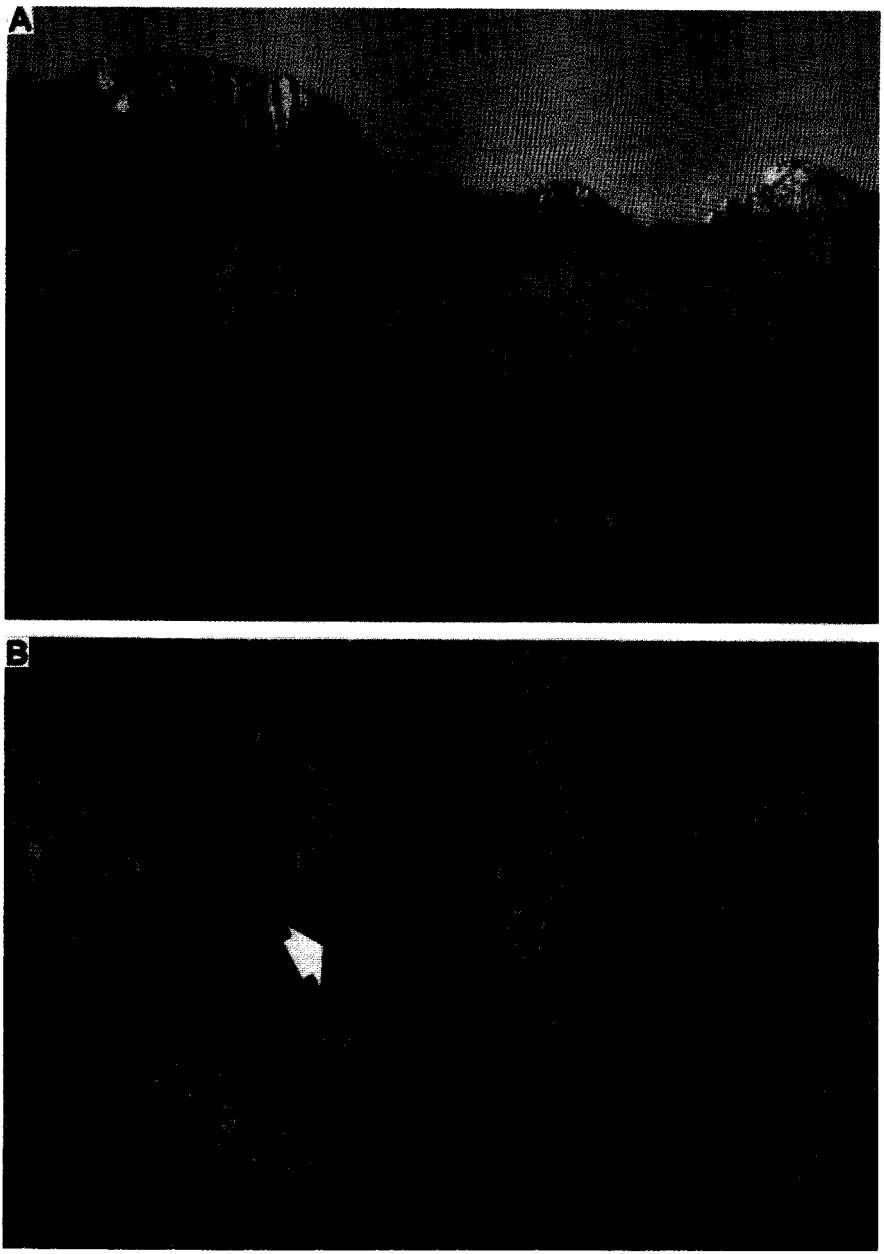

FIG. 1. The upper photograph (A) shows a stand of dead ponderosa pines killed by western pine beetles. This site is located in the Davis Mountains of west Texas. The lower print (B) shows an aerial color-infrared video image of a stand of dead ponderosa pines killed by western pine beetles. The arrow points to the yellowish gray image response of the infested trees. The GPS data is shown on the bottom of the scene.

An aerial CIR digital video image of a stand of dead ponderosa pines infested with western pine beetles in the study site is shown in Fig. 1B. The arrow points to the distinct yellowish-gray image response of the infested trees. Healthy ponderosa pines and other tree species have various magenta and red image tones. The GPS data are displayed at the bottom of the scene. The integration of the latitude-longitude coordinates with the video image is useful to locate the pine beetle infestations over remote and inaccessible areas. The distinct signature of the dead ponderosa pine trees was due to their yellow-orange to orange-brown foliage color, as depicted in Fig. 1A. Other stands of dead ponderosa pines infested with western pine beetles had a similar CIR video image and could be readily distinguished from surrounding vegetation. Ponderosa pines under moderate stress from western pine beetle infestation could also be detected with the video imagery. These trees had a predominance of yellow to yellow-tan foliage (dying trees) and a pinkish-white CIR video image response (not shown) (Everitt et al. 1997). Analysis of the aerial video imagery identified what appeared to be 50 infestations of western pine beetles in ponderosa pines within the study site. Ground surveys confirmed the presence of western pine beetles in ponderosa pines at 49 of these locations. The remaining location was a stand of pinyon pines, ***Pinus edulis*** Engelm., that were infested with a second species of pine beetle, ***Ips confusus*** LeConte. The dead pinyon pines had an orange-brown foliage color similar to that of the dead ponderosa pines and, consequently, had a video image response similar to that of the latter. A GPS was used in some of the ground surveys to help locate georeferenced coordinates of pine beetle infestations obtained from the aerial video imagery.

The GPS latitude/longitude data obtained from the video imagery were integrated with GIS technology to georeference western pine beetle infestations in ponderosa pines in the study site. Fig. 2 (upper right) shows a GIS map of Jeff Davis County with the study site denoted in the lower center portion of the map. The triangles depict GPS latitude/longitude coordinates for western pine beetle infestations within the study site. The locations of infestations are overlapped because of the small map scale. Fig. 2 (center) shows a more detailed GIS map of the study site depicting the locations (triangles) where western pine beetle infestations occurred. Many of the locations are overlapped due to the small map scale. However, this map is useful because one can associate general land use characteristics (i.e., hydrography, roads, towns) with the GPS marked locations where western pine beetle infestations occur. A detailed GIS map showing all of the locations where the 49 infestations occurred is shown in the lower portion of Fig. 2. Dirt roads are represented by bold lines, while hydrographic features have finer lines.

These results showed that aerial CIR digital videography can be used successfully to detect western pine beetle infestations in ponderosa pines in the Davis Mountains of west Texas. Videography offers the advantages of rapid acquisition of data with short turn-around time, 100% observation of the scene, and a procedure that is considerably less costly than ground surveys (Tueller 1982, Everitt et al. 1994b). This

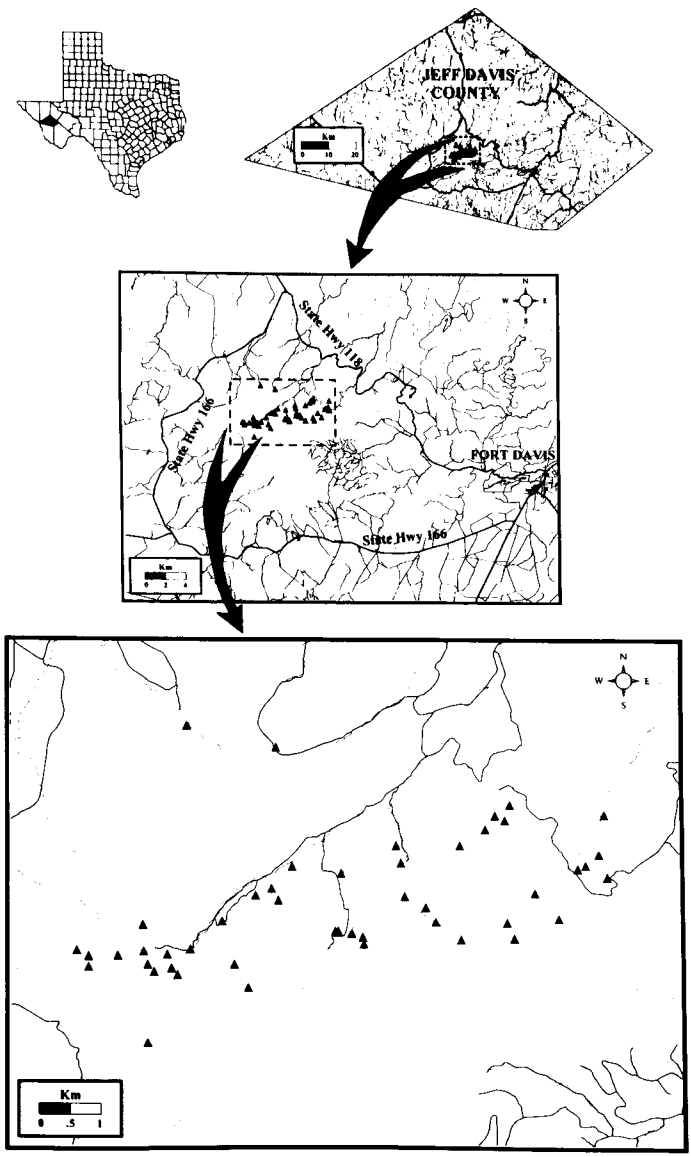

FIG. 2. GIS map (upper) of Jeff Davis County, Texas with the study site denoted in the lower center portion of the map. A large scale GIS map (center) of the study site showing the locations (triangles) where western pine beetle infestations occur. A large scale detailed GIS map (lower) of the study site depicting all 49 locations (triangles) where western pine beetle infestations occur.

technique also provides an efficient means to survey the rugged and mountainous terrain.

The integration of videography, GPS, and GIS technologies are valuable tools for mapping the distribution of western pine beetle infestations over often inaccessible areas. Maps can be produced that can aid the natural resource manager to locate pine beetle infestations over a large area so that the infested trees can be cut down to help reduce the spread of this pest (Billings 1980). The video imagery can serve as a permanent geographically located image data base to monitor future spread of western pine beetle infestations over time. The joint use of these technologies provides previously unavailable information about the extent and spatial dynamics of western pine beetle infestations in this area. It is anticipated that the integration of these technologies can also be used for a variety of other natural resource management applications.

ACKNOWLEDGEMENTS

Thanks are extended to Buck Cavazos and David Escobar for processing the video imagery and to Angie Cardoza for word processing. We also thank Donald McIvor, ranch owner, and Randy Glover, ranch foreman, for permission to enter and use the study area. We remind readers that the study area is private property and not accessible without the written consent of the landowner.

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RESPONSE OF RED IMPORTED FIRE ANT TO MAGNETIC FIELDS IN THE NEST ENVIRONMENT

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ABSTRACT

Magnetic fields (MFs) were applied to laboratory colonies of the red imported fire ant, *Solenopsis invicta* Buren, to determine if ant behavior was affected. Weak, alternating-current electromagnetic fields caused fire ant workers to deposit eggs and larvae near the MF source in experimental colony containers. Ants did not react to stronger, static MFs, indicating MF type may be important to ant response. Our results reveal that fire ants may use MF information in nesting activities and in orientation.

INTRODUCTION

Conflicting data exist concerning ants' ability to sense magnetic field (MF) information. Rosengren and Fortelius (1986) tested the red wood ant, *Formica rufa*, to determine its use of geomagnetic cues. The horizontal component of a MF surrounding an experimental foraging arena was redirected by 60^0 counter-clockwise from the ambient Earth MF using a Heimholtz coil. Ants did not respond asymmetrically or preferentially in their movement choices. Apparently, the ants did not use magnetic cues to retain nest sites or foraging routes, instead relying on sight and odor cues. However, Anderson and Vander Meer (1993) concluded that the foraging activity of the red imported fire ant, *Solenopsis invicta* Buren, was influenced by MF cues. In their research, ants were first acclimated to either a normal (Earth MF) or artificially reversed (180^0 change from Earth's MF) magnetic environment, after which they were allowed to find newly introduced baits and establish trails in environments of opposite MF polarity. Ants in these trials took significantly longer to create return trails than in control environments where the acclimation stage MF did not change upon bait introduction. Anderson and Vander Meer (1993) concluded that the red imported fire ant had MF orientation abilities. Additionally, fire ants (Eagleson 1940; MacKay et al. 1989, 1990) and other ants (Jolivet 1986, Little 1984) accumulate in and damage active electrical equipment with which electromagnetic fields (EMFs) are associated.

Because MFs influence moth (*Noctua pronuba*) navigation (Baker and Mather 1982), beetle larvae (*Tenebrio molitor*) orientation (Arendse 1978), honey bee (*Apis mellifera*) behavior (Gould 1984), and wasp (*Vespa orientalis*) nest architecture (Kisliuk and Ishay

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1977), a multifaceted ant response to MFs is possible. MFs may affect ants in diverse behavioral areas rather than simply in navigational and orientational realms, as demonstrated in other social hymenopterans. Lindauer and Martin (1972) demonstrated that swarming honey bees without visual or tactile cues used MF information to align newly constructed combs in the same direction as was the parent comb. De Jong (1982) manipulated an artificial MF enclosing bee swarms and controlled the directional component of their new nests, revealing that nest construction was apparently based on MF information. Kisliuk and Ishay (1977) introduced an additional MF into the nest-building environment of a common Middle Eastern wasp, *Vespa orientalis*. Juvenile worker wasps built irregular nests which originated in regions of high MF strength and were directed towards decreasing field intensity.

Previous observations by the authors (unpublished data) suggested that fire ant workers moved colony brood towards EMFs. The objective of this research was to determine whether MFs applied to laboratory fire ant colonies influenced the placement of brood (eggs and larvae collectively) by colony workers.

MATERIALS AND METHODS

Polygynous red imported fire ant colonies were collected from the campus of Texas Tech University, Lubbock, Texas during the summer of 1995. Ants were placed in 40 x 27 x 15 cm plastic foraging trays, each of which contained a 12 x 12 x 3.5 cm clear, sealed plastic "brood box" which housed queens, brood, and nursery workers. Each brood box had a floor of solid dental plaster, which retained moisture and maintained a humid micro-environment, and was externally covered with strips of black electrical tape that left only the top surface transparent. Ants and brood were clearly visible from above within brood boxes, without disturbing activity inside. Five entry holes (one hole at each corner and one in the center) were drilled in each transparent box top. Hole arrangement prevented any locational bias in ant numbers caused by entry or exit points. Ant colonies were fed liquified dog food (Sportsman's Choice, North Arkansas Wholesale, Bentonville, AR), given water ad libitum, and maintained at an ambient 24^0 C and 70 % R. H. Five colonies of approximately equal size (mean population = 650 ants, with 8 queens, 60 eggs and larvae) were observed for responses to two different types of artificially generated MFs.

Each colony container was enclosed in brown wrapping paper and covered with an opaque plastic top, creating a darkened colony environment without shadow or light cues and maintaining uniform temperatures and humidities in all tested colonies. Black tape wrapping the brood box sides also removed any visual cues for ants occupying the box itself. Each transparent brood box top was marked with a wax pencil to create 16, 3 x 3 cm blocks in a grid arrangement on the transparent top. When viewed from above with the colony container top momentarily removed, brood numbers were determined in each 3 x 3 cm square which made up the total area of the brood box (Fig. 1).

Two MFs differing in both strength and field characteristics were used. One apparatus was a 36 x 14 x 13 cm wooden box which housed an internal solenoid consisting of approximately 600 coils of copper electrical wire. This electromagnet (EMF) device was placed on a styrofoam base next to the outside surface of one side of the colony container, pointing into the container but separated by a 1-cm-thick piece of styrofoam. When powered, the EMF penetrated the ant colony container, creating the strongest MF on the closest (approximately 6 cm) side of the brood box to the device (Fig. 1). Powered by a 120-Volt alternating-current (VAC)/60-Hertz (Hz) wall socket source, this apparatus created an AC-MF which changed polarity 60 times per second. Transversely measured with a Gauss-meter (F.W. Bell Inc., Model 4048, Orlando, FL), MF strength was 0.560 Gauss (n = 10 measurements, SE = 0.19 G) at the inside surface of the nearest brood box wall (Fig. 1). Potential thermal, acoustic, and electric field cues from the EMF device were negated by the

styrofoam separators, internal device construction, and spacing between device and brood box during experimentation.

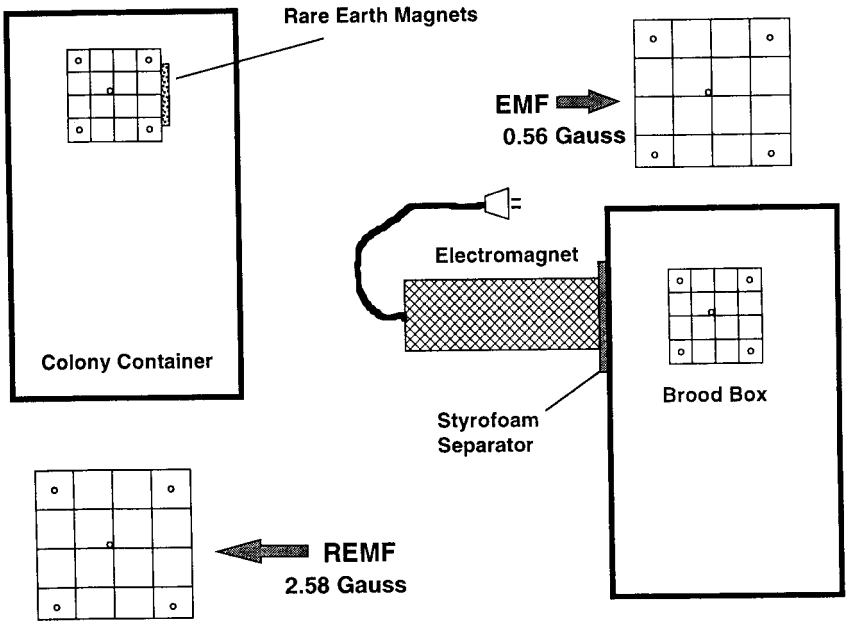

FIG. 1 Fire ant colony containers, magnetic field apparatuses and mean treatment-magnetic field intensities (rounded to hundredth Gauss)

The second MF was generated by four rare-earth (RE) magnets (i.e., lodestone bar-magnets), each 1 cm in diameter and 0.5 cm thick. Magnets were stacked in a 2 x 2 orientation, generating a static MF of 2.582 Gauss (n = 10 measurements, SE = 0.13 G; both polarities). The RE magnets, however, were strapped by rubber bands directly to one side of the brood box and created the strongest MF on that side of the box (Fig. 1). The three other sides of both the REMF- and EMF-treated brood boxes were negligibly magnetized corresponding to the strength of the ambient Earth MF (average MF strength at surface of other brood box walls was 0.404 G, SE = 0.0057 G, n = 36 measurements).

Magnetic fields were applied (as an REMF or EMF "treatment") to one randomly assigned side of a brood box for a 24-hr period, then brood numbers were rated for each of the sixteen blocks of the brood box. A ranking system was used where "0" indicated no brood (eggs or larvae) accumulation in a block, "1" equalled 1-5 brood in a block, "2" equalled 6-10 brood, "3" equalled 11-15 brood, etc., to a ranking of "9" indicating 41-45 brood per block. A "10" indicated a dense aggregation of brood corresponding to more than 45 eggs or larvae in a block. After ranking and recording brood numbers in each block of the box, the brood box was reoriented randomly inside the container, and the MF treatment was applied to a different

side. This procedure was repeated ten consecutive times (days) for each magnetic source and a control situation (no MF) for each of five colonies, allowing each colony exposure to three, ten-day treatments. Temperatures were recorded (with a standard thermocouple to 0.25^0 sensitivity) on the external and internal surface of each brood box side. Constant humidity was measured and maintained in each sealed brood box by water sprays every two days in each ten-day cycle.

Brood rankings of the four peripheral blocks nearest the applied treatment were summed and converted to a percentage (index B) of total brood ranking for the entire box (all 16 blocks). The B index thus provided a measure of the percentage of brood in each colony that had been moved toward a MF source by nursery workers in response to each treatment. We employed non-parametric statistical analyses to analyze colony responses to MF treatments because small sample sizes precluded assumptions about data normality and variance. The Wilcoxon Rank Sum Test (WRST) was used to detect significant differences in the mean B indices associated with each treatment. Mean B indices under each MF treatment for each colony were also plotted against their controls.

RESULTS AND DISCUSSION

Nursery workers in all colonies "stacked" and clearly deposited brood in large clumps (often containing more than 30 eggs and larvae) on the side of the brood box closest to the EMF during that ten-day treatment period. Significant differences among treatments were detected by the WRST: the mean B index of the EMF treatments was larger than that of the REMF ($T = 40$, $P << 0.025$) and control ($T = 40$, $P << 0.025$) treatments. There was, however, no difference in the mean B index of the REMF and control treatments ($T = 30$, $P >> 0.10$). Very small brood clusters (often less than ten eggs and larvae) were scattered randomly throughout the peripheral blocks in REMF and control treatments and were always placed against box walls. No temperature or humidity differences were measured when comparing any of the MF sides to non-MF sides. Thus, the application of either MF type did not alter the microclimate of the colony containers.

Fig. 2 shows the ten-day responses of experimental colony three to the treatments and is representative of all colonies tested. Three colonies showed a decrease in response to the EMF over time which may indicate acclimation. No trends were noted in ant response to REMF or control treatments.

Our results support the hypothesized use of MF information by the red imported fire ant. In the absence of light, acoustic, and temperature cues, fire ant nursery workers consistently moved colony eggs and larvae towards the source of a weak (0.560 G), but AC-driven EMF. Workers did not move brood towards the strong (2.582 G), but static MF of the rare-earth magnets. If MF strength was an influence on fire ant behavior, one would expect a stronger response to a strong MF and a proportional response to a lesser MF when compared to a non-MF control situation. Our results do not bear this out and may indicate that MF type (i.e., fluctuating versus static) is more important than MF strength in influencing fire ant behavior. However, future research must explore the effect on fire ants of MFs of similiar strength, but differing type, and the effect of the interplay of MF strength and source.

Workers responded to AC-MFs on a 60-Hz cycle, which is the same resonance used in a typical organism's neuronal system (Kaufman and Michaelson 1974). The 60-Hz cycle is also in harmonic resonance with the 1200 - 4800-Hz AC frequencies which were attractive to ants in electrical experiments by MacKay et al. (1989). Thus, a particular resonance and frequency, such as 60-Hz or 1200-Hz, may elicit a high degree of response from ants. However, distance from MF source must also influence ant response since MF strength rapidly decreases with increasing distance from the magnetic source.

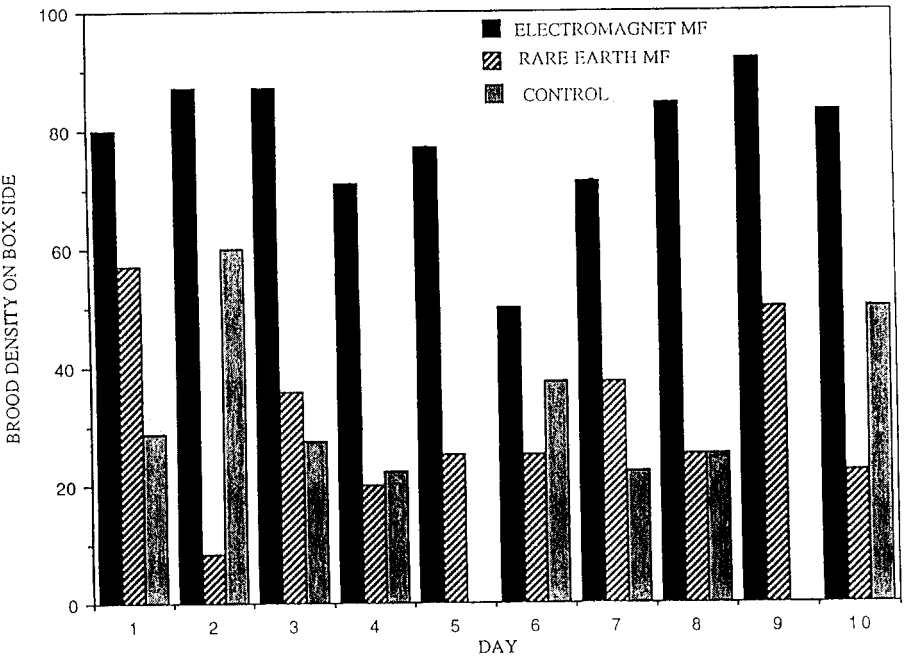

FIG. 2 Fire ant colony brood rankings influenced by magnetic fields (experimental colony three)

Common electrical apparatuses are powered with 120 VAC at 60-Hz that generate the same type and magnitude MF as our experimental EMF. Although research by MacKay et al. (1989, 1992a, 1992b) did not show MFs as "attractive" to fire ant workers, the effects of MFs on intracolony behaviors were not examined. In the field environment, however, fire ants are routinely observed nesting in such electrical apparatuses (MacKay et al. 1989, Vinson and MacKay 1990). Thus, MFs may play a role in the ant-electricity phenomenon.

Gould (1984) argued that it would be of great significance and usefulness in nest and comb-building insects if an "agreement" existed as to the direction and orientation of their nests and combs before and during construction by large numbers of workers. Perhaps MF information from the Earth is an ideal template in nest architecture and activity. For example, all of an ant colony's nest-building workforce would be coordinated to build underground tunnels, chambers, exits and entries according to one "set of directions." MF cues also may provide information concerning the location of queens, larvae, pupae, and food stores in a subterranean environment that lacks visual cues, however rife with chemical signals. Our results support the hypothesis of the voluntary positioning of eggs and larvae within the fire ant colony based on external MF information.

ACKNOWLEDGMENT

This research was a portion of the senior author's M.S. Thesis at Texas Tech University. Research was funded by the State of Texas Line Item for Red Imported Fire Ant Research. This is contribution number T-4-407, College of Agricultural Sciences and Natural Resources, Texas Tech University.

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PRESERVATION OF COUMAPHOS IN CATTLE DIPPING VATS

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ABSTRACT

A method to prevent the formation of potasan in coumaphos suspensions was tested in cattle dipping vats near the Texas-Mexico border. The suspensions were acidified to below pH 5.5 by the addition of triple superphosphate fertilizer (TSP) at the initial charging and the vats were then operated in the usual manner. A vat in Falcon, TX, was used to dip 4,325 head of cattle over a 396-d period and a vat in Zapata, TX, was used to dip 2,733 cattle over a 681-d period before they needed to be cleaned and recharged. Potasan, a toxic by-product of coumaphos produced by microbial activity, was not formed in either vat. In another study, potasan formed in vats after the addition of TSP was discontinued, allowing the pH to increase. Addition of TSP to reduce the pH of the vat contents to below pH 5.5 quickly prevented further potasan formation. Although the use of TSP in dipping vats containing coumaphos increased the rate of sedimentation of active ingredient in freshly made material, this problem was not considered unmanageable and was alleviated as vats were used.

INTRODUCTION

The ticks *Boophilus annulatus* (Say) and *Boophilus microplus* (Canestrini) are the only agents for transmission of *Babesia bovis* (Babes) in the US. A tick eradication campaign begun in 1907 through a combination of federal, state, and local resources resulted in elimination of *Boophilus* ticks and bovine babesiosis from the US by 1940 (Graham and Hourigan 1977, Kuttler 1988). However, since *Boophilus* ticks and babesiosis remain endemic in Mexico, the US is subject to re-infestation at any time. Cattle imported from Mexico must be treated with acaricide and be certified tick-free before entry into the US. In addition, cattle raised within a quarantine zone along the US side of the border with Mexico, where tick exposure is possible due to stray livestock and game animals crossing the border, must be treated and certified tick-free before being moved to the interior. If a tick infestation is discovered in a herd within the quarantine zone, the herd is placed in a mandatory treatment program for at least 9 months to assure all ticks within the infested area are killed.

The primary means for the treatment of cattle at US ports of entry and for cattle within the quarantine zone is dipping. A typical dipping vat is a long concrete trough, built into the ground, that is 2 m deep (*ca.* 7 ft) at the end at which cattle are introduced and which slopes up at the far end so that cattle can climb out after swimming the length of the vat. Once cattle have

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exited the vat, they usually are left for a time on a concrete drain pad so excess pesticide that drips off can be collected and returned to the vat. Over the years crude oil, arsenicals, toxaphene, and DDT have been used in dipping vats associated with tick eradication efforts (Graham and Hourrigan 1977). However, the organophosphate pesticide coumaphos [O-(3-chloro-4-methyl-2-oxo-2*H*-1-benzopyran-7-yl) *O,O*-diethylphosphorothioate] has been used almost exclusively along the Texas-Mexico border since 1968 (Graham and Hourrigan 1977, personal communication with APHIS-VS).

Volumes of dipping vats range from 10,500 to over 19,000 liters with the average vat currently used in the APHIS-VS tick eradication program containing just over 14,000 liters. Vats are charged initially with coumaphos at a rate of 1650 to 3000 mg/liter in water. Water and/or coumaphos are added periodically to maintain the level of pesticide within the acceptable range. There are several conditions placed on the management of coumaphos-containing dipping vats that limit the efficiency of use of the pesticide. Vats are cleaned and recharged every 2 yr unless one of several other factors triggers disposal before that time. These factors include a limit on the number of head that can be dipped in a vat of a particular size (generally the limit is two head per 3.78 liters of liquid in the vat), the accumulation of sediments from manure or dirt carried into the vat, or the accumulation of potasan [O-(4-methyl-2-oxo-2*H*-1-benzopyran-7-yl) *O,O*-diethylphosphorothioate]. Potasan is a transformation product of coumaphos formed through a reductive dechlorination of coumaphos by anaerobic microbes in the vat (Shelton and Karns 1988) and is believed to be more toxic to cattle than is coumaphos (APHIS-VS, personal communication). If the potasan level in a vat exceeds 300 mg/liter, the pesticide solution must be removed and the vat cleaned and recharged. The current method for disposal of spent coumaphos solutions is to place them in evaporation pits near the vats; the dried coumaphos residues remain in the soil. Recently, methods for the biodegradation of these wastes have been developed (Karns et al. 1995, Mulbry et al. 1996).

The tick eradication program can generate up to 2-5 x 10^5 liters of high level coumaphos waste from operations in the US alone. It is not known how much waste is generated in Mexico, but it is probably a much higher amount. We have previously published the results of a test in an unused dipping vat showing that reducing the pH of the vat liquid can prevent the accumulation of potasan thus possibly extending the useful life of the coumaphos charge (Karns et al. 1995). This would allow maximum use of each coumaphos charge and has the added benefit of leaving a waste amenable to disposal by biodegradation of the coumaphos (Karns et al. 1995). Previous work has shown that acidifying coumaphos solutions had no adverse effects on cattle or on the efficacy of coumaphos in killing ticks (Davey et al. 1995). In this report, we present the results from two working program vats that show that triple superphosphate fertilizer (TSP) can be used to reduce the pH of working vats in order to extend the effective life of coumaphos in the vats.

MATERIALS AND METHODS

Cattle dipping vats that are part of the tick eradication program were charged with water and coumaphos (Co-Ral flowable liquid, 500 g/liter, Bayer Animal Health) by APHIS-VS personnel according to standard protocols. Vat #2 at the city of Zapata, Zapata County, TX, received 13,570 liters of water and 45 liters of Co-Ral on 8 December 1993. Eighty-two kilograms of TSP (0-44-0) were added to the vat to reduce the pH. Vat #9 at Falcon in Zapata County, TX, was charged with 14,295 liters of water and 47 liters of Co-Ral on 18 October 1993. This vat also received 82 kg TSP to reduce the pH. The vats were stirred and cattle were dipped according to normal program procedures. At approximately 1-wk intervals, samples

were taken from stirred vats for monitoring pH and coumaphos and potasan concentrations. The pH was measured in well mixed samples with a glass pH combination electrode with a calomel reference. When water and Co-Ral were added to vats to replace loss of material carried out on cattle, TSP was added at the same rate of 23 kg/3780 liters (50 lb/1000 gallons) of liquid. The rate of addition of TSP was altered in some cases when measurements indicated the need for additional pH reduction. At the conclusion of these tests, Vat #9 at Falcon was recharged and again monitored as described in results. The program vat at the Cattle Fever Tick Research Lab (CFTRL) in Mission, TX, was also used in these studies and, except where noted, was charged and maintained as described above.

Well-mixed cattle dip (100 ml) from Zapata #2 and Falcon #9 vats (samples from day 121 and 173, respectively) or virgin cattle-dip made from Co-Ral flowable liquid and Beltsville, MD, tap water was placed into graduated cylinders and allowed to settle for up to 26 h. At various times, a Pipeteman pipetor was inserted into the liquid until the tip of the pipetor was at the 80 ml mark of the cylinder. A sample (100 µl) was withdrawn and diluted in methanol (900 µl) to dissolve the coumaphos. Coumaphos concentrations were determined by HPLC as described below. The same method was used to test the sedimentation of coumaphos in samples from the Acuna vat located in Mexico, across the border from Del Rio, TX. This vat contained TSP at the recommended rate and serviced over 7,000 head of cattle prior to the extraction of the sample tested.

Samples of cattle-dip from dipping vats were shaken vigorously to suspend solids that had settled to the bottom during shipping and storage. Samples (200 µl) were removed and added to 800 µl of methanol in a 1.5 ml microcentrifuge tube. Diluted samples were mixed and centrifuged at 13,000 x g for 2 min to remove solids. Supernatants were analyzed by High Performance Liquid Chromatography on a NovaPak C-18 radial compression cartridge column (Waters Assoc., Milford, MA) in a solvent system of 80% methanol in 0.01 N phosphoric acid (v/v) at a flow rate of 2 ml/min. Coumaphos and potasan were detected by absorbance at 320 nm with a Waters 490E or 996 detector. The amounts of potasan and coumaphos were calculated by comparison to standards of known concentration.

RESULTS AND DISCUSSION

The vats at Zapata and Falcon were chosen for this test because of their proximity to the Cattle Fever Tick Research Lab (where samples were collected for shipping), willingness of personnel in the county office to participate in the experiment, and the fact that both these vats had shown the propensity for potasan accumulation in previous cycles of use (M. McMeans, USDA/APHIS-VS, personal communication). In the cycle previous to this test, Vat #9 at Falcon had accumulated potasan above the allowable limit after only 3 months of use, during which time only 489 head of cattle were dipped. Vat #2 at Zapata had experienced potasan accumulation problems in 1992 after dipping 816 head of cattle but was preserved by the use of copper sulfate and used for an 18-month period covering 1992-1993. Although copper sulfate can be used as a general biocide to prevent potasan accumulation, its presence precludes the use of microbial processes for the disposal of waste and so is to be avoided if possible (Karns et al. 1995). As shown in Fig. 1, the addition of TSP fertilizer to vat #9 at Falcon prevented potasan formation over the 396-d period of vat use. Over 4,000 head of cattle were dipped during this period with no reported adverse effects. The pH of the dip-vat solution was *ca.* 3.8 when the vat was initially charged but rose to 5.0-5.5 and stabilized. As the vats were used, the pH rose slowly to values close to pH 5.5, which was the maximum level that demonstrated control of potasan formation in laboratory tests, probably due to urine and feces deposited in the vat during dipping operations. In addition, some TSP was probably removed

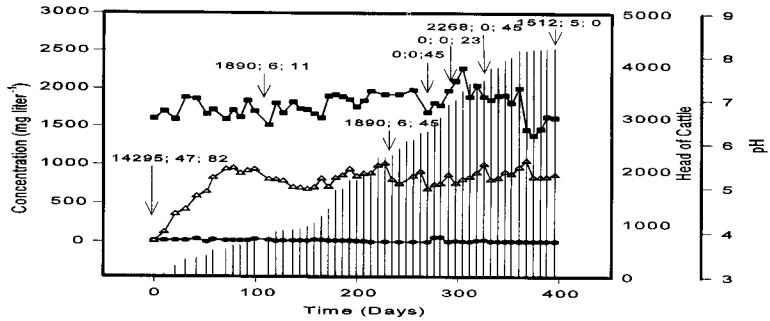

FIG. 1. Performance of CoRal coumaphos suspension in vat #9 at Falcon, Zapata County, TX, for the 396-d period from 18 Oct 1993 to 18 Nov 1994. Arrows designate times at which additions were made to the vat with the numbers above each arrow showing the amounts of water (in liters), CoRal Flowable Liquid (in liters) and triple superphosphate (in kg) [in that order] added to the vat. Symbols: (▵) pH of the vat contents, (■) coumaphos concentration, and (●) potasan concentration. Vertical lines show the total number of cattle dipped up to the day indicated.

from the vat by the treated animals. The addition of TSP fertilizer at a rate of 23 kg/3780 liter (50 lb/1000 gal) of water added to replace volume lost due to evaporation or removal by animals was sufficient to maintain a pH value below 5.5. The dip in the Falcon vat was finally disposed of after 396 d because of the accumulation of sediment.

The results for Vat #2 in the city of Zapata were similar to those for the vat in Falcon except that the time frame for use of the vat was longer and fewer animals were dipped (2,733 head, Fig. 2). The same protocol for TSP addition at times of replenishment was followed. This vat was emptied and cleaned after 681 d of use.

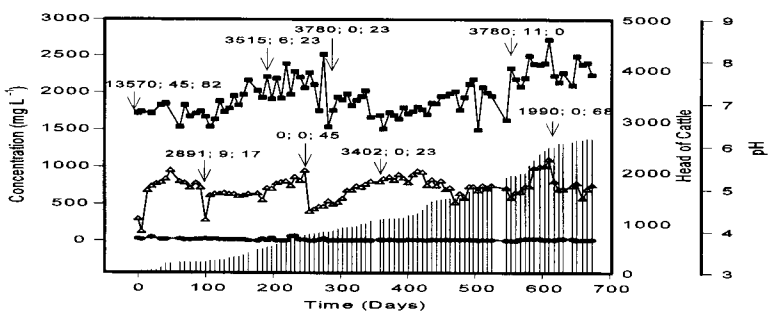

FIG. 2. Performance of CoRal coumaphos suspension in vat #2 at Zapata City, Zapata County, TX, from 8 Dec 1993 to 7 Sept 1995. Arrows designate times at which additions were made to the vat with the numbers above each arrow showing the amounts of water (in liters), CoRal Flowable Liquid (in liters) and triple superphosphate (in kg) [in that order] added to the vat. Symbols: (▵) pH of the vat contents, (■) coumaphos concentration, and (●) potasan concentration. Vertical lines show the total number of cattle dipped up to the day indicated.

Although the vats used in the previous study had prior histories of potasan formation, there was no guarantee that potasan-forming microbes were present in the vat at the time of these tests; therefore, these tests did not prove that the reduction of the pH in the vat had a direct effect on potasan formation. In an attempt to show that the addition of TSP to reduce the pH of the dipping vat contents indeed inhibits potasan formation, two vats (Vat #9 at Falcon upon recharge and the program vat at the Cattle Fever Tick Research Lab [CFTRL]) were monitored over the period between December 1994 to December 1996. Both vats were initially charged with coumaphos and TSP according to protocols described previously and maintained as described for the first year of use. At that time, further additions of TSP to control pH were ceased. As shown in Fig. 3 for the Falcon vat and Fig. 4 for the CFTRL vat, the pH of the contents began to rise after the cessation of TSP addition. The pH level in each vat reached between pH 6 and 7 at the onset of spring when the temperature of the vat contents reached levels more conducive to rapid microbial activity. At this time, the potasan levels in the vat rose rapidly. The addition of TSP to drop the pH to a level below 5.5 quickly stopped the accumulation of potasan in both vats, conclusively showing that the addition of TSP to vats inhibits the conversion of coumaphos to potasan.

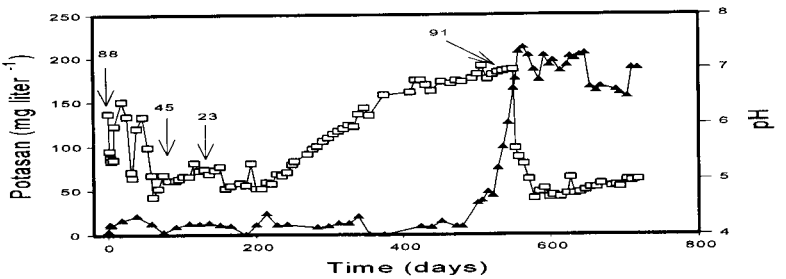

FIG. 3. Potasan formation in Vat#9 at Falcon during the period of Dec 1994-1996. Symbols: (▲) potasan, (□) pH. Arrows indicate the addition of TSP to the vat with the amount (in kg) shown at the tail of the arrow.

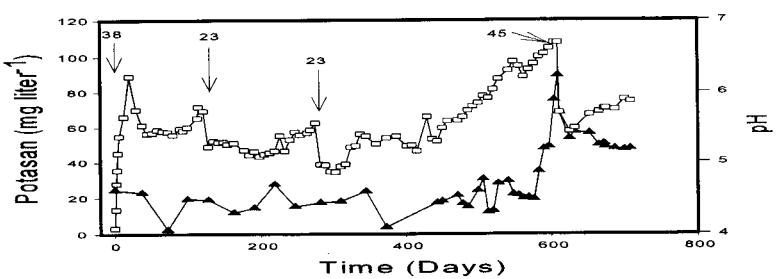

FIG. 4. Potasan formation in the CFTRL program vat during the period of Dec 1994 to Nov 1996. Symbols: (▲) potasan (□) pH. Arrows indicate the addition of TSP to the vat with the amount (in kg) shown at the tail of the arrow.

Although the data indicate that the addition of TSP fertilizer to lower the pH of the dip-vat contents prevented accumulation of potasan, communications with APHIS-VS field personnel indicated some operational difficulties caused by this practice. Excessive sedimentation of the dip-vat contents was reported by some operators. If the practice of adding fertilizer to the vat caused too rapid a sedimentation of coumaphos, it might cause a decrease in efficacy.

The results of experiments designed to test the effect of TSP on the sedimentation rate of coumaphos are shown in Figs. 5 and 6. In freshly made coumaphos samples, there was a much more rapid rate of sedimentation of coumaphos when TSP was added (Fig. 5). However, in samples of used cattle dip, the effect of TSP on sedimentation was much less pronounced (Fig. 6), with the very dirty material from the Acuna vat showing almost no sedimentation of the active ingredient. Triple superphosphate is a form of calcium phosphate that is enriched in phosphate content. Since calcium salts are used in the wastewater treatment industry to flocculate solids in wastewater, they probably have the same effect on the particulate materials in coumaphos. The sedimentation of coumaphos in the dip has not affected operation of the vats as the normal mixing procedures and the action of cattle in the vats has proven sufficient to keep the coumaphos well distributed (APHIS-VS personal communication); however, the flocculation caused by calcium in the vat has affected the measurement of sediment accumulation used by program tick inspectors as a measure of the condition of vat contents. At various intervals, inspectors check the amount of dirt and manure that has accumulated in a vat by placing 100 ml of well-mixed cattle dip in a graduated cylinder and allowing particulates to settle out for at least 8 h. If the amount of solids in the bottom of the cylinder exceeds 10 ml (10% by volume), the vat is considered too dirty and must be cleaned and recharged. Addition of TSP to used dip-vat material that had not been previously treated caused the solids to pack less densely at the bottom of the cylinder and thus give erroneously high readings. In tests with material from several vats, the addition of TSP caused the sediment to take up 33% more volume than it did in untreated material (J. Karns, unpublished data). Thus, multiplying the

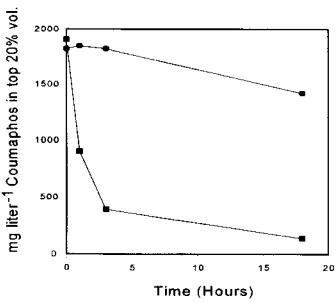

FIG. 5. Settling of coumaphos in fresh CoRal suspensions with (■) and without (●) triple superphosphate treatment.

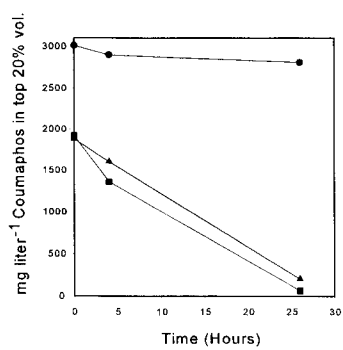

FIG. 6. Settling of coumaphos in used suspensions from the vats at Acuna (●), Zapata #2 (▲), and Falcon #9 (■) that have been treated with triple superphosphate.

volume of sediment in a TSP-treated vat by 0.67 gives a value comparable to those obtained in untreated material.

Because of the problem with sediment readings and the expense of shipping TSP fertilizer to remote areas, other acids were tested as a means of reducing the pH of coumaphos cattle dips for prevention of potasan formation. Phosphoric, hydrochloric, sulfuric, and citric acids all were effective in reducing the pH of coumaphos suspensions in laboratory tests. None of these acids had any negative chemical effect on the coumaphos in the dip nor did they have any effect on the sedimentation of coumaphos or solids in the dip. However, when phosphoric and citric acids were tested in vats in the field, it was impossible to keep the pH below the 5.5 level determined to be effective in preventing potasan formation. Apparently these acids reacted with the concrete in the vats and were rapidly neutralized. Laboratory tests in which fragments of concrete were added to acid-treated coumaphos suspensions confirmed that the pH rose consistently in the presence of concrete (J. Karns, unpublished data). Thus, other acids were ineffective in maintaining low pH in working cattle-dipping vats.

The present study shows that TSP fertilizer can be used by field personnel to preserve coumaphos-containing cattle dips with no significant adverse effects on dipping operations. Even though the two vats used in this study had previously accumulated potasan during normal operations, precipitating disposal of the contents, it is possible that they would not have done it this time. Because we were unable to set up control vats to show that potasan formation would have occurred in these particular vats without the addition of TSP, we must allow our previous study (Karns et al. 1995) to stand as proof of the preservative nature of the TSP treatment. However, the observation that the addition of TSP quickly stopped the accumulation of potasan in the Falcon and CFTRL vats strongly supports the conclusion that TSP is effective in controlling the microbes that convert coumaphos to potasan. This study will aid in the development of protocols for working vats in which cattle are actually dipped. Of particular note is that TSP fertilizer needs to be added whenever replenishment of the dipping vats takes place to maintain the pH at a level that will insure prevention of potasan formation. We currently recommend that TSP fertilizer be added to dipping vats at a rate of 23 kg/3,780 liter (50 lb per 1,000 gal) of vat contents at the time of charging to give an initial pH between 4.5 and 5.5. Every time water is added to the vat to make up volume lost due to dipping cattle or evaporation, TSP should be added at the same rate of 23 kg/3780 liter of water. In treated vats, we also recommend that the results from sedimentation tests in the field be multiplied by a factor of 0.67 to compensate for the effects of calcium phosphate on the density of the solids in the vat.

Previous studies have shown that coumaphos wastes in cattle dips that have been preserved with TSP fertilizer can be biodegraded and hence eliminated without harm to the environment (Karns et al. 1995). We are currently working on pilot-scale reactors for use in the field as waste treatment devices. Through the combined efforts of entomologists, microbiologists, and tick inspectors, we have devised a system for the minimization of wastes generated by an arthropod eradication program and have assured the biodegradability of the wastes that are generated. Cost savings for the Tick Eradication Program should be realized due to less pesticide required to carry out the mandate of the program while the environmental benefits should become evident through the waste minimization and development of viable methods for biodegradation of wastes.

ACKNOWLEDGMENT

We thank Mr. McMeans and the tick inspectors of Zapata County, Texas, for gathering samples and Mr. Adolpho Pena for transporting samples to CFTRL. We thank Mr. Norman Meres for assisting with the chemical analysis of samples.

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EVALUATION OF AN AIR-ASSISTED GROUND SPRAYER FOR CONTROL OF BOLL WEEVIL (COLEOPTERA: CURCULIONIDAE) AND BEET ARMYWORM (LEPIDOPTERA: NOCTUIDAE)

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ABSTRACT

Control of boll weevils, *Anthonomus grandis grandis* Boheman, and beet armyworms, *Spodoptera exigua* (Hübner), in cotton using air-assisted ground application of insecticides was studied in field and laboratory bioassays, and by insecticide residue analysis with gas chromatography. A Hardi Twin air-assisted ground sprayer was used to apply malathion (1.12 kg [AI]/ha) and Spod-X LC (247 ml/ha). Laboratory bioassays of individual leaves and squares using boll weevils were used to compare applications with and without air-assistance. A field bioassay also was conducted by caging boll weevils on individual plants. Bioassays of cotton treated with Spod-X LC were conducted by caging beet armyworm larvae on the undersides of leaves at mid-canopy. In these tests, the effectiveness of angling the air curtain was compared to application with the air oriented straight down. Air-assistance did not significantly increase boll weevil mortality in the bioassays of individual leaves, nor were there differences in malathion residues on leaves at top and mid-canopy. Bioassays of squares, caged plant bioassays, and residue analysis of squares showed enhanced efficacy when air-assistance was used. Application with air-assistance enhanced beet armyworm mortality. Angling the air curtain forward 30° increased beet armyworm mortality above that of the other treatments in one test and produced numerically higher, though not significant, mortality in another test. Air-assistance shows potential for enhancing the control of the boll weevil and beet armyworm.

INTRODUCTION

The most effective means of controlling any insect pest with insecticides is by a timely and uniform spray application. Decreased spray deposition on the plant diminishes the effectiveness of insecticides and generally is the result of application method and environmental factors, chiefly wind, high temperature, and humidity. Ware et al. (1970) determined that less than 50% of the insecticides applied by aircraft in Arizona reach their

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target. Though environmental factors are less influential during ground application compared to that by aircraft, off-target movement is still a problem. Tests in Arizona showed that the percentage of the spray deposited in short cotton (74 cm) was 39 % on the plant and 34% on soil; and in rank cotton (124 cm), 83% was deposited on the plant and 6% on the soil (Ware et al. 1975). In some cases, insecticide failures thought to be due to insect resistance may have actually been caused by application during adverse environmental conditions or poor application methods.

One promising method of improved application by ground is through the use of air-assisted sprayers. Air-assistance is attracting much attention due to its potential for reducing drift (Cooke and Hislop 1987, Gaultney et al. 1996, Howard et al. 1994, Taylor and Andersen 1989) and increasing canopy penetration (Bode 1988, Quanquin et al. 1989, Womac et al. 1992). Watson and Wolff (1985) compared percentage coverage of applications made with an aircraft, a hydraulic ground sprayer, and a hydraulic ground sprayer equipped with an air carrier system. The air carrier system improved deposition by as much as 400 and 900% on soybeans and corn, respectively. There are several models of air-assisted ground sprayers presently manufactured for use in row crops. Each uses air to force the spray down into the canopy. One model, the Hardi Twin (Hardi International, Davenport, IA), has the capability of angling the air and the nozzle forward or backward 30°. Air-assisted sprayers are not currently being used for insect control in cotton. However, given the large volume of insecticide used in cotton and the difficulty associated with controlling insect pests feeding in protected areas of the cotton plant (i.e., squares and the underside of leaves), this new technology may be well suited for control of cotton insect pests. Manor et al. (1989) showed that a Degania (John Bean Sprayers, Jonesboro, AR) air-assisted sprayer increased coverage in cotton for control of sweetpotato whitefly, *Bemisia tabici* (Gennadius); however, control was not different from conventional hydraulic nozzle application. Manor et al. (1991) then developed a "canopy air jet" to penetrate the cotton canopy from all sides. The canopy air jet provided much greater coverage than conventional over-the-top application. Womac et al. (1992) showed increased deposits of bifenthrin on cotton leaves and squares during application with a Hardi Twin air-assisted sprayer. In a separate test comparing air-assisted application of thiodicarb with that of a conventional dropped nozzle sprayer with three nozzles (one over-the-top and two dropped) per row, they found that mortality of beet armyworm, *Spodoptera exigua* (Hübner), larvae caged on the leaf underside was not increased by air-assistance. The morphology of the cotton plant and the behavior of cotton insect pests present a challenge for application technology. The most troublesome pests in cotton are those whose feeding and oviposition behavior keep them and their offspring protected from insecticide residues. For example, whiteflies, aphids, spider mites, and beet armyworms all inhabit the underside of the cotton leaf. The tobacco budworm, *Heliothis virescens* (F.); cotton bollworm, *Helicoverpa zea* (Boddie); and tarnished plant bug, *Lygus lineolaris* (Palisot de Beauvois), feed on the flower bud protected by bracts; boll weevil, *Anthonomus grandis grandis* (Boheman), and pink bollworm, *Pectinophora gossypiella* (Saunders), larvae develop inside the flower bud. Therefore, it is little wonder that the most troublesome pests of cotton are those that inhabit areas of the plant that are difficult for insecticides to reach. This research was aimed at determining how well air-assisted application penetrated the cotton canopy and deposited insecticide on squares and the underside of cotton leaves. Two serious pests of cotton, the boll weevil and the beet armyworm, were selected as bioassay organisms for determining how well air-assistance targeted these protected parts of the cotton plant. In addition, insecticide residue analyses were conducted to determine efficiency of deposition, canopy penetration, and longevity on the plant.

MATERIALS AND METHODS

All tests were conducted during August 1996 in cotton planted in 40-inch rows at Stoneville, MS. All applications were made with a PTO driven Hardi Twin air-assisted ground sprayer mounted on a high-clearance tractor.

Boll Weevil Tests. Malathion (Cythion 5 EC, Cyanamid, Wayne, NJ) was applied at a 1.12 kg (AI)/ha rate using a Hardi Twin sprayer with and without air-assistance on 7 August. The spray parameters were as follows: nozzle, Hardi 4110-08 (0.31 liter/min at 310.3 kPa); pressure, 310.3 kPa; speed, 8.0 km/h; volume, 28.0 liter/ha; air-speed, full (35-40 m/s); air angle, straight down. Wind speed during application was 0-9.6 kph (0-3 m/s). Application was made to plots 12 (1.0 m) rows × 61 m of DPL 5415 cotton in full canopy planted 6 May 1996. There was a 12 row buffer between each replicate.

Bioassay. Leaves from the upper and mid canopy were collected at 0, 24, 48, and 72 hours after application and placed in petri dishes (15 × 100 mm) containing one boll weevil (4-5 d old). Boll weevils were obtained from a colony maintained at the Gast Rearing Facility, USDA-ARS, Mississippi State, MS. Mortality was determined after 24 and 48 hours by pinching the weevil's rostrum. Those that did not move were recorded as dead. In addition to bioassays of individual leaves, plant-cage bioassays were conducted in the field. Fibre-air Plant Sleeves (20.3 × 48.3 × 55.9 cm, Kleen Test Products, Brown Deer, WI) were used to cage weevils on the upper third of individual plants. These plant sleeves gave the weevils freedom to feed on the terminal and 4-5 squares. Ten cages per replicate were used with 5 weevils in each cage. Cages were placed in plots 24 h after treatment. Weevils remained on plants in the cages for 24 h, at which time mortality was determined. Surviving weevils were transferred to petri dishes and held for 48- and 72-h mortality recordings.

Squares (4-8 mm) from the upper canopy were collected and bioassayed in 35.0 ml plastic diet cups containing a 0.64 mm layer of gelled agar in the bottom. One adult boll weevil (4-5 d old) was placed in each cup. Mortality was recorded at 24 and 48 h.

Residue. Cotton leaves were collected from the fourth node down from the terminal of ten plants in each replicate. Leaves were placed in plastic bags on ice and transported to the laboratory. Malathion residues were removed with 3 ml of 100% ethanol from the upper and lower surfaces of cotton leaves using Dual Side Leaf Washers (Carlton 1992). Aliquots (2 ml) were placed in auto-sampler vials for analysis by gas chromatography. Squares from the upper third of the plant were collected in the field, transported on ice to the laboratory, weighed, and placed in beakers containing 10 ml of 100% ethanol. The squares were shaken for 5 m at 150 cps to remove malathion residues. The rinseate was evaporated to 2.5-5 ml, after which a 2.5-ml aliquot was placed in an autosampler vial for analysis by gas chromatography.

Residue analyses were performed on a Hewlett-Packard 5890 gas chromatograph equipped with a flame photometric detector and auto-sampler. The gas chromatograph was operated with Hewlett-Packard's Chemstation software. The parameters of our residue analysis method were as follows: injector temperature, 200 C; detector temperature, 200 C; oven program, 120 C initial temperature with a 25 C/min increase to 250 C for 1 min, then a 25 C/min increase to 280 C for 4 min. A Hewlett-Packard Ultra-1 cross-linked methyl silicone gum phase column (25m × 0.32 mm × 0.52 μm) with a 2.65 ml/min flow of helium was used. Retention time of malathion was 5.597 min.

Beet Armyworm Test. Spod-X LC (247 ml/ha, Biosys, Columbia, MD), a beet armyworm nucleopolyhedrovirus, was applied to cotton in six row (1.0 m) x 30.4 m plots using a Hardi Twin air-assisted sprayer with only half the boom turned on. There was a six row buffer between each replicate. Two separate tests were conducted on 14 and 15

August. The treatments in each test were: air straight down, air back 30°, air forward 30°, and no air-assistance. The spray parameters were as follows: nozzle, Hardi 4110-08 (0.68 liter/min at 310.3 kPa); pressure, 344.7 kPa; speed, 6.4 km/h; volume, 94 liter/ha; air-speed, full (35-40 m/s); boom height, 40-50 cm above the crop. Wind speed during application was 0-9.6 kph (0-3 m/s). Third- to fourth-instar beet armyworm larvae, obtained from Biosys (Decatur, IL), were caged for 48 h on the undersides of leaves at mid canopy immediately after the spray had dried. Larvae were confined in hair-clip cages constructed from 1" diameter PVC pipe and fine mesh cloth. After 48 h, larvae were collected, brought to the laboratory, and transferred to artificial diet (35-ml plastic cups) and held at 27°C for 7 d, at which time mortality was recorded.

Experimental Design. The experimental design of all mortality tests for the treatment effect, with three levels, was a randomized complete block with four replicates per treatment. Since mortality and residue measurements were made at the top and mid-canopy, canopy location (top and mid) was a sub-plot. In the analysis of residue data, leaf surface (upper and lower) was a sub sub-plot. Additional analysis, combined over time, for mortality and residue was performed. Because the time*treatment interaction was significant in all tests, time was treated as an additional split. Percentage mortality data were adjusted for stabilizing variance by an arcsin transformation and then analyzed using PROC GLM (SAS Institute 1990). Means were separated by least significant difference where appropriate. Residue data were analyzed using PROC MIXED (SAS Institute 1990), and means were separated using the PDIFF option.

RESULTS

Boll Weevil Test. Immediately after application, the percentage mortalities (48 h) among weevils placed on leaves from upper canopy treated with and without the air-curtain were 80 and 62%, respectively. Weevils on treated leaves collected from the mid canopy had mortalities of 52% with air and 42% without air-assistance. However, neither of these mean comparisons were significant at P=0.05 (Table 1). Immediately after treatment, there were no significant differences in malathion residue ($\mu g/cm^2$) on leaves treated with and without air-assistance at either the upper or mid canopy (Table 1). Malathion residues on leaves from the upper canopy treated without air-assistance (2.07 $\mu g/cm^2$) were numerically higher than leaves treated with air-assistance (0.78 $\mu g/cm^2$). Main effect means for canopy location were significantly different (F=7.6, df=1,6, P=0.033), with higher residues detected on upper canopy leaves (1.42 $\mu g/cm^2$) than on mid-canopy leaves 0(0.72 $\mu g/cm^2$). The interaction between treatment and leaf surface was not significant. However, higher (F=22.92, df=1,6, P=0.003) residues, averaged over treatments, were found on the upper leaf surface (1.7 $\mu g/cm$) than on the lower surface (0.41 $\mu g/cm$). Malathion rapidly degraded after 24 h. No detectible levels of malathion were found at 24 and 48 h after treatment and boll weevil mortality dropped to 5-25% on treated leaves.

The results of a bioassay of squares collected immediately after application showed significantly higher percent mortality (F=18.47, df=2, 8, P=0.0006) on squares treated with air-assistance (51%) than on squares treated without air-assistance (17%) (Table 2). Furthermore, immediately after treatment, there were significantly greater residues (F=11.85, df=1, 3, P=0.04) on squares treated with air-assistance (45.0 mg/g of square) than on squares treated with no air-assistance (18.0 mg/g of square) (Table 2). Air-assistance had no effect on the longevity of malathion on squares. Boll weevil mortality and residues on squares at 24 and 48 h after treatment were not significantly different. The results of the bioassay in which weevils were caged on plants in the field 24 h after treatment showed that both 48 and 72 h weevil mortalities were higher (F=22.36, df=2, 6,

P=0.0001) in the air-assistance treatment (28 and 36%, respectively) than without air-assistance (16 and 21%, respectively) (Table 3).

TABLE 1. Percentage Mortality (48 h) of Boll Weevils and Malathion Residues on Leaves Collected Immediately After Applications With and Without Air-assistance.

	Hours after treatment					
	0		24		48	
Treatment	Residue[a]	Mortality	Residue	Mortality	Residue	Mortality
			Top Leaf			
No Air	2.07	62	0	8	0	8
Air	0.78	80	0	10	0	25
Control	---	0	---	2	---	5
			Mid Leaf			
No Air	0.82	42	0	5	0	12
Air	0.64	52	0	10	0	15
Control	---	0	---	8	---	10

[a]µg /cm^2.

TABLE 2. Percentage Mortality of Boll Weevils and Malathion Residues on Squares Treated With and Without Air-Assistance.

	Hours after treatment					
	0		24		48	
Treatment	Residue[a]	Mortality[b]	Residue	Mortality	Residue	Mortality
No Air	18.0 b	17 b	3.3 a	10 a	0.2 a	11 a
Air	45.0 a	51 a	1.6 a	10 a	0.4 a	4 a
Control		2 c		4 a		2 a

[a]Residue = µg/gram of square tissue.

[b]Means in a column followed by the same letter are not significantly different (P = 0.05) as determined by least significant difference.

Beet Armyworm Tests. Results from the two tests were significantly different (F=4.24, df=1, 31; P=0.0481). Therefore, Tests 1 and 2 were analyzed separately. In the first test, bioassay results showed that when the air was oriented forward 30°, beet armyworm mortality (32%) was higher (F=15, df=15, 4, 32; P=0.0001) than when the air was either oriented back 30° (15%) or turned off (15%) (Table 4). There were no differences in armyworm mortality in sprays applied with air oriented straight down (23%) or back 30° (15%). Furthermore, there were nosignificant differences in armyworm mortality in sprays applied back 30° or with no air (15%).

In the second test, all treatments with air-assistance resulted in higher (F=7.29, df=4, 12, P=0.0015) beet armyworm mortality than without air-assistance. Although not

significant, air forward 30° again resulted in the highest mortality (40%), followed by air down (35%), and air back 30° (32%) (Table 4).

TABLE 3. Percentage Mortality of Boll Weevils Caged on Cotton Plants 24 h After Treatment With Malathion.

	Hours after exposure		
Treatment	24[a]	48	72
No Air	8 a	16 b	21 b
Air	12 a	28 a	36 a
Control	4 a	10 c	2 c

[a]Means in a column followed by the same letter are not significantly (P = 0.05) different as determined by least significant difference.

TABLE 4. Percentage Mortality of Beet Armyworm Larvae Caged on the Underside of Cotton Leaves Treated With Spod-X LC Using Air-Assisted Application With Variation in the Angle of the Air Curtain.

Treatment	Test 1[a]	Test 2
Air Forward 30°	32 a	40 a
Air Straight Down	23 ab	35 a
Air Back 30°	15 bc	32 a
No Air	15 bc	15 b
Control	2 c	0 c

[a]Means in a column followed by the same letter are not significantly different (P = 0.05) as determined by least significant difference.

DISCUSSION

Higher mortalities occurred in all bioassays when application was made with air-assistance. Significant differences were observed in the cage bioassay and the bioassay of individual squares, while numerically higher mortalities were found for the air-assistance treatment when individual leaves were bioassayed. Higher malathion residues were obtained in residue analysis of squares treated with air-assistance, but not in residue analysis of leaves. Also, residue analysis did not show significant differences in canopy penetration between the treatments. While no significant increases in deposits on leaves occurred from application with air-assistance as determined by residue analysis, air-assistance was shown to increase the mortality of boll weevils caged on plants in the field and on squares bioassayed in the laboratory. Boll weevils caged on plants are in a more natural environment and have less restrictions on mobility and thus have a greater probability of encountering malathion residues deposited on stems and squares. Also, in preliminary tests in which fluorescent dye was applied to cotton plants, it was observed that more dye penetrated between the bracts and was deposited on the inner surface of
bracts and on flower uds when the air was turned on than when application was made without air; however, residueswere not quantified.

Beet armyworm mortality also was increased by the use of air-assistance, especially when the air was angled forward 30°. Air-assistance seems to have deposited greater amounts of virus on the underside of leaves since this was where the larvae were caged. Taylor and Andersen (1989) reported an increase in plant deposits of 74% for fine droplet sprays angled forward with air- assistance compared to medium droplet sprays applied

straight down without air-assistance. Also, Hislop et al. (1995) showed that compared with a medium droplet spray applied straight down without air-assistance, forward-angled air-assisted very fine/fine droplet sprays increased deposits on spring wheat by 71%.

The results of this research indicate that air-assistance can significantly enhance the efficacy of insecticides for control of two cotton pests that are difficult to control with conventional application methods.

ACKNOWLEDGMENT

Debra Gary conducted residue analyses using gas chromatography and assisted in bioassays. Debbie Boykin provided guidance in the analysis of data.

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NEW CAVE COLLEMBOLA FROM MEXICO AND BELIZE[1]

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ABSTRACT

Two new species of cave Collembola, one from each of the genera *Typhlogastrura* Bonet (Hypogastruridae) and *Trogolaphysa* Mills (Paronellidae), the first from Mexico and the second from Belize, are described, illustrated and compared with their closest relatives. An additional new record of *Heteromurus nitidus* (Templeton) from a Mexican cave is given, and a record for *Trogolaphysa* sp. from Guatemala is included.

INTRODUCTION

Few papers dealing with Collembola from Neotropical caves have appeared since the contribution of Palacios-Vargas (1989). The most recent species comprehensive work is on the genus *Arrhopalites* Börner (Palacios-Vargas & Zeppelini, 1995) which is very widely distributed in the area with several species being highly adapted to cavernicolous life.

The genus *Typhlogastrura* is distributed mainly in the Palearctic Region, with 12 known species, plus the new one herein described. The genus was revised by Thibaud (1980). Most of the species live in caves. As far as known, their distribution extends to the midnorthern part of Mexico. *Trogolaphysa* now includes 23 described species from the Neotropical Region (cf. Palacios Vargas et al. 1985, Thibaud & Najt 1988, Yoshii 1988). Nine species occur in Mexico below latitude 20° North. This is one of the most interesting genera of Collembola because of their adaptations (troglomorphs) to cave life, but there are still several species with eyes and pigment well developed in such environments and others that dwell on the surface.

[1] Project: Cave fauna from Mexico and Central America. Partial support from Dirección General de Asuntos del Personal Académico, UNAM, México and invited Professor at the Museum National d'Histoire Naturelle de Paris (Laboratoire d'Entomologie).

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In this paper, we have studied some material from caves that were loaned to us by Dr. Kenneth A. Christiansen (Grinnell College, Iowa, USA). There are two new species, one from each of the genera *Typhlogastrura* (Hypogastruridae) and *Trogolaphysa* (Paronellidae). Both species are interesting because of their morphological adaptations unique to the genera. Additional records for another species in the region also are given.

Typhlogastrura Bonet, 1930

Species in the genus usually lack pigment; eyes reduced or absent; postantennal organ with 3-11 tubercules arranged in a circle; antennal segment IV with 5 - 17 sensilla, an apical simple, bi- or trilobulate bulb; unguis long and thin, with an internal tooth, unguiculus with basal lamella and filament about ½ length of unguis; tenaculum with generally 4+4 teeth; furca well developed, dentes with usually 7-8 setae, mucro type *armata* and long anal spines. Chaetotaxy type A (Thibaud, 1980): on meso- and metathorax seta p2 is in position m2, and on abdominal tergite IV p1 is a microseta and p2 is a macroseta.

Typhlogastrura elsarzolae sp. nov.

(Figs. 1 - 7)

DESCRIPTION: Length (n=2): 1.5 mm. (1.35 - 1.67). Color in alcohol white. Ocular area without any pigmentation. All dorsal setae slightly crenulate (Fig. 1). Ratio head: antenna= 1.0: 1.4. Ant. I with 7 setae; Ant. II with 13 setae; Ant. III with 18 setae, 2 guard sensillae, two microsensilla and one ventral microsensillum. Ant. IV with 9 sensilla, one microsensillum and one subapical sensorial organ; subapical bulb simple (Fig. 2). With 1+1 cornulae. Ocular area with only two setae. Postantennal organ with 5-6 vesicles (Fig. 3). Tibiotarsus III with 18 setae. Unguis as typical for the genus, with a median tooth and two basal teeth; one small external tooth also present (Fig. 4). Ratio unguis III: dens = 1.3. Unguiculus with basal lamella and its filament about 3/5 length of unguis. Ventral tube with 4 + 4 setae. Tenaculum with 4 + 4 teeth. Dens about 48 µm, with seven setae (Fig.5). Mucro about 20 µm, spoon like. Ratio dens: mucro as 2.4. Anal spines 75 µm, about 1.1 length of unguis III, on separate papillae (Figs. 1 and 7). Female genital plate with 8 circumgenital pairs of setae and 2 eugenital setae (Fig. 6).

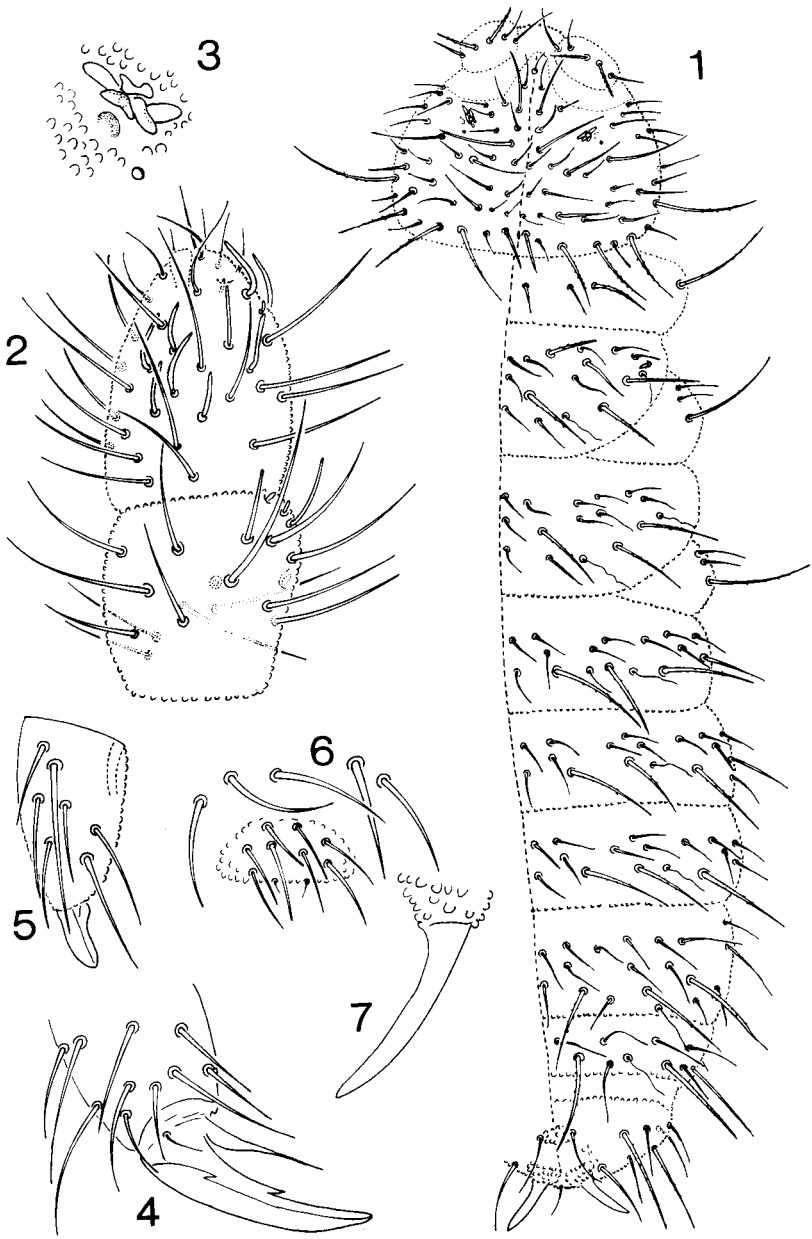
The chaetotaxy of the head, thorax and abdomen is illustrated in Fig. 1.

VARIATION: Some asymmetries of chaetotaxy were observed such as setae p1 lacking on one side of Th. I of one specimen. One specimen lacks the eye and postantennal organ on one side.

ETYMOLOGY: This species is dedicated to Lic. Elsa del Carmen ARZOLA for her help on this project.

TYPE MATERIAL: The holotype female will be deposited in the Collembola collection (Cat. 1968) of the Laboratorio de Ecología y Sistemática de Microartrópodos; paratype female will be kept at the Laboratoire d'Entomologie, París.

TYPE LOCALITY: MEXICO: Nuevo León: Pozo Primero de Septiembre, 21-XI-1993, P. Sprouse Col.



FIGS. 1-7. *Typhlogastrura elsarzolae* sp. nov. 1, dorsal chaetotaxy; 2, antennal segments III and IV, dorsal view; 3, postantennal organ, accessory boss and corneula; 4, apex of tibiotarsus III, unguis and unguiculus; 5, dens and mucro; 6, genital plate of female; 7, anal spine.

DISCUSSION: This is the second species of *Typhlogastrura* known from Mexico, the first, *T. veracruzana* Palacios-Vargas & Thibaud (1985) was described from a cave from Veracruz State.

T. elsarzolae differs from *T. veracruzana* in having only 9 sensilla on Ant. IV (vs. 11 - 14), apical bulb simple (vs. trilobulate), and having only 7 dental setae. The new species has one corneula on each side of the head. *T. veracruzana* generally has no corneula, however when one is present, it is in a different position as illustrated by Palacios-Vargas & Thibaud (1985).

Trogolaphysa Mills, 1938

Diagnosis (after Thibaud & Najt, 1988). Entomobryomorph. With or without pigment. Without postantennal organ. Eyes reduced or completely absent. Two pairs of trichobothria on the head (only one illustrated on Fig. 8) and 2-3, 3, 3 pairs on Abd. II-IV. With setae and scales. Tenent hair capitate, clavate or acuminate. Abdominal segment IV four times the length of Abd. III. Dens with 1-2 rows of ciliate spines. Mucro with 3-5 teeth. Ant. IV may be annulate, but never subdivided in two subsegments. Labial triangle with five anterior smooth setae and a posterior row with setae M1, M2/m2, r, L1/l1, L2. Most of the species known from caves.

Trogolaphysa belizeana sp. nov.

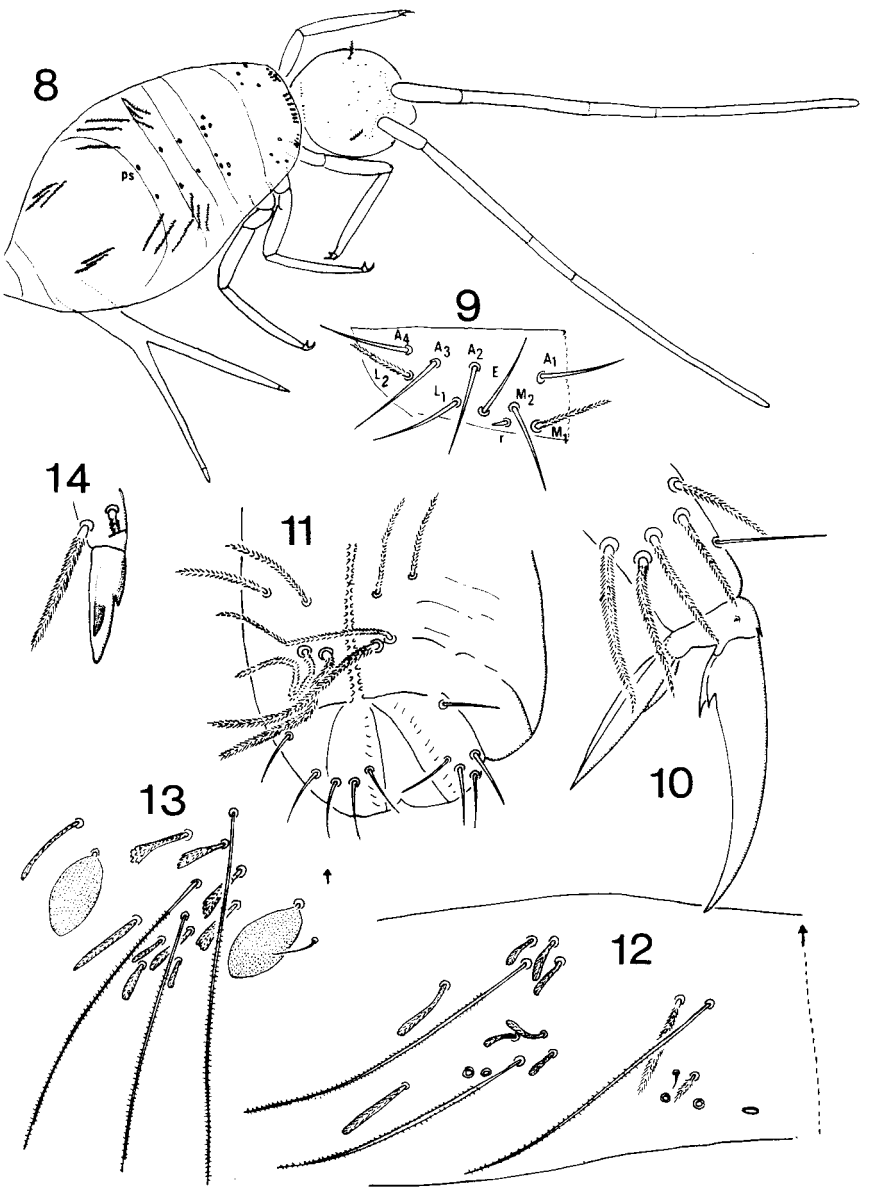
(Figs. 8-14)

DESCRIPTION: Length at most 2.6 mm; without any pigmentation and eyes. Ant. I and II with scales, setae and long tubular sensilla. Base of Ant. I with 3 setae. Ant. IV with unclear annulations and without apical bulb. Ratio head: antenna = 1:4 (Fig. 8). Ratio Ant. I; II; III; IV = 1: 4; 7; 12. Labrum with 4/5,5,4 smooth setae, ventral lobes crenulate. Labium with A1 to A5 smooth, M1 and L2 ciliate, M2, E and L1 smooth, « r » reduced (Fig. 9). Legs without scales. Trochanteral organ with 18 setae. Unguis long and slender, without median tooth, only with two basal teeth and one small external tooth (Fig. 10). Unguiculus I with small serrations, II and III smooth. Pretarsal seta reduced. Tenent hair acuminate. Ratio unguis: unguiculus; tenent hair = 1: 0.62; 0.41. Ventral tube with four pairs of ciliate macrosetae and five pairs of smooth setae on anterior apex (Fig.11), posterior surface with only ciliate mesosetae. Tenaculum with 4 + 4 teeth and one setae on corpus. Ratio manubrium: dens; mucro = 1:1.5;0.12. Manubrium with setae and scales. Dens with setae, scales and two rows of spines, with about 30 each. Mucro with only 3 teeth (Fig. 14). Ratio mucro: unguis III = 1:1.8. Habitus with the trichobothria is represented in Figure 8; details of the chaetotaxy of abdominal segments II and IV are given in Figs. 12 and 13.

ETYMOLOGY: Named aften Belize, the country from which it is known.

TYPE MATERIAL: Holotype female and two paratypes at Laboratorio de Ecología y Sistemática de Microartrópodos; three paratypes at Laboratoire d'Entomologie Musèum Natural d'Histoire Naturelle, Paris.

TYPE LOCALITY: BELIZE: Cayo; cave Actun Chapal (7 Km SE Benque Viejo del Carmen). 10-XII-1992. W. R. Elliot Col.



FIGS. 8-14. *Trogolaphysa belizeana* sp. nov. 8, habitus with trichobothria, insertion of macrosetae and position of pseudopores (ps); 9, labial chaetotaxy; 10, apex of tibiotarsus III, unguis and unguiculus; 11, apex of ventral tube, anterior view; 12, partial chatotaxy of abdominal segment II, left side; 13, partial chaetotaxy of abdominal segment IV, left side; 14, apex of dens and mucro.

DISCUSSION: *Trogolaphysa belizeana* is close to *T. ecuatoricus* Palacios-Vargas *et al.* (1985) and *T. haiticus* (Palacios-Vargas et al. 1985) and *T. bessoni* Thibaud & Najt (1988). The three species share the lack of eyes, pigment, and the ventral middle tooth on the unguis. *T. belizeana* can be differentiated by the presence of three tricobothrium on abd. II, only three teeth on the mucro, by the long antenna and the presence of only M1 and L2 ciliate on the labial triangle.

The chaetotaxy of the second abdominal tergum in this species is unlike any described so far for members of this genus. In addition the presence of macrochaeta on the third thoracic tergum and the labial setae, most of which are smooth, make this a very interesting species.

Among all the blind species of *Trogolaphysa* from Central America, *T. belizeana* seems to be the most highly adapted to cave life, because of the foot complex and the length of the antenna.

Trogolaphysa sp.

There is an undescribed species with 3 corneulae on each side of the head, two of them in anterior position are very close to each other. The foot complex is not very modifed, as the unguis still has a very well developed median tooth. All the posterior labial setae (except « r ») are ciliate as in *T. hondurasensis* (Palacios-Vargas et al. 1985).
Locality: GUATEMALA: Petén; Cave Kaxon Pec. Dos Pilas, Sagaxche. A. Cobb Col. May 1993, one specimen.

NEW RECORDS

Heteromurus nitidus (Templeton, 1835)

MEXICO: Nuevo León: La Escondida; Pozo Primero de Septiembre, 21-XI-1993, P. Sprouse Col. Six specimens.

We have compared Mexican specimens with those from Europe and have found no differences. Denis (1938) studied collections from 17 localities in Italy and found some variation in the numbers of eyes and teeth of the unguis, but considered them to be the same species with intraspecific variations. In addition, we can state that the labial triangle has setae A1 to A5 smooth and setae « r » is well developed (R); all the labial setae are smooth. Our record represents the first record of this species for Mexico.

ACKNOWLEDGMENTS

The authors appreciate the gift of specimens by Dr. Kenneth A. Christiansen (Grinnell College, Iowa, USA).

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PATTERNS OF HOST UTILIZATION BY *CATOLACCUS GRANDIS*,[1] AN EXOTIC PARASITE OF BOLL WEEVIL:[2] EFFECTS OF HOST MICROHABITAT

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ABSTRACT

Research was conducted to investigate the effects of host microhabitat on the incidence of oviposition by *Catolaccus grandis* (Burks), an exotic parasite of boll weevil, *Anthonomus grandis* Boheman. Third-instar boll weevil larvae and pupae infesting cotton squares were shown to be highly susceptible to attack by *C. grandis*, regardless of location within the cotton habitat (i.e., within the canopy or on the soil surface following abscision). In contrast, the susceptibility of immature host stages infesting cotton bolls was shown to be largely predicated on the physical condition of the individual fruiting form. Third-instar boll weevil larvae and pupae infesting bolls characterized by separated carpel segments or other types of surface damage (which presumably provide access to the parasite's ovipositor) were readily parasitized, whereas contemporaries infesting fresh (undamaged) bolls appear to have been largely protected from attack by this parasite species.

INTRODUCTION

Recent studies have clarified several important aspects of host utilization by *Catolaccus grandis*, an exotic parasite of boll weevil, *Anthonomus grandis* Boheman. A preference by *C. grandis* for third-instar boll weevil larvae has long been recognized and has formed the basis for current *in vivo* rearing procedures (Cate 1987, Morales-Ramos *et al.* 1992, Roberson and Harsh 1993). However, field evaluations conducted in southern Texas and elsewhere have repeatedly demonstrated the propensity of *C. grandis* to parasitize boll weevil pupae in natural infestations (e.g., Summy *et al.* 1995). The remaining life stages are generally parasitized to only a limited extent, but may be exploited for host-feeding purposes (Morales-Ramos and Cate 1992, Summy *et al.* 1995).

Although oviposition by *C. grandis* is largely restricted to third-instar boll weevil larvae and pupae, both of these stages are commonly distributed in at least four distinct microhabitats within the cotton environment. Immature boll weevils may develop within either flower buds (squares) or the fruit (bolls), either of which may be attached to fruiting branches within the canopy or may occur on the soil surface (squares, in particular, tend to abscise following damage caused by boll weevil oviposition or feeding activities). Evaluation of parasite augmentation efforts in southern Texas have consistently revealed two distinct trends regarding the distribution of parasitism by *C. grandis* in natural host infestations: 1) susceptible host stages infesting squares are generally parasitized to a

[1]Hymenoptera: Pteromalidae.
[2]Coleoptera: Curculionidae.

considerably greater extent than contemporaries infesting bolls, and 2) of the segment of available hosts occurring in squares, those occurring on the soil surface (i.e., within abscised squares) are generally parasitized to a considerably greater extent than contemporaries distributed within the canopy. The latter trend is consistent with an earlier report by Johnson *et al.* (1973), who suggested that *C. grandis* tends to concentrate searching activities on the soil surface. However, field studies conducted in several areas have demonstrated the ability of *C. grandis* to effectively search for hosts within the canopy (Morales-Ramos and King 1991, Morales-Ramos *et al.* 1994), and at least one recent study suggests that the parasite may preferentially search the canopy (Tillman 1993).

In order to explain such differences, and to gain a better understanding of the effects of host microhabitat on the incidence of oviposition by *C. grandis*, we conducted a series of studies designed to monitor the spatial distribution of parasitism within natural host infestations, and to evaluate the ability and propensity of *C. grandis* to parasitize hosts occurring in 1) cotton squares versus bolls, and 2) squares distributed within the canopy versus abscised counterparts occurring on the soil surface.

MATERIALS AND METHODS

Augmentative releases of *C. grandis* were conducted in two sites: 1) a 0.8-ha cotton plot located on ARS facilities near Weslaco in Hidalgo County, and 2) a 1.6-ha field of cotton regrowth located near Cavazos in Cameron County. The sequence of parasite releases in the Weslaco site included an initial release of 1,000 mated female *C. grandis* on 14 Oct 1994, followed by subsequent releases of 2,000 female parasites on 17, 20, 24 and 26 Oct (9,000 total). An initial release of 2,000 female parasites in the Cavazos site on 7 Nov was followed by a subsequent release of 2,000 females on 9 Nov (4,000 total). In each release site, a random sampling procedure was employed to estimate densities of immature boll weevils infesting squares and bolls within the canopy and on the soil surface, and the intensity of parasitism by *C. grandis* and indigenous parasite species occurring in each stratum. All squares and bolls (attached and abscised) occurring within each of 5-10 randomly selected 1.0-m^2 sample points were collected at weekly intervals. Such samples were dissected (under a stereomicroscope) and total numbers of live and dead forms of each life stage (including those parasitized by *C. grandis*) occurring in each type of fruiting form were recorded.

The ability of *C. grandis* to parasitize hosts occurring in squares versus bolls was evaluated in a series of choice and no-choice oviposition tests conducted under laboratory conditions. In choice tests, groups of 15 squares and 10 bolls infested by third-instar boll weevil larvae or pupae were placed within plexiglas oviposition cages (Morales-Ramos *et al.* 1992). In no-choice tests, only a single type of fruiting form was placed within a given cage (i.e., groups of infested squares and bolls were placed in separate cages). In both cases, infested plant material was exposed to ca. 350 mated female *C. grandis* for a period of ca. 8 h under constant conditions (27.8 ± 2°C; 70 ± 5% RH; 14L:10D photoperiod). Squares and bolls exposed in this manner were then removed and dissected (under a stereomicroscope) to measure the incidence of parasitism and total numbers of eggs per host within each group. Each test was replicated five times, and treatment means were compared using Fisher's Exact test (SAS Institute 1985).

The propensity of *C. grandis* to parasitize immature boll weevils infesting squares within the canopy versus contemporaries distributed on the soil surface (i.e., in abscised squares) was evaluated in a series of experiments conducted in the release sites at Weslaco and Cavazos. Groups of 25 infested cotton squares attached to 1.0-m strings were installed in pairs (one string within the canopy; one on the soil surface beneath plants) at randomly selected points within each field (8 in Weslaco; 5 in Cavazos) and were left *in situ* for a

period of 7-14 days. Exposed squares were then returned to the laboratory and dissected (under a stereomicroscope) to measure the incidence of parasitism within each group. Treatment means were compared using Student's-*t* test.

RESULTS AND DISCUSSION

In each of the two release sites, augmentative releases of *C. grandis* were accompanied by an appreciable increase in the incidence of parasitism within a relatively brief time interval (Tables 1 and 2). As in previous studies (e.g., Summy *et al.* 1995), parasitism by *C. grandis* was restricted to third-instar boll weevil larvae and pupae, and was concentrated among host individuals infesting abscised cotton squares (99.6 and 93.9% of parasitized hosts detected in the Weslaco and Cavazos fields, respectively). Third-instar host larvae and pupae infesting squares were relatively abundant within the canopy but were parasitized to only a limited extent (0-11.1% at Weslaco; 0-11.8% at Cavazos), as were individuals infesting bolls distributed within the canopy and on the soil surface (0-22% at Weslaco; 0% at Cavazos).

A series of oviposition experiments revealed a dramatic difference in the intensity of parasitism occurring among host individuals infesting squares versus bolls (Table 3). In choice tests, parasitism was concentrated among third-instar host larvae and pupae infesting squares, most of which were superparasitized (100% parasitism; 27.9 eggs/host). No parasitism was detected among contemporaries infesting bolls ($P<0.001$). In the no-choice situation, a relatively high incidence of parasitism was evident among susceptible host stages infesting squares (100% parasitism; 30.6 eggs/host), whereas contemporaries infesting bolls were parasitized to only a limited extent (7.6% parasitism; 27.5 eggs/parasitized host) ($P<0.001$). Careful examination of bolls in which parasitism by *C. grandis* was evident invariably revealed some type of surface damage (e.g., separated carpel segments) which presumably provided access for the parasite's ovipositor. A subsequent comparison involving groups of infested bolls categorized as "cracked" (carpel segments separated artificially) and

TABLE 1. Distribution of Parasitism by *Catolaccus grandis* in a Boll Weevil Infestation Near Weslaco, TX, 1994.

	Attached (Canopy)		Abscised (Soil Surface)	
Date	Hosts[a] ($\bar{x} \pm$ SEM)	% Parasitized	Hosts[a] ($\bar{x} \pm$ SEM)	% Parasitized
		Squares		
10/14	6.9 ± 2.0	0.0	2.5 ± 0.5	1.6
10/20	1.9 ± 0.6	0.0	4.4 ± 0.7	22.0
10/27	0.2 ± 0.2	0.0	3.6 ± 1.3	27.0
10/31	0.8 ± 0.3	11.1	5.2 ± 1.2	52.5
		Bolls		
10/14	0.0 ± 0.0	----	0.2 ± 0.1	0.0
10/20	0.0 ± 0.0	----	0.1 ± 0.04	14.2
10/27	0.2 ± 0.2	0.0	0.2 ± 0.2	0.0
10/31	0.8 ± 0.7	0.0	0.8 ± 0.3	22.0

[a]Aggregate of third-instar weevil larvae and pupae.

TABLE 2. Distribution of Parasitism by *Catolaccus grandis* in a Boll Weevil Infestation Near Cavazos, TX, 1994.

Date	Attached (Canopy) Hosts[a] (x̄ ± SEM)	% Parasitized	Abscised (Soil Surface) Hosts[a] (x̄ ± SEM)	% Parasitized
		Squares		
11/07	0.9 ± 0.6	0.0	4.4 ± 0.8	0.0
11/14	5.1 ± 2.3	11.8	11.9 ± 2.5	78.2
		Bolls		
11/07	0.0 ± 0.0	----	0.1 ± 0.1	0.0
11/14	0.1 ± 0.1	0.0	0.0 ± 0.0	----

[a]Aggregate of third-instar weevil larvae and pupae.

"uncracked" (carpel segments not separated) revealed a relatively high incidence of parasitism within the former group (71.5% parasitism: 1.8 eggs/host) and no evidence of parasitism within the latter group ($P<0.001$) (Table 4). Collectively, these results suggested that the relatively thick carpel walls of undamaged bolls may effectively protect immature boll weevils from attack by *C. grandis*, either by constituting a mechanical barrier to the parasite's ovipositor, or by obscuring some type of cue required for oviposition by this parasite species.

TABLE 3. Distribution of Parasitism Among Boll Weevil Cohorts Exposed to *Catolaccus grandis* Under Laboratory Conditions.

Fruiting form	Hosts[a]	% Parasitized[b] (x̄ ± SEM)	Eggs/host[c] (x̄ ± SEM)
		Choice	
Squares	59	100.0 ± 0.0 a	27.9 ± 2.2
Bolls	118	0.0 ± 0.0 b	-----
		No-Choice	
Squares	62	100.0 ± 0.0 a	30.6 ± 2.9
Bolls[d]	150	7.6 ± 5.3 b	27.5 ± 0.5

[a]Aggregate of third-instar larvae and pupae.
[b]Means within columns followed by same letter not significantly different at 5% probability level (Fisher's Exact Test).
[c]Parasitized forms only.
[d]Without exception, bolls in which parasitism was evident exhibited separated carpel walls or other types of surface damage that presumably provided access for parasite's ovipositor.

TABLE 4. Distribution of Parasitism Among Boll Weevil Cohorts Infesting Cotton Bolls Exposed to *Catolaccus grandis* Under Laboratory Conditions.

Fruiting form	Hosts[a]	% Parasitized[b] ($\bar{x} \pm$ SEM)	Eggs/host[c] ($\bar{x} \pm$ SEM)
Cracked	62	71.5 ± 2.9 a	1.8 ± 0.5
Uncracked	50	0.0 ± 0.0 b	-----

[a]Aggregate of third-instar weevil larvae and pupae.
[b]Means within columns followed by same letter not significantly different at 5% probability level (Fisher's Exact Test).
[c]Parasitized forms only.

Although parasitism in each of the release sites was concentrated among hosts infesting abscised squares (Tables 1 and 2), field experiments conducted in both locations provided no evidence of any searching preference by *C. grandis* for the soil surface relative to the canopy (Table 5). In the Weslaco site, the incidence of parasitized hosts infesting squares forced to remain within the canopy was comparable to that occurring among counterparts located on the soil surface (77.7 and 65.2% parasitism, respectively) (t=1.341; df=14; P>0.05). Similar results were obtained in the Cavazos site, where the incidence of parasitism within the canopy was similar to that occurring on the soil surface (83.1 and 84.8%, respectively) (t=0.122; df=8; P>0.05).

Collectively, these observations provide a plausible explanation for the concentration of attacks by *C. grandis* among host individuals infesting abscised squares (relative to contemporaries infesting squares within the canopy) that is commonly evident in parasite release sites. Cotton squares damaged as a result of boll weevil oviposition exhibit a pronounced tendency to abscise, which generally occurs before the immature boll weevil has developed to the third larval instar, or shortly thereafter. In the former case, exposure of susceptible host stages to parasite activity occurs exclusively on the soil surface; in the latter, the exposure time within the canopy tends to be relatively brief. Thus, the majority

TABLE 5. Distribution of Parasitism by *Catolaccus grandis* Among Host Individuals Infesting Attached and Abscised Cotton Squares, Weslaco and Cavazos, Texas, 1994.

Location	No. Hosts	% Parasitized[a] ($\bar{x} \pm$ SEM)
	Weslaco	
Attached	138	77.7 ± 7.4 a
Abscised	140	65.2 ± 5.7 a
	Cavazos	
Attached	47	83.1 ± 5.8 a
Abscised	38	84.8 ± 13.0 a

[a]Means followed by same letter are not significantly different at 5% significance level (Student's-t test).

of the available host stages are exposed to parasite activity for the greatest period of time on the soil surface (i.e., within abscised squares). However, the minority of host individuals infesting squares that remain within the canopy for the entire developmental period are clearly susceptible to attack by *C. grandis* and hence, do not appear to inhabit a *bona fide* ecological "refuge."

Several conclusions may be drawn from these data regarding the effects of host microhabitat on the intensity of parasitism by *C. grandis*. Third-instar boll weevil larvae and pupae infesting cotton squares appear to be highly susceptible to attack by *C. grandis*, regardless of location within the cotton habitat (i.e., within the canopy or on the soil surface). In contrast, the susceptibility of immature host stages developing in bolls appears to be largely predicated on the physical condition of the individual fruiting form. Third-instar boll weevil larvae and pupae infesting bolls characterized by separated carpel segments or other types of surface damage (which presumably provide access to the parasite's ovipositor) are parasitized readily, whereas contemporaries infesting fresh (undamaged) bolls appear to be largely protected from attack by this parasite species.

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PATTERNS OF HOST UTILIZATION BY *CATOLACCUS GRANDIS*,[1] AN EXOTIC PARASITE OF BOLL WEEVIL:[2] EFFECTS OF HOST STAGE

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ABSTRACT

A series of experiments were conducted under laboratory conditions to clarify ovipositional preferences by *Catolaccus grandis* (Burks), an exotic parasite of boll weevil, *Anthonomus grandis* Boheman, and to determine the suitability of various host stages for development of immature parasites. In both choice and no-choice ovipositional experiments, female *C. grandis* exhibited a pronounced preference for (but did not appear to discriminate between) third-instar boll weevil larvae and pupae. Parasitism of younger and older host stages was either nonexistent (eggs and first-instar larvae) or occurred at extremely low levels (second-instar larvae and unemerged teneral adults). Immature *C. grandis* developed with equal facility on third-instar boll weevil larvae and pupae, to a lesser extent on second-instar host larvae, and failed to develop on the remaining life stages of the host. The relationship between ovipositional preferences of *C. grandis* and its potential efficacy under field conditions is discussed.

INTRODUCTION

The exotic parasite *Catolaccus grandis* (Burks) is currently considered the most promising natural enemy candidate for augmentative biological control of boll weevil, *Anthonomus grandis* Boheman (King *et al.* 1993). Augmentation of *C. grandis* at rates ranging between 600-2,500 female parasites/ha per week have resulted in degrees of host suppression ranging from moderate to intense (Summy *et al.* 1995, Morales-Ramos *et al.* 1995a). Augmentative releases of *C. grandis* have been shown to be compatible with potentially disruptive practices such as the use of mechanical cultivation and early-season application of broad-spectrum insecticides, and have been successfully integrated into the production of "short-season" cotton in the Lower Rio Grande Valley of Texas (King *et al.* 1993, Summy *et al.* 1994).

The potential efficacy of *C. grandis* is governed by certain biological attributes which influence the ability of the parasite to exploit its host within the target environment. One of the most fundamental of these traits is the ability to utilize various stages of the host for oviposition and development. Current literature suggests that the preferred host stage for oviposition by *C. grandis* is the third instar larva (Johnson *et al.* 1973), which has been used extensively in current

[1] Hymenoptera: Pteromalidae.
[2] Coleoptera: Curculionidae.

in vivo rearing efforts (Cate 1987, Morales-Ramos *et al.* 1992, Roberson and Harsh 1993, Greenberg *et al.* 1995). However, field evaluations conducted in the Lower Rio Grande Valley of Texas have generally revealed an intense degree of parasitism among other life stages of the host, particularly pupae (Morales-Ramos *et al.* 1995a, Summy *et al.* 1995). In at least one study, significant numbers of second-instar boll weevils were parasitized by *C. grandis* (Morales-Ramos *et al.* 1994). Although the ability of *C. grandis* to utilize third-instar boll weevil larvae has been well-documented, ovipositional preferences by the parasite and the suitability of various host stages for parasite development have not been determined.

In order to better understand the patterns of host utilization by *C. grandis*, we conducted a series of laboratory experiments designed to evaluate: 1) ovipositional preferences by *C. grandis* for immature stages of the host, and 2) the suitability of the various host stages for development of immature parasites.

MATERIALS AND METHODS

Parasites used in these experiments were reared *in vivo* using procedures developed by Cate (1987) and modified by Morales-Ramos *et al.* (1992) and Roberson and Harsh (1993). Parafilm™ sheets each containing ca. 130 third-stage boll weevil larvae (obtained from the R. T. Gast Insect Rearing Facility, Starkville, MS) were exposed to ca. 350 mated female parasites for a period of ca. 3 hours under constant insectary conditions (26 ± 1°C; 65 ± 5% RH; 12:12 h L:D). Sheets exposed in this manner were then placed within ventilated emergence containers for the duration of the parasite developmental period (12-14 d). Following emergence, adult parasites were placed within plexiglas cages and allowed to oviposit for a period of 2-5 d prior to use in laboratory experiments.

Preferences by *C. grandis* for the various host developmental stages were evaluated in a series of choice and no-choice oviposition experiments conducted under laboratory conditions. In choice tests, an open petri dish containing 20 infested squares categorized as "fresh" (infested primarily by first- and second-instar host larvae) was placed adjacent to a counterpart containing 20 "dried" squares (infested primarily by third-instar host larvae and pupae) within 40x40x26-cm plexiglas rearing cages (Morales-Ramos *et al.* 1992). In the no-choice situation, subsets of each pair (i.e., dishes containing either 20 "fresh" or 20 "dried" squares) were placed within separate cages. In both types of experiment, host cohorts were exposed to ca. 100 mated female *C. grandis* for a period of ca. 24 h under constant conditions (27.8 ± 2°C; 70 ± 5% RH; 14L:10D photoperiod). In a separate no-choice experiment, parasite densities were varied from 10 to 100 parasites per cage. Each group of cohorts exposed in this manner were then removed and dissected (under a stereomicroscope) to determine the incidence of parasitism among individuals of each host stage and total numbers of parasite eggs per host. Five replicates of the choice test and three of each of the no-choice tests were conducted during 22-28 November 1994. Numbers of parasitized and unparasitized hosts within the various cohorts were compared using Fisher's exact test (SAS Institute 1985).

The suitability of various host stages for development of immature parasites was evaluated in two experiments. In the first, groups of hosts containing 12-14 individuals of each life stage (the three larval instars and pupae) were encapsulated within Parafilm™ cells and exposed to mated female parasites (at a ratio of one parasite per five hosts) for a period of approximately 24 h under constant insectary conditions (26± 1°C; 65 ± 5% RH; 12L:12D photoperiod). Parasitized hosts were examined under a stereomicroscope and all but one parasite egg was removed from each parasitized form (larvae of *C. grandis* are cannibalistic, and only one parasite is generally capable of completing development on a given host). Numbers of parasites surviving to the pupal stage on each host stage was recorded, and pupal weights of female

parasites were measured using a Mettler™ AT200 balance. In the second experiment, a single *C. grandis* egg was placed on individuals of these same host stages, which were then encapsulated within Parafilm cells and monitored using procedures described previously. Estimates of the various parameters obtained in these experiments were compared using Tukey's studentized range test (SAS Institute 1985).

RESULTS AND DISCUSSION

When provided a mixture of all immature host stages, *C. grandis* exhibited a pronounced ovipositional preference for the older host stages (Table 1). Parasitism was concentrated among third-instar larvae and pupae (96.0 and 100% parasitism, respectively; $P>0.05$), and was minimal or nonexistent among eggs (0.0%) and individuals of the first two larval instars (0.0 and 5.3%, respectively; $P<0.001$). Examination of parasitized third-instar larvae and pupae revealed a high incidence of superparasitism (100%) among both stages (14.3 and 29.8 eggs/host, respectively).

An ovipositional preference by *C. grandis* for older host stages became even more apparent in a series of no-choice oviposition tests (Table 2). When exposed to host cohorts composed entirely of third-instar larvae and pupae, parasitism of both stages was intense (100% parasitism of each stage; $P>0.05$), and all of the host individuals were superparasitized (23.5 and 26.5 eggs/host on third-instar host larvae and pupae, respectively). In contrast, parasitism occurring among host cohorts infesting fresh squares (younger stages predominant) was restricted to a few third-instar larvae which had been inadvertently included in the samples (Table 2). All of the latter were parasitized, and most (80.0%) had been superparasitized (3.6 eggs/host) ($P<0.001$).

A comparison of host cohorts exposed to variable densities of parasites revealed no evidence of discrimination by *C. grandis* among the two oldest immature host stages (Table 3). At a parasite density of 100 females per cage, 100% of the third-instar host larvae and pupae were parasitized ($P>0.05$), and most were also superparasitized (98.1 and 90.0%, respectively). The overall incidence parasitism was somewhat lower at a parasite density of 10 females per cage, although ratios of parasitized third-instar larvae and pupae were similar (92.2 and 83.3%, respectively) ($P>0.05$), and a relatively high incidence of superparasitism was evident among both stages (73.9 and 65.0% for third-instar larvae and pupae, respectively).

In each of the two previous experiments, a relatively low incidence of parasitism among

TABLE 1. Distribution of Parasitism Among Immature Boll Weevils Exposed to *Catolaccus grandis* in Choice Tests Conducted Under Laboratory Conditions.

Host[a] Stage	Hosts per replicate ($\bar{x}$ ± SEM)	% Parasitized[b]	% Super-parasitized[c]	Eggs per parasitized host ($\bar{x}$ ± SEM)
Eggs	2.8 ± 0.9	0.0 a	-----	-----
L(1)	4.2 ± 1.7	0.0 a	-----	-----
L(2)	8.8 ± 1.5	5.3 a	0.0	1.0 ± 0.0
L(3)	18.2 ± 0.9	96.0 b	100.0	14.3 ± 1.2
P	1.0 ± 0.6	100.0 b	100.0	29.8 ± 8.4

[a]L=larval stage (instar within parenthesis); P=pupal stage.

[b]Values within columns followed by same letter not significantly different at 5% probability level (Fisher's exact test).

[c]More than one parasite eggs per host.

TABLE 2. Distribution of Parasitism Among Boll Weevil Cohorts Exposed to *Catolaccus grandis* in No-Choice Tests Conducted Under Laboratory Conditions.

Host[a] Stage	Hosts per replicate (x̄ ± SEM)	% Parasitized[b]	% Super-parasitized[c]	Eggs per parasitized host (x̄ ± SEM)
		Group A[d]		
Eggs	3.3 ± 0.7	0.0 a	-----	-----
L(1)	6.7 ± 0.3	0.0 a	-----	-----
L(2)	8.3 ± 1.2	0.0 a	-----	-----
L(3)	0.7 ± 0.7	100.0 b	80.0	3.6 ± 1.3
		Group B[e]		
L(3)	8.3 ± 1.2	100.0 a	100.0	23.5 ± 2.7
P	5.0 ± 1.0	100.0 a	100.0	26.5 ± 3.2

[a]L=larval stage (instar within parenthesis); P=pupal stage.
[b]Values within columns for each group followed by same letter not significantly different at 5% probability level (Fisher's exact test).
[c]More than one parasite eggs per host.
[d]Infesting fresh squares; younger host stages predominant.
[e]Infesting dried squares; older host stages predominant.

newly-eclosed (teneral) adult boll weevils was evident (Table 3). Preliminary observations revealed evidence of partial or complete paralysis among all adults extracted from squares in which one or more parasite eggs had been deposited. In subsequent observations, teneral adult weevils parasitized by *C. grandis* invariably exhibited a similar syndrome and were apparently unable to emerge from squares or to feed on the latter when extracted artificially (in contrast to unparasitized adults, which emerged readily and fed voraciously on squares following emergence). Within a period of seven days, the incidence of mortality among parasitized adults (n=7; 85.8% mortality) was substantially greater than that occurring among contemporaries in controls (n=7; 0% mortality) and among individuals that had been exposed to parasite activity but had not been parasitized (n=10; 0% mortality) ($P<0.001$). The paralysis syndrome and relatively high incidence of mortality occurring among adult boll weevils parasitized by *C. grandis* were presumably manifestations of an "envenomization" effect that has been documented among immature boll weevils attacked by this parasite species (Morales-Ramos *et al.* 1995b). Parasitism of adult boll weevils by *C. grandis* has been observed under field conditions on at least one occasion (in a release site near El Ranchito in Cameron County, Texas during 1994; K. R. Summy, unpublished data) but appears to be relatively uncommon and its significance in biological control of the pest has not been determined.

Results of an ovipositional experiment conducted under standard *in vivo* rearing conditions revealed preferences similar to those discussed previously and demonstrated the ability of *C. grandis* to develop successfully on several host stages (Table 4). When presented a mixture (choice) of boll weevil larvae and pupae encapsulated within Parafilm cells, *C. grandis* concentrated attacks among third-instar larvae and pupae (83.7 and 51.5% parasitism, respectively), parasitized second-instar larvae to a limited extent (12.5% parasitism), and failed to parasitize first-instar hosts ($F=147.2$; df=3,8; $P<0.001$). Survival of immature parasites (from

TABLE 3. Distribution of Parasitism Among Boll Weevil Cohorts Exposed to Selected Densities of *Catolaccus grandis* Under Laboratory Conditions.

Host[a] Stage	Hosts per replicate ($\bar{x} \pm$ SEM)	% Parasitized[b]	% Super-parasitized[c]	Eggs per parasitized host ($\bar{x} \pm$ SEM)
		Group A[d]		
L(3)	16.7 ± 1.9	92.2 a	73.9	2.4 ± 0.2
P	4.7 ± 2.6	83.3 a	65.0	2.2 ± 0.5
A	1.0 ± 1.0	67.0 a	50.0	2.5 ± 1.5
		Group B[e]		
L(3)	17.3 ± 1.2	100.0 a	98.1	12.6 ± 1.4
P	7.0 ± 1.0	100.0 a	90.5	13.7 ± 1.9
A	3.3 ± 1.8	30.0 b	100.0	4.6 ± 1.4

[a]L=larval stage (instar within parenthesis); P=pupal stage; A=unemerged (teneral) adults.
[b]Values within columns for each group followed by same letter not significantly different at 5% probability level (Fisher's exact test).
[c]More than one parasite eggs per host.
[d]10 female parasites/cage.
[e]100 female parasites/cage.

the egg to pupal stage) occurred at similar levels among individuals developing on third-instar larvae and pupae (64.1 and 59.8%, respectively) ($P>0.05$) and was significantly lower among counterparts developing on second-instar host larvae (41.7%) ($F=6.0$; df=2,5; $P=0.047$). Pupal weights of female parasites were greatest among individuals that developed on third-instar host larvae ($\bar{x}$ = 6.5 mg) and least among counterparts reared on second-instar hosts ($\bar{x}$ = 5.0 mg) ($F=75.8$; df=2,22; $P<0.001$). The latter trend suggested a somewhat greater potential fecundity of parasites developing on third-instar host larvae relative to counterparts reared on second-instar larvae and pupae (see Greenberg et al. 1995).

Similar patterns were evident in a subsequent experiment in which parasite eggs were artificially implanted on boll weevil larvae and pupae (Table 5). Survival of immature parasites (from the egg to pupal stage) was similar among individuals developing on third-instar larvae and pupae (33.0 and 27.7%, respectively), occurred at significantly lower levels among counterparts developing on second-instar larvae (12.5%), and was not evident among individuals developing on first-instar hosts ($F=5.8$; df=2,6; $P=0.039$). The somewhat higher incidence of parasite mortality that was evident in the second experiment probably related to the fact that immature boll weevils had not been immobilized by adult parasites (i.e., by the envenomization effect) and hence, may have injured or destroyed appreciable numbers of immature parasites. As in the previous experiment, pupal weights of female parasites were greatest among individuals reared on third-instar boll weevils ($\bar{x}$ = 6.4 mg), but did not differ among counterparts reared on second-instar host larvae and pupae ($\bar{x}$ = 4.9 and 5.5 mg, respectively ($F=23.6$; df=2,12; $P<0.001$).

Several conclusions may be drawn from these data regarding the selection and utilization of various host stages by *C. grandis*. When exposed to immature boll weevils occurring within their natural microhabitat (cotton squares), *C. grandis* exhibits a pronounced preference for (but does not appear to discriminate between) third-instar larvae and pupae, both of which are suitable

TABLE 4. Survival and Development of *Catolaccus grandis* on Immature Boll Weevils Under Laboratory Conditions.

Host[a] Stage	No. Hosts[b]	% Hosts[c] Parasitized	% Survival to[c] Pupal Stage	Pupal[c] Weight (mg)
L(1)	36	0.0 a	----	----
L(2)	40	12.5 b	41.7 a	5.0 a
L(3)	37	83.7 d	64.1 b	6.5 c
P	39	51.5 c	59.8 b	6.0 b

[a]L=larval stage (instar within parenthesis); P=pupal stage.
[b]Based on three replicates each containing 12-14 individuals of each life stage enclosed within Parafilm cells. Eggs deposited by female wasps.
[c]Means within column followed by same letter not significantly different at 5% significance level (Tukey's studentized range test).

for development of immature parasites. The remaining host stages are generally parasitized to only a limited extent, and several appear to be unsuitable for parasite development (i.e., eggs, first-instar larvae and teneral adults). Despite their limited role as oviposition sites, several of these latter stages may be used extensively for host-feeding purposes, an event that invariably results in death of the host (Morales-Ramos and Cate 1992, Summy *et al.* 1995).

Two traits discussed herein provide a partial explanation for the demonstrated effectiveness of *C. grandis* when augmented in sufficient quantities in natural host infestations. By concentrating attacks among the older host stages, *C. grandis* inflicts an incidence of host mortality that is *a priori* more suppressive in nature than a similar incidence of mortality occurring among younger host stages (see discussion of "indispensible" or "irreplaceable" mortality in Southwood [1978]). Second, the suitability of several host stages for oviposition and development serves to lengthen the temporal "window" during the developmental period in which the host remains susceptible to attack by the parasite.

TABLE 5. Survival and Development of *Catolaccus grandis* Artificially Implanted on Immature Boll Weevils Under Laboratory Conditions.

Host[a] Stage	No. Hosts[b]	% Survival to[c] Pupal Stage	Pupal[c] Weight (mg)
L(1)	38	0.0 a	----
L(2)	33	12.5 b	4.9 a
L(3)	36	33.0 c	6.4 b
P	33	27.7 c	5.5 a

[a]L=larval stage (instar within parenthesis); P=pupal stage.
[b]Based on three replicates each containing 12-14 individuals of each host life stage encapsulated within Parafilm cells. Single parasite egg placed on each host using camel's hair brush.
[c]Means within column followed by same letter not significantly different at 5% significance level (Tukey's studentized range test).

ACKNOWLEDGMENT

We wish to thank J. M. Caballero and J. Cavazos for technical assistance in these studies.

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EL COMPORTAMIENTO DEPREDADOR DE PACHYCONDYLA HARPAX[1] FABR. SOBRE GNATHAMITERMES TUBIFORMANS[2] Buckley EN CONDICIONES DE CAUTIVERIO.

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RESUMEN

Las forrajeras de tres nidos de *Pachycondyla harpax* mostraron que el 100% de las presas capturadas en la naturaleza pertenecieron a las termitas *Gnathamitermes tubiformans*, siendo el 95% pertenecientes a la casta obrera y el 5% a la soldado. El comportamiento de depredación observado en tres nidos y bajo condiciones de laboratorio mostró dos grupos de secuencias. La secuencia más corta (secuencia principal) tuvo un máximo de 5 pasos mientras que las secuencias con mayor número de pasos (secuencias alternas) variaron de 6 a 15 pasos, teniendo la secuencia principal mayor cantidad de registros (73.2%) que las alternas (26.8%).

ABSTRACT

Foragers of three ant nests of *Pachycondyla harpax* showed that these ants mainly prey in nature on the termites *Gnathamitermes tubiformans* (95% workers and 5% soldiers). Predatory behavior was registered in three nests under laboratory conditions obtaining two main sequences. The shortest sequence (main sequence) had a maximum of 5 steps while the sequences with more steps (alterantive sequences) varied from 6 up to 15 steps. The main sequence accounted for 73.2% of all the observations while alternative sequences showed only 26.8%.

INTRODUCCION

Pachycondyla harpax Fabr. es una ponerina distribuída desde Texas hasta Brasil, Bolivia y Paraguay. Su hábitat va desde los climas semiáridos hasta los tropicales. Los nidos se encuentran bajo las piedras o troncos, frecuentemente a la sombra de árboles o arbustos y raramente a campo abierto, aunque en las partes tropicales pueden hacerlo en madera en descomposición o en tallos huecos. La entrada al nido es casi inconspicua, haciendo difícil su localización. La estructura del nido es muy primitiva y consiste en pocas galerías simples, algunas de las cuales corren a lo largo de la superficie del suelo inmediatamente bajo las piedras o troncos, mientras que

[1] Hymenoptera: Ponerinae
[2] Isoptera: Termitidae

otras se extienden en el suelo a profundidades hasta de 30 cm. El número estimado de individuos por nido fluctúa de 15 a 100. En la mayoría de los nidos se encuentran sólo individuos sin alas, que no se distinguen ni de forma ni tamaño de las pocas aladas, y que pueden poner huevos. En cautiverio, *P. harpax* se alimenta de yema de huevo o azúcar pero no de termitas (Wheeler, 1990).

En las hormigas carnívoras, la búsqueda y obtención de alimento puede ser por medio del reclutamiento masivo de otros individuos del nido y que comprende una serie de comportamientos coordinados complejos. Este es el caso de las más evolucionadas, las cuales pueden obtener presas de gran tamaño (Wilson, 1971). Puede ser también una búsqueda y obtención por medio de un reclutamiento arcaicio (Lachaud, 1985), o puede ser individual, como es el caso de la mayoría de las hormigas consideradas como primitivas. En éstas, la secuencia general del comportamiento depredador consiste en la detección, localización, aproximación, antenación, ataque, levantamiento de presa, picadura y transporte. Tal es el caso de la hormiga ponerina *Odontomachus troglodytes* (Dejean y Bashingwa, 1985), (Lachaud y Déjean, 1994) y de la mirmicina *Serrastruma serrula*, siendo tal secuencia adaptable a las circunstancias que se presentan en la caza (Dejean, 1980).

Pueden ajustar su estrategia de caza a cada tipo de presa (Dejean y Corbara, 1990; Dejean, 1991; Dejean et al, 1993). Cuando son especialistas, como el caso de *P. commutata* (la cual se especializa en termitas de un mismo grupo), emplean una estrategia definida de depredación (Mill, 1984). La edad y experiencia juegan así mismo un papel importante (Dejean y Lachaud, 1992).

Los reportes de la dieta de *P. harpax* no mencionan a las termitas como alimentación en condiciones de cautiverio (Wheeler, 1900), y el mismo Wheeler (1936) no las incluye en ninguno de los 5 grupos de relación hormigas-termitas. Por lo anterior, consideramos importante describir la estrategia de depredación que utiliza esta hormiga sobre *Gnathamitermes tubiformans*, termitas desérticas que se distribuyen desde Texas hasta México y que se alimentan de pastos, raíces y otra vegetación superficial alrededor de la cual construyen tubos durante la noche (Weesner,1965), y poder así incrementar los pocos datos que se tienen de la especie.

METODOLOGIA.

1 Las hormigas y termitas utilizadas fueron obtenidas de los terrenos semiáridos de la zona noreste de la Ciudad Universitaria, en San Nicolás de los Garza, N. L. México.

Tres colonias de *P. harpax* fueron localizadas y observadas previamente a la fase experimental para conocer las presas que capturan en condiciones naturales. Para conocer su dieta se les retiraron las presas a las obreras que se dirigían al nido. Posteriormente se colectaron y se transportaron al laboratorio. Cada colonia fue instalada en un nido de yeso de 27 x 20 x 5 cm cubierto con un vidrio y con una arena exterior para la caza de 74 x 33 x 7 cm cubierta con vidrio.

En la colonia 1 se colectaron 43 obreras; en la 2, 30 obreras y en la 3, 35 obreras, 5 larvas y 10 pupas. Fueron mantenidas con gajos de naranja y obreras de termitas.

2 Las obreras de *G. tubiformans* se utilizaron como presas por haberse observado que fueron la principal fuente de

alimento capturado y transportado al nido en el campo. Poco tiempo antes de la fase experimental, se colectaban las termitas bajo montículos de pasto en los terrenos de la Universidad y se llevaban al laboratorio.

3 La metodología estuvo dividida en dos fases:

a) La primera (de campo) consistió en tres observaciones de 24 hrs contínuas hecha a cada una de las colonias para el registro de los tipos de presas que captura *P. harpax*. Estos ensayos fueron efectuados durante junio, 1994.

b) La segunda (de laboratorio) consistió en la observación y toma de datos para la determinación de la secuencia del comportamiento depredador. Dichas observaciones fueron llevadas a cabo durante junio y julio, 1994.

El número de termitas utilizadas en cada observación, el tiempo (en días) durante el cual se estuvieron introduciendo termitas a las diferentes colonias y el tiempo diario de observación estuvieron en función de la intensidad de la depredación mostrada por las hormigas.

Las termitas se colocaron en grupos (no más de 10 individuos) a una distancia de 20-25 cm de la entrada del nido. Las observaciones se hicieron diariamente durante 29 días.

Para evaluar el comportamiento depredador se hizo un registro secuencial de los comportamientos presentados desde la búsqueda de la presa hasta su localización, captura, transporte e introducción al nido, basándose en las observaciones hechas a cada cazadora de cada colonia. Estas fueron marcadas para su identificación individual adhiriendo con cyanoacrilato una pastilla plástica de diferente color y forma en el tórax de cada hormiga, (técnica modificada de Fresneau, 1977). En el caso de muerte de una cazadora marcada, sus secuencias fueron eliminadas. El número de secuencias límite para cada individuo fue de 10 y el número de individuos considerado en cada colonia para ser sometido a análisis se tomó en función del número de cazadoras que completaron 10 secuencias por colonia.

Este estudio se realizó bajo un fotoperíodo de 12/12 y a una temperatura de 25 ± 2° C. durante 29 días consecutivos.

La eventual diferencia en la estrategia de depredación entre las colonias se pondría en evidencia mediante un análisis de varianza y análisis discriminante analizados con el programa SPSS.

RESULTADOS

Observaciones de campo.

1 El 100% de las presas observadas para cada nido pertenecieron a la especie *G. tubiformans* siendo el 95% pertenecientes a la casta obrera y el 5% a la casta soldado.

2 La actividad de forrajeo para esta fecha fue contínua, comenzando pocas horas después de anochecer (21:00 hrs), disminuyendo durante el amanecer y cesando completamente pocas horas después de éste (9:00-10:00 hrs).

Observaciones de laboratorio.

El número de individuos que completaron 10 secuencias para cada nido durante la toma de datos fueron los siguientes: Colonia 1, (9); colonia 2, (6); colonia 3, (10).

De las observaciones hechas a estos individuos se obtuvo una secuencia general (la cual representa la suma de secuencias hechas por cada individuo). Esta muestra el orden seguido por cada uno de los pasos que comprenden el comportamiento de depredación y para los que únicamente se incluyeron aquellos

valores iguales o superiores al 5% (Fig. 1).

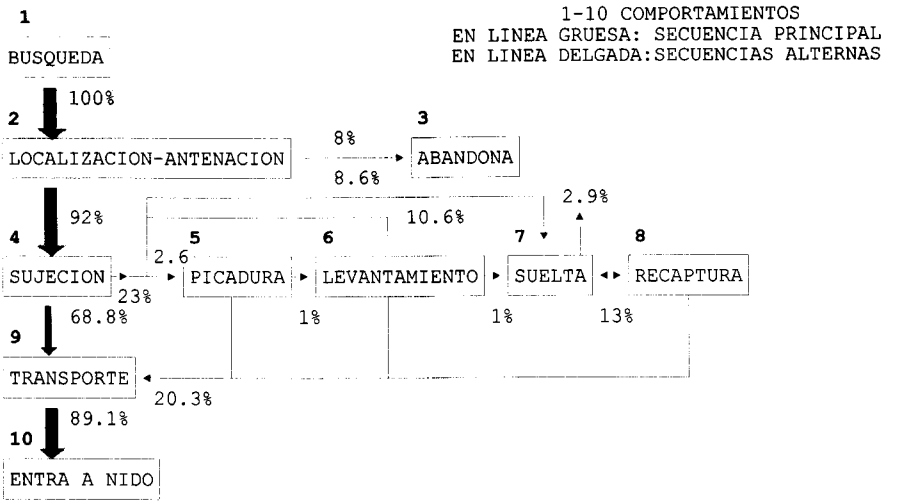

FIG. 1 Secuencia general del comportamiento de depredación sobre obreras de *Gnathamitermes tubiformans* seguida por 25 cazadoras de *Pachycondyla harpax* pertenecientes a tres colonias y que completaron 10 secuencias cada una.

En el seguimiento general de los comportamientos de la depredación se observó que la secuencia más corta para todas las colonias fue de 5 pasos y se le denominó principal:1-2-4-9-10, mientras la más larga fue de 15 pasos: 1-2-4-7-8-6-9-7-8-6-9-7-8-9-10 (ver Fig. 1). A todas las secuencias mayores de 5 pasos se les denominó secuencias alternas.

Los valores porcentuales obtenidos para cada paso dentro de la secuencia general nos indican que la secuencia principal fue recorrida por las depredadoras en un 73.2% mientras que las alternas lo fueron en un 26.8%. Algunos de estos pasos sólo fueron presentados por uno o dos individuos de una de las tres colonias.

También se observó que la secuencia principal fue más frecuente en los últimos registros mientras las alternas en los primeros. Para medir lo anterior, se tomaron en cuenta las primeras cinco secuencias de las 10 totales correspondientes a cada individuo y las últimas cinco, cuantificando el número de secuencias principales (5 pasos) y alternas (más de 5 pasos) para cada caso.

En los primeros cinco registros, la principal representó el 24% contra el 76% de las alternas. En cambio para las últimas cinco, se obtuvo que el 56% pertenecen a las secuencias con cinco pasos (principal) mientras el 44% representó a las secuencias con 6 o más pasos (alterna). Comparando las primeras 5 secuencias contra las 5 últimas obtenemos un valor de X^2= P < 0.025. Hubo casos excepcionales (2) cuyas 10 secuencias fueron de 5 pasos. En contraste, 2 forrajeras (nido 1 Y 3) cuyos porcentajes de secuencias alternas fueron mayores del 80%.

Para comparar los porcentajes de las frecuencias entre los pasos del comportamiento depredador entre las tres colonias, se realizó un análisis de varianza con los datos transformados mediante el arcoseno, encontrándose una F= 0.05 y P> 0.90, no encontrando diferencia significativa entre las medias de las secuencias de pasos en las colonias (Tabla 1 y Tabla 2).

TABLA 1. Estadística descriptiva de las frecuencias de pasos del comportamiento depredador en las colonias de *P. harpax* .

MINIMO: número de registros más bajo.
MAXIMO: número mayor de registros de individuos que pasaron del desplazamiento al contacto con termitas.

NIDO	MINIMO	MAXIMO	MEDIA	DESVIACION ESTANDAR
1	0.00	98	15.11	26.27
2	0.00	58	9.36	18.49
3	0.00	104	16.54	32.55

TABLA 2. Clasificación de la frecuencia de los pasos seguidos mediante el análisis discriminante entre las tres colonias.

NUMERO DE CASOS: se incluyó el número máximo de combinaciones de pasos presentadas por los individuos depredadores.

GRUPOS ACTUALES	NUMERO DE CASOS	DATOS REAGRUPADOS		
		N 1	N 2	N 3
1	28	2	18	8
2	28	2	21	5
3	28	3	19	6

Se observa que en la colonia 2 la secuencia de pasos es más homogénea, siendo la colonia con menor número de depredadoras, mientras que en las colonias 1 y 3 fueron más heterogéneas debido a que tienen ambos un mayor número de depredadoras, lo que propicia una mayor variabilidad en los tipos de secuencias en la depredación.

DISCUSIONES

Observaciones de campo.

Aunque el 100% de las presas capturadas por *P. harpax* haya sido sobre termitas de la especie *G. tubiformans* debemos considerar la posible existencia de un factor oportunístico en función de la abundancia, disponibilidad y características de las presas para una estación del año determinada. Variaciones similares han sido observadas en *P. villosa* en función a la velocidad de la presa y al tipo de forrajeo de la misma (Dejean y Corbara, 1990).

Las termitas *Nasutitermes corniger* y *G. tubiformans*, son uno de los insectos más abundantes en el área de estudio. La primera ha desarrollado sistemas antidepredadores eficientes

que consisten en la colocación de los soldados situados a los lados de las obreras cuando éstas se desplazan en la superficie del suelo y que repelen los ataques de *P. harpax* mediante la proyección de excreciones adhesivas (observaciones personales), fenómeno que ha sido igualmente descrito en *P. villosa* (Déjean y Corbara, 1990). En *G. tubiformans* la defensa radica en sus mandíbulas fuertemente esclerotizadas, principalmente en la casta soldado, pero que no impiden que *P. harpax* pueda depredar efectivamente sobre éstas. En consecuencia, este último género de termitas parece ser la presa más accesible, cuando menos durante el período de observación, y especialmente la casta obrera la cual es mucho más abundante que la soldado, por estar más expuestas por su tipo de forrajeo en la superficie (Weesner, 1965).

Según Wheeler (1900), las colonias de *P. harpax* tienen pocos individuos (15 a 100), por lo que presentan un comportamiento de forrajeo individual, lo cual les permite cazar presas como las termitas.

En las observaciones en campo *P. harpax* forrajeó durante la noche, pero en días nublados la actividad se prolongó a lo largo del día y pudo verse forrajear a las 13.30 hrs. con una temperatura en el suelo a la sombra de 28° C aunque al sol se estuviera a 35° C.

Fase de laboratorio.

La secuencia general de depredación obtenida para *P. harpax*, se puede resumir en un total de 10 pasos, de los cuales 5 son básicos por la alta frecuencia con que se repitieron: búsqueda, localización-antenación, sujeción, transporte y entrada al nido. Esta secuencia es muy parecida a la de otras ponerinas consistentes en: búsqueda, localización, antenación, sujeción, picadura y transporte (Dejean, 1987; Dejean y Bashingwa, 1985, Dejean y Corbara, 1990), aunque existen pequeñas variables interespecíficas. La picadura no fue muy común dado el tamaño pequeño de la termita obrera (0.4 cm) con respecto al tamaño de la hormiga (1 cm) y a la escasa resistencia que presentaron las termitas. Se incluyó la secuencia de entrada al nido porque hubo individuos que habiendo capturado a la termita la abandonaban sin introducirla al nido, por lo que las consideramos como secuencias incompletas. Aparentemente el abandono de la presa es común cuando se trata de termitas: *P. villosa* lo hace un 9.6% con obreras de *Nasutitermes; P. soror* un 4% cuando las presas son soldados de *Cubitermes; P. harpax* un 8% con obreras de *Gnathamitermes*, y *B. senaarensis* un 2% cuando se trata de obreras de *Macrotermes*, no así cuando la presa son obreras de *Cubitermes*, (Déjean y Corbara, 1990; Déjean, 1991), aunque lo anterior parece tener más relación directa con el tamaño que con el tipo de presa (Lachaud y Déjean, 1994).

La secuencia más corta de cinco pasos evolucionó en el transcurso de las depredaciones. Esto sugiere una tendencia de *P. harpax* para explotar el recurso en forma óptima, con lo cual puede lograr una economía de energía. Las formas de hacerlo pueden variar de un grupo a otro. La ponerina *Paltothyreus tarsatus* logra dicha explotación en forma óptima gracias a su flexibilidad conductual individual, la cual es determinante en la estrategia de forrajeo a nivel de nido (Dejean et al, 1993). *Pachycondyla soror* ajusta su secuencia en función del tipo de presa (Dejean, 1991) y *Pachycondyla commutata* alterna estrategias de forrajeo individual o masivo (lo que logra mediante reclutamiento) en función de la cantidad de presas

disponibles (Mill, 1984).

En las secuencias alternas los pasos que presentaron una cantidad menor de frecuencias fueron presentados por uno o varios individuos de uno de los tres nidos. Estos pasos con porcentajes bajos y ejecutados por un número determinado de individuos pueden ser atribuidos a una variación interindividual y a una ontogenia particular.

La secuencia principal (1-2-4-9-10), aparte de ser la más recorrida (73.2%), tuvo mayor frecuencia en los últimos cinco registros de los diez correspendientes para cada individuo, contrastando de un 24% en los cinco registros iniciales contra un 56% en los cinco registros finales. Aunado a esto se registró un descenso en los porcentajes de las secuencias alternas variando de un 76% a un 44%. Este aumento en la frecuencia de la secuencia principal en los últimos registros señala que *P. harpax* se torna más eficiente a medida que va teniendo mayor cantidad de encuentros con sus presas.

Es posible que dicha eficiencia pudiera estar unida a un factor de aprendizaje y memorización ya que conforme *P. harpax* tiene más enfrentamientos con su presa, memoriza el sitio donde las presas se encuentran habitualmente (las termitas siempre fueron colocadas en el mismo lugar). Aprende también las posibles vías de huida que su presa pudiera tener, adquiriendo así mayor experiencia en la forma de captura, al igual que en su orientación espacial con respecto al nido, similar a lo observado para *Serrastruma serrula* por Dejean, (1980).

El hecho de que las presas siempre hayan sido colocadas en el mismo sitio tuvo un papel importante en los resultados. En estudios preliminares a este trabajo, el área donde se colocaron las presas fue cambiada en varias ocasiones obteniendo después de esto, para todos los casos, una mayor cantidad de tiempo y de pasos invertidos en la búsqueda y el transporte de la presa. A su vez se notaron más erráticas en cuanto a la orientación del área de forrajeo con respecto al nido, tardando hasta una hora en encontrar la entrada del mismo. Como consecuencia la depredadora soltaba y recapturaba a la presa en más de una ocasión para explorar el área a fondo. Un comportamiento similar fue observado por Hölldobler (1984) en la hormiga ponerina *P. tarsatus* por lo cual emitió la hipótesis de que la desorientación estaba basada en una perturbación del campo visual.

Los estudios preliminares realizados en laboratorio, previos a la fase experimental, mostraron que *P. harpax* es capaz de alimentarse sobre otro tipo de presas como larvas le lepidópteros y grillos parcialmente dañados, tanto en fase de larva como adultos. Se obtuvo sólo una modificacion en la frecuencia de los pasos que componen la secuencia general, tales como picadura, soltar y recapturar presa o bien, invirtiendo más tiempo en el transporte de la misma, sin afectar el número de pasos que forman dicha secuencia.

En la secuencia general, las frecuencias registradas en cada paso para cada una de las colonias fueron diferentes, esto debido a las diferencias que pueden existir entre ellas. Los análisis utilizados revelaron que las diferencias presentes entre las tres colonias no afectaron significativamente a la secuencia de depredación general por lo que se concluye que las tres colonias están sometidas bajo el mismo patrón conductual de depredación.

AGRADECIMIENTOS

Al Dr. William MacKay por la identificación de las hormigas y termitas, así como a sus comentarios sobre el manuscrito, y a al Dr. Jean Paul Lachaud por la revisión y sugerencias al mismo.

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SOUTHWESTERN ENTOMOLOGICAL SOCIETY

PERSPECTIVE

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

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ECOLOGY OF THE BOLL WEEVIL[1] IN THE UNITED STATES COTTONBELT

Don R. Rummel[2] and K. R. Summy[3]

Following the first confirmed boll weevil, *Anthonomus grandis* Boheman, infestation near Brownsville, Texas in 1894, the species demonstrated considerable migratory ability by rapidly spreading eastward throughout the southern U. S. Cotton Belt. Hunter and Pierce (1912) reported that boll weevil infestations spread at a rate of 40 - 60 miles annually. Of equal note was the rapid northward spread of the boll weevil from the subtropical Texas Rio Grande Valley into temperate cotton production areas of north Texas and southern Oklahoma. By 1903, boll weevil infestations were found near the southern boundary of Dallas county in north central Texas, and in 1904, infestations were located north of Dallas county. The southeastern portion of Oklahoma was infested with boll weevils in 1905, and infestations appeared firmly established in central Arkansas by 1909 (Hunter and Pierce 1913). The rapid northward spread of the insect demonstrated that a lengthy period of adaptation was not required for the boll weevil to thrive in areas with relatively cold winters. Published studies of the movement of the boll weevil through the cottonbelt indicate that the population detected near Brownsville, TX in 1894 probably possessed the physiological requirements to survive the winter in all but the most northern cotton production areas. Natural selection has however, apparently resulted in boll weevil populations better adapted to drier and colder conditions. This is evidenced by the fact that the establishment of boll weevil populations in the western and most northern cotton growing areas of Texas was quite slow when compared to the eastward movement of the species.

Hunter and Pierce (1912) reported that the western boundary of the boll weevil infested area was reasonably stable by 1906 and was defined by a line extending northeastward from Del Rio, TX across central Texas into southern Oklahoma. The western limits of the range of the boll weevil remained relatively stable for almost 50 years indicating that there were environmental restrictions to the establishment of boll weevil populations in west Texas. Low rainfall and higher elevations were the most often cited reasons for the failure of the boll weevil to become quickly established in west Texas cotton production areas (Pierce 1913, Hunter 1914). Recent studies indicate that less abundant, and less favorable winter habitat, and somewhat colder winters were equally important in restricting the westward spread of the boll weevil (Rummel 1970).

However, the establishment of resident boll weevil populations in some areas of west Texas was apparently aided by certain changes in land use patterns such as the introduction of shelterbelt, or windbreak plantings in the 1930's (Slosser and Boring 1980) and Conservation Reserve Program grass plantings in the 1980's (Carroll et al. 1993). The recent establishment of a boll weevil population in Texas southern High Plains counties indicates that the species continues to adapt and increase it's range (Adkisson et al. 1997). However, there does appear to be some limit to the northern expansion of the boll weevil population. Overwinter survival of the boll weevil in the cotton producing counties of the Texas Panhandle is marginal, with infestations decreasing greatly following cold winters. Cotton production in the central and northern counties of the Texas Panhandle is very limited, but even if significant cotton acreage still existed in those areas it appears that the boll weevil would have difficulty establishing resident populations.

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When we make allowances for differences in cultural practices, and local and regional differences in weather, the ecology of the boll weevil appears quite similar across the U. S. Cottonbelt. Only when we leave the temperate cotton growing region and enter the sub-tropical Rio Grande valley of Texas do we note marked differences in boll weevil behavior. In the present paper we attempt to summarize and interpret ecological studies of the boll weevil conducted over the past 100 years.

ECOLOGY IN THE TEMPERATE ZONE

Diapause. Early researchers recognized that the boll weevil survived the winter in a state of comparative if not complete inactivity (Hinds and Yothers 1909). Realizing that the overwintering period was a vulnerable time in the seasonal cycle of the boll weevil, considerable effort was devoted to the study of this phenomenon (Hunter and Hinds 1905, Sanderson 1907, Hinds and Yothers 1909, Malley 1901). However, most early research devoted to boll weevil hibernation involved determining the preferred types of winter habitat, the effects of various environmental factors on survival, and when boll weevils began entering and leaving hibernation sites. These studies served as the basis for cultural control recommendations such as early harvest and stalk destruction to reduce the size of the overwintering boll weevil population.

In their landmark publication, "Diapause in *Anthonomus grandis* Boh.", Brazzel and Newsom (1959) reported and described diapause in the boll weevil. They reported that diapause in the boll weevil was characterized by cessation of gametogenesis, atrophy of gonads, increase in fat content, decrease in water content, and decrease in respiratory rate. The study of Brazzel and Newsom (1959) demonstrated that in temperate cotton production areas only boll weevils in diapause survived the winter. They reported that diapausing boll weevils were found in overwinter habitat each month of the year except June and July, and speculated that if extensive sampling of winter habitat were conducted during these months diapausing boll weevils would be found.

Even though considerable research has been conducted, diapause remains one of the least understood and most important parts of the seasonal biology of the boll weevil. Initiation of diapause in the boll weevil has been related to several environmental factors. Earl and Newsom (1964) reported that exposure of immature forms to short photoperiods of LD 11:13 induced diapause while a photoperiod of LD 13:11 suppressed diapause. Lloyd et al. (1967) implicated short photoperiod in the initiation of diapause along with exposure of adults to night temperatures of 10°C, boll feeding in the larval stage, limited square feeding in the adult stage, and boll feeding in the adult stage.

While numerous environmental factors have been reported to play a role in the initiation of diapause it is obvious that not all implicated factors are present when diapausing boll weevils began to appear in cotton fields. Sterling and Adkisson (1974) reported a low incidence of diapause in the first boll weevil generation during mid-July in the Texas Rolling Plains and found diapausing individuals at all succeeding dates with greatest numbers detected during October. These authors concluded that in their study area , diapausing boll weevils developed during every generation.

A gradual increase in the incidence of diapause as the cotton season progresses appears standard throughout the cottonbelt and is generally associated with maturing of the cotton plant (Lloyd and Merkl 1961, Beckham 1962, Lloyd et al. 1964, Mitchell and Mistric 1965, Sterling and Adkisson 1966, Rummel 1970). Phillips (1976) concluded that the boll weevil in its northward movement adapted to photoperiod as an indicator of season but retained the capacity to "read" its host, a characteristic that probably evolved around the occurrence of dry and wet seasons in its area of origin.

Some differences in the diapause response of boll weevils have been detected among different populations. Sterling and Adkisson (1966) reported that boll weevils along the boundary of the Texas High Plains entered diapause considerably earlier in the fall and in greater percentages than those in central Texas. They concluded that the boll weevil had become adapted to the environment of the High Plains area and a distinctive photoperiodic race had developed. Cole and Adkisson (1983) also found that the High Plains boll weevil strain entered diapause earlier and in greater numbers than the central Texas strain.

Late Season Dispersal and Entry Into Winter Habitat. Early experiments with pheromone traps indicated a definite seasonal pattern in boll weevil movement. Peak

capture of overwintered boll weevils on pheromone traps usually occurred in late May or early June followed by a sharp decline with little response during much of the mid-season period. Boll weevil response to pheromone traps then began to increase rapidly about mid-August with greatest numbers captured during late September or October (Bottrell et al. 1970, Hardee et al. 1970, Ridgway et al. 1971). It is generally considered that the late season response of boll weevils to pheromone traps is a reflection of the dispersal of reproductive and diapausing boll weevils in search of new breeding and overwintering sites. The direction of dispersal during late season seems random except as influenced by wind (Moody et al. 1993). Rummel et al. (1977) reported a significant seasonal variation in the height of boll weevil flight between the spring and fall migratory periods. During the late season migratory period, significantly greater numbers of boll weevils were captured at high levels on pheromone traps and in aircraft towed nets than in the spring. The seasonal variation in the height of flight appears to reflect the different ecological roles filled by overwintered and late season migratory boll weevils. The increased number of boll weevils flying at high levels during the fall is an indication of the tendency toward long-range migratory flights which disperse the population into new breeding and overwintering areas (Rummel et al. 1977).

The ability of boll weevils to migrate long distances has been documented by several researchers. Davich et al. (1970) reported capturing boll weevils on pheromone traps up to 45 miles from the nearest cotton. Johnson et al. (1975) recaptured marked boll weevils at distances of 1 to 33 miles from their release point. Beerwinkle et al. (1996) reported a fairly uniform distribution of late-season migratory boll weevils up to 20 k (12.4 miles) from source cotton. Guerra (1988) reported that several marked boll weevils released in Mexico were recovered in pheromone traps near Brownsville, Texas, a distance of approximately 162 miles from the release point.

It is the shorter range dispersal within cotton growing areas however, which has the greatest impact on boll weevil management. Rummel and Adkisson (1970) reported that during the spring following a diapause control program which greatly suppressed the potential overwintering population, most fields infested with overwintered boll weevils were located within 0.5 mile of favorable overwintering habitat. They concluded that most diapausing boll weevils dispersed relatively short distances from their field of origin, and that overwintered boll weevil infestations were influenced greatly by the distance of favorable overwintering habitat from the cotton field. Ridgway et al. (1971) studied the late season dispersal of marked boll weevils from an isolated cotton field and reported that the majority of boll weevils recovered were taken in pheromone traps within 4.5 miles of the field. Studies conducted by Moody et al. (1993) support the theory that most diapausing boll weevils migrate relatively short distances from their field of origin. These authors studied late season migration of boll weevils from small isolated cotton fields by marking weevils in the field and recovering migrants with pheromone traps and through examination of leaf litter in overwintering habitat. Moody et al. (1993) reported that more than half of the migratory boll weevils recovered were captured on pheromone traps located within 2 miles of the field, and over 90% of the marked migratory boll weevils were captured within 4 miles of the source field. Almost all marked boll weevils recovered from overwinter habitat were found within 0.5 mile of the source field. While there was a definite trend toward fewer numbers of marked weevils as distance from the source field increased, some weevils were captured out to 10 miles (Moody et al. 1993).

Moody et al. (1993) found that the migratory boll weevil population during late summer and fall was divided into two segments that were discrete in time. During the late summer and early fall, the majority of the migrating boll weevil population was composed of reproductive individuals, with peak migration of diapausing individuals occurring approximately 45 days later. These authors concluded that there was ample time for early reproductive migrants to reproduce after reaching the limits of their dispersal flight. Therefore, the diapausing progeny of these early migrants could theoretically double the range of dispersal from the original source field.

Hardee et al. (1970) stated that the causal factors involved in late season boll weevil dispersal were not fully understood but several factors were probably involved. A decrease in available food due to maturity of the crop, and increasing population density are generally assumed to play some role in the onset of late season dispersal (Hinds and Yothers 1909, Fenton and Dunnam 1928). However, there is a definite seasonality to the dispersal

phenomenon which often seems independent of these factors. The onset of boll weevil dispersal during late season, sometimes in the absence of obvious causal factors, may be a reflection of the phenomenon described by Andrewartha and Birch (1964) as "an innate tendency toward dispersal". It is evident that the boll weevil has a well adapted dispersal mechanism of both short and long range flights which ensures that the population is exposed to new breeding and overwintering sites. The preponderance of data suggest that diapausing boll weevils move the shortest distances while the longest migratory flights are made by reproductive individuals.

The early studies of Brazzel and Newsom (1959) indicate that some movement of diapausing boll weevils into winter habitat begins before mid-August. The onset of boll weevil migration during late summer (Hardee et al. 1970, Ridgway et al. 1971, Moody et al. 1993) does not however, necessarily indicate that significant numbers of weevils are moving into winter habitat at that time. Some researchers do feel that early movement into winter habitat is an important consideration in late season management programs. Mitchell et al. (1973) stated that it was extremely important that boll weevil diapause suppression programs be started early enough to prevent weevils from entering overwintering sites in August. Most data indicate however, that the actual settling of diapausing boll weevils into winter habitat is a gradual process which increases as the fall season progresses and is closely correlated with decreasing temperature (Sanderson 1907, Hinds and Yothers 1909, Wade and Rummel 1978). Wade and Rummel (1978) reported that only a small portion of the potential overwintering population effected early entry into winter habitat, but the entry rate increased rapidly as the fall period progressed. They stated that the greatest numbers of overwintering boll weevils were found in winter habitat immediately following the first plant killing freeze. Thus, when cold temperatures occur early in the fall, greater numbers of boll weevils will enter winter habitat early.

Winter Survival. Hunter and Pierce (1912) pointed out that boll weevils seeking shelter from the cold would enter all kinds of places which might afford protection. It was quickly recognized by early researchers that few boll weevils successfully overwintered in dried bolls or plant debris found within the cotton field. Wooded areas near cotton, which provided an accumulation of dead leaves and other plant debris were recognized as the primary boll weevil overwintering sites (Hunter and Hinds 1905, Sanderson 1907, Hinds and Yothers 1909, Hunter and Pierce 1912). Within different areas of the cottonbelt certain types of habitat have been noted which provide especially favorable overwintering sites for the boll weevil. In the coastal area of Texas and the southern portions of the gulf states, Spanish moss was considered excellent shelter for overwintering boll weevils by Hunter and Pierce (1912), while sand shinnery oak, *Quercus havaardii* Rydb., is considered highly favorable winter habitat in the Texas Rolling Plains (Adkisson et al. 1965). Whether or not a particular type of habitat is conducive to good winter survival for the boll weevil appears to depend mostly upon the insulating properties of the habitat (Carroll et al. 1993).

Boll weevil overwinter survival rates of less than 1.0% to over 50% have been reported from numerous studies conducted across the cottonbelt (Sanderson 1907, Newell and Dougherty 1910, Fenton and Dunnam 1929, Sterling 1971, Rummel and Carroll 1983, Rummel and Carroll, unpublished data). The great variation in boll weevil winter survival reported from different studies is a reflection of variation in the factors which influence boll weevil winter mortality among years and study sites. Exposure to low temperature is recognized as the major factor affecting boll weevil survival during the winter (Gaines 1953, Smith and Scales 1965, Pfrimmer and Merkl 1981). Price et al. (1985) reported that in the Texas Rolling Plains, 91.6% of the variation in boll weevil winter mortality was explained by length of time in overwintering habitat, winter habitat temperature, and the rainfall by habitat (leaf litter) temperature interaction. Slosser et al. (1996) reported that diapausing and nondiapausing boll weevils could be separated by exposure to - 7.5° C (18.5° F) for 6 hours. After this exposure, diapausing adults were alive and able to walk, while nondiapausing adults were either dead or unable to stand.

While excessive moisture in winter habitat will increase mortality (Taft and Hopkins 1966), a certain level of moisture is necessary for optimum boll weevil winter survival. Leggett and Fye (1969) reported that a much higher percentage of diapausing boll weevils survived under wet conditions than under dry conditions. Parajulle et al. (1996) stated that a simultaneous occurrence of both high temperature and high rainfall was more conducive for overwintering survival than the occurrence of either situation alone. The temperature and

moisture interaction as it applies to boll weevil winter mortality has not been fully explored. However, the preponderance of data indicates a rather straightforward relationship. Cold, dry winter conditions result in the greatest boll weevil mortality, while moderate temperatures and adequate moisture in overwinter habitat provide optimum conditions for boll weevil survival.

Some factors affecting overwinter boll weevil survival are more difficult to measure than winter severity. Brazzel and Newsom (1959) described fat body accumulation as one of the major characteristics of boll weevil diapause and indicated that boll weevils required about two weeks of feeding to accumulate the fat reserves necessary for winter survival. The accumulation of large fat reserves is generally considered necessary for boll weevils to survive long periods of harsh winter weather. Studies conducted by Mitchell and Taft (1966), however, indicated that boll weevils needed very little fat reserve to survive the winter. They suggested that the accumulation of large fat deposits by diapausing boll weevils was coincidental to the occurrence of diapause and occurred primarily because diapausing weevils continued to feed at a time when their rate of metabolism was considerably reduced. Fat accumulation alone is apparently not a reliable indicator of winter survival potential. Boll weevils exhibiting both oogenesis and hypertrophy of the fat body are commonly found in field populations during the fall period. The most common explanation of this phenomenon is that such individuals are in the process of absorbing the eggs, accumulating fat, and entering diapause (Walker 1967). Walker (1967) stated that boll weevils possessing both greatly developed fat bodies and mature eggs were most likely breaking diapause and becoming reproductive. However, recent work by Palmer and Cate (1992) showed that even limited reproductive output by female boll weevils precluded winter survival. The work of Palmer and Cate (1992) indicates that regardless of fat body development, any boll weevil showing evidence of reproductive activity has little chance of winter survival and cannot be considered as being in diapause.

It is obvious that varying levels of fat are found in diapausing boll weevils. As indicated by Mitchell and Taft (1966), individuals with limited fat body development may survive the winter under the right conditions. However, most data indicate that fat body accumulation in diapausing boll weevils is important in winter survival. Rummel and Carroll (1993) stated that from the survival standpoint, the vigor of diapausing boll weevils at the time they entered winter habitat was as important as winter severity. They considered the requisites for the production of vigorous diapausing boll weevils to be 1) abundant food, 2) high quality food, and 3) adequate feeding time. The fulfillment of these requirement results in strong, fat, diapausing boll weevils which have high survival potential (Rummel and Carroll 1993).

The duration of exposure in overwintering habitat has greater influence on mortality than freezing temperature (Price et al. 1985). A major determinant in the time diapausing boll weevils spend in winter habitat is the time of entry. Considerable data exist showing that weevils entering winter habitat during the mid to late fall period tend to exhibit higher winter survival rates than early entering individuals (Hunter and Hinds 1905, Hinds and Yothers 1909). Wade and Rummel (1978) and Rummel and Carroll (1983) reported that diapausing boll weevils placed in winter habitat in late August and early to mid-September consistently had lower survival rates than weevils entering winter habitat in October. From a biological standpoint, this pattern makes sense. Boll weevils entering winter habitat early in the fall have a longer exposure period to winter habitat conditions and thus have a reduced probability of surviving long enough to emerge and find their host plant the following spring. Stone et al. (1990) reported that the occurrence of low temperatures during the later portion of the overwintering period may exact greater mortality than early cold periods. In laboratory studies, Slosser et al. (1996) found that young diapausing adult boll weevils (36 days old) were more tolerant of freezing temperatures than were older (110 day old) adults. Thus, boll weevils which enter winter habitat early probably use more of their fat reserves and therefore, are more susceptible to late winter cold weather than weevils which enter late in the fall. Regardless of the time of entry into winter habitat, boll weevils must be physiologically suited for survival if they are to successfully overwinter. Therefore, the vigor (Rummel and Carroll 1993) of weevils at the time they enter winter habitat may override the time of entry as a survival factor.

Spring - Summer Emergence. Based upon a 27-year study, Davis et al. (1967) concluded that during most years a high percentage of overwintered boll weevils had

emerged from overwinter sites in central Texas by mid-June. They pointed out however, that variation in rainfall and spring temperatures probably accounted for differences in emergence patterns among years. Carroll and Rummel (1985) reported that the response of overwintered boll weevils to pheromone traps during the spring and early summer was an accurate reflection of weevil emergence from winter habitat. The accuracy of pheromone traps in reflecting spring - summer emergence has enabled researchers to more clearly define the emergence profile of overwintered boll weevils. Overwintered boll weevil emergence patterns showing peak emergence during the late May to mid-June period seem to be common across the cottonbelt. However, emergence patterns in all areas of the cottonbelt sometimes vary greatly among years. McKibben et al. (1992) reported variations in overwintered boll weevil emergence patterns in Mississippi and pointed out the management problems which arise when significant overwinter emergence overlaps the cotton fruiting period. An understanding of the factors which influence overwintered boll weevil emergence is an important part of the overall understanding of boll weevil ecology.

The spring - summer emergence profile of overwintered boll weevils seems to be greatly influenced by factors involved in winter mortality. Gaines (1935) reported that overwintered boll weevil emergence periods were shortest following winters of lowest survival and longest following winters with high survival. Jones and Sterling ((1979a) reported earlier initiation of emergence and an extended emergence period after mild winter conditions compared with a severe winter. Stone et al. (1990) stated that the coincidence of high winter survival followed by extended spring emergence of overwintered boll weevils suggested that the two processes were linked. Rummel and Carroll (1993) reported that a 13-year study of boll weevil winter survival and spring - summer emergence in the Texas Rolling Plains strongly supported such a linkage. Parajulee et al. (1996) concluded that temperature during diapause affected both overwintering survival and spring-summer emergence. These authors stated that the higher the temperature above the lower threshold of boll weevil activity (>6.1° C), the higher the winter survival, and earlier the initiation of spring emergence. Conversely, the greater the degree-days below 0.0°C the lower the winter survival and later the initiation of emergence from winter habitat. While the causal factors are not fully understood, this relationship seems rather straightforward. When the boll weevil winter survival rate is low, the subsequent spring - summer emergence profile is abbreviated, while a high level of winter survival results in an extended overwintered boll weevil emergence period.

The relationship between the time diapausing boll weevils enter winter habitat and the timing of emergence in the spring is disputed in the literature. Some studies have found no relationship between time of entry into winter habitat and the spring -summer emergence pattern (Calhoun 1931, Sterling 1971, Taft et al. 1973), while others (Mitchell et al. 1973, Jones and Sterling 1979a) reported that weevils entering winter habitat early in the fall emerged later the following spring and summer. Hunter and Hinds (1905) stated that the weevils which survived the winter and successfully attacked cotton the following spring were primarily weevils which developed late in the fall. Wade and Rummel (1978) and Rummel and Carroll (1983) reported that boll weevils entering winter habitat later in the fall tended to have a higher survival rate and emerge later during the subsequent spring than early entering boll weevils. Differing opinions on the relationship between the time diapausing boll weevils enter winter habitat and the timing of emergence may arise largely because of different techniques used in the studies. Rummel and Carroll (1993) argued that when the age structure of test weevils was carefully controlled, and boll weevil cohorts of a known age were released into winter habitat on a weekly basis, the pattern of lower survival and earlier emergence of early cohorts was quite clear. However, differences in survival and time of emergence did not appear among all temporal cohorts. Usually there were no significant differences in survival and emergence time among early, mid- or late October cohorts. However, boll weevils placed in winter habitat in late August and early to mid-September consistently had lower survival rates and emerged earlier than October cohorts. In some, but not all years, early November cohorts exhibited better survival and later emergence than early-mid October cohorts (Rummel and Carroll 1993). The linkage between the time diapausing boll weevils enter winter habitat and the timing of emergence seems as strong as that between time of entry and winter survival. The factor common to both appears to be the time of exposure in winter habitat.

However, the association between the time of boll weevil entry into winter habitat and the timing of spring - summer emergence is not completely straightforward. Regardless of the time of entry into winter habitat, boll weevils must be physiologically prepared if they are to survive the winter. Late entering boll weevils which do not possess the necessary vigor (Rummel and Carroll 1993) will have lower survival rates and survivors will likely be forced into earlier emergence than physiologically fit weevils which enter winter habitat earlier in the fall.

Temperature and rainfall influence the emergence pattern of overwintered boll weevils (Parencia et al. 1964, Smith et al. 1965, Mitchell et al. 1972). The influence of temperature during the overwintered boll weevil emergence period seems straightforward; emergence is accelerated by higher temperatures and depressed by low temperature. Stone et al. (1990) concluded that accumulation of degree-days was an appropriate time-scale for predicting overwintered boll weevil emergence. The influence of moisture on overwintered boll weevil emergence is less well defined. Mitchell et al. (1972) reported that early in the emergence period, temperature was the dominant factor but that the influence of rainfall increased as temperature increased. Experience has shown that during the overwintered boll weevil emergence period, boll weevil response to pheromone traps usually increases sharply following rainfall. This phenomenon is most evident when periods of dry weather during the emergence period are interrupted by rainfall.

The interaction of habitat type and temperature also has an important influence on overwintered boll weevil emergence. Hunter and Pierce (1912) reported that boll weevils which sought winter shelter in cool, shaded timbered areas or in moss in swamp land remained in hibernation longer than weevils overwintering in more open areas. Slosser et al. (1984a, 1984b) and Slosser and Fuchs (1991) compared overwintered boll weevil survival and spring emergence in open habitat types to habitat which provided a dense canopy cover. They concluded that leaf litter in the more open habitats received greater amounts of solar radiation, which resulted in higher temperatures and accelerated emergence of overwintered boll weevils in the spring. Carroll et al. (1993) reported the same findings after comparing overwintered boll weevil emergence from grasses and shinnery oak mottes. Therefore, the emergence profile of overwintered boll weevils and the time of infestation in cotton may be greatly influenced by the type of habitat near cotton.

The longevity of overwintered boll weevils after emergence from winter habitat is an important factor influencing population development in cotton. Fenton and Dunnam (1929) recognized that in many instances significant numbers of overwintered weevils emerged too far in advance of squaring to contribute to reproduction. These authors coined the term "effective emergence" to denote that portion of the overwintered boll weevil population which emerged late enough or survived long enough to reproduce in cotton.

Studies of the longevity of overwintered boll weevils after emergence have produced widely varying results, probably because of different experimental techniques. Hunter and Hinds (1905) stated that fully one-third of the emerging overwintered boll weevil population could be expected to live for a period of time longer than that occurring between the planting of cotton and the formation of squares. This conclusion was based upon laboratory studies in which weevils feeding on cotton plant terminals lived an average of 45 days. Hunter and Hinds (1905) also reported a life span of 80 days for males and 70 days for female boll weevils fed on squares in the laboratory. However, Fenton and Dunnam (1929) found in field studies that male and female weevils feeding on squaring cotton lived an average of 19.4 and 16.1 days respectively, and weevils restricted to seedling cotton had an average life span of only 8.1 days.

Fye et al. (1959) caged overwintered weevils on seedling cotton in the field and as the cotton grew, surviving weevils were transferred to older plants. Using this technique, Fye et al. (1959) established an average longevity for overwintered boll weevils of 21.9 days. Rummel and Carroll (1985) used techniques modeled after those of Fye et al. (1959) and reported that 60% of newly emerged overwintered boll weevils died within 10 days, and 90% within 20 days when provided with seedling cotton as the sole food source. These authors stated that only those weevils feeding on squaring cotton exhibited a high percentage of survival beyond 20 days.

The preponderance of data indicate that unless squares are available for food, emerged overwintered boll weevils are relatively short lived. Therefore, in temperate cotton growing regions, a sizable portion of overwintered boll weevil emergence may be suicidal each year.

In the Texas Rolling Plains where the majority of the cotton acreage is planted during the later part of May, it is estimated that during most years, 10 percent or less of the emerging overwintered boll weevil population constitutes effective emergence (Wade and Rummel 1978, Rummel and Carroll 1983).

However, there is evidence that in the absence of cotton, boll weevils may utilize polyphagous pollen feeding as a survival strategy. Rummel et al. (1978) discovered that during the spring, overwintered boll weevils in the Texas Rolling Plains readily fed on the pollen of a native flowering plant, Yellow woolywhite, *Hymenopappus flavescens* Gray, before cotton was available. In some tests, these authors found that feeding on the pollen of yellow woolywhite significantly extended the life span of newly emerged overwintered boll weevils. Benedict et al. (1991) reported evidence of polyphagous pollen feeding by boll weevils in northeastern Mexico and in the Lower Gulf Coast and Rio Grande Valley areas of Texas. Recently, Jones and Coppedge (1996) reported that in south Texas, emerging overwintered boll weevils were found feeding on a wide variety of wild pollens. Jones et al. (1993) identified numerous non-cotton feeding hosts of the boll weevil in northeastern Mexico. These authors concluded that weevils emerging from overwintered habitat might survive by feeding on the pollen of available plants until cotton was available. Jones et al. (1993) also speculated that boll weevils dispersing from cotton at the time of harvest might augment stored fat reserves by feeding on non-cotton pollen, thus increasing overwintering survival as well as the distance of dispersal.

Colonization of Cotton. The question of whether boll weevils are attracted to cotton or find their host plant through random search continues to be a subject of debate. Hunter and Pierce (1912) concluded that the boll weevil apparently had no sense by which it could locate cotton. These authors stated that the general aimless flight of thousands of individuals seemed sufficient to account for the infestation of new cotton fields. Hunter and Pierce (1912) did consider however, that the fact that boll weevils were rarely found on plants other than cotton, might indicate some attraction to cotton. Following the discovery that the male boll weevil produced a sex pheromone (Cross and Mitchell 1966, Keller et al. 1964), studies by Hardee et al. (1969) showed that the pheromone also acted as an aggregating agent and was the major factor involved in boll weevil colonization in cotton. However, other studies have shown that a boll weevil attractant of at least short range activity is present in cotton (Keller et al. 1963, 1965, Neff and Vanderzant 1963, Hardee et al 1966a, 1966b, Daum et al. 1967, Minyard et al. 1969). In addition, field studies in which emerging overwintered boll weevils and migrating boll weevils were able to locate small, isolated plots of squaring cotton support the argument for the presence of a boll weevil attractant in cotton (Parencia et al. 1964, Beckham and Morgan 1960).

Studies by Hardee et al. (1969) indicated that cotton volatiles exerted little if any influence on the migratory behavior of boll weevils, and that pheromone produced by male boll weevils after chance entry into cotton initiated the colonization process. Hardee et al. (1969) pointed out that boll weevils generally overwintered near cotton and that emerging overwintered boll weevils would have little difficulty locating cotton whether or not they were attracted by plant volatiles. The reverse of this argument however, is that the boll weevil did not evolve in cultivated cotton and the requirement to locate wild cotton plants which might often be sparse and scattered would place the species at a distinct evolutionary disadvantage. Whatever the cause of initial entry, it is evident that colonization of cotton by overwintered boll weevils is closely related to plant phenology, with infestation increasing as square size and density increase (Walker and Bottrell 1970, Roach et al., 1971, White and Rummel 1978).

Several studies in which overwintered boll weevil movement into small, isolated cotton plots was carefully documented, indicate that boll weevils are attracted to fruiting cotton (Walker 1966, Walker and Bottrell 1970, White and Rummel 1978). White and Rummel (1978) stated that initial overwintered boll weevil entry into cotton appeared to be a positive response to squaring cotton with attraction greatly intensified by pheromone production after male boll weevils fed on squares. However, White and Rummel (1978) also found that when overwintered boll weevils were immediately removed from isolated cotton plots, thus removing the pheromone source, the colonization process was disrupted. While there is no question that pheromone is the dominant factor in the colonization process of overwintered boll weevils, the preponderance of data indicate that some form of plant attraction also plays a role. Recent studies of specialized neurons for pheromones and host

plant odors in the boll weevil support this position. Dickens (1990) demonstrated that the boll weevil has receptor neurons responsive not only to components of its aggregation pheromone, but also to host plant volatiles, and concluded that boll weevils can detect their host plant from some distance.

Rates of Increase. The explosive nature of boll weevil infestations in cultivated cotton was noted by numerous early researchers. Hunter and Pierce (1912) estimated that one pair of overwintered adults could produce 3,001,250 offspring in four generations. These estimates were based on an average 35-fold increase between generations and made no allowance for natural mortality. Knipling (1959) estimated the rate of increase of boll weevil populations to be 2.5, 5.0, and 7.5 -fold for low, average and high rates of increase depending upon environmental conditions. The generalized estimates of Knipling (1959) fit rather well with studies conducted in various areas of the cottonbelt. Walker and Hanna (1963) reported a 3-fold increase of first generation boll weevils over the overwintered generation, and a 7 fold increase between the first and second generation in a central Texas study, and Lloyd et al. (1964) reported an average population increase of 5-fold per generation in Mississippi. In a study conducted in the Texas Rolling Plains, Sterling (1969) stated that boll weevils increased at a rate of 2.7 fold/week in fields considered to have near optimum conditions for population growth. Rummel (1970) reported that an overwintered boll weevil population estimated at 33 weevils/acre increased at a rate of 1.6 fold/week in dryland cotton fields where the conditions for boll weevil development were considered sub-optimum because of low square density. However, this rate of increase was sufficient to produce a relatively large diapausing population by the time cotton growth was terminated by frost.

The role of biotic agents in the regulation of boll weevil populations is important, but usually subordinate to that of abiotic agents. Numerous native parasites and predators are known to attack the boll weevil but their actions seldom suppress populations to the point of economic control (Lincoln and Graves 1978). In a cottonbelt wide survey, Chestnut and Cross (1971) reported that 12.77% of the productive squares and 5.56% of total squares infested by boll weevil were parasitized. In a similar study in insecticide free fields in the Texas Rolling Plains, Meinke and Slosser (1981) reported a boll weevil parasitism rate of 19.3 and 7.8 % for productive and total squares respectively. Sturm and Sterling (1990) reported that the cause of immature boll weevil mortality varied with geographic location in Texas. Predation was the dominant natural mortality factor in the eastcostal region, while mortality was equally divided among parasitism, predation and square desiccation in the northcentral region. In the westcentral region of the state, square desiccation accounted for the majority of immature boll weevil mortality. Among the three geographic locations, Sturm and Sterling (1990) recorded natural immature boll weevil mortality of 64-82%. They concluded however, that this level of mortality was not generally enough to prevent economic losses from the insect unless weevil numbers were very low. The red imported fire ant, *Solenopsis invicta*, is under certain conditions, a highly effective predator of the boll weevil, often preventing the development of economically damaging infestations (Jones and Sterling 1979b, Sterling 1978, Sterling et al. 1984). A few pathogens of the boll weevil have been observed in the field (Cross 1983), but their role in population regulation appears minor.

Isley (1932) stated that in Arkansas, the rate of increase and abundance of the boll weevil was determined primarily by weather. Sterling (1969) reported that the abundance of squares and bolls as determined by soil fertility, weather and agronomic practices was the key factor in the rate of increase and the abundance of boll weevils in cotton fields. The boll weevil carrying capacity of a cotton field was considered by Sterling (1969) to be a function of fruit abundance. Therefore, fields with different carrying capacities would produce different boll weevil population levels, even though the rate of increase might be the same. Large populations of overwintered boll weevils often limit square production, thereby limiting oviposition sites. In studies conducted in central Texas, overwintered boll weevil populations of approximately 200/acre produced larger F1 populations than overwintered populations which exceeded 500/acre (Walker 1966). Environmental resistance to boll weevil population development, primarily dominated by weather, varies among cotton growing regions and accounts for differences in reports of boll weevil growth rates. For example, studies conducted by Walker (1966) in a central Texas river bottom cotton production system indicated that overwintered populations of 50 to 100 boll weevils/acre

resulted in heavy, damaging late season infestations. However, in dryland cotton in the Texas Rolling plains, Slosser et al. (1991) found that populations of up to 532 overwintered boll weevils/acre could be tolerated before insecticidal treatment became profitable. In general, if environmental conditions are favorable for cotton growth, abiotic resistance to boll weevil population development is minimal and damaging infestations usually develop.

ECOLOGY IN THE SUBTROPICAL ZONE

Due to its strategic location, the Lower Rio Grande Valley (LRGV) of Texas appears to have been the first area of the U. S. Cotton Belt colonized by the boll weevil during its northward range expansion that began about 1892 (Hunter and Hinds 1904). The boll weevil population that occurs in this region is somewhat unique in that it exists in a climatic zone transitional between the warm-temperate environment of south-central Texas and the tropics of northern Mexico (Orton et al. 1967), and is located in the general region where the "southeastern" and "Mexican" forms of boll weevil intergrade (Burke et al. 1986). Based on the relative frequencies of polymorphisms and rare alleles, the boll weevil population of the LRGV region appears to be one of the most genetically variable populations occurring in the United States (Terranova et al. 1991).

The boll weevil life system in the subtropical LRGV region shares a number of important similarities with populations occurring in temperate zones. As in other areas of the U. S. Cotton Belt, reproduction occurs primarily on cultivated cotton, although at least three alternate reproductive hosts are known to occur in the LRGV region (Lukefahr and Martin 1962, Cross et al. 1975, Burke and Clark 1976). Overwintered adult weevils are commonly observed in commercial cotton plantings prior to square formation, and may continue to colonize such cotton for an extended time period (Coppedge et al. 1996). Indigenous natural enemies associated with boll weevil in the LRGV region attack the pest only facultatively and have generally been relatively ineffective in biological control of the pest (Cate et al. 1990). Moreover, the impact of important abiotic mortality factors may be reduced appreciably by certain cultural practices (e.g., irrigation, which tends to minimize the incidence of mortality caused by square desiccation). As a result, immature boll weevils developing on cotton commonly exhibit a relatively high incidence of survivorship (Sturm et al. 1989), and rates of increase in commercial cotton plantings may approach 30-fold between generations (Summy et al. 1988a, 1988b, 1992c). With the advent of cotton plant maturity and senescence, adult boll weevils initiate a general dispersal from cotton into other types of habitat (Wolfenbarger et al. 1976, Guerra and Garcia 1982, Guerra et al. 1982a, Guerra 1988, Wright and Chandler 1989), where they are destined to either perish or to overwinter successfully by one of several means. At this point, many of the reported peculiarities of the LRGV population become apparent.

The Diapause Problem. Several studies have reported classic symptoms of diapause in adult boll weevils from the LRGV region. Based on levels of succinate-cytochrome c reductase (SCR) in thoracic and abdominal tissue, Keeley et al. (1977) concluded that adult weevils overwintering in the LRGV region (latitude 25o 55' N) appear to exhibit a more intense state of diapause than counterparts occurring in more northerly latitudes. Moreover, adult weevils exhibiting traits that have traditionally been associated with diapause (i.e., enlarged fat body and atrophied reproductive systems) have been collected from pheromone traps and samples of ground trash throughout the postharvest fallow season, which extends from September through February (Wolfenbarger et al. 1976, Graham et al. 1978, 1979, Guerra et al. 1982a).

Nevertheless, the nature of boll weevil diapause in the LRGV region is somewhat conjectural, and several studies suggest that the physiology of adults overwintering in this region may differ in several respects from those occurring in the temperate zone. Based on rates of oxygen consumption, Guerra et al. (1982b) suggested that overwintering adult weevils in the LRGV region remain physiologically active (i.e., potentially reproductive) and enter a state of "quiescence" rather than diapause. A similar strategy was proposed for adult weevils overwintering in the Mexican tropics (Guerra et al. 1984). While conceding the existence of diapause in the LRGV population, Keeley et al. (1977) reported significantly lower levels of lipid content and abdominal succinate - cytochrome c reductase activity with respect to counterparts from more northerly latitudes. Based on these findings, the latter authors stated that lipid content (generally considered a reliable indicator of

diapause in temperate zones) was inconclusive with respect to this population. More recently, Rankin et al. (1994) documented a direct relationship between the amount of fat body reserves and the propensity of adults from the LRGV region to engage in long-duration flight, but found no relationship between size of the fat body and ovarian development. Based on these results, the latter authors suggested that an enlarged fat body per se appears to be largely unreliable as a criterion for diapause in the LRGV population.

Regardless of the physiological mechanisms involved, survivorship studies conducted in the LRGV region have clearly demonstrated the ability of overwintering adults to survive extended periods under apparently adverse conditions. In outdoor feeding experiments conducted during 1981-1983, adults of both sexes exhibited an ability to survive periods up to 307 days when provided a continuous diet of cotton fruiting forms, and up to 162 additional days following the termination of a limited (21-day) feeding regimen (Summy et al. 1992a). In one series of these experiments, a segment of the adults which had survived to the mid-winter period appear to have entered a state of adult dormancy typical of that occurring in the temperate zone, although such behavior was not evident among any cohort monitored during the following year. Cohorts exposed to abnormally low temperatures during the severe freeze of 24-25 December 1983 (58 hrs <0oC; -8.9oC minimum) exhibited a relatively low incidence of mortality (22.8-35.4%), which suggested a considerable degree of cold-hardiness among these individuals (Summy et al. 1988b, 1992a). The ability of adult boll weevils overwintering in the LRGV region to survive prolonged periods under conditions of apparent starvation has been documented in several subsequent studies (Summy et al. 1995, 1997).

Distribution, Reproductive Status and Age Structure of the Overwintering Weevil Population. In virtually all areas of the U. S. Cotton Belt, the overwintering boll weevil population has been generally perceived as consisting primarily or entirely of diapausing (nonreproductive) adults that enter a state of extended dormancy within distinct types of noncotton overwintering habitat (see previous discussion). Such does not appear to be the case in the LRGV region, however, where surveys have consistently revealed an apparent rarity or absence of dormant adult boll weevils within stands of deciduous leaf trash and other habitat types that are generally regarded as preferred overwintering sites in temperate areas (Graham et al. 1978, 1979, Guerra et al. 1982a, Summy et al. 1988b, 1992a). For example, Graham et al. (1978) reported densities of 0.195 weevils/m2 in surveys conducted during 1973-1974 (most of which were detected in grassy areas), and substantially lower densities during the following two seasons (0.002 and 0.027 adults/m2, respectively). Densities comparable to those reported by Graham et al. (1978) were evident in two subsequent surveys conducted in the LRGV region (Guerra et al. 1982a, Summy et al. 1988b). Although a correlation between damage to cultivated cotton and proximity to sites of preferred overwintering habitat has been identified in certain temperate areas (Rummel and Adkisson 1970), attempts to identify such a relationship in the LRGV region have been unsuccessful (J. R. Cate and K. R. Summy, unpublished data; see also Summy et al. 1992a).

One possible explanation for these discrepancies was provided by Keeley et al. (1977), who noted relatively high succinate - cytochrome c reductase activities in thoracic tissue of adult weevils from the LRGV region, and suggested that such individuals might remain active to a considerably greater extent that counterparts occurring in temperate regions. Although the validity of this hypothesis has not been determined, pheromone traps monitored in the LRGV region have consistently indicated a substantial incidence of flight activity throughout the extended fallow period (Wolfenbarger et al. 1976, Graham et al. 1978, 1979, Guerra and Garcia 1982, Guerra et al. 1982a, Summy et al. 1992a, 1992b). Collection of adult boll weevils from pheromone traps located at a considerable distance from cotton suggests that much of this flight activity involves long-distance dispersal, a trend that has been corroborated with mark-recapture studies (Guerra 1988). Moreover, the large variety of pollens that have been detected in the gut of adult weevils collected in pheromone traps suggests a fundamental importance of foraging behavior (on plants other than cotton) in the overwinter survival of adult boll weevils in the LRGV region and areas located to the south (Jones et al. 1992, 1993, Jones and Coppedge 1996).

For many years, fields of undestroyed cotton have provided the overwintering boll weevil population of the LRGV region with a potentially vast and nearly optimal habitat in which the pest may overwinter in large numbers by several means. Although crop production regulations currently applicable to the LRGV region mandate the destruction of

cotton stalks prior to 1 September and prohibit planting until 1 February (Texas Department of Agriculture 1997), a significant number of cotton fields have traditionally remained intact during the period following harvest (Summy and King 1992). For example, aerial surveillance of Cameron, Hidalgo and Willacy Counties detected ca. 11,150 ha of undestroyed cotton following the 1982 production season, and >20,000 ha following the production seasons of 1984-1986 (Summy et al. 1988b). Under the relatively mild climatic conditions that are generally prevalent in the LRGV region during the fall and winter periods (Orton et al. 1967), cotton plants may fully express their perennial growth habit and tend to be prolific in the production of fruiting forms, i.e., feeding and oviposition sites for boll weevil (Summy et al. 1988a).

The ability of overwintering boll weevils (or at least a segment of the overwintering population) to reproduce continuously during most, if not all, of the September - February fallow season has been demonstrated in several studies (Guerra et al. 1982a, Summy et al. 1988a, 1988b, 1992b). The extent of reproduction that may occur within stands of undestroyed cotton was exemplified in studies that indicated an estimated production of ca. 483,000 adult weevils per ha of cotton regrowth during a 25-wk period characterized by relatively dry conditions, and ca. 1.6 million adults per ha during a 31-wk period in which significant amounts of precipitation occurred (Summy et al. 1988a, 1988b). In addition to the sheer numbers of adult weevils produced, reproduction of this magnitude tends to generate high-density weevil infestations composed primarily of immature stages, an overwintering population structure that differs fundamentally from that occurring in most areas of the U. S. Cotton Belt. Surveys conducted in stands of cotton regrowth near Brownsville during the relatively mild fallow season of 1984 exemplified the relatively high densities (7,900 - 1.1 million weevils per ha) and preponderance of immature stages (53.4-97.4%) that appears to characterize such infestations (Summy et al. 1988b).

Effects of subfreezing temperatures on boll weevil infestations occurring on fallow-season cotton in the LRGV region were documented in surveys conducted at two locations during the winter of 1984 (Summy et al. 1988b, 1992b). Death of infested cotton plants during early-January was accompanied by a rapid desiccation of extant squares and bolls, an event that served to curtail subsequent oviposition by overwintering boll weevils. A continuation of development by immature stages infesting desiccated fruiting forms (particularly bolls) produced a gradual change in the age structure of the infestations, i.e., from a preponderance of immature stages at the time of plant death (ca. 54,000 weevils per ha; 94.6% immature stages) to a uniform adult age structure by early-April (200 weevils per ha; 100% adults). During the subsequent 4-month period, pheromone trap catches in the vicinity of the freeze-killed cotton were substantially greater than those occurring within a 173 km2 control area devoid of cotton (mean totals of 798.0 and 120.0 adults/trap/month, respectively), and contained a significantly higher ratio of teneral (soft, reddish) adults (means of 16.0 and 5.8% tenerals, respectively). Based on these observations, Summy et al. (1988b, 1992b) concluded that sporadic freezes of the type encountered during 6-11 January 1985 (-1.7oC minimum) serve largely to destroy cotton host plants, an event that tends to alter both the reproductive status and the age structure of overwintering weevil infestations (i.e., from reproductive infestations composed primarily of immature stages to infestations composed primarily or entirely of nonreproducing adults). Moreover, contents of pheromone traps provided evidence that many of the soft reddish (teneral) adults that are commonly collected from pheromone traps during the winter and spring periods (and have traditionally been considered evidence of recent reproduction; see Guerra et al. 1982a) are in reality relatively old individuals only recently emerged from cotton residue.

One of the most cryptic and potentially important overwintering habitats of boll weevil in the LRGV region involves infested cotton residue buried beneath the soil surface (Summy et al. 1988b, 1992b). While the destruction of cotton canopies during the late-summer period (i.e., by conventional shredding operations) may destroy significant numbers of immature boll weevils as a result of exposure to lethal soil temperatures, this does not generally occur during the cooler fall and winter periods (Summy et al. 1986). Immature boll weevils infesting bolls incorporated into soil by conventional plowing operations may continue development to the adult stage, and have been shown to infiltrate up to 20 cm of topsoil following eclosion and emergence (Fye et al. 1970, Summy et al. 1985). The significance of buried cotton residue as overwintering habitat for boll weevil (or conversely, the futility of attempting to destroy dense weevil infestations that have been allowed to

develop during the fallow season) has been recognized for nearly a century. One of the earliest statements to this effect was that of Hunter (1909), who noted that "...The point may be raised that winter plowing, burying the weevils found in trash on the surface, might have the same effect as burning. On the contrary, experiments have shown that weevils can easily make their way through several inches of soil. Consequently, such work in general is as likely to protect as to destroy the weevils." The validity and significance of Hunter's admonition was exemplified by more recent studies that documented an estimated emergence of ca. 396,000 adult boll weevils during a period of ca. 30 days (13 March - 18 April, 1985) from a 243-ha block of cotton fields that had been "destroyed" by conventional cultivation during the previous December (Summy et al. 1988b, 1992b).

The ability of boll weevils to overwinter as either adults or immature stages within desiccated cotton bolls has been documented in temperate zones (Cowan et al. 1963, Walker and Shipp 1963), and appears to be particularly commonplace in areas characterized by relatively mild climatic conditions and poor cultural controls, e.g., Arizona and much of southern Texas (Fye et al. 1970, Bergman et al. 1983, Bariola et al. 1984, Guerra et al. 1984, Henneberry et al. 1989, Summy et al. 1988b, 1992b). Although the underlying physiological mechanisms of this strategy have not been clarified (c.g., see Guerra et al. [1984] and Bergman et al. [1983] for a divergence of opinion regarding the diapause status of such individuals), boll weevils developing to the adult stage are clearly capable of surviving a prolonged period of entrapment without suffering any appreciable reduction in fitness (Summy et al. 1992b). For example, mean survival times of adult weevils extracted from desiccated bolls following a 7-month holding period in the laboratory ranged between 4-162 days for males (=108.3 days) and 5-125 days for females (=76.0 days). Oviposition first became evident approximately 11 days after exposure to squares, and continued for a period of 108 days. Of the total eggs produced during this period (103.5 eggs per female), 82.9% were determined to be viable (Summy et al. 1992b).

Significance of Alternate Reproductive Hosts. Although boll weevil reproduction in southern Texas occurs primarily on cultivated cotton, at least three alternate reproductive hosts are known to occur in the LRGV region (Lukefahr and Martin 1962, Cross et al. 1975, Burke and Clark 1976). The most abundant and important of these wild hosts is the sulfur mallow, *Cienfuegosia drummondii* (A. Gray) Lewt., an exotic species that was presumably introduced into southern Texas from South America and is currently distributed from Cameron County in the LRGV region to Calhoun County in the Texas Coastal Bend (Burke and Clark 1976). Stands of sulfur mallow are located primarily in the coastal region bordering the Gulf of Mexico, and tend to be most abundant on poorly-drained heavy clay soil types. Fruiting patterns of the plant tend to be erratic and are fundamentally influenced by precipitation and soil moisture. Although quantitative estimates of boll weevil reproduction occurring in stands of sulfur mallow are not available, the plant is potentially important as it provides a means for boll weevils to reproduce during periods in which cotton is normally absent (i.e., during the postharvest fallow season), and many of the known stands occur in ecologically-sensitive areas in which insecticidal use is either unfeasible or prohibited (Burke and Clark 1976).

Other Peculiarities. Although many of the idiosyncrasies associated with the LRGV boll weevil population undoubtedly reflect the effects of environmental conditions, others appear to have a genetic basis. In addition to the reported differences in the physiology of overwintering adults (see previous discussion), elevated levels of pesticide tolerances have been reported in this region on several occasions. In surveys designed to compare the toxicity of malathion to boll weevil populations in nine states, the highest LD50 values were obtained from samples collected in the LRGV region (Rathinam 1979). More recently, Teague et al. (1983) compared the toxicity of azinphosmethyl to boll weevils from the LRGV region and other areas of Texas. Results indicated that 7- to 10-day old LRGV adults fed squares were 3- to 6-x more tolerant to azinphosmethyl than counterparts from the Brazos Valley of Texas and a laboratory colony. Such tolerance was not evident among cohorts of younger adults fed artificial diet, or among cohorts from any area that were exposed to methyl parathion. An elevated level of tolerance to azinphosmethyl was evident in studies conducted in the LRGV region several years later (Wolfenbarger et al. 1986). Although control failures involving azinphosmethyl have not materialized in the LRGV or other areas of Texas, the data of Teague et al. (1983) suggest that at one point in time (the

early 1980's), producers in the LRGV region faced the specter of resistance to one of the principal organophosphate materials used to control their primary cotton pest.

SUMMARY

Despite its tropical origin, the boll weevil has successfully invaded and colonized a vast range that currently extends from the southern United States to central South America. The spectacular ecological success of this insect has been predicated in part on the existence or evolution of certain biological processes (e.g., diapause) that facilitate survival during periods of adverse conditions, and allow the insect to maintain viable populations under a wide range of environmental conditions. Because suppression of damaging boll weevil infestations on commercial cotton has proven to be difficult in virtually all areas of the pest's range, the development of tactics designed to minimize survival during periods of adversity (e.g., the fallow season) has become a major goal of our current management and eradication efforts. An adequate understanding of the boll weevil life system as it occurs within each of the major cotton-production zones, and how life history strategies of the pest may vary geographically, is critical to the development and successful implementation of such tactics.

Within most areas of the U. S. Cotton Belt, diapause appears to be the principal survival strategy that allows boll weevils to successfully overwinter. Despite regional differences in climate and cultural practices, the boll weevil diapause phenomenon appears to be initiated and manifested in a similar manner in most areas of the temperate zone. Following an initial feeding period (on cotton), adult boll weevils disperse into certain types of preferred overwintering habitat, enter a period of extended dormancy, and exit the following spring and summer in a fairly predictable "emergence profile." Knowledge of such behavior has been used to considerable advantage in the development of effective pest management strategies for boll weevil, including techniques designed to monitor early-season flight activity (e.g., the trap-index system developed by Rummel et al. (1980)) and noninsecticidal strategies designed to suppress the "overwintered" boll weevil population (e.g., the uniform delayed planting scheme used in the Texas Rolling Plains).

Although certain aspects of boll weevil biology appear to be basically similar throughout its range, several important changes become evident along the latitudinal gradient that extends from the temperate zone of North America into the tropics of Central and South America. As indicated earlier, differences in the physiology of overwintering adult weevils have been identified in temperate versus subtropical and tropical environments, and may account for many of the apparent differences in behavior that appear to exist along this gradient (e.g. the continuous flight activity that is generally evident in subtropical and tropical environments throughout the fallow season, and the apparent rarity or absence of dormant adult weevils in stands of deciduous leaf trash and similar habitats). On the other hand, many of the reported peculiarities of the LRGV weevil population appear to be the direct result of poor cultural practices, e. g., the traditional presence of undestroyed cotton during the fallow season, which has fundamentally altered the distribution, reproductive status, and age structure of the overwintered weevil population. The manner in which these parameters might change if the overwintering weevil population in the LRGV region were truly deprived of cotton for an extended period has been impossible to ascertain, and will remain speculative until the chronic stalk destruction problem has been resolved.

During the past century, a wealth of information has become available regarding the biology and ecology of boll weevil in key areas of its range. Nevertheless, much remains to be learned of this important cotton pest (e.g., the biochemical basis of boll weevil diapause is poorly understood, and the ecology of populations occurring in tropical zones has only recently received adequate attention). A continuation of sound research in these critical areas will add to our scientific knowledge of the boll weevil as a biological organism, and should improve our ability to manage (and possibly eradicate) the pest from various parts of its range.

ACKNOWLEDGEMENTS

The authors express their appreciation to M. D. Arnold and S. C. Carroll for their assistance in the preparation of this manuscript.

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EFFECT OF INSECT PREDATORS AND A PYRETHROID INSECTICIDE ON COTTON APHID, *APHIS GOSSYPII* GLOVER[1], POPULATION DENSITY

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ABSTRACT

Cotton plants enclosed in predator exclusion cages were compared with uncaged plants to examine the impact of predaceous arthropods on cotton aphid, *Aphis gossypii* Glover, populations. Effects of the pyrethroid insecticide cyhalothrin (Karate®) on *A. gossypii* population development also were examined. Rapid increases in cotton aphid numbers, resulting in significant yield reductions, occurred on plants when predators were excluded. These aphid populations declined only after exceeding the carrying capacity of the plants. Cotton aphid infestations on uncaged plants and on plants to which predators were introduced or allowed access were quickly controlled by naturally occurring predator populations. When predators were excluded, cotton aphid numbers increased faster and to higher levels on cyhalothrin treated plants than on untreated plants. The more rapid increase in aphid populations was not due to the absence of predators, and was therefore attributed to a physiological interaction between cyhalothrin and cotton leaves or cotton aphids that resulted in increased aphid reproductive activity.

INTRODUCTION

The pest status of *Aphis gossypii* Glover on cotton in the Texas High Plains varies greatly. The cotton aphid may be considered a fluctuating pest because it is a severe pest during years in which outbreaks occur and a secondary pest in other years (Leser et al. 1992). The cotton aphid is sometimes considered beneficial (Leser 1994, Fuchs and Minzenmayer 1995) because it provides a food source for arthropods which prey on pests such as the cotton bollworm, *Helicoverpa zea* (Boddie). Currently, there are no accurate methods of predicting the density to which aphid populations will develop.

The economic threshold for *A. gossypii* in Texas High Plains cotton has been set at 50 aphids per leaf. However, this is not intended to be a precise level at which producers should take action, but rather a warning of potential economic loss (Leser 1994). Producers and consultants are urged to take other factors, such as beneficial arthropod populations, insecticide efficacy, and potential resurgence of aphid populations into account before applying an aphicide.

The influence of predators on cotton aphid population development is not well defined. Leser et al. (1992) stated that although many beneficial arthropods prey on cotton aphids, predator populations in the Texas High Plains typically average less than 1% of aphid populations. 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

[1] Homoptera: Aphididae

[2] Texas Agricultural Extension Service, 209 South 5th Street, Brownfield, TX 79316.

occasional economic damage. However, 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.

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, Brown and Reed 1992, Kidd et al. 1996). Insecticide applications targeting other pests, such as *H. zea*, 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 50-75% 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 the elimination of natural enemies with pesticides did not appear to cause the initial population increase of cotton aphids in the Texas High and Rolling Plains. 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.

Little is known about the impact of predators on cotton aphid populations, and there are no accurate methods of predicting cotton aphid infestations. A more accurate understanding of the influence of predators on cotton aphid population dynamics is needed to establish effective control tactics. Pyrethroid insecticides are commonly used to control cotton bollworm populations and cotton aphid populations tend to increase rapidly following these applications. 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. The objective of the present study was to investigate the impact of naturally occurring insect predators and a pyrethroid insecticide on cotton aphid populations in cotton.

MATERIALS AND METHODS

To determine the impact of insect predators on cotton aphid populations, predator exclusion cages were placed over single cotton plants and aphid population densities were compared with those on single uncaged plants during the 1993 growing season at the Texas Agricultural Research and Extension Center, Lubbock, TX. Wooden framed 68.58 cm^2 predator exclusion cages with removable lids were constructed and covered with 32 x 32 Lumite® synthetic mesh screen (Style #50060, Lumite Division of Synthetic Industries, Gainsville, GA). GSC 71+ cotton was planted on 3 June and 14 individual plants were selected for the test on 26 July. Plants were spaced 6 m apart in two adjacent 1-m (40 in.) rows. Plant selection was based on plant hardiness, size, fruit-set and the total number of aphids on the entire plant. Unusually hardy or stunted plants, as well as plants that lacked a natural aphid population, were not selected. Adjacent plants were removed from the 1-m area on each side of the selected plants. Inspections were made and the total number of aphids on each entire plant was recorded. All arthropods other than cotton aphids were removed from the plants before positioning the cages on 26 July.

The experiment was arranged in a randomized block design consisting of two treatments and seven blocks. Plots were sampled on a weekly basis from 2 August through 24 September. The total number of aphids on one randomly selected full size upper and middle leaf per plant was counted during each inspection. Predators, including lady beetle adults and larvae, primarily *Hippodamia* spp.; lacewing larvae, primarily *Chrysoperla* spp.; minute pirate bugs, *Orius* spp.; and big-eyed bugs, *Geocoris* spp., were sampled weekly on the uncaged plants. Whole plant examinations of caged plants were made on each sample date

and at least three additional times per week. All predaceous arthropods that had gained entry were removed. Cages were removed on 27 September. All plants were mapped and bolls were hand-harvested on 12 November. Seed cotton was ginned and individual plant yields were recorded.

This experiment was expanded in 1994 to include the eight treatments arranged in a randomized block design consisting of six blocks as follows. (1) Uncaged control: Aphid and predator populations were allowed to develop undisturbed. (2) Caged control: This treatment consisted of caged plants from which predators were excluded. (3) Uncaged treated control. (4) Caged treated control: All aphids were removed from these plants before setting the cages in place. The aphid population density in these treatments was maintained at the lowest possible level throughout the duration of the experiment. When the aphid population density began to increase, aphids were physically removed from the plant or controlled with a mixture of dicrotophos (Bidrin®) at a rate of 0.25 lbs AI/ac and M-Pede® at a rate of 1 qt/ac. (5) Uncaged pyrethroid treated. (6) Caged pyrethroid treated: Cyhalothrin (Karate®) at a rate of 0.025 lbs AI/ac was applied to these treatments on 26 July. (7) Caged predator access: This treatment consisted of caged plants to which predators were allowed access through an opening around the lower portion of the cage. (8) Caged introduced predators: This treatment consisted of caged plants that received two introductions of five lady beetle larvae, *Hippodamia* spp. Lady beetle larvae were collected from naturally occurring populations on cotton plants within the immediate area and introduced on 5 and 12 August.

All-Tex Atlas cotton was planted on 25 May. Plants were selected on the same basis used in 1993 and all insecticides were applied with a backpack sprayer. A Campbell Scientific CR10 weather data logger was placed in the field on 27 July and used to monitor solar intensity and ambient temperature inside and outside cages for the duration of the experiment. Predator exclusion cages were set in place on 25 and 26 July. Initial cotton aphid counts were made on a whole plant basis and all predaceous arthropods were removed before setting cages in place. Additional cotton aphids, collected from bordering plants, were introduced onto plants that had significantly lower starting counts. Plots were sampled seven times on a weekly basis from 1 August through 16 September. The total number of aphids on one upper, middle, and lower leaf of each plant was counted on each sample date. Sample size was reduced to two leaves per plant on 9 September due to excessive leaf drop in caged pyrethroid treated and caged control treatments. Treated control treatments received insecticide applications on 3 and 10 August. Insect predator populations were monitored on uncaged plants by counting the total number of selected predators on entire plants. Cages were removed on 27 September. All plants were mapped and bolls were progressively hand-harvested on five dates from 1 through 22 November. The purpose of this intermittent harvest was to prevent loss of lint due to damp weather conditions. Specific boll positions and seed cotton weights were recorded. Bolls were ginned and individual plant yields were recorded.

Fiber quality, including micronaire, length, uniformity, and strength, was determined by analysis of sub-samples of each treatment in 1993 and 1994. All fiber quality and boll position data were evaluated on an observational basis. Cotton lint yield and aphid and predator population density data were subjected to analysis of variance, and means were separated using Duncan's new multiple range test (DNMRT) in 1993. Because of missing data, yield and aphid population density means were separated with the least significant difference (LSD) test in 1994 (SAS Institute 1985).

RESULTS AND DISCUSSION

1993 Experiment. Cotton aphid population development on plants in predator exclusion cages and on uncaged plants is shown in Fig. 1. Caged plants averaged 29.4 aphids per plant, and uncaged plants averaged 30.7 aphids per plant at the time the test was initiated on 26 July. Differences in mean number of aphids per leaf were statistically significant on all dates following the 2 August inspection ($P < 0.05$, DNMRT). Caged plants averaged 17 aphids per leaf on 2 August, but infestations quickly increased to 635 aphids per leaf on 13 August and 1470 aphids per leaf on 19 August. Cotton aphid population density peaked at 1,677 aphids per leaf on 3 September, then declined and leveled off at 840 aphids per leaf on 24 September when inspections were terminated. This decline appeared to be a consequence of cotton aphid populations exceeding the carrying capacity of the plants.

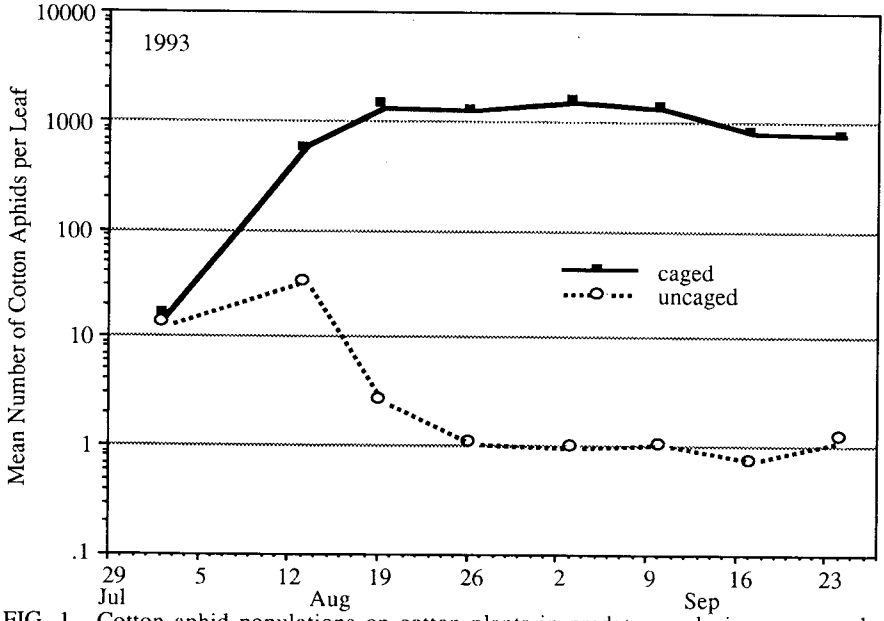

FIG. 1. Cotton aphid populations on cotton plants in predator exclusion cages and on uncaged cotton plants near Lubbock, TX, 1993.

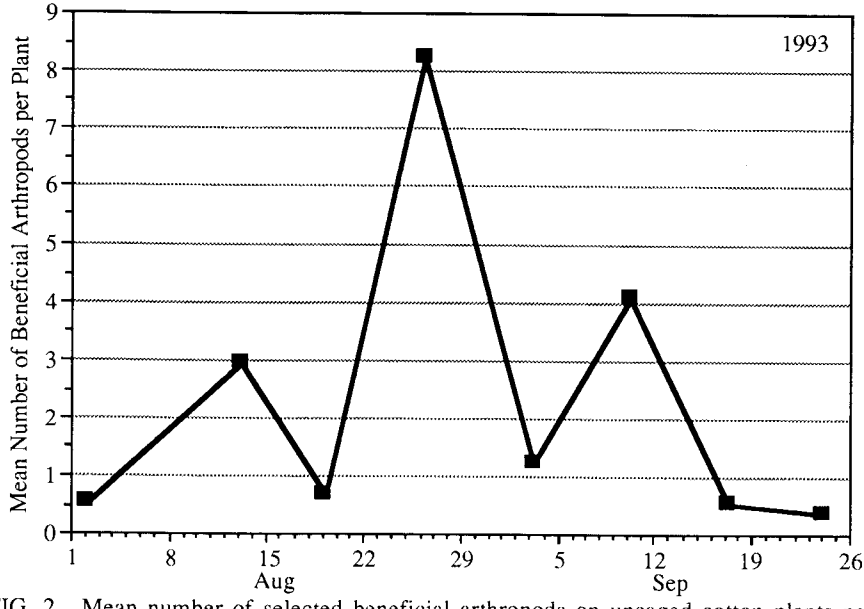

FIG. 2. Mean number of selected beneficial arthropods on uncaged cotton plants near Lubbock, TX, 1993 (lady beetles, primarily *Hippodamia* spp.; lacewing larvae, primarily *Chrysoperla* spp.; minute pirate bugs, *Orius* spp.; and big-eyed bugs, *Geocoris* spp.).

Cotton aphid populations on uncaged plants increased to a peak of 34 aphids per leaf on 13 August, then declined to <3 aphids per leaf on 19 August and remained below this level for the duration of the experiment. The low aphid population density on uncaged plants was attributed to the impact of predaceous arthropods.

Population density of selected beneficial arthropods on uncaged plants is shown in Fig. 2. Although no predators were detected on 26 July, populations increased to three beneficial arthropods per plant on 13 August. This increase coincided with increasing aphid populations. Predator populations reached a peak of eight per plant on 26 August and declined to less than one per plant on the final sample date (24 September). The increase and decline of predator populations appeared to be a direct response to the increase and decline of aphid populations.

High aphid population densities on plants in predator exclusion cages had a significant negative effect on cotton lint yield ($P < 0.05$, DNMRT). Uncaged plants produced an average of 24.8 grams of lint per plant and caged plants produced an average of 2.2 grams of lint per plant (Table 1). Compared with uncaged plants, caged plants set less fruit and shed a considerable number of squares, bolls, and leaves. Caged plants were notably shorter and less vigorous than uncaged plants. Decreased yield and altered growth and development of caged plants were attributed to high cotton aphid population densities resulting from the exclusion of beneficial arthropods.

TABLE 1. Cotton Lint Yield from Plants in Predator Exclusion Cages and Uncaged Plants Exposed to Field Environment Near Lubbock, TX, 1993.

Treatment	Mean grams lint/plant [a]
Caged	2.2 a
Uncaged	24.8 b

[a] Means followed by the same letter are not significantly different ($P < 0.05$, DNMRT).

Fiber quality was negatively affected by high aphid numbers on plants in predator exclusion cages (Table 2). Fiber strength of lint from caged plants was 17.00 g/tex, compared with 27.75 g/tex from uncaged plants. Micronaire of lint harvested from caged and uncaged plants was 2.23 and 3.60, respectively. Fiber uniformity and length of lint from caged plants were also numerically less than those from uncaged plants. Reduced quality of fiber from caged plants was proportional to aphid infestations and was attributed to the effects of cotton aphids on cotton plant and fiber development. High aphid numbers appeared to cause plants to abort some early squares and bolls, and slowed the development of bolls, resulting in immature, low quality fiber.

TABLE 2. Fiber Quality Analysis Means from Caged and Uncaged Control Plants Near Lubbock, Texas, 1993.

	Sample mean [a]			
Treatment	Length (in.)	Micronaire	Uniformity ratio	Strength (g/tex)
Caged	0.948	2.23	0.693	17.00
Uncaged	0.992	3.60	0.820	27.75

[a] Mean of three sub-samples.

1994 Experiment. Cotton aphid population development on plants in the 1994 predator exclusion cage test is shown in Fig. 3. Starting counts of each treatment ranged from a mean of 11.7 to 36.3 aphids per plant, and averaged 21.3 aphids per plant. No significant differences in aphid populations were detected among treatment means until 9 August, when aphid numbers on caged pyrethroid treated plants were significantly greater than those of

caged introduced predator plants ($P < 0.05$, LSD). Aphid numbers in the caged control and the caged pyrethroid treated treatments were significantly greater than all other treatment means from 31 August through 9 September. A dramatic decrease in cotton aphid populations occurred on 20 August on plants to which predators had access. Sampling was continued in these treatments through 9 September so statistical comparisons could be performed. Since it was evident that no resurgence in these populations had occurred, only the caged pyrethroid treated and caged control treatments were sampled on 16 September.

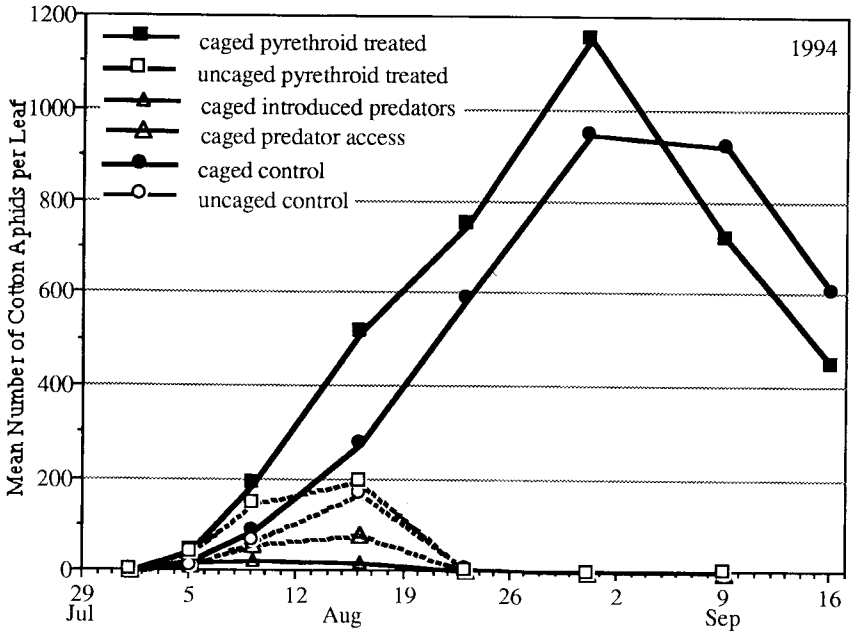

FIG. 3. Mean number of cotton aphids per leaf on caged and uncaged cotton plants exposed to various treatments near Lubbock, TX, 1994.

Aphid populations on caged and uncaged pyrethroid treated plants began to increase rapidly following the cyhalothrin application on 26 July (Fig. 3). The initial increases of aphid numbers in these two treatments were very similar and appeared to be directly influenced by cyhalothrin. Aphid numbers on uncaged pyrethroid treated plants exceeded 152 per leaf on 9 August and peaked at 200 per leaf on 16 August. This population quickly declined to less than six aphids per leaf on 23 August and remained below this level for the duration of the experiment. Aphid populations on caged pyrethroid treated plants increased faster and reached higher numbers than those of all other treatments. More than 194 aphids per leaf were present on 9 August and the highest population density was recorded on 31 August when aphid numbers exceeded 1156 per leaf. The aphid population appeared to be in excess of the carrying capacity of the plants and declined to 725 aphids per leaf on 9 September. Over 467 aphids per leaf were present when inspections were terminated on 16 September. The carrying capacity of the plants appeared to be the only factor regulating the development of this aphid population.

Inspections of caged and uncaged control plants on 1 August detected 3.87 and 3.89 aphids per leaf, respectively (Fig. 3). Aphid populations on uncaged plants increased gradually to a peak of 171 aphids per leaf on 16 August. On this date, caged control plants averaged more than 280 aphids per leaf. The following week, only ten aphids per leaf were present on uncaged plants, while caged plants supported more than 594 per leaf. Following this inspection, aphid populations on uncaged plants remained below two aphids per leaf until

inspections were terminated. Aphid numbers on caged plants continued to increase to a peak of 949 per leaf on 31 August, and more than 611 per leaf were present when inspections were terminated on 16 September. Though the cage environment may have had some influence on aphid population development, the difference in aphid numbers on caged and uncaged plants was primarily attributed to the lack of beneficial arthropods on the caged plants.

Development of aphid populations on plants in predator access cages was similar to those on uncaged control plants (Fig. 3), though they peaked at only 82 aphids per leaf on 16 August. This difference of 89 aphids per leaf was not significantly different from populations on uncaged control plants. Aphid population density was believed to be held at a lower level on plants in predator access cages because of the inability of predators to find their way out through the opening in the lower portion of the cage. Predators appeared to move upward when attempting to exit the cages, and were therefore confined to specific plants for an extended period of time. In this treatment, aphid numbers decreased to four per leaf on 23 August and remained below this level until inspections were terminated.

Inspections detected 19.5 aphids per leaf on plants in the caged introduced predators treatment (Fig. 3) before introduction of the first five lady beetle larvae on 5 August. Aphid population density increased slightly to a peak of 25 aphids per leaf on 9 August. Five additional lady beetle larvae were introduced on 12 August, and aphid numbers decreased to 20.5 per leaf on 16 August. Aphid numbers decreased to five per leaf on 23 August and remained below this level until inspections were terminated. Though the number of predators introduced was considered high given the existing aphid population density, lady beetle larvae effectively reduced aphid numbers and held them at sub-economic levels.

Population density of selected beneficial arthropods, shown in Fig. 4, was monitored in the uncaged control, caged predator access, and uncaged pyrethroid treated treatments. A statistical difference was detected on only one date, 31 August, when significantly more beneficial arthropods were present on plants in predator access cages than on plants in other treatments ($P < 0.05$, DNMRT). Lady beetle populations were monitored in the caged introduced predators treatment, but these data were excluded from the statistical analysis.

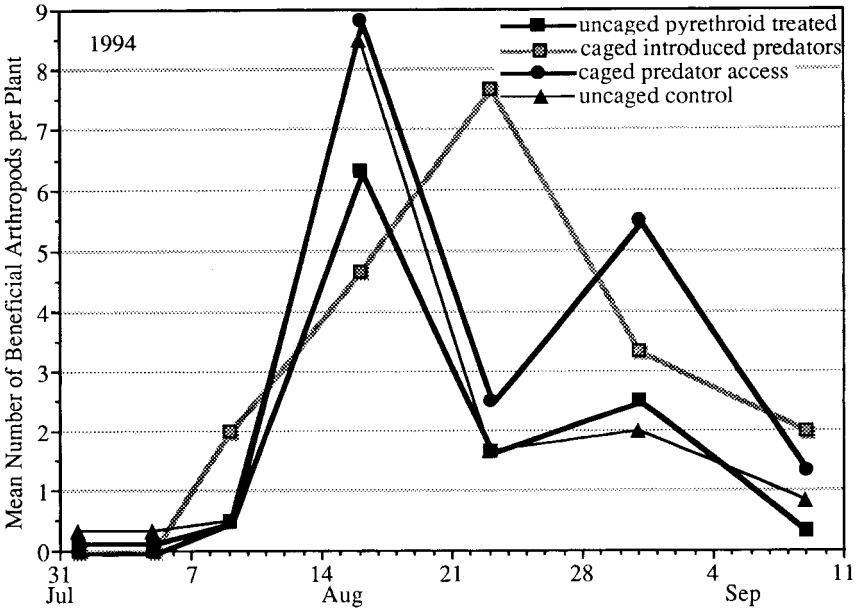

FIG. 4. Mean number of selected beneficial arthropods on caged and uncaged plants exposed to various treatments near Lubbock, TX, 1994 (lady beetles, primarily *Hippodamia* spp.; lacewing larvae, primarily *Chrysoperla* spp.; minute pirate bugs, *Orius* spp.; big-eyed bugs, *Geocoris* spp.; and syrphid fly larvae, *Syrphus* spp. and others.).

The first introduction of five lady beetle larvae was on 5 August, when an average of 19.5 aphids per leaf were present. Though aphid numbers increased to a peak of 25 per leaf, only two lady beetles per plant were found on 9 August (Fig. 4). These lady beetles were primarily non-feeding pupae. On 12 August, five additional larvae were introduced. An average of 4.7 larvae per plant were present on 16 August, when aphid numbers averaged 20.5 per leaf. These lady beetles were primarily larvae, but all developmental stages were present on this date. The peak lady beetle population of 7.7 per plant was detected on 23 August, when only five aphids per leaf remained. Lady beetle numbers gradually declined to an average of two per plant on the final sample date.

Lady beetles did not effectively reduce aphid populations until all developmental stages were present. It appeared that either multiple introductions or an introduction of lady beetles in more than one developmental stage would be most effective in reducing aphid population density.

Uncaged pyrethroid treated plants received an application of cyhalothrin on 26 July, and no predators were detected on these plants until 9 August, when they averaged 0.5 per plant (Fig. 4). Predator population density increased to a peak of 6.3 per plant on 16 August before declining to 1.7 per plant on 23 August. A slight increase was noted on 31 August, before numbers decreased to 0.3 predators per plant on 9 September. The initial lack of predators appeared to be due to the residual activity of cyhalothrin. In absence of a significant predator population, aphid numbers increased quickly. Predators appeared to move back to these plants and controlled the aphid infestation when the residual activity of cyhalothrin had dissipated.

Predator populations on uncaged control plants averaged 0.3 per plant on 1 and 5 August (Fig. 4). Numbers increased slightly to 0.5 predators per plant on 9 August and peaked at 8.5 per plant on 16 August, before declining to 1.7 per plant on 23 August. A slight increase was noted on 31 August, and numbers decreased to 0.8 per plant on 9 September.

Average predator population density on plants in predator access cages remained at 0.2 per plant until 9 August, when inspections revealed 0.5 predators per plant (Fig. 4). On 16 August, the number of predators on these plants peaked at 8.8 per plant, before dropping to 2.5 per plant on 23 August. As on uncaged pyrethroid treated plants and uncaged control plants, an increase in predator numbers was noted on 31 August. However, the number of predators on plants in predator access cages increased significantly on this date to 5.5 per plant. The number of predators per plant decreased to 1.3 per plant on 9 September.

Predator populations in all treatments appeared to be dependent upon aphid populations and followed fluctuations in aphid population densities. Both aphid and predator numbers peaked in all three treatments on 16 August. Lady beetles, which comprised approximately 80% of the predators found, appeared to be primarily responsible for these fluctuations. Increases in predator numbers on 31 August appeared to be the result of increased reproductive activity during the week of 16 August (Fig. 4). Lady beetles present on 16 August were primarily in the larval stage. Given the relatively high temperature and low humidity in late August, lady beetle larvae present on 16 August could have reached the adult stage by 23 August. Because of the reduced aphid numbers present at that time, the majority of these adults probably dispersed in search of prey. However, some of these adult lady beetles may have mated and deposited eggs before dispersal. These eggs would have hatched during the following week, resulting in the increased predator population density on 31 August. In predator access cages, the inability of predators to disperse resulted in a higher number of predators on the last three sample dates.

Cotton lint yields from plants in the predator exclusion cage test are shown in Table 3. These results concur with plant map data. Caged, treated control plants were taller, had more nodes, and set more first and third position bolls than other plants (Table 4). Uncaged treated control plants set the greatest total number of bolls, and the most second position bolls. Uncaged treated control plants produced an average of 24.60 grams of lint, which was more than that of all other treatments. Yields of caged pyrethroid treated and caged control plants were significantly less than yields of all other treatments ($P < 0.05$, LSD). These plants produced the lowest number of bolls in all positions and averaged only 4.85 and 6.22 grams of lint per plant, respectively. Caged control plants produced an average of 9.88 grams of lint less than uncaged control plants and 18.38 grams less than uncaged treated control plants. Yields of all plants were inversely proportional to the seasonal mean number of aphids per leaf (Fig. 5), and reduced lint production was attributed to the effects of aphid infestations.

TABLE 3. Cotton Lint Yield from Caged and Uncaged Plants Receiving Various Treatments Near Lubbock, TX, 1994.

Treatment	Mean grams lint/plant [c]
Uncaged treated control [a]	24.60 a
Caged introduced predators [b]	23.87 a b
Caged treated control [a]	21.68 a b c
Caged predator access [c]	18.98 a b c
Uncaged pyrethroid treated [d]	17.53 b c
Uncaged control	16.10 c
Caged control	6.22 d
Caged pyrethroid treated [d]	4.85 d

[a] Treated with dicrotophos (Bidrin®) @ 0.25 lbs AI/ac plus M-Pede @ 1.0 qt/ac on 3 and 10 August.

[b] Five lady beetle larvae, *Hippodamia* spp., were introduced on 5 and 12 August.

[c] Caged plants to which predators had access.

[d] Treated with cyhalothrin (Karate®) @ 0.025 lbs AI/ac on 26 July.

[e] Means followed by the same letter are not significantly different ($P < 0.05$, DNMRT).

TABLE 4. Mean Plant Growth Characteristics from Caged and Uncaged Plants Exposed to Various Treatments near Lubbock, TX, 1994.

	Mean per plant						
				Boll position			
Treatment [a]	Height	Nodes	No. bolls	1st	2nd	3rd+	Veg.
Uncaged treated control [b]	24.38	19.83	12.17	8.00	3.17	0.83	0.17
Caged treated control [b]	27.10	21.00	11.20	8.40	1.80	1.00	0.00
Caged introduced predators [c]	26.38	20.50	11.17	7.67	2.67	0.83	0.00
Caged predator access [d]	24.13	19.67	9.67	6.50	2.33	0.83	0.00
Uncaged pyrethroid treated [e]	22.38	19.17	8.67	6.33	2.00	0.17	0.17
Uncaged control	22.13	18.17	7.83	6.33	1.50	0.00	0.00
Caged control	24.80	20.00	4.80	4.60	0.20	0.00	0.00
Caged pyrethroid treated [e]	25.42	19.83	4.50	4.00	0.50	0.00	0.00

[a] Arranged in descending order of total number of bolls.

[b] Treated with dicrotophos (Bidrin®) @ 0.25 lbs AI/ac plus M-Pede @ 1.0 qt/ac on 3 and 10 August.

[c] Five lady beetle larvae, *Hippodamia* spp., were introduced on 5 and 12 August.

[d] Caged plants to which predators had access.

[d] Treated with cyhalothrin (Karate®) @ 0.025 lbs AI/ac on 26 July.

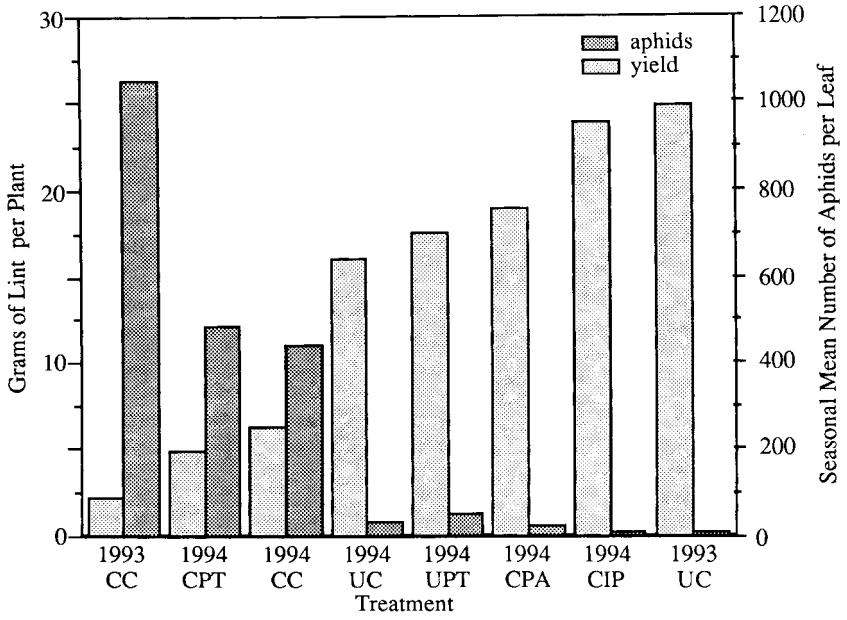

FIG. 5. Seasonal mean number of aphids per leaf and cotton lint yield from caged and uncaged plants exposed to various treatments near Lubbock, TX, 1993 and 1994. CC, caged control; CPT, caged pyrethroid treated; UC, uncaged control; UPT, uncaged pyrethroid treated; CPA, caged predator access; CTC, caged treated control; CIP, caged introduced predators; UTC, uncaged treated control.

Micronaire and uniformity were negatively affected by high aphid numbers on caged pyrethroid treated and caged control plants (Table 5). Micronaire averaged 2.20 and 2.70, respectively, compared with a combined average micronaire of 4.52 in all other plants. Uniformity ratio of lint from caged pyrethroid treated plants averaged 0.810, and that from caged control plants averaged 0.815. Uniformity ratio of lint from other treatments averaged 0.827 to 0.850. However, strength appeared to increase with aphid numbers. Lint from caged pyrethroid treated plants and caged control plants averaged 38.00 and 34.75 g/tex, respectively, compared with a combined average strength of 28.83 grams per tex in all other treatments. The unusually high strength may have been an instrument anomaly caused by the low micronaire, which results in decreased cellulose and increased primary wall in the fiber.

The average daily temperature inside cages was approximately 0.76°C higher than the temperature outside cages (Fig. 6). This could have caused an increase in caged plant development, but such an increase was not observed. The lack of an increase in plant development may have been due to reduced solar radiation inside cages (Fig. 7). Caged plants received an average of 6.64 $mJ/m^2/d$ less than uncaged plants. Though temperature and light intensity may have influenced cotton aphid population development, increases in aphid numbers were primarily attributed to the absence of beneficial arthropods, and decreased lint production was attributed to the effects of aphid infestations.

The response of aphid populations to cyhalothrin did not appear to be related solely to the abundance of aphid predators. Results of the present study show that while predator populations may be suppressed by early season cyhalothrin applications, this is not the only cause of aphid population increases following a cyhalothrin application. In the absence of predators, aphid populations increased faster and to higher levels on pyrethroid treated plants than on untreated plants. Cyhalothrin apparently causes a physiological change in cotton leaves or cotton aphids, which promotes aphid reproductive activity. Further research would be required to determine the exact site of this physiological alteration.

TABLE 5. Fiber Quality Analysis Means from Caged and Uncaged Plants Receiving Various Treatments near Lubbock, TX, 1994.

Treatment	Sample mean [a]			
	Length (in.)	Micronaire	Uniformity ratio	Strength (g/tex)
Uncaged treated control [b]	1.042	4.57	0.850	29.00
Caged introduced predators [c]	1.104	3.93	0.847	33.00
Caged treated control [b]	1.135	4.63	0.833	26.33
Caged predator access [d]	1.083	4.40	0.833	29.33
Uncaged pyrethroid treated [e]	0.990	4.67	0.827	28.00
Uncaged control	1.063	4.93	0.827	27.33
Caged control	1.156	2.70	0.815	34.75
Caged pyrethroid treated [e]	0.938	2.20	0.810	38.00

[a] Mean of three sub-samples.

[b] Treated with dicrotophos (Bidrin®) @ 0.25 lbs AI/ac plus M-Pede @ 1.0 qt/ac on 3 and 10 August.

[c] Five lady beetle larvae, *Hippodamia* spp., were introduced on 5 and 12 August.

[d] Caged plants to which predators had access.

[e] Treated with cyhalothrin (Karate®) @ 0.025 lbs AI/ac on 26 July.

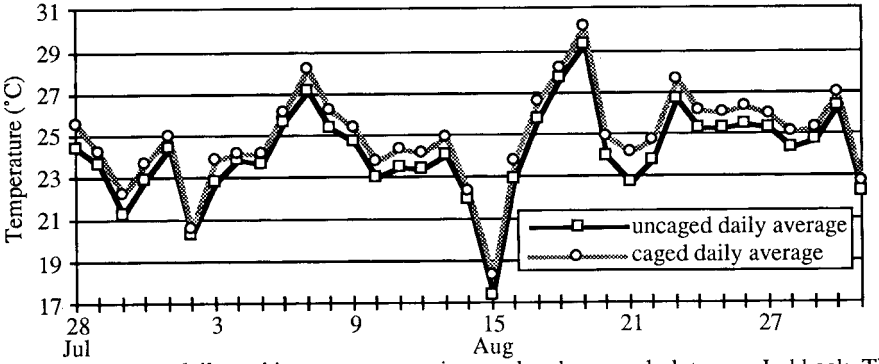

FIG. 6. Average daily ambient temperature in caged and uncaged plots near Lubbock, TX, 1994.

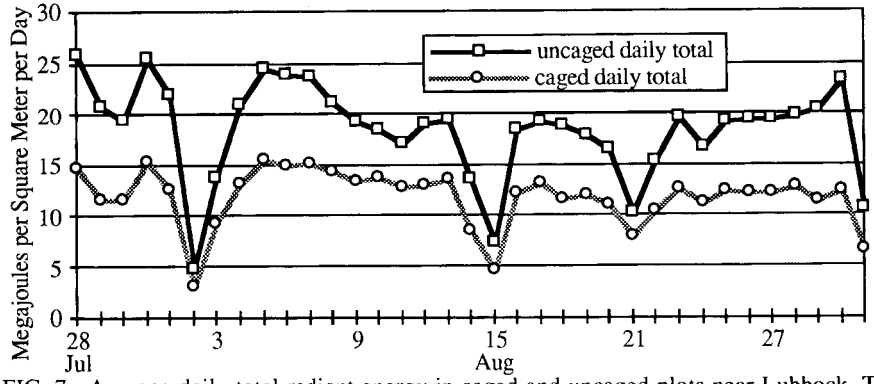

FIG. 7. Average daily total radiant energy in caged and uncaged plots near Lubbock, TX, 1994.

Though cyhalothrin applications appeared to stimulate aphid infestations, results of this study indicate that natural beneficial arthropod populations clearly have a significant impact on the suppression of infestations. Cotton aphid infestations which resulted in major yield reductions, occurred in the absence of cyhalothrin when predators were excluded and aphid populations were allowed to reach high densities. In addition, predator populations developed in response to increasing aphid populations, and initial increases in aphid population densities did not appear to be dependent on the presence or population density of predators. The decline of cotton aphid populations on uncaged plants was consistently attributed to the impact of beneficial arthropods, and when predators were excluded, aphid populations declined only after exceeding the carrying capacity of the plants.

Lady beetles, primarily *Hippodamia convergens* Guérin-Méneville, were the most abundant and effective of the aphid predators monitored. Data from the caged introduced predators treatment indicate that lady beetles can effectively suppress aphid infestations when present in high numbers. However, multiple introductions of high numbers, or populations in more than one developmental stage may be required to effectively suppress aphid infestations. Since multiple introductions of large numbers of predators are not economically justifiable, cotton producers should increase efforts to preserve naturally occurring beneficial arthropod populations. Manipulation of natural beneficial populations by providing advantageous habitats, such as planting windrow crops, may be helpful in harboring and sustaining adequate aphid predator populations. However, further research is needed to determine the impact of this type of cropping system.

Naturally occurring aphid predator populations typically suppress cotton aphid population development in the Texas High Plains, though their impact is often overshadowed by occasional aphid outbreaks. Early season aphid populations have the reproductive capacity to develop at rates much higher than that of their predators when environmental conditions are favorable, and periodic outbreaks are likely to occur. However, control tactics should not be based on atypical conditions. Aphid predators, especially lady beetles, appear to be primarily responsible for the prevention or suppression of economically damaging aphid populations. When not disrupted, predatory arthropods will usually suppress aphid populations, and insecticide applications will not be necessary.

Results of this study clearly show that cotton aphids can have a negative impact on cotton lint production. Lint production was inversely proportional to seasonal aphid population densities (Fig. 5). Lint production increased when aphid populations were controlled or limited through the use of insecticides or beneficial arthropods. 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, the duration of an aphid infestation appeared to be the most influential on lint production.

Successful cotton aphid management strategies 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 tactic 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 development of damaging cotton aphid infestations.

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 support.

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HOST ACTIVITY SPECTRUM OF THE CRYIIA *BACILLUS THURINGIENSIS* SUBSP. *KURSTAKI* PROTEIN: EFFECTS ON LEPIDOPTERA, DIPTERA, AND NON-TARGET ARTHROPODS

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ABSTRACT

Purified CryIIA insecticidal protein from *Bacillus thuringiensis* subsp. *kurstaki* was tested against one isopod (Crustacea, Isopoda) and 35 insect species representing the orders Coleoptera, Collembola, Diptera, Hemiptera, Homoptera, Hymenoptera, Isoptera, Lepidoptera, Neuroptera, and Orthoptera. All tests were conducted using diet-incorporation feeding bioassays. Only Lepidoptera and Diptera (Culicidae) were sensitive to the CryIIA protein. The most susceptible Lepidoptera species, *Anticarsia gemmatalis* was >12500-fold more sensitive (LC_{50} = 0.016 µg/ml) than *Agrotis ipsilon* and *Spodoptera frugipera* (LC_{50} values >200 µg/ml). Sublethal concentrations of CryIIA protein reduced the larval growth of all Lepidoptera species. Among the Culicidae, the most susceptible species, *Anopheles quadrimaculatus* (LC_{50} = 0.037 µg/ml) was >500-fold more sensitive than *Culex pipiens* (LC_{50} >200 µg/ml). Other Diptera (*Musca* and *Drosophila*) and non-Lepidoptera species were not affected by ingestion of the CryIIA protein. These data confirm that CryIIA protein has biological activity specific for Lepidoptera and Diptera, and demonstrate that considerable variability in species susceptibility exists within these orders.

INTRODUCTION

Bollgard® transgenic cotton expresses the CryIA(c) protein from *Bacillus thuringiensis* subsp. *kurstaki* that is toxic to pest Lepidoptera species in the *Heliothis/Helicoverpa* complex and to *Pectinophora gossypiella* (Saunders) (Perlak et al. 1990). Purified CryIA(c) protein, equivalent to the protein in transgenic cotton, has biological activity highly specific to Lepidoptera and is therefore safe for beneficial and other nontarget insect species (MacIntosh et al. 1990, Sims 1995). A second *B. thuringiensis* protein, CryIIA, is being developed to improve the existing effectiveness of Bollgard cotton and as a potential component of a multiple-gene strategy to delay insect resistance (see Tabashnik 1994). CryIIA protein is an appropriate choice for a multiple-gene strategy because it shares only 37% amino acid sequence homology with CryIA proteins (Donovan et al. 1988) and has a unique insect midgut binding mode of action (English et al. 1994).

CryIIA protein displays an unusual dual toxicity to larvae of both Lepidoptera and Diptera (mosquitoes) (Yamamoto and McLaughlin 1981, Donovan et al. 1988, Moar et al. 1994), but the effects of CryIIA protein on beneficial species such as insect pollinators, parasitoids, predators, and soil decomposers (e.g., Collembola, earthworms) have not been reported. Nontarget invertebrates can be exposed to the CryIIA insecticidal protein in transgenic cotton in several ways. For example, transgenic pollen and nectar can be consumed by nontarget species. Because CryIIA protein cannot be detected (<86 ng/g) in the pollen or nectar of transgenic cotton, this exposure is expected to be minimal. Protection of insecticidal proteins within plant matrices may prolong their environmental persistence and increase their bioavailability (Jepson et al. 1994). These insecticidal proteins will be added to soil as a component of post-harvest plant tissue (Sims and Holden 1996). Prior to eventual degradation, the proteins may be consumed

by nontarget soil invertebrates such as earthworms and Collembola, which are important components of soil decomposition processes (Calow 1993).

This study was conducted at Monsanto Co., Chesterfield, MO during 1995-96 to confirm the host activity spectrum of the CryIIA protein and to verify the safety of transgenic plants to beneficial and non-target insects. CryIIA protein was tested against 35 species of insects representing ten insect Orders: Coleoptera, Collembola, Diptera, Hemiptera, Homoptera, Hymenoptera, Isoptera, Lepidoptera, Neuroptera and Orthoptera and one species of terrestrial Isopod (Crustacea, Isopoda). The results are compared to other published information on insect sensitivity to the CryIIA protein and implications for resistance management using CryIIA protein are discussed.

MATERIALS AND METHODS

Test Substance. CryIIA protein was produced by fermentation of a recombinant *Escherichia coli* containing the *cryIIA* gene. CryIIA protein was purified from crude refractile bodies using nonchromatographic methods and lyophilized. The final lyophilized preparation was ≈ 60% (wt/wt) pure CryIIA as determined by BCA assay and ELISA and was equivalent to the CryIIA protein produced in transgenic cotton plants. It was similar in length (634 amino acids) and nearly identical in amino acid sequence (one amino acid change near the N-terminal end) to the protein encoded by the *cryIIA* gene described by Donovan et al. (1988). Confirmation of protein size was determined by SDS-PAGE analysis.

Test Insects. Test insects were obtained from the following sources: Lloyd Browne, Easton, PA (LB); California Agricultural Research, Inc., Kerman, CA [CA]; Carolina Biological Supply Co., Burlington, NC [CB]; Colorado Dept. Agric., Palisade, CO [CD]; Ecogen, Langhorne, PA [EC]; French Agric. Res., Inc., Lamberton, MN [FR]; GAST Laboratory, USDA-ARS, Mississippi State, MS [GA]; Monsanto, Chesterfield, MO [MO]; New Jersey Dept. Agric., Trenton, NJ [NJ]; Notre Dame Univ., Notre Dame, IN [ND]; Rincon-Vitova Insectaries, Ventura, CA [RV]; Univ. Arizona, Tucson, AZ [UA]; BIRL, USDA-ARS, Newark, DE [BI]; SIML, USDA-ARS, Stoneville, MS [SI].

Insect Diets. Test species were exposed using appropriate diets that were readily consumed and provided adequate nutrition for the duration of the test. Published or commercially available diets used were the soybean-based multi-species Lepidoptera diet (King and Hartley 1992), pink bollworm diet (Bartlett and Wolf 1985), Homoptera diet (Walters et al. 1990), *Drosophila* diet (Carolina Biological Inc., Burlington, N.C.) and Southern corn rootworm diet (Marrone et al. 1985). Other diets are described under the relevant species.

Incorporation of CryIIA Protein Into Diet. Except for tests on species of Culicidae, CryIIA protein was incorporated into test diet and fed to larvae or adults. A concentrate of the test material was diluted with insect diet to achieve the final test concentration. Typically, 6 ml samples of protein in distilled water, or distilled water alone (control), were incorporated into 24 ml of insect diet within 50 ml centrifuge tubes. Treated diet was poured into custom made 96-well insect assay trays (Jarold Mfg. Co., St. Louis, MO) or microtitre plates and allowed to cool and harden. Control diet contained the same ingredients as the treated diet but no CryIIA protein. One neonate larvae was placed into each well and heat sealed with ventilated Mylar® plastic film. For Hymenoptera tests, CryIIA protein was mixed into honey or carbohydrate solutions (adults) or water (larvae) and offered to the insects. Additional details of the test procedure used with each species are described below.

Dosage Level and Frequency of Administration. Species expected to be insensitive to CryIIA protein were evaluated by scoring survival folowing exposure to a single high dose (≥50 ppm) concentration. In some studies, tests were scored when control mortality >20-30% while for other species, a fixed test duration was more appropriate. Assays were incubated at 20-28°C.

Coleoptera. Anthonomus grandis Boheman [GA], *Leptinotarsa decemlineata* (Say) [NJ], *Diabrotica undecimpunctata howardi* Barber [MO], *Diabrotica virgifera* LeConte [FR], and *Hippodamia convergens* Guérin-Méneville [CB]. *H. convergens* adults, in replicated (six) groups of 25 beetles, were fed a 50% sucrose solution containing 50 ppm CryIIA protein and evaluated when control mortality >20%. Test conditions were 26-

28°C with RH >40% and a 12:12 LD photoperiod. *A. grandis* ova were mixed into a 0.15% agar solution, pipetted (2-6 ova per well) onto Lepidoptera diet surface, and dried under a BioHood prior to covering with Mylar and incubation at 28°C. Evaluation was made by scoring the number of wells, per replicate, containing at least one living *A. grandis* larva. *L. decemlineata*, *D undecimpunctata howardi* and *D. virgifera* were tested by exposing neonate larvae to CryIIA protein dried onto the surface of Southern corn rootworm diet (Marrone et al. 1985) at 9-15 µg/cm^2. Tests were scored following 7 d incubation at 28°C.

Lepidoptera. Agrotis ipsilon (Hufnagel) [EC], *Spodoptera frugiperda* (Smith) [EC], *Spodoptera exigua* (Hübner) [SI], *Helicoverpa zea* (Boddie) [EC], *Manduca sexta* (L.) [CB], *Trichoplusia ni* Hübner [LB], *Heliothis virescens* (F.) [SI], *Ostrinia nubilalis* (Hübner) [FR], *Pseudoplusia includens* (Walker) [SI], *Anticarsia gemmatalis* Hübner {SI], and *Pectinophora gossypiella* (Saunders) [UA]. CryIIA protein concentrations were incorporated into Lepidoptera diet and tested against neonate larvae. Survival and weights of test larvae were determined 5-7 d after test initiation. *P. gossypiella* was tested by incorporating CryIIA protein into pink bollworm diet (Bartlett and Wolf 1985) and determining the proportion of larvae in the four instar classes following 11 d exposure to each concentration.

Diptera. Musca domestica L. [CB], *Drosophila melanogaster* Meigen [CB], *Aedes aegypti* L. [ND], *Aedes triseriatus* (Say) [ND], *Anopheles quadrimaculatus* Say [ND], and *Culex pipiens* L. [CB]. Tests for *M. domestica* and *D. melanogaster* were conducted at ≈25°C within individual wells of 32-well diet trays (C-D International, Pitman, NJ). *M. domestica* larvae were tested on diet consisting of powdered milk (80 g), baby formula (80 g), dried yeast (20 g), methyl paraben (0.3 g), streptomycin sulfate (0.1 g), agar (15 g), and water 930 ml. Four neonate larvae were added to wells containing 5 ml of treated or control diet; there were eight wells in each of the 5 replicates. Mortality was scored after 10 d when most survivors had pupated. *D. melanogaster* ova (4/well) were added to treated or control *Drosophila* diet (Carolina Biological, Burlington, NC) with eight wells in each of the 3 replicates. Mortality was scored after 6 d. For mosquito assays, CryIIA protein was suspended in approximately 2 ml of distilled water within 3.7 ml capacity glass vials. Ten 3rd-4th instar larvae were added to each vial. There were three replicate vials per concentration and the test was repeated on three separate days. The assay was maintained at 23°C. Survival of test larvae was determined 24 h after test initiation for *A. quadrimaculatus* and 48 h after test initiation for the remaining species.

Orthoptera. Blattella germanica (L.) [CB] and *Acheta domesticus* (L.) [CB]. CryIIA protein was incorporated into Lepidoptera diet and poured into 50-well insect assay trays. *B. germanica* nymphs (3rd-4th instar) were added to individual wells (one per well) and sealed in contact with the diet using ventilated Mylar membrane. The assay was maintained at ca. 28°C and evaluated for roach survivorship after 5 d. *A. domesticus* nymphs were tested in a similar manner.

Homoptera/Hemiptera. Rhopalosiphum padi (L.) [MO], *Bemisia tabaci* Gennadius [MO], and *Oncopeltus fasciatus* (Dallas) [CB]. CryIIA protein was incorporated into a minimal aphid diet (Walters et al. 1990). Test diet was added to wells of 24-well culture plates and covered with Parafilm® membrane. *R. padi* (mixed 1st-4th instars) or *B. tabaci* (adults) were added to another matching 24-well plate at a rate of approximately 12 per well (aphids) or 29-66 per well (whiteflies). The plate with treated diet was inverted over the plate containing test insects. Wells of the opposing plates were aligned, and the plates were joined using tape. Insects fed through the overhead membrane on the treated diet above. The assay was maintained at 22°-24°C and evaluated at 3 d for survival. *O. fasciatus* nymphs (2nd-3rd instars) were tested by adding CryIIA protein to finely ground raw sunflower (30%, w/w) in 2% agar and covering the mix with a stretched Parafilm membrane sachet. The bugs readily fed through this membrane and the test was scored at 14 d.

Isoptera. Reticulitermes flavipes (Kollar) [CB]. *R. flavipes* workers were exposed to 200 µg/ml CryIIA protein mixed into 2% agar plugs containing 0.67 g powdered cellulose (Alphacel) and 150 µg FD&C #1 blue dye (Hilton-Davis, Cincinnati, OH) per ml. Test arenas were 100 mm diameter petri dishes with water-saturated plaster of paris bottoms. There were 30 termites in each of four treatment and control replications, and the test duration was 15 d.

Isopoda. Porcellio scaber Latreille [CB]. Individuals from a mixed cohort of adult and juvenile woodlice were randomly allocated to 100 mm diameter petri dishes with damp filter paper bottoms. Ten Isopods in each of five treatment and control replications were fed Lepidoptera diet. Treated diet contained 200 µg/ml CryIIA protein.

Collembola. Folsomia candida (Willem) [MO] and *Xenylla grisea* Axelson [MO]. Test diets were prepared by suspending 1.0 g of Bakers yeast in 3.0 ml of distilled water containing 200 µg of CryIIA protein. This suspension was frozen and lyophilized to dryness (S. R. Sims, unpublished data). Tests were conducted in 60 x 20 mm plastic petri dishes with plaster of paris + charcoal bottoms (9:1 ratio). Immature *F. candida* 8-12 d old and *X. grisea* 7-11 d old were used to initiate the tests. Ten individual insects were used per replicate (petri dish); there were five replicates, per treatment or control, for *F. candida* and six replicates for *X. grisea*. Test duration was 21 d at approximately 19°C and a 0:24 LD photoperiod. Fresh test diet was added on 0, 7, and 14 d; additional water was added on 7 d and 14 d to maintain saturation of the plaster of paris substrate. The tests were scored by counting the number of surviving adults and progeny (*F. candida*) or total number of individuals (*X. grisea*).

Neuroptera. Chrysopa carnea [RV]. CryIIA protein, in reverse-osmosis water, was mixed with eggs of *Sitotroga cerealella* (Olivier) to obtain a test concentration of 50 µg/g. Treated eggs were fed to individual larvae confined to one-ounce semi-transparent plastic cups with semi-transparent lids (Solo Cup Co., Baltimore, MD). There were 30 insects per treatment and control group. Test conditions were 21-22°C with a relative humidity of 60 ±11(SD)% and a 12:12 LD photoperiod. Fresh diet was prepared weekly and larvae had *ad libitum* access to diet throughout the test. Observations of mortality and/or abnormal behavior were made daily until pupation in the control >50%.

Hymenoptera. Apis mellifera [CA]. Larval honeybees were tested as described by Sims (1995). Two-three day-old larvae within larval cells on brood frames were dosed with 3.0 µl of 50 µg/ml CryIIA protein in distilled water. A 3.0 µl dose of 50 µg/ml potassium arsenate was used as a positive control and distilled water alone was the negative control. Larval survival from dosing to adult emergence was compared between the treatments.

Adult honeybees were tested in a manner similar to that described by Sims (1995). An "even age" group of adult bees was obtained by allowing adults to emerge from brood frames for approximately 3 d under controlled environmental conditions. Test containers were 12.7 cm^3 and constructed of 3.2 mesh/cm hardware cloth. Test diet, prepared on study day 0, was a 30% (w/v) fructose:water mixture containing 50 µg/ml CryIIA protein; control diet was 30% fructose alone. Test diet was introduced into the cages using a glass shell vial with two 1.0 mm holes drilled into the lid. Each test container contained approximately 50 bees; there were three replicates per treatment and control. Test conditions were 22-26°C with a relative humidity of 35-47%. Old diet was replaced with fresh diet every 48 h. Adult bees were observed twice on the day of test initiation for mortality and signs of toxicity and once each day thereafter. The study was terminated when control mortality >20%.

Hymenoptera. Macrocentrus ancylivorus Rohwer [CD], *Meteorus pulchricornis* (Wesmael) [BI], and *Nasonia vitripennis* (Walker) [CB]. *N. vitripennis* testing used a 50 µg/ml CryIIA protein concentration in a 50% sucrose solution. This solution was fed to six replicated groups of 25 adult wasps confined to 1 pint (87 mm diameter x 85 mm high) rolled paper containers. A cotton swab coated with test diet was inserted into the side of each container. Test conditions were 26-28°C with relative humidity >40% and a 12:12 LD photoperiod. Treated diet was refrigerated; old test diet was replaced with fresh diet daily. Observations of mortality and abnormal behavior were made daily until negative control mortality >20%. Test conditions for *M. ancylivorus* and *M. pulchricornis* were similar except that temperature was approximately 25°C and photoperiod was 14:10 LD. CryIIA protein was mixed into a 50% honey solution at a concentration of 200 µg/mL. *M. ancylivorus* was tested in five replicate groups of 20 adult wasps; *M. pulchricornis* was tested in three replicate groups of eight adult wasps. Observations of mortality and/or abnormal behavior were made daily until control mortality >30%.

Statistical Analysis. LC_{50} evaluations of CryIIA-sensitive species, were performed using Probit analysis. EC_{50} values (the effective concentration reducing larval weight by

50% relative to the control) were calculated using nonlinear regression analysis as described by Sims and Berberich (1996). For nonsensitive species, the percentage mortality for each replicate was arcsine transformed prior to comparison of treatment and control groups by analysis of variance. Statistics were conducted using SAS (SAS Institute Inc., Cary, NC) or Pesticide Research Manager (Gyllings Data Management, Inc., Brooking, SD).

RESULTS AND DISCUSSION

All 11 Lepidoptera species tested were sensitive to the CryIIA protein (Table 1). *A. gemmatalis* was the most sensitive species whereas *A. ipsilon* and *S. frugiperda* showed little mortality at 200 µg/ml but were stunted by sublethal concentrations (Table 1). Species susceptibility to CryIIA, ranked by LC_{50} values, was consistent with the EC_{50} ranking. *A. ipsilon* and *S. frugiperda* could not be ranked according to LC_{50} response but EC_{50} comparison clearly showed that *A. ipsilon* was the least sensitive of all Lepidoptera tested (Table 1). The LC_{50}/EC_{50} ratio was low for the most sensitive species but was >200 for *S. exigua* and *H. zea*. Of the lepidopteran pests targetted for control by CryIIA protein in transgenic cotton, *P. gossypiella* and *H. virescens* were extremely sensitive whereas *H. zea* was somewhat less sensitive to CryIIA compared to CryIA(c) protein (Stone and Sims 1993).

Among the Diptera, *M. domestica and D. melanogaster* larvae were not sensitive to CryIIA protein (Table 1). The previously reported susceptibility of larval Culicidae to CryIIA protein was confirmed but there was considerable inter-species variability. For example, *A. quadrimaculatus* was >500-fold more sensitive than *C. pipiens*. The distinct LC_{50} estimates for *A. aegypti* and *A. triseriatus* indicate that significant differences in sensitivity can exist among congeneric species of Culicidae.

TABLE 1. Sensitivity of Lepidoptera and Diptera Species to Purified CryIIA Protein in Test Diet (µg/ml)

Species	LC_{50} (SEM)	EC_{50} (SEM)	LC_{50}/EC_{50}
LEPIDOPTERA			
Agrotis ipsilon	> 200.0[a]	54.5 (7.3)	-
Spodoptera frugiperda	> 200.0	0.730 (0.245)	-
Spodoptera exigua	79.2 (67.3)	0.355 (0.047)	223
Helicoverpa zea	14.5 (12.7)	0.038 (0.008)	382
Manduca sexta	0.186 (0.112)	0.013 (0.002)	14
Trichoplusia ni	0.128 (0.068)	0.017 (0.002)	8
Heliothis virescens	0.114 (0.016)	0.006 (0.001)	19
Ostrinia nubilalis	0.060 (0.039)	0.025 (0.008)	2
Pseudoplusia includens	0.055 (0.010)	0.018 (0.002)	3
Pectinophora gossypiella	-	0.012 (0.005)[b]	-
Anticarsia gemmatalis	0.016 (0.001)	0.006 (0.001)	3
DIPTERA			
Musca domestica L.	> 100.0	-	-
Drosophila melanogaster	> 100.0	-	-
Culex pipiens[c]	> 200.0	-	-
Aedes aegypti[c]	37.06 (9.70)	-	-
Aedes triseriatus[c]	2.84 (0.74)	-	-
Anopheles quadrimaculatus[c]	0.37 (0.03)	-	-

[a] Values with > showed less than 50% toxicity at the concentration listed.
[b] Concentration at which 50% of test larvae were in the 3rd or 4th instar (estimated using regression analysis).
[c] Concentration was µg CryIIA protein per ml of test water.

All other species of nontarget and beneficial insects showed no significant toxic effects from ingestion of the CryIIA protein (Table 2). Surviving nontarget and beneficial insects exposed to high concentrations of the CryIIA protein were normal in appearance and behavior during the tests.

TABLE 2. Sensitivity of Insect and Isopod Species to CryIIA Protein

Species	CryIIA dose (µg/ml)	Test length (days)	% Mortality Check	% Mortality Test	*p*
HYMENOPTERA					
Apis mellifera - adults	50	21	20.3	24.3	>0.05
Apis mellifera - larvae	50	18[a]	16.3	12.5	>0.05
Macrocentrus ancylivorus	200	8	30.7	25.8	>0.05
Meteorus pulchricornis	200	15	34.2	50.0	>0.05
Nasonia vitripennis	50	21	21.3	32.0	>0.05
COLEOPTERA					
Hippodamia convergens	50	8	25.3	37.3	>0.05
Anthonomus grandis	100	7	10.4	18.2	>0.05
Diabrotica undecimpunctata howardi	9 µg/cm^2	5	13.7	8.2	>0.05
Diabrotica virgifera	15 µg/cm^2	7	10.4	12.4	>0.05
Leptinotarsa decemlineata	9 µg/cm^2	7	3.6	5.9	>0.05
HEMIPTERA/HOMOPTERA					
Oncopeltus fasciatus	200	14	28.7	19.6	>0.05
Rhopalosiphum padi	200	3	6.6	6.1	>0.05
Bemisia tabaci	200	3	7.1	5.3	>0.05
ORTHOPTERA					
Acheta domesticus	100	5	16.6	20.0	>0.05
Blattella germanica	100	14	6.7	10.0	>0.05
COLLEMBOLA					
Folsomia candida	200	21	0.0	4.0	>0.05
Xenylla grisea	200	21	0.0	0.0	>0.05
ISOPTERA					
Reticulitermes flavipes	200	15	22.6	19.3	>0.05
NEUROPTERA					
Chrysopa carnea	50	12	6.7	3.3	>0.05
ISOPODA					
Porcellio scaber	200	12	25.3	33.8	>0.05

[a] Mortality from dosage (2-3 day old larvae) to adult emergence

Toxicology tests can identify potential ecological risks created by exposure of beneficial nontarget insects to insecticidal proteins produced by transgenic crop varieties (Urban and Cook 1986). The degree of risk for individual species is a function of both toxicity and level of exposure to plant parts containing the protein. Using these criteria, there is little or no expected ecological risk of CryIIA protein to beneficial insects. Representative beneficial species such as *A. mellifera, H. convergens,* and *C. carnea* were unaffected by ingestion of high concentrations of CryIIA protein. In addition, exposure of beneficial insects will be minimal because the concentration of CryIIA protein in the pollen and nectar of transgenic cotton is below the limit of detection (86 ng/g) using a validated ELISA (G. Rogan, unpublished data). These findings are consistent with previous work showing that *B. thuringiensis* insecticidal proteins have no deleterious effect on beneficial/nontarget insects, including predators and parasitoids of pest Lepidoptera or *A. mellifera* (Flexner et al. 1986, Kreig and Langenbruch 1981, Cantwell et al. 1972, Vinson 1989, Melin and Cozzi 1989, Sims 1995). Vinson (1989) and Melin and Cozzi (1989) reviewed the extensive literature that has established the safety of microbial *B. thuringiensis* and encoded proteins to an array of beneficial insects.

Previous work demonstrated that the CryIIA protein is selective for Lepidoptera and Diptera (Yamamoto and McLaughlin 1981, Donovan et al. 1988, Moar et al. 1994). Among the Diptera, CryIIA activity appears to be specific for mosquito larvae in the

familiy Culicidae. One report (Hodgman et al. 1993) described a *B. thuringiensis* isolate, producing CryIAb, CryIB, and CryIIA proteins, that was active against *Musca domestica, Culex fatigans, A. aegypti.* However, the CryIIA protein purified from this isolate was not active against *M. domestica,* a result consistent with the findings of this study. Few other examples of Diptera-active *B. thuringiensis* endotoxins exist other than CryIIA and the CryIV proteins from *B. thuringiensis* var. *israelensis* (Aronson et al. 1986, Höfte and Whiteley 1989). Haider et al. (1986) described a 130-kDa CryIA protoxin from *B. thuringiensis* subsp. *aizawai* which was proteolytically converted to a toxic 52-kDa product in the gut of *A. aegypti* larvae. Smith et al. (1996) reported on the dual Lepidoptera and Diptera activity of a 135-kilodalton delta endotoxin from *B. thuringiensis* subsp. *aizawai* which appeared to be independent of a species-specific gut activation procedure.

Widner and Whiteley (1989) described the cloning and sequencing of the homologous *cryB1* and *cryB2* crystal protein genes from *B. thuringiensis* subsp. *kurstaki.* These genes were renamed *cryIIA* and *cryIIB* by Höfte and Whiteley (1989). Both genes encode polypeptides of 633 amino acid residues that have molecular masses of approximately 71 kDa and are 87% homologous (Dankocsik et al. 1990). Because the CryIIB protein is not toxic to dipteran larvae, studies have been conducted to determine the specificity region(s) of the CryIIA protein responsible for Diptera activity (Widner and Whiteley 1990, Liang and Dean 1994). Specificity regions 1 and 2 of domain II (amino acids 278-412) are involved with activity against *A. aegypti* larvae. Although it is outside the specificity region of domain II, a sequence of 215 amino acids (residues 61-275) in the CryIIA protein is 30% homologous to a sequence of 211 amino acids (residues 45-255) in the dipteran-active 72 kDa CryD protein from *B. thuringiensis* subsp. *israelensis* (Donovan et al. 1988).

Sublethal concentrations of *B. thuringiensis* insecticidal proteins reduce the growth rate of Lepidoptera larvae in a predictable manner (Dulmage and Martinez 1973, Sims and Berberich 1996). EC_{50} values were determined for all Lepidoptera tested for comparison with corresponding LC_{50} values (Table 1). In general, species sensitivity ranked by LC_{50} values was consistent with the EC_{50} ranking. Although the physiological basis for this mortality/stunting relationship is unknown, analysis of growth inhibition can form the basis for an effective monitoring program used to detect changes in population susceptibility to *B. thuringiensis* proteins (Sims et al. 1996).

Resistance management theory suggests that a mixture of two or more insecticidal proteins in the same plant line (gene pyramiding) might delay the development of resistance more effectively than single toxins used in a spatial mosaic or sequential manner (Gill et al. 1992, Tabashnik et al 1991, Roush 1989). If there are low initial gene frequencies of the resistance genes and mixtures are used in combination with non-transgenic refuges then rare resistant genotypes are likely to be diluted by mating with susceptible individuals. Mixtures are expected to be most effective when each individual toxin produces high larval mortality and there is a lack of cross-resistance between the toxins. The susceptibility of lepidopteran pests of cotton to the CryIIA and CryIA(c) proteins is consistent with the requirement for high, and equivalent, individual protein bioactivity. *P. gossypiella* appears to be somewhat more sensitive to CryIIA than to CryIA(c) protein, *H. virescens* is equally sensitive to both proteins, and *H. zea* is only 2 to 5-fold less sensitive to CryIIA (Stone and Sims 1993, English et al. 1994, T. Watson, unpublished data). The ideal protein toxins to use in a mixture should be as distinct as possible to minimize the chances for cross-resistance, i.e. when selection with one or more distinct proteins reduces susceptibility to other proteins (Gould et al. 1995). Although the characteristics of CryIIA and CryIA(c) are theoretically consistent with these requirements by having minimal sequence homology and unique modes of action (Donovan et al. 1988, English et al. 1994), several reports on insects resistant to *B. thuringiensis* proteins indicate that cross-resistance patterns cannot be generalized (Tabashnik et al. 1996). Laboratory selected strains of *H. virescens* resistant to CryIA(c) protein and *S. exigua* resistant to CryIC protein show broad spectrum cross-resistance to numerous *B. thuringiensis* proteins including CryIIA (Gould et al. 1992, Moar et al. 1995). This cross-resistance was not associated with significantly reduced binding to midgut receptor sites. In examples where reduced binding has been implicated as the basis for resistance, less cross-resistance to the CryIIA protein was observed. For

example, a unique *H. virescens* strain (YHD2) selected for a high level of resistance to CryIA(c) had little cross-resistance to CryIIA (Gould et al. 1995). Similarly, two strains of *Plutella xylostella* selected for resistance to CryIA proteins under field conditions had little or no cross-resistance to CryIIA (Tabashnik et al 1993, Tang et al. 1996). The variability in cross-resistance response to *B. thuringiensis* insecticidal proteins suggests that the value of gene pyramiding for resistance management will not be fully understood prior to commercial introduction and long-term use of multi-gene plant varieties.

ACKNOWLEDGMENT

I thank V. Maggi and M. Beevers (California Agricultural Research) and S. J. Palmer (Wildlife International Ltd.) for conducting the honeybee and selected beneficial insect studies, J. W. Martin (Monsanto) for technical assistance, and W. J. Moar for review of the manuscript.

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PYRETHROID RESISTANCE ASSOCIATED WITH DECREASED BIOTIC FITNESS IN HORN FLIES (DIPTERA: MUSCIDAE)

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ABSTRACT

Development rates, relative fecundities and relative biotic potentials of one strain of susceptible and one strain of pyrethroid-resistant horn flies, *Haematobia irritans* (L.), were measured under insecticide-free conditions. Resistant flies pupated significantly less successfully, and their rate of adults produced/100 eggs was significantly less than that for susceptible flies. The relative biotic potential of the resistant strain was 0.57, and it developed more slowly than the susceptible. Results suggest that the decrease in pyrethroid resistance that has been observed in early season field populations is a result of a competitive disadvantage of resistant flies in the absence of pyrethroids. Resurgence of resistant populations may be forestalled by treating with pyrethroids only when economic thresholds are reached, and by seasonal alteration of pyrethroids with insecticides that have different modes of action.

MATERIALS AND METHODS

Susceptible (S) horn flies were obtained from laboratory colonies at the Food Animal Protection Research Laboratory (FAPRL) in College Station, Texas, and the Knipling-Bushland Livestock Insects Research Laboratory (K-BLIRL) in Kerrville, Texas. This strain has been maintained without host contact for over 25 years at the latter facility; flies from the FAPRL were progeny of this colony and also were maintained separately from a host. Laboratory flies had not been exposed to any insecticides (although the larval media and blood were not routinely screened for residues) and were susceptible to all classes of insecticides.

Resistant (R) horn flies were obtained from the Knipling-Bushland Livestock Insects Research Laboratory. This strain of flies had been reared for over 100 generations in isolation on a caged steer. To ensure homozygosity for resistance, a 0.5% solution of technical grade permethrin in acetonitrile was applied five days/week in 4-ml aliquots to the shoulders and back of the caged steer. Pupae and adult flies were obtained from this colony and transported to the FAPRL where their progeny were reared without host contact. Five to seven generations of the R strain were reared in the manner described below prior to beginning the study to allow adaptation to the altered rearing conditions. Data were not collected until the R population had stabilized and was self-sustaining.

Both S and R strains were reared and maintained similarly at the FAPRL under conditions described by Bay and Harris (1978). R flies were not pressured with any insecticide during this study; however, their resistance level and the susceptibility of the

S strain were monitored following a modification of the method described by Plapp (1971). The inner surfaces of 20-ml glass scintillation counting vials were coated with an insecticide residue. Residues were applied by pipetting insecticide in acetone into the vials and adding acetone to yield a total volume of 0.5-1.0 ml. Acetone alone was used in controls. Vials were manually rolled on their sides until the acetone evaporated. Dried vials were placed upright in a vented hood for at least 5 min to remove all traces of vaporized acetone before the flies were introduced. Vials were stoppered with either cotton or clean caps. The two different types of covers did not alter pyrethroid toxicity. The resistance level of R flies did not change to permethrin or to cypermethrin. LD_{50} values for the S strain after 1-h exposure were 0.355 and 0.030 ug/vial for permethrin and cypermethrin, respectively. For the R strain, LD_{50} values were 12.5 and 4.30 ug/vial, respectively, for the same pyrethorids and exposure interval.

Eggs were collected from females, 5 to 7 days post-emergence, within 6 h of oviposition and counted. A maximum of 200 eggs were transferred to 1-quart, plastic containers and reared as previously described. Approximately 24 h prior to emergence, pupae were collected and counted. Any mortality as a result of handling was recorded. Emerging adults were sexed and counted approximately every 12 h until emergence was completed. Earliest emergence, mean emergence/day and average development time were calculated for each sex and strain. Susceptible horn flies produced large numbers of progeny, so fewer replicates with large sample sizes were utilized (3 to 13). In contrast, R flies, producing fewer progeny, necessitated many replicates (10 to 25) with small sample sizes. Data were analyzed with a modified Student's distribution (t'test) (Snedecor and Cochran 1980).

Same age virgin females were collected, counted and mated. At five to seven days post-emergence, females were exposed to larval medium for 24 h. Blood meals were available to the females during this period. Emerging offspring were counted and sexed at least once every 12 h until emergence ceased. Fecundity studies were replicated 3 to 14 times. Data were analyzed by the modified t'test (Snedecor and Cochran 1980).

Precise calculations of the intrinsic rate of increase (R) require measurements of the mean generation time and total lifetime fecundity (Andrewartha and Birch 1954). Female horn flies oviposit up to 29 days after emergence and can produce as many as 200 eggs in a lifetime. Adults can live in excess of 1 month (Palmer and Bay 1983). Since it is the first progeny produced during the adult life span that have the greatest effect on a population's growth and its phenotypic ratio (Lewontin 1965, Price 1976 as cited by Roush and Plapp 1982), we did not determine total lifetime fecundity. Instead, daily fecundity was measured during the period of peak oviposition, which occurs at five to nine days post-emergence (Schmidt et al. 1973), Palmer and Bay 1983). Mean generation time was not measured, because, in a species in which all life stages are present at once, it is development time, not generation time, that correlates positively with fitness (Lewontin 1974). Therefore, fecundity and biotic potential were calculated on a daily basis for the primary ovipositional period, as described by Roush and Plapp (1982), where daily fecundity = (number of adult progeny produced)/(female) (day) and daily biotic potential = ($\log_e$ fecundity)/(average development time).

RESULTS AND DISCUSSION

Emergence was completed within 72 h of its initiation in both strains (Table 1). Both S males and females tended to emerge sooner and develop faster than their R counterparts, but the differences were not statistically significant ($P>0.10$). Females of

both strains emerged slightly sooner than males, which agrees with previous observations (McLintock and Depner 1954).

Table 1. Comparison of emergence and development rates by sex of susceptible and resistant horn flies.

	M ±SD[a]			
	Male		Female	
	Susceptible[b]	Resistant[c]	Susceptible[b]	Resistant[c]
First Emergence (days)	8.83 ± 0.41	9.04 ± 0.65	8.58 ± 0.38	8.91 ± 0.66
% Emerged				
Day 1	45.8 ± 36.8	30.1 ± 28.2	79.5 ± 24.8	63.8 ± 27.6
Day 2	50.5 ± 34.1	64.1 ± 25.8	20.0 ± 24.3	35.0 ± 27.6
Day 3	3.72 ± 8.30	5.80 ± 11.8	0.52 ± 1.27	1.23 ± 2.99
Average Development Time (in days)	9.49 ± 0.50	10.2 ± 0.80	9.33 ± 0.47	9.63 ± 0.75

[a] Data showed no significant differences (t'test, P>0.10).
[b] n = 6 generations.
[c] n = 11 generations, except for average development time where n = 14 generations.

Pupation and emergence rates, adults produced/100 eggs, sex ratios, fecundities and biotic potentials for each strain are reported in Table 2. Pupation and emergence rates for the S strain were analogous to those reported previously for this strain under similar rearing conditions (Bay and Harris 1978, Bridges et al. 1984). Differences between the S and R strains were highly significant for both pupation rate and adults produced/100 eggs (P<0.0005). Susceptible flies pupated nearly twice as successfully as R flies and produced more than twice as many progeny. Susceptible flies also had a slightly more successful emergence rate than R flies. The sex ratio for both strains was effectively 1:1. The daily fecundity for S females was higher than for R females. Biotic potentials appeared to be too low for either strain to continue to exist; however, these are daily biotic potentials, and both strains could easily increase their numbers by several fold during the possible one-month period of reproductivity. Comparison of the two strains indicated that the R flies had only 57% of the daily biotic potential of the S flies. It is noteworthy that the R strain's daily biotic potential was much less than that of the S strain. Although the R strain was allowed to become adapted to the rearing conditions, it may not have been as well-adapted as the S strain, which had been reared in the laboratory considerably longer. However, since data were recorded for over 25 consecutive generations and none of the parameters measured varied significantly between the first and last generation, it is unlikely that maladaption to the rearing conditions alone could account for the much lower biotic potential of the R strain. In addition, field studies have indicated that the reduced biotic potential of the R strain may be an accurate reflection of the environmental fitness of resistant populations. Early season application of a pyrethroid eliminates most of a horn fly population. The population rebounds slowly and by late season, repeated pyrethroid applications no longer provide satisfactory control (Sheppard and Hinkle 1985). Overall, results of this study validate Sheppard's (1987) suggestion that pyrethroid-resistance may be associated with decreased biotic fitness in horn flies. Slower developing phenotypes, such as the R

Table 2. Comparison of pupation, emergence, and reproductive successes, sex ratio, fecundity and biotic potential of susceptible and resistant horn flies.

Parameter	Mean ± SD[a]	
	Susceptible	Resistant
%Pupation	66.8 ± 9.75* (n = 3)[b]	34.6 ± 15.5* (n = 10)
%Emergence	92.7 ± 5.29 (n = 13)	76.5 ± 22.6 (n = 23)
Adults produced/100 eggs	60.2 ± 6.25* (n = 3)	27.3 ± 14.7* (n = 11)
% Female	45.1 ± 10.6 (n = 9)	50.0 ± 15.8 (n = 25)
Daily Fecundity (F)[c]	2.67 ± 2.32 (n = 3)	1.78 ± 1.55 (n = 14)
Daily biotic potential (BP)[d]	0.105	0.060
BP relative to Lab strain[e]	1.00	0.57

[a] Data followed by an asterisk are significantly different (t'test, $P<0.0005$).
[b] n = number of generations
[c] F = (# adult progeny)/(female-day)
[d] BP = ($\log_e$ fecundity of strain)/(average female development time of strain)
[e] BP relative to lab strain = (BP of strain)/(BP of Lab strain)

strain, are at a competitive disadvantage in a large population (Dobzhansky et al. 1942), and will account for only a small proportion of that population. Under insecticide-free conditions, the R strain is at a disadvantage; however, treatment with a pyrethroid effectively removes the competition and the disadvantage. Population rebound by the R strain may be slow, but will succeed as long as it retains the effective advantage provided by the presence of the pyrethroid.

Population resurgence may be enhanced if heterozygous resistant-susceptible (R-S) flies survive pyrethroid treatment. We did not measure the biotic potential of the R-S hybrid, but as a rule, heterozygotes tend to develop faster than homozygotes (Dobzhansky et al. 1942). For example, resistant-susceptible hybrids for metabolic resistance in house flies show no fitness disadvantages, yet they are resistant (Roush and Plapp 1982). Horn fly R-S hybrids are also resistant. The intermediate level of resistance that they possess is enhanced by backcrossing to the resistant parent (McDonald and Schmidt, 1987). If the biotic potential for the R-S hybrid is even slightly higher than that of the R strain, the population will recover more quickly.

The lower biotic potential of the R strain also helps explain why resistance is apparently lost during the winter in the absence of pyrethroids. Once the insecticide is removed, the competitive disadvantage returns. Progeny containing susceptible alleles and migrant susceptible flies are primarily responsible for establishing the early season

population; however, the proportion of flies containing resistant alleles is larger the second spring, allowing for the more rapid evolution of resistance observed during succeeding seasons (Sheppard and Hinkle 1987).

In order to prevent the establishment of a resistant population, pyrethroid application should be delayed as long as possible. By delaying treatment until the fly density reaches the economic threshold (ca. 250 flies per animal in Texas), the competitive disadvantage of the resistant flies is maintained and an uncontrollable fly population is less likely to develop.

Insecticides with different modes of action should be used in alternate seasons. Horn flies resistant to one insecticide tend to be susceptible to insecticides with a different mechanism of action (Sheppard and Marchiondo 1987, Byford et al. 1988). Combining pesticides with two different modes of action and applying them together is not recommended since insect populations can have more than one type of resistance present simultaneously. Eventually, it is possible that no pesticide would provide effective, economical control. Therefore, we recommend alternating insecticides seasonally by their mode of action to prevent resistant flies of the previous season from quickly re-establishing an uncontrollable population.

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ANATOMY AND INNERVATION OF THE FLEXOR TIBIALIS MUSCLE OF THE COCKROACH *LEUCOPHAEA MADERAE* (FABRICIUS)

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ABSTRACT

The flexor tibialis is a large muscle positioned along the posterior border of the femur in the metathoracic segment of the Maderia cockroach, *Leucophaea maderae* (Fabricius). The large muscle consists of eight muscle groups or bundles originating on a long central apodeme in the femur and inserting on the lateral cuticular faces of the femur and along the boundary between the femur and the trochanter. This muscle is innervated by branches of the fifth nerve which arises from the metathoracic ganglion. Many flexor muscle fibers attached to the central apodeme have ultrastructural features that resemble the intercalated discs of heart muscle. The myofibrils in cross-section are bound by the distinctive fenestrations of the sarcoplasmic reticulum. Although the characteristic thick and thin filaments within the myofibrils were evident, there are 11 to 12 thin filaments around each thick one instead of the 6 to 1 ratio usually found in skeletal muscle. Nerve-muscle junctions and small tracheae were detected in the intermyofibrillar spaces.

INTRODUCTION

One of the more important trends in insect neuroscience in recent years has been the discovery of a number of chemical messengers in the central nervous system. Most of these messengers are neuropeptides that regulate developmental, metabolic, and behavioral processes (Kelly et al. 1994). The wide distribution and multifunctional nature of these neuropeptides have encouraged the development of prospective target cell and tissue preparations to evaluate the range and character of a given response to a specific neuropeptide.

In the Maderia cockroach, *Leucophaea maderae* (Fabricius), more than a dozen peptides have been found that alter the character of muscle activity in a variety of visceral organs of this insect (Cook and Wagner 1990). The effects of these myotropins on skeletal muscle preparations from this cockroach have not been determined. Although various leg muscle preparations from the locust *Locusta migratoria* (Migratorioides) (Hoyle 1955) and the cockroach *Periplaneta americana* L. (Washio and Koga 1990) have been studied, neither the anatomy nor the functional details of the large flexor tibialis muscle in *L. maderae* has been fully described. Hagopian (1966) did report on some of the ultrastructural features of muscle fibrils found in the femur of the Maderia cockroach, but he gave no details on the origin and insertion of the many fiber bundles that comprise the flexor tibialis muscle. Therefore, we have given a broader description of the principal anatomical features of this large muscle and its innervation as well as some of its histological and ultrastructural aspects.

Moreover, preliminary experiments indicated that such a preparation could be used to measure the pharmacological effects of chemical messengers on this skeletal muscle (Cook and Staneart 1991).

MATERIALS AND METHODS

The *L. maderae* cockroaches used for this study were taken from stock colonies maintained at 27°C and RH of 40%. The insects were fed dry dog food and water *ad libitum*. All isolated muscles were perfused with a saline solution that had the following composition (in mM): NaCl 156, KCl 2.7, $CaCl_2$ 1.8, glucose 22. The pH of the saline solution was adjusted to 6.8 with 0.1 M NaOH.

Adult male cockroaches were decapitated and the metathoracic legs were removed by severing the leg at the membrane between the second episternal plate and the first and second trochanteral plates on the ventral surface of the metathoracic segment as described by the nomenclature of Carbonell (1947). The femur of each leg was drawn across a shallow basin made of soft dental wax and placed in such a manner to allow the flexor tibialis in the femur to have a dorsal exposure. The femur was secured in place by fastening dental wax against the coxa and across the extended tibial segment. After the cuticle of the posterior surface of the femur was carefully removed from this preparation, the anatomical features of the entire flexor tibialis could be studied in dorsal aspect.

The metathoracic ganglion and the nerves that arise from it to innervate the legs were readily exposed by the removal of the basisternal plate on the ventral surface of the segment. Once this was accomplished, specific branch nerves were traced to their terminations by carefully removing the cuticle and by parting or cutting muscle groups.

The femur from the legs of male cockroaches was cut with a razor blade into 1-2 mm thick cross sections. Cross sections were immediately placed in a fixative solution composed of 1% acrolein and 2.5% glutaraldehyde in a 0.1 M sodium cacodylate buffer (pH 7.4) for 1 h at room temperature or overnight in the cold (4°C). Specimens were rinsed in the buffer three times (10 min each) in the cold (4°C) and postfixed in 1% OsO_4, buffered with 0.1 M sodium cacodylate, for 2 h in the cold (4°C). Tissues were dehydrated in a series of aqueous acetone mixtures: 30, 50, 70, 95, and 100% (three times in 100%) before embedding in Spurr's resin as modified by Mollenhauer (1986). Tissues were sectioned with a Reichert Ultracut S microtome either at 1 µm and stained with azure ß-methylene blue or thin-sectioned (0.06 μm) and stained with lead citrate. Appropriate specimens were viewed under the light microscope (Nomarski differential interference contrast) or under a Hitachi H 7000 electron microscope at 75 kV.

RESULTS AND DISCUSSION

Although the flexor tibialis in the living state assumes a position along the posterior border of the femur, it is shown in dorsal aspect in Figs. 1A and B. This complex muscle consists of eight major fiber bundles, five of which are shown. The three additional bundles not shown are those that correspond to muscle groups 3, 4 and 5 along the ventral side of the femur.

All of the fiber bundles of the flexor tibialis originate on a long central apodeme (Ap) in the femur, which extends from the tibia (Ti) to the asterisk in Fig. 1A; however, insertions for these fibers occur at a variety of points as shown. Central muscle bundles 1 and 2 insert on the cuticle along the anterior border of the trochanter (Tr). Muscle fibers 3a-e insert along the anterior (3a & b - dashed ovals) and lateral borders (3c-e) of the femur (Fm). Muscle bundles 4 and 5 simply insert on the cuticle of the lateral face of the femur. The fan shaped

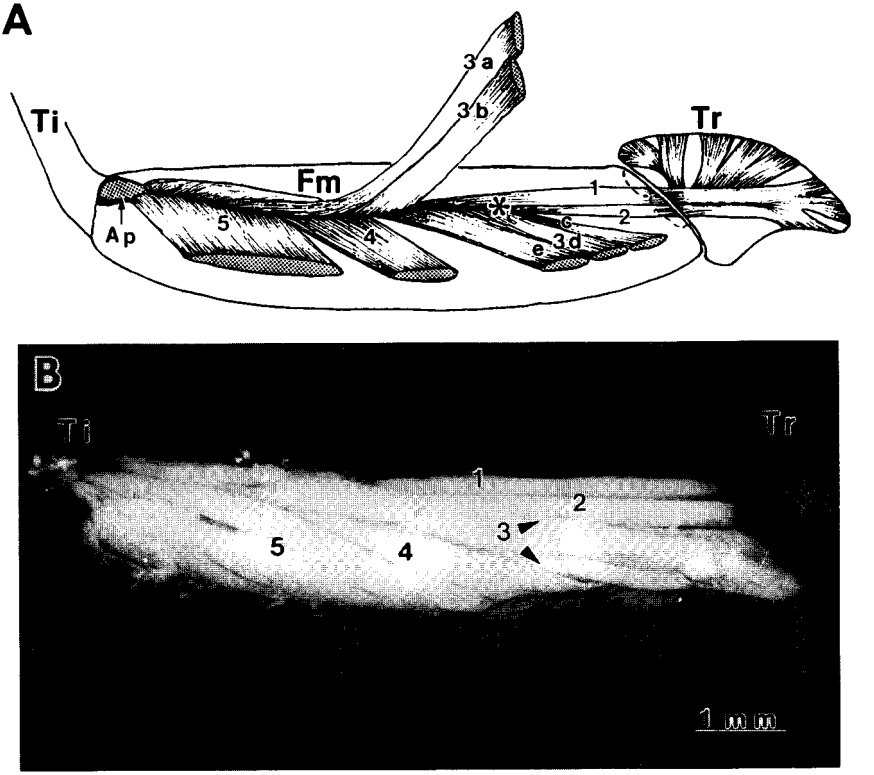


FIG. 1 The flexor tibialis positioned along the posterior border of the femur in the living state is shown in dorsal lateral aspect. A) Drawing of the distribution of the major muscle bundles of the flexor tibialis within the femur (Fm) and the trochanter (Tr):Ti = tibia; Ap = apodeme; * end of apodeme. B) Photomicrograph of the flexor tibialis in lateral aspect after the cuticular face of the femur has been removed. The numbers correspond to those shown in A.

muscles shown in dorsal aspect in the trochanter arise from two cuticular apodemes, which in turn originate from the inner edge of the anterior border of the femur. These muscles insert on the dorsal (posterior) cuticular face of the trochanter.

The innervation of the cockroach leg in *Periplaneta americana* (L). was described first by Pringle (1939). He found that four of the seven nerves that arise from the metathoracic ganglion (MtThG) enter the leg. Like *P. americana*, nerves 3b and 5 innervate the muscles of the femur in the Maderia cockroach (Figs. 2A, B). After following separate paths through the coxa (Cx), these two nerves fuse in the trochanter and again separate in the femur as shown in Fig. 2B. The flexor tibialis receives its innervation from a series of small nerves that arise from a major branch of the fifth nerve in the femur, while the other branch of the same nerve largely carries sensory axons. The extensor tibialis receives its innervation from a small branch of nerve 3b. The innervation to both muscles in the femur was confirmed by physiological stimulation.

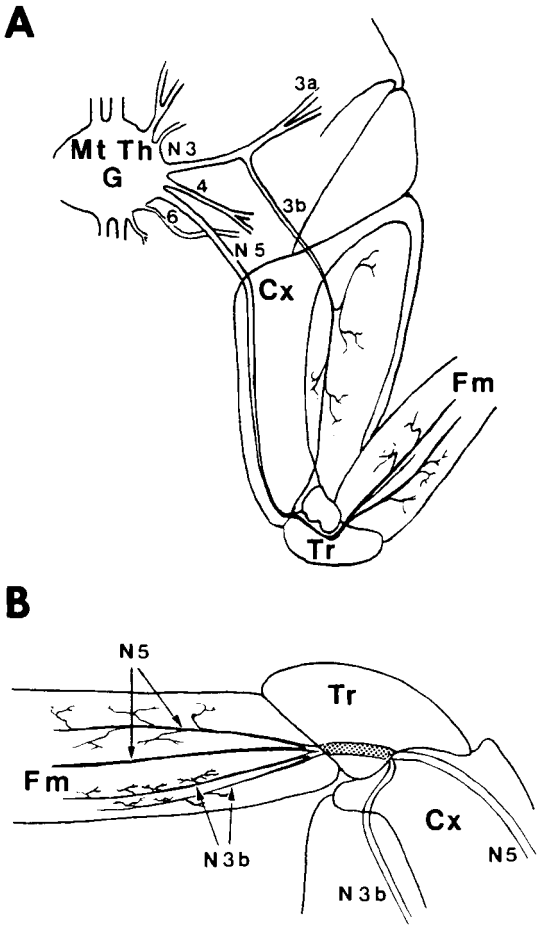
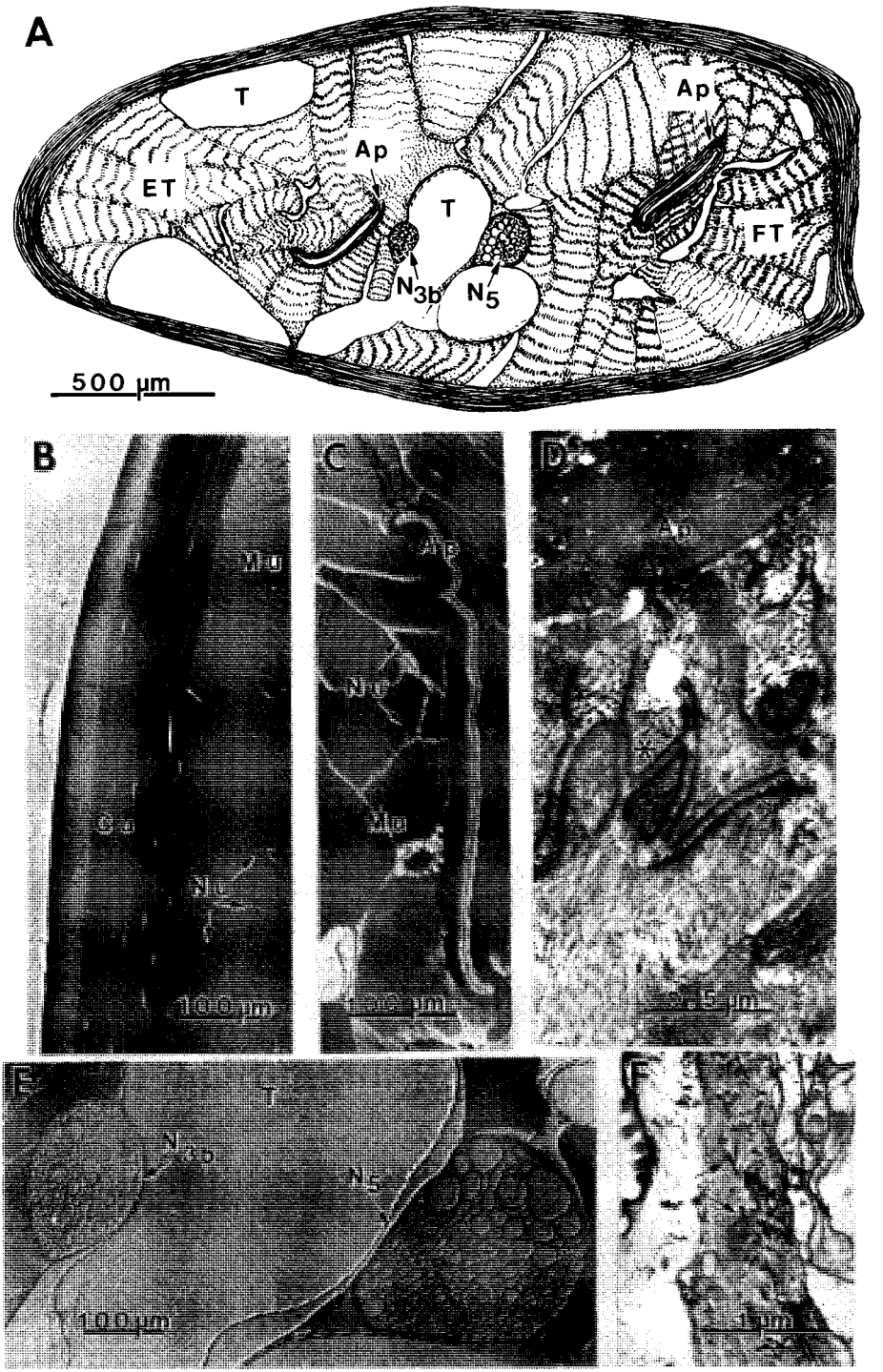


FIG. 2. Pattern of innervation for the femur (Fm). A) Principal nerves that arise from the metathroracic ganglion (MtThG) and proceed into the coxal (Cx) region (ventral view). B) Fusion of nerves 3b and 5 as they pass through the Trochanter (Tr) and the distribution of branches of these nerves within the femur.

FIG. 3. Histological and ultrastructural features of the femur in cross-section. A) Summary drawing of the femur showing the arrangement of principal muscles, nerves, and tracheae: T = trachea; ET = extensor tibialis; Ap = apodeme; N = nerve; FT = flexor tibialis. B) Light micrograph of the interface between the flexor muscle and the cuticle of the femur: Cu = cuticle; Mu = muscle; Nu = nuclei. C) Cross-section of the central apodeme of the flexor tibialis: Ap = apodeme; Mu = muscle; Nu = nuclei. D) Electronmicrograph of the region of muscle attachment to the apodeme: * = the distinctive thick double membrane network; Ap = apodeme. E) Cross-sectional relationship between nerves 3b and 5 that shows axonal profiles: T = trachea; N = nerve. F) Electronmicrograph of the nerve trunk perineurium that reveals the presence of collagen crystals (arrows).


A
T
Ap
ET
Ap
T
FT
N3b
N5
500 µm
B
C
D
E
F
100 µm

The histological features of the femur are shown in cross section in Figs. 3A through E. The drawing (Fig. 3A) illustrates the arrangement of the principal tissue elements found within the femur. The muscle fibers that surround the central apodeme (Ap) to the right of the fifth nerve comprise the flexor tibialis, while the muscle elements to the left of nerve 3b constitute the extensor tibialis. The open spaces between the two nerves correspond to large tracheal trunks (T) that course through the entire central region of the femur.

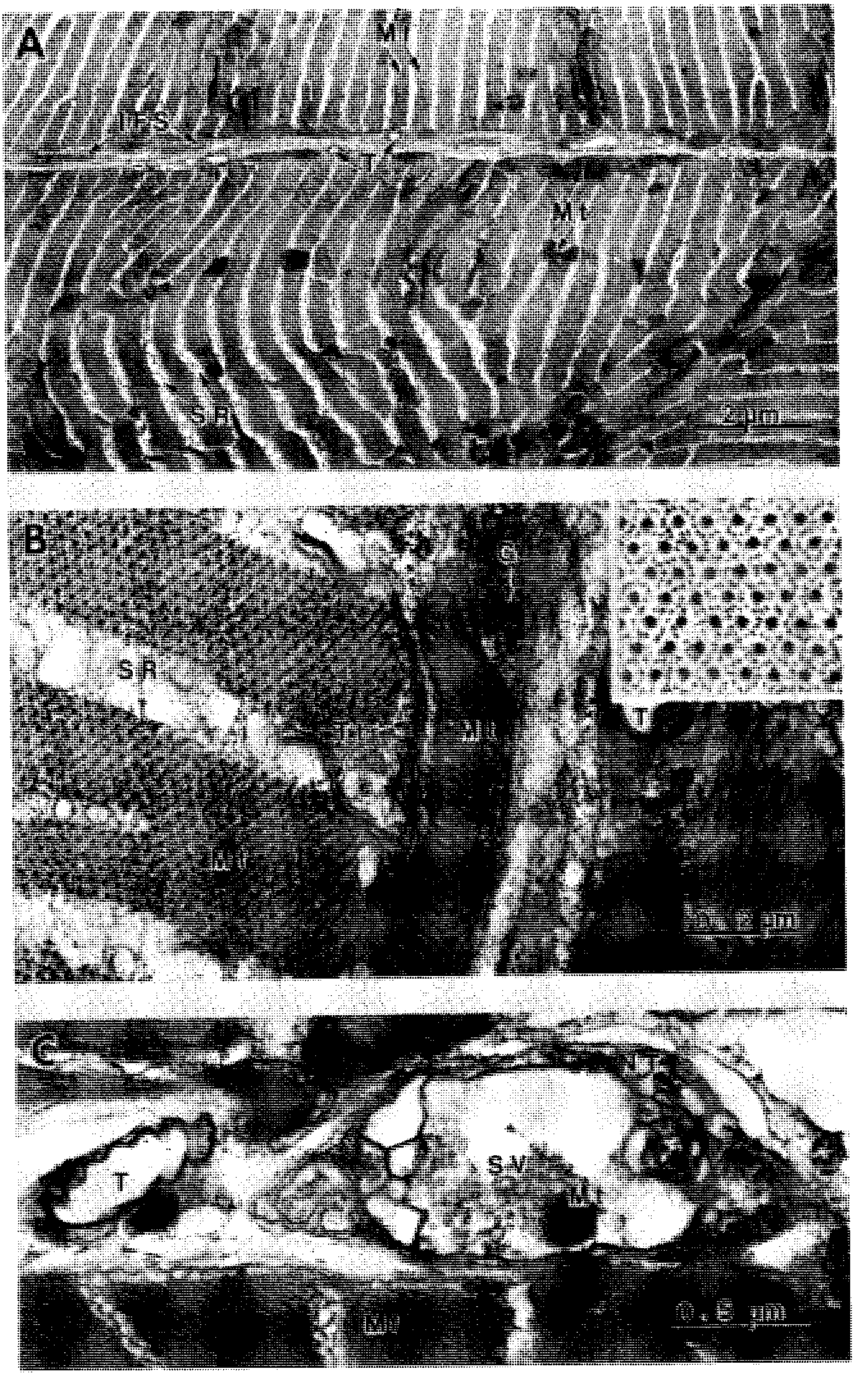
The histological details of the regions of origin and insertion for muscle fibers of the flexor tibialis are shown in Figs. 3B and C. A region of muscle attachment to the cuticular surface (Cu) of the femur is shown in Fig. 3B, while the micrograph in Fig. 3C depicts the central apodeme where muscle fibers of flexor tibialis originate. Many fibers at their attachment to this central apodeme have characteristic ultrastructural features (Fig. 3D*). Hoyle (1983) has described these structures as "a zig-zag thickened double-membrane that closely resembles the intercalated discs of heart muscle." He found that these special structures were interposed between antennular muscles and tonofilaments from the cuticle in certain copepods.

Axonal profiles for both nerves 3b and 5 are shown in Fig. 3E. An electron micrograph of the perineural sheath that surrounds these nerves is illustrated in Fig. 3F. Oblique profiles of collagen crystals are evident in the fine granular base material of this sheath (Smith and Treherne 1963). Muscle fibers of the flexor tibialis are classed as microfibrillar muscles with closely packed cylindrical fibers. Each fiber is ensheathed in a relatively tough structureless membrane, the sarcolemma, and the nuclei of the sarcoplasm are either scattered throughout the substance of the fiber or disposed immediately beneath the sarcolemma (Wigglesworth 1972).

The cross-sectional arrangement of myofibrils (Mf) within several muscle bundles of the flexor tibialis are shown in Fig. 4A. Each myofibril is bound by the distinctive fenestrations of the sarcoplasmic reticulum (SR). The scattered mitochondria (Mt) range in shape from oval to elongate. At higher magnifications, elements of the T-tubule (Tt) system could be recognized in association with the sarcoplasmic reticulum (SR) (Fig. 4B). Moreover, the characteristic thick and thin filaments within myofibrils could be detected. Although the obliqueness of the section prevents a clear profile of the filament arrangements, it is evident that more than six thin filaments surrounded each thick filament. The inset in Fig. 4B illustrates the fact that there can be as many as 11 to 12 thin filaments around each thick filament. This observation confirms Hagopian's (1966) report of the same phenomenon in the flexor tibialis of the Maderia cockroach.

Nerve terminals were detected in the intermyofibrillar spaces near small tracheae. In the example shown in Fig. 4C, a close apposition exists between the nerve terminal membrane and the muscle sarcolemma that lies beneath. A large cluster of synaptic vesicles (SV) is also present adjacent to the terminal membrane.

FIG. 4. Ultrastructural characteristics of the flexor tibialis muscle. A) Panoramic view of the arrangement of myofibrils (Mf) of the flexor tibialis in cross-section which shows the distinctive fenestrations of the sarcoplasmic reticulum (SR) and the distribution of mitochondria (Mt). IFS = interfibrillar space; T = trachae. B) Higher magnification of the boundary region between the myofibrials (Mf) and the interfibrillar space. Elements of the T-tubule (Tt) system are evident along with sarcoplasmic reticulum (SR), mitochondria (Mt), glycogen granules (G) and trachae (T). The inset shows myofilament profiles with 11 to 12 thin filaments surrounding each thick filament. C) A nerve terminal (NT) in the interfibrillar space is in close apposition to the sarcolemma of a myofibril (Mf). SV = synaptic vesicles; Mt = mitochondria, T = trachae.


A
Mt
TFS
T
Mt
SR
2 µm
B
SR
Mt
T
C
T
SV
M
Mt
0.5 µm

The anatomical features described in this report identify specific muscle bundles within the flexor muscle. Such a description provides an opportunity to position recording microelectrodes in the muscle with greater precision and to explore the electrical properties of the entire muscle in a systematic manner. Moreover, the ultrastructural details provide the basis for a precise understanding of prospective sites of drug action. Finally, this study will allow the investigator to make comparisons between skeletal and visceral muscle responses for certain key neuropeptides found in the Maderia cockroach.

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POLLEN FEEDING BY THE BOLL WEEVIL (COLEOPTERA: CURCULIONIDAE) FOLLOWING COTTON HARVEST IN EAST CENTRAL TEXAS

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ABSTRACT

Alternate food plants of adult boll weevils, *Anthonomus grandis* Boheman, in Brazos County of East Central Texas were determined by identifying pollen grains ingested by weevils captured in pheromone traps from September 1988 through June 1989. Survival of spring-emerged boll weevils when fed two of the most commonly encountered pollen types were compared with control groups in field cages. A total of 3,760 pollen grains from a wide variety of species of plants was identified from dissected boll weevils captured at various distances from cultivated cotton fields. The most common pollen grains isolated from weevil digestive tracts were: *Quercus* sp. (oak), *Phoradendron tomentosum* (DC) A. Gray (mistletoe), *Ilex* sp. (yaupon), *Callirhöe involucrata* (Torr.) A.Gray (wine cup), and various unidentified species of the families Compositae and Leguminosae. No significant differences were found between male and females in either the percentages of individuals with ingested grains or in the mean number of grains per individual. Likewise, weevils with different reproductive states and weevils with different levels of stored fat had similar quantities and percentages of ingested pollen suggesting pollen feeding is a common behavior regardless of sex or physiological status. Boll weevils fed pollen of oak or wine cup in field cages had significantly greater survival than individuals reared on water alone. These results suggest that pollen feeding may be an important factor in spring survival of the boll weevils emerging from overwintering sites in East Central Texas, and perhaps also in other areas of the southern United States.

RESUMEN

Se determinaron las plantas silvestres de las que se alimenta el picudo del algodonero, *Anthonomus grandis* Boheman, después de la cosecha de algodón en el Valle de Brazos en el centro-este de Texas. La determinación se llevó a cabo por medio de la identificación de granos de polen de los tractos alimenticios de picudos, capturados con trampas de feromona colocados a varias distancias de los campos de algodón entre septiembre de 1988 y junio de 1989. Además, se comparó la sobrevivencia de *A. grandis* emergidos que se alimentaron con de polen de : *Quercus stellata* Wang. y *Callirhöe involucrata* (Torr.) A. Gray con individuos ofrecidos solamente agua (control) en jaulas de campo. Fueron encontrados un total de 3,760 granos de polen correspondientes a una gran

diversidad de plantas. Los tipos de polen mas comunes fueron *Quercus* sp. (encino), *Phoradendron tomentosum (DC)* A. Gray, *Ilex* sp., *Callirhöe involucrata* (Torr.) A. Gray y especies no identificadas de Compositae y Leguminosae. El porcentaje de picudos con polen en los intestinos y los números de granos aislados por individuos no fueron significativamente diferente entre machos y hembras, dentro de individuos con estados reproductivos diferentes, ni dentro de individuos con diferentes niveles de grasas almacenadas. Los picudos alimentados con polen de *Quercus* o de *C. involucrata* tuvieron significativamente mayor tasa de sobrevivencia que los individuos alimentados solo con agua, lo que sugiere que la alimentación de pollen de plantas silvestres puede ser un factor importante en la sobrevivencia de individuos de *A. grandis* que emergen en la primavera en el centro-este de Texas, y también en otras regiones del Sur de los Estados Unidos.

INTRODUCTION

The boll weevil, *Anthonomus grandis* Boheman, overwinters in the southern United States in the adult stage. Survival of overwintering boll weevils is a critical determinant in the severity of infestations of the subsequent cotton season. Reducing densities of overwintering boll weevil populations has long been advocated as a key management strategy for controlling the pest (Mally 1902, Hunter 1904) and is a principal element in present area-wide suppression programs. The nature and extent of feeding by the weevil prior to entering overwintering habitats may be an important factor in overwinter survival (Rummel and Carroll 1985). Studies of the effect of diet and food availability on overwintering survival of the boll weevil have primarily focused on use of cultivated cotton as a food for adult weevils (Fenton and Dunnam 1929, Fye et al. 1959, White and Rummel 1978). However, it has long been known that adult boll weevils feed on plants other than cotton (Coad 1914, Gaines 1934, Walker 1959, Parrot et al. 1966, Cross et al. 1975, Rummel et al. 1978, Burke et al. 1986, Palumbo et al. 1990, Benedict et al. 1991, Jones et al. 1993). These plants may provide energy and nutrients that increase boll weevil survival during the absence of cultivated cotton.

Through the use of pollen isolation techniques, the extent and diversity of plants upon which adult boll weevils feed has recently been elucidated. Benedict et al. (1991) and Jones et al. (1992, 1993) found that boll weevils captured at various distances from cultivated cotton in tropical and subtropical habitats had pollen grains from a large number of species of plants and often contained large quantities of pollen grains in the alimentary canal. Furthermore, these authors found that boll weevils which fed on buds and flowers of various wild plants often survived at rates comparable to those feeding on flower and fruit of cotton. Accordingly, it has been postulated that pollen feeding on non-host plants is a principal survival mechanism of the weevil in tropical and subtropical habitats when the host plants are vegetative (Jones et al. 1993). The extent and seasonal occurrence of pollen feeding in temperate habitats where boll weevils are less active during winter months is not clear. The objectives of the following study were: 1) to identify plants other than cotton on which the boll weevil feeds and to determine the seasonal occurrence of such feeding in Brazos County of East Central Texas, and 2) evaluate the possible importance of alternate food plants by comparing survival of adult boll weevils in field cages fed pollen from two common plants, *Quercus stellata* Wang and *Callirhöe involucrata* (Torr.) A. Gray.

MATERIALS AND METHODS

Trap Studies. Hardee ® boll weevil pheromone traps (Consep Membranes, Dend Oregon) were modified to collect and preserve attracted weevils as described by Jones et al. (1992). Traps were baited with grandlure pheromone strips (Hercon ®, South Plainfield, New Jersey). Fourteen of these modified Hardee traps were placed in various habitats along roads in the Brazos County, Texas, in 1988 and 1989. Traps were placed from 500 m to 23 km northeast of cultivated cotton fields in relatively undisturbed wooded areas as well as in pastures and disturbed locations. Traps were checked from 23 September 1988 to 25 May 1989 at one- or two-week intervals, at which times weevils were removed from traps, counted, and placed in labeled vials containing 7% formalin. Fresh grand-lure strips were placed in traps at biweekly intervals.

In the laboratory, captured weevils were washed in 70% alcohol and dissected. A sample of 507 weevils (n = 47, 95, 42, 42, 47, 2, 50, 134, 43, respectively, for September through May) of the captured boll weevils was analyzed for the presence of pollen in their digestive tracts. Weevils were processed for pollen analysis in two ways: 1) individually (one digestive tract per tube, n =367), or 2) in pooled samples (more than one digestive tract per test tube, n =140) of 8 to 20 individuals from the same trap and sample date. For each weevil, the sex, fat content ("low," "medium," or "high") and gonad size were recorded.

Additionally, the digestive tract of each dissected weevil was removed and examined for the presence of pollen. This process began by removing the digestive tract and placing it in a 6 ml test tube. The tract was washed twice in the tube, first with 95% ethyl alcohol and then with glacial acetic acid, with each wash followed by centrifugation. After drawing off the glacial acetic acid, 3 ml of fresh acetolysis mixture (1:9 sulfuric acid/acetic anhydride) was added to the tube. The tube was then placed with other samples in a test tube rack and heated in a water bath at just below 100° C for 10 to 15 minutes. The tubes were then subjected to three washes, each being followed by centrifugation in the following sequence: glacial acetic acid, 95% alcohol, and distilled water. To insure that all pollen grains were recovered, samples were examined under a dissecting microscope and individual grains transferred with a hand-blown micropipet onto standard glass slides. Pollen grains were mounted on slides using glycerin and labeled as to date and trap location. Pollen grains were examined with a phase contrast microscopy and identified using standard identification guides (Kapp 1969) and by comparison with reference collections made during this study and with those maintained in the Pollen Identification Laboratory, Department of Anthropology, Texas A&M University.

Data from weevils caught in traps and pollen grain totals were analyzed using statistical analysis system software package (SAS Institute 1985) to determine if pollen feeding was more common during a specific season of the year or to weevils of certain physiological states. Chi-square statistics were calculated to compare the percentage of weevils containing ingested pollen among different categories, including sex and fat content. The mean numbers of pollen grains per weevil between sexes, among fat categories and among months were compared using a one-way analysis of variance. The models used were: 1) log number of pollen grains per weevil = month of capture; 2) log number of pollen grains per weevil = month of capture + sex; and 3) log number of pollen grains per weevil = month of capture + fat category. Mean separation of the number of pollen grains per weevil for the different variables were made using Tukey's studentized range test (Steel and Torrie 1980).

The mean, standard deviation, and index of clumping (David and Moore 1954) were calculated for each pollen type identified. The index of clumping (I_{DM}) was calculated for the distribution of pollen grains within individual weevils using the following formula: I_{DM} = (variance of mean number of grains per weevil /mean number of grains per weevil)-1. I_{DM} values of 0 indicate a Poisson (random distribution) of individuals, whereas values above zero indicate an aggregated distribution.

Field Cage Studies. Adult boll weevils were held in field cages provided with two of the most commonly recovered pollen types from trapped weevils (oak and wine cup) to determine if feeding on these pollens affected survival of boll weevils under ambient conditions in East Central Texas. Cotton bolls infested with boll weevils were collected on 31 October 1988 from untreated cotton research fields on the Texas A&M University Research Farm in Burleson County, Texas. Boll weevils emerging from bolls during the following three weeks were placed in overwintering cages (35 by 33 by 25 cm) and provided with water and excelsior for protection. Cages were held at ambient temperatures near a small research plot in College Station, Texas. Boll weevils that became active after 1 April were placed in individual 200-ml plastic cups with screened tops. Each weevil was marked on the elytra with xylol-based paint coded as to the week in which the weevil emerged. Each cup contained a small amount of excelsior, water source with a wick, and either oak pollen or wine cup pollen as a food source, or water only (control). Pollen grains were placed in 2-ml vial caps and fresh pollen was provided every three days. Oak pollen, *Quercus stellata* Wang., was collected from flowering oak trees using a hand-held, battery operated vacuum cleaner ("Dust-buster®"). Wine cup, *Callirhöe involucrata* (Torr.) A. Gray, pollen was hand collected from individual flowers.

Weevils within cups were held at ambient temperatures and humidity on a covered table (1 m high) on the edge of a small research field in College Station, Texas. Mortality of weevils was recorded daily for all treatments, and dead weevils were removed upon discovery.

Survival of boll weevils in the two feeding trials was compared using the general linear model procedure of the SAS (SAS Institute 1985) software package. A one way analysis of variance was conducted using two variables: food treatment (pollen treatment or water only) and the week the weevil became active in spring. The numbers of individuals in the first trial offered oak pollen was 25 whereas the control group (water only) consisted of 26 individuals. Individuals in the second trial offered wine cup pollen was 14 individuals with the control group consisting of 26 individuals. The model used was: log (days alive after emergence) = food treatment + week became active in spring. Mean separation of survival times from these field cage studies were compared using Tukey's studentized range test (Steel and Torrie 1980).

RESULTS AND DISCUSSION

Field Studies. Results indicated that the boll weevil commonly feeds on pollen of many plants other than cotton in Brazos County. Approximately half of all dissected weevils had ingested pollen (51.1%, n=367), and a total of 3,760 pollen grains was isolated from individual and pooled weevil samples. Pollen was recovered from the digestive tracts of weevils from all habitats and months when weevils were captured, except February when only two individuals were trapped.

Seasonal changes were found in the percentages of weevils that had ingested pollen. During the fall months, when the number of weevils caught in traps was highest (Fig. 1),

more than half of the dissected weevils had ingested pollen (Table 1). This percentage dropped significantly during the winter months (Table 1) when few weevils were trapped and flowering plants were scarce. The percentage of weevils with ingested pollen and the number of pollen grains per weevils was highest during "spring emergence" during April and May (Table 1) when flowering plants were abundant and weevils were relatively active (Fig. 1).

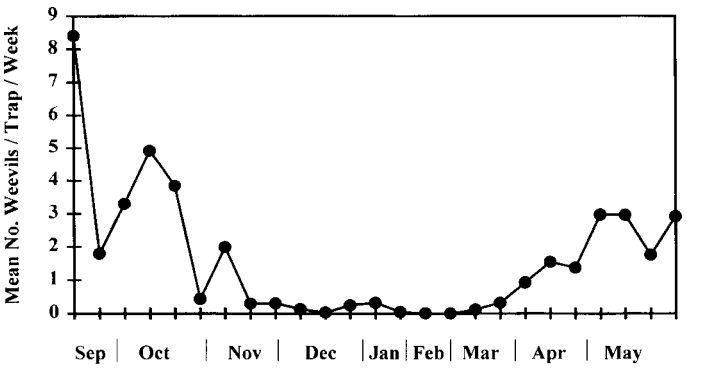

FIG. 1. Mean number of boll weevils captured in pheromone traps following cotton harvest during 1988 and 1989 in Brazos County, Texas (n= 2902 captured weevils).

A wide variety of pollen was identified from trapped weevils (Table 2). Pollen of at least 24 species of plants within 17 identified families were recovered. The species with the greatest number of isolated grains were: oak (*Quercus* sp.); mistletoe (*Phoradendron tomentosum* [DC] A. Gray) ; yaupon (*Ilex* sp.); wine cup (*Callirhöe involucrata* [Torr.] A. Gray); and various unidentified species of the families Compositae and Leguminosae. These species of plants also had the highest clumping indices (Table 2), suggesting that weevils actively fed on pollen of these plants and that the presence of these grains in the digestive tracts was not the result of incidental ingestion.

The appearance of the most common pollen grains ingested by weevils reflected the phenology of these plants in East Central Texas. Highest counts of composite and legume pollen were recovered during fall months (65% and 84%, respectively, of the total number of composite and legume pollen grains recovered) when species of these families were the principal flowering plants available. As expected, pollen of oak, yaupon and wine cup were recovered exclusively during the spring months when these plants were flowering.

The recovery of oak pollen from trapped weevils is significant because leaf-litter of oaks is reported as a preferred overwintering habitat of the boll weevil (Adkisson et al. 1965, Rummel and Adkisson 1970). Oaks flower in early spring in East Central Texas and produce copious quantities of pollen. Thus, weevils emerging from oak litter at this time have ready access to a virtually unlimited supply of pollen from these trees.

The appearance of mistletoe pollen in the weevils was of considerable interest because this parasitic plant blooms principally during the winter months (Correll and

Johnston 1979). Mistletoe is one of only a few plants that flower during January in East Central Texas, yet boll weevils were apparently able to locate and feed on its tiny, relatively inaccessible flowers. Mistletoe pollen accounted for 53.7% of the pollen isolated from weevils in January. Whether these weevils were individuals which had remained after emergence from cotton or were inactive for a period and emerged at some later date is unknown. The virtual absence of stored fat observed in these weevils suggested that exhaustion of energy reserves may have caused these individuals to leave overwintering

TABLE 1. Comparison of the Percentages of Boll Weevils with Ingested Pollen and the Numbers of Pollen Grains per Weevil Captured in Pheromone Traps During 1988-1989 in Brazos County, Texas by Categories of Sex, Fat Body Reserves and Season of Capture.

Variable	No. boll weevils dissected	Total number of grains recovered	Pollen grains / weevil, when pollen present (mean ± sd) [a,b]	% with ingested pollen [c]
		Season Captured		
Fall (Sep., Oct., Nov.)	84	131	2.98 ± 2.84 a (n=44)	52.4*
Winter (Dec., Jan., Feb.)	91	352	12.13 ± 36.78 a (n=29)	31.9*
Spring (Mar., Apr., May)	192	1170	10.35 ± 33.89 a (n=113)	58.9*
		Sex		
Female	235	1298	10.63 ± 36.00 a (n=122)	51.9
Male	132	355	5.54 ± 13.26 a (n=64)	48.5
		Fat Body Reserves		
None	28	173	13.10 ± 22.75 a (n=13)	46.4
Medium	226	890	7.48 ± 26.52 a (n=119)	52.7
High	113	590	10.93 ± 38.62 a (n=54)	47.8

[a] Means with the same letter within a variable group not significantly different using one way analysis of variance (log transformed): (F=2.45, df=15, $p > 0.05$; F=0.56, df=20, $p > 0.05$; F= 1.13, df=2, $p > 0.05$, respectively for the variable sex, fat body reserves, and season captured).

[b] Analysis excluded one outlier with 1550 grains in digestive tract.

[c] Proportions with asterisk significantly different within category (X^2 =0.398, $p > 0.05$; X^2 =0.627, $p > 0.05$; and X^2 =18.11 $p < 0.01$; respectively, for the variable sex, fat body reserves, and season captured).(X^2= 0.398, df=1, $P > 0.05$; and X^2= 0.933, df=2, $P > 0.05$; respectively).

TABLE 2. Pollen Grains Recovered from Boll Weevils Trapped During 1988-89, in Brazos County, Texas.

Plant Family	Species	No. grains recovered [a]	% pollen total	Pollen grains/weevil, when present (Mean ± std)	Clumping Index [b]
	GYMNOSPERMAE				
PINACEAE	*Pinus* sp.	8	0.21	1.0 ± 0.0	-
	MONOCOTYLEDONEAE				
TYPHACEAE	*Typhus* sp.	1	0.03	-	-
GRAMININAE	*Zea mays* L.	33	0.88	1.4 ± 0.9	-0.6
	unidentified spp.	57	1.52	1.7 ± 1.9	2.2
LILIACEAE	unidentified sp	11	0.29	2.8 ± 3.1	3.5
	DICOTYLEDONEAE				
SALICACEAE	*Salix* sp.	4	0.11	2.0 ± 1.0	0.0
JUNGLANDACEAE	*Carya* sp.	5	0.13	1.0 ± 0.0	-
FAGACEAE	*Quercus* sp.	131	3.48	2.8 ± 7.6	16.7
ULMACEAE	*Celtis* sp.	15	0.40	1.1 ± 0.1	-0.9
VISCACEAE	*Phoradendron tomentosum* (DC) Gray	68	1.81	34.0 ± 29.0	24.9
CHENOPODIUM/ AMARANTHACEAE	unidentified spp.	6	0.16	1.0 ± 0.0	-
AQUIFOLIACEAE	*Ilex* sp.	191	5.08	12.7 ± 22.5	39.8
ROSACEAE	unidentified sp.	8	0.21	1.6 ± 0.4	0.1
LEGUMINOSAE:	*Melilotus* sp. (?)	61	1.62	30.5 ± 40.0	52.2
	unidentified sp.	1791	47.62	119.4 ± 199.3	334.1
MALVACEAE	*Callirhöe involucrata* (Torr.) Gray	278	7.39	92.7 ± 158.5	271.0
	Gossypium hirsutum L.	18	0.48	1.5 ± 0.8	0.4
	unidentified spp.	50	1.33	2.6 ± 6.1	14.5
ONAGRACEAE:	*Oenothera* sp.	6	0.16	1.0 ± 0.0	-
CUCURBITACEAE	unidentified sp.	1	0.03	-	-
COMPOSITAE	"High spine" [c]	265	7.05	11.1 ± 35.1	111.0
	"Low spine" [c]	580	15.42	12.3 ± 43.7	155.8
	Taraxacum sp.	3	0.08	1.0 ± 0.0	-
Unidentified Dicots		160	4.25	4.1 ± 5.5	7.5
Fungal Spores		9	0.39	2.5 ± 3.2	4.0
COMBINED TOTAL FOR ALL POLLEN GRAINS		3760	100.00	9.8 ± 83.6	713.0

[a] Includes pooled samples (n = 125 weevils) and individual samples (n = 367)

[b] Clumping Index (I_{DM} = (variance/mean) -1); 0 indicates random (non-clumped) distribution whereas values above zero indicate an aggregated (clumped) distribution (David and Moore 1954).

[c] Qualitative terms used to describe the size of external exine spines in relation to size of pollen grain.

sites in search of food. However, given the small numbers of weevils trapped during winter months and the low probability of these early emerging individuals surviving to spring, mistletoe is probably inconsequential in maintaining populations of weevils during winter months. Nevertheless, the ability of boll weevils to locate and feed on mistletoe pollen is of interest. Understanding the capabilities of the boll weevil in locating feeding hosts provides further evidence that boll weevils actively look for pollen as a food source even during periods when pollen is scarce.

Pollen feeding is apparently not a behavior restricted to weevils of a specific sex or a physiological state (Table 1). The overall percentage of individuals containing pollen did not vary significantly between males and females or among weevils with different levels of stored fat. There also was no significant difference in the percentage of reproductive females containing pollen when compared with non-reproductive females (X^2= 1.647, df=1, $P > 0.05$). The amounts of pollen per individual also did not vary significantly between males and females or among weevils with different levels of fat content although the mean number of grains per weevil was often considerable (Table 1). Mean separation was confounded by the high variation in the number of pollen grains per weevil (mean = 8.70 $\pm$ 83.49 grains/individual ; range 0-1550, n=368). This variation was apparently the result of a small proportion of the captured weevils having fed heavily on pollen just prior to capture. Other captured weevils may have fed as heavily on pollen sources as these individuals but evacuated the gut prior to capture. Evidence for this came from the observation of a weevil captured in April with several grains of *Oenothera* sp. adhering to the elytra, indicating visitation to *Oenothera* flowers. However, this individual had no pollen grains in the digestive tract. Further study is needed on the digestive processes involved in the breakdown of pollen grains in the alimentary tract of the boll weevil.

Field Cage Studies. Weevils emerging during the spring survived significantly longer when fed oak pollen or wine cup pollen compared to those offered only water (Table 3). The mean survival of pollen-fed individuals was higher than the maximum longevity of their respective control group. Twenty-four percent of the individuals fed oak pollen and 28% of fed wine cup pollen survived more that 30 days. Eight percent (n=2) of the oak fed individuals survived more than 70 days, at which time, cultivated cotton in the Brazos River Valley had flower buds suitable for boll weevil oviposition.

TABLE 3. Comparison of the Mean Survival (Days) of Spring-Emerged Boll Weevils Held with Oak Pollen and Water, Wine Cup Pollen and Water or Water Only (Control).

Treatment	*n*	Mean Survival[a], d	Max Survival, d
		Trial 1	
Oak pollen	25	21.4 a	74
Control (Water only)	26	7.5 b	18
		Trial 2	
Wine cup pollen	14	19.2 a	61
Control (Water only)	24	5.2 b	13

a Treatments with same letter not significantly different using Tukey's studentized range test (Steel and Torrie 1980) of log transformed data (F=5.82; df = 3, 47; $P < 0.01$; and F=7.43; df = 5, 32; $P < 0.01$,; for trials 1 and 2, respectively).

Field cage trials were not directly analogous to field conditions because neither oak nor wine cup pollen was available for the extended time periods offered weevils in cages. In addition, results from identification of pollen from trapped weevils in the field indicated that weevils were not restricted to a single pollen source as food and may have fed on several pollens in a short time period. Such mixtures of pollen may be superior food sources to single pollen diets (Jones et al. 1993), and survival rates in the field could possibly be greater than those represented in field cages.

In conclusion, the boll weevil is of tropical origin and has only invaded Texas and the southeastern United States within the past 100 years (Burke et al. 1986). Like many pollen feeding beetles in the tropics, the boll weevil feeds on pollen from many species of plants when its reproductive host is not in flower. Results presented here indicate that pollen feeding by the boll weevil is also a common behavior in the temperate habitats of East Central Texas. It also suggests that this behavior probably occurs in boll weevil populations in other temperate cotton growing areas of the southern United States.

Results from this study greatly increase the number of known species of plants that the boll weevil will feed upon in temperate habitats. These findings are especially important in areas where cotton fields are surrounded by wooded, non-agricultural habitats. Boll weevils have large numbers of potential pollen sources in these habitats from late March until May. Field studies indicated that feeding on diverse pollen by weevils was a common behavior during these months, and field cage studies provided evidence that this feeding can increase survival of boll weevils emerging from winter habitats. These conclusions suggest that estimates of the survival rates of boll weevils emerging in spring based on the mortality of individuals without access to pollen sources may significantly underestimate the survival of the boll weevil in many areas of the southern United States where large numbers of potential food plants are found in flower several months before cultivated cotton becomes reproductive.

The findings presented here and previous studies (Benedict et al. 1991, Jones et al. 1993) are important in revising our understanding of the host location behavior of the boll weevil. Although, the boll weevil has been classified as narrowly oligophagous, (Cross et al. 1975), this classification refers to reproductive plant selection and not in describing selection of adult food plants. The wide range of adult food plants versus the narrow range of reproductive hosts indicates that feeding response by the boll weevil may not always be linked with reproductive behavior and oviposition. This may explain why some species of plants elicit greater feeding response than cotton but are not plants that the boll weevil selects for oviposition (Thompson et al. 1970, Parrot et al. 1989). The wide range of food plants of adult boll weevils may also help explain why these weevils possess sensory neurons sensitive to "green leaf volatiles" common to many plants (Dickens 1986, 1990). The large number and high sensibility of these neurons on the antennae of boll weevils probably allow individuals to locate suitable food plants from a wide range of plant families. However, volatiles from plants used as reproductive hosts of the boll weevil, male-produced pheromone, or their combination are more attractive to boll weevils than plant odor alone (Dickens 1986). This suggests that, in the absence of suitable hosts for reproduction and oviposition, the green leaf volatiles of many plants will attract boll weevils that feed on their flowers. However, boll weevils may be preferably attracted to reproductive hosts and their associated chemical cues when present. Further study is needed to clarify differences in the cues which elicit feeding alone in the boll weevil with that of feeding which leads to oviposition.

ACKNOWLEDGMENT

This study was conducted with funds provided by the Department of Entomology at Texas A&M University in the laboratory of James R. Cate. I thank James Cate and Pete Krauter for ideas and comments concerning this research. I also thank Vaughn M. Bryant, Jr. and John Jones of the Pollen Identification Laboratory of the Anthropology Department of Texas A&M University for help in identification of pollen. This research was supported by the Texas Agricultural Experiment Station and was completed at the Universidad Autónoma de Querétaro, Querétaro, México.

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BIOTYPE E GREENBUG REPRODUCTION AND DEVELOPMENT THROUGH THREE GENERATIONS ON RESISTANT AND SUSCEPTIBLE WINTER WHEAT GENOTYPES

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ABSTRACT

Biotype E greenbugs, *Schizaphis graminum* (Rondani), reared through three generations on a resistant winter wheat germplasm line, TXGBE273, that differed genetically from the susceptible wheat cultivar 'TAM 105' at the *Gb2* and *Gb3* greenbug-resistance loci, showed a significant reduction in total number of nymphs produced per adult (23.3, 26.9, and 26.2 vs. 63.7, 61.7 and 55.8, respectively, for three generations), average nymphs per adult per day (0.9, 0.9, and 1.0 vs. 2.1, 2.1 and 1.7, respectively, for three generations), total lifespan (29.9, 31.1 and 27 d vs. 33.2, 34 and 33 d, respectively, for three generations) and adult lifespan (24.5, 25.5 and 21.7 d vs. 29.0, 29.7 and 28.9 d, respectively, for three generations) compared to greenbugs reared exclusively on TAM 105. Nymphal period for these same greenbugs reared on TXGBE273 was significantly longer than those reared on TAM 105 (5.4, 5.6, and 5.3 d vs. 4.2, 4.2 and 4.1 d, respectively, for three generations). The same significant differences were noted in estimates of the intrinsic rate of increase (0.192, 0.182, and 0.185 vs. 0.312, 0.305, and 0.304, respectively, for three generations), and similar trends were noted in mean generation time and net reproductive rate. The effects of the resistance factors on reproduction and lifespan remained constant across generations and were not found to carry over from one generation to the next. When greenbugs were reared for two generations on TXGBE273 and then returned to TAM 105 for the third generation, the aphids rebounded to reproductive and lifespan parameters that were near those observed using only TAM 105 as the host, although still significantly lower in all categories other than average nymphs per adult per day (1.8 and 1.7, respectively, for greenbugs from TXGBE273 back to TAM 105 and those reared exclusively on TAM 105).

INTRODUCTION

Lazar et al. (1995) reported on the antibiotic effects of a resistant line of hard red winter wheat, *Triticum aestivum* L., on biotype E greenbug, *Schizaphis graminum* (Rondani), fecundity. This wheat line, TXGBE273, differs from the biotype C and E greenbug-susceptible wheat cultivar 'TAM 105' at the *Gb2* and *Gb3* loci that confer biotype C and E greenbug resistance, respectively.

In a continuation of the research by Lazar et al. (1995), the experiments reported here extend the earlier study to compare greenbug biotype E growth and development

over three generations on resistant TXGBE273 and susceptible TAM 105 winter wheat hosts. The experiments were designed to determine whether effects on the greenbug population reared on TXGBE273 could be ameliorated by returning a portion of the population to TAM 105 after being reared for two generations on TXGBE273.

MATERIALS AND METHODS

Two wheat genotypes were used in the experiments: one variety, TAM 105, and one breeding line, TXGBE273. These genotypes were chosen for their close genetic relationship and their differing alleles at two loci conferring resistance or susceptibility to greenbug biotype E. Resistance at each locus is dominant for susceptibility (Porter et al. 1994). TAM 105 (genotype gb2gb2gb3gb3) is susceptible to both biotypes C and E; TXGBE273 (genotype Gb2Gb2Gb3Gb3) is resistant to both greenbug biotypes.

Biotype E greenbugs were reared on seedlings of the susceptible host, TAM 105. Using a small, fine-haired brush, single adult aphids were transferred to individually caged leaves on single seedlings at the two-leaf stage of development. Each seedling was grown in a 150 x 25 mm glass test tube containing a vermiculite medium flooded with 1/4 strength Hoagland's solution. The cages were held in an incubator with mixed fluorescent and incandescent light (approximately 300 $\mu Em^{-2}s^{-1}$) on a 12-hr photophase. Temperature was held constant at 24° C. Single, first-instar nymphs from these aphids were caged on leaves of plants of each wheat genotype. Each aphid/plant combination was considered a replication. Experiments were performed sequentially for a total of 50 replications of each host genotype. Nymphs were observed daily and molting was recorded to mark passage between stadia until the adult stage was reached. The adults were then observed daily until they died. Each day, new nymphs produced by the adults were recorded and removed from the plant.

Analyses of variance on developmental data were performed using the method of least squares (SAS Institute 1995). Where significant F tests were obtained, mean separations were made through least significant difference (LSD) tests (Steel and Torrie 1960). Intrinsic rates of increase (r_m) were calculated by the method of Wyatte and White (1977). The precision of the r_m estimates was calculated using a jackknife procedure described by Dixon (1993). This procedure uses pseudovalues generated from the data set that were compared to the actual estimate, giving an indication of the bias associated with the computed estimate of r_m. In all cases, the bias did not exceed 0.0017, indicating that the estimates of r_m were essentially unbiased. Standard errors for the r_m estimates were calculated using a bootstrap procedure also described by Dixon (1993). Confidence intervals were constructed using standard t-test methods.

RESULTS AND DISCUSSION

Mean values for the total number of nymphs produced per adult, nymphs per day, total lifespan, nymphal duration and adult lifespan are presented in Table 1. Within each generation, total nymphs per adult, mean nymphs per day, total lifespan and adult lifespan were significantly greater for greenbugs reared on TAM 105 than for those reared on TXGBE273. Nymphal duration was significantly shorter for greenbugs reared on TAM 105 than those reared on TXGBE273. In the third generation, greenbugs reared on TXGBE273 for two generations and then returned to TAM 105 (labeled as "Return" in Table 1) produced significantly fewer nymphs and had significantly shorter adult and

TABLE 1. Total Numbers of Nymphs per Adult, Mean Nymphs per Adult per Day, and Lifespan Parameters for Biotype E Greenbugs on Two Winter Wheat Lines.

Parameter	Generation						
	First		Second		Third		
	TAM 105	TXGBE273	TAM 105	TXGBE273	TAM 105	TXGBE273	Return [e]
				Mean ± (se)			
Total nymphs/adult	63.7 (2.5) a[a]	23.3 (1.7) b	61.7 (1.7) a	26.9 (1.8) b	55.8 (2.6) a	26.2 (2.0) c	47.3 (2.5) b
Mean nymphs/day	2.1 (0.1) a	0.9 (0.1) b	2.1 (0.1) a	0.9 (0.1) b	1.7 (0.1) a	1.0 (0.1) b	1.8 (0.1) a
Total lifespan [b]	33.2 (1.1) a	29.9 (1.1) b	34.0 (0.5) a	31.1 (1.1) b	33.0 (0.9) a	27.0 (1.4) c	30.0 (0.9) b
Adult lifespan [b]	29.0 (1.1) a	24.5 (1.1) b	29.7 (0.5) a	25.5 (1.1) b	28.9 (0.9) a	21.7 (1.4) c	25.6 (0.9) b
Nymphal duration [b]	4.2 (0.1) a	5.4 (0.2) b	4.3 (0.1) a	5.6 (0.2) b	4.1 (0.1) a	5.3 (0.2) b	4.4 (0.2) a
n_0 [c]	50	48	50	47	47	41	39
n_1 [d]	50	47	50	46	46	39	39

[a] Means followed by the same lower case letter in a row within a generation are not significantly different (LSD, p=0.05). For the first generation, $t = 1.99$, df=95; for second generation t=1.99, df=94; for third generation t=1.98, df=123.

[b] Days.

[c] Total greenbug nymphs in experiment.

[d] Number of original nymphs reaching the adult stage. This figure was used for statistical analyses.

[e] Greenbugs reared on TXGBE273 for two generations and then returned to TAM 105.

total lifespans than those reared exclusively on TAM 105 (47.3, 25.6, and 30.0 for TXGBE273 vs. 55.8, 28.9 and 33.0 for TAM 105, respectively). Mean nymphs per day and nymphal duration were equivalent to those of greenbugs reared on TAM 105 (1.8 and 4.4 for TXGBE273 vs. 1.7 and 4.1 for TAM 105, respectively). In comparison to greenbugs reared exclusively on TXGBE273, aphids in the "return" treatment produced significantly more nymphs (47.3 vs. 26.2), had a significantly higher mean number of nymphs per day (1.8 vs. 1.0), and had significantly longer total and adult lifespans (30.0 and 25.6 vs. 27.0 and 21.7, respectively) than those reared exclusively on TXGBE273. Nymphal duration was significantly shorter for the "return" greenbugs than those reared exclusively on TXGBE273 (4.4 vs. 5.3). Generally, the data collected from greenbugs reared on TXGBE273 for two generations and then returned to TAM 105 for one generation provided parameter estimates intermediate to those generated by data collected from aphids reared exclusively on TAM 105 or TXGBE273, and the differences were statistically significant in most cases. Significant differences were not found in the beginning numbers of nymphs in a treatment and the number surviving to the adult stage regardless of generation or host plant type.

Results similar to those described above were found in the estimates for r_m, net reproductive rate (R_0), and mean generation time (T) (Table 2). In each generation, the r_m estimate for greenbugs reared on TAM 105 was significantly larger than those reared on TXGBE273 (0.312, 0.305, 0.304 vs. 0.192, 0.182, 0.185). The r_m estimates for greenbugs reared on a given wheat genotype across generations were not significantly different for either TAM 105 or TXGBE273. Although a valid separation test is not available to evaluate R_0 and T, the values associated with these estimates followed the r_m estimates, an expected outcome because R_0 and T were derived from r_m. Greenbugs in the "return" category had an r_m estimate that was identical to the r_m estimate of greenbugs reared exclusively on TAM 105. Differences in the number of replications between Table 1 and Table 2 (n_1 and n, respectively) are due to removing data from the r_m analyses for greenbugs that reached the adult stage but did not produce nymphs.

As with the research reported by Lazar et al. (1995), our results indicated the winter wheat line TXGBE273 has a significant impact on greenbug biotype E lifespan and reproduction compared to greenbugs reared on the susceptible cultivar TAM 105. Because TAM 105 (genotype gb2gb2gb3gb3) and TXGBE273 (genotype Gb2Gb2Gb3Gb3) are closely related, but differ in the *Gb2* and *Gb3* loci, it is assumed these loci confer resistance that can be classified as antibiosis because reproduction and lifespan of greenbug feeding on TXGBE273 was deleteriously affected.

Although the difference in lifespan and reproduction was significant between the two genotypes, we found it interesting that at least through three generations, biotype E greenbugs responded to an individual genotype in a consistent manner, i.e., the reproductive rate and lifespan parameters did not differ across generations within a genotype. If the antibiotic effects imposed by TXGBE273 were cumulative, one would expect r_m to decrease through progressive generations. This was not the case, however, and when greenbugs reared on the resistant line for two generations were returned to susceptible TAM 105 for the third generation, they regained their reproductive capacities to the extent that the r_m estimates were equivalent to greenbugs reared exclusively on TAM 105 for three generations. Although "returned" greenbugs still produced significantly fewer total nymphs per adult than those reared exclusively on TAM 105, the average number of nymphs per adult per day was higher, although not statistically higher, because of the significantly shorter adult lifespan of aphids returned to TAM 105.

TABLE 2. Intrinsic Rate of Increase (r_m), Net Reproductive Rate (R_0) and Mean Generation Time (T) for Biotype E Greenbugs Reared on Two Winter Wheat Lines.

Parameter	Generation						
	First		Second		Third		
	TAM 105	TXGBE273	TAM 105	TXGBE273	TAM 105	TXGBE273	Return [d]
r_m [a]	0.312 a	0.192 b	0.305 a	0.182 b	0.304 a	0.185 b	0.304 a
Std. error [b]	0.018	0.014	0.014	0.016	0.010	0.018	0.010
R_0	60.5	23.5	60.6	24.4	54.2	21.7	46.6
T	14.1	17.3	14.3	17.9	13.8	17.0	14.5
n [c]	50	46	50	45	46	39	39
			Jackknife estimates [b]				
Pseudovalue	0.312	0.192	0.304	0.181	0.304	0.185	0.280
Bias	-0.0003	-0.0006	0.0017	0.0005	-0.0001	0.0005	-0.0007

[a] r_m estimates followed by the same letter within a generation are not significantly different by t-test, p=0.05.

[b] After Dixon 1993. Std. errors generated through a bootstrap method.

[c] Number of greenbugs surviving to adult stage.

[d] Greenbugs reared on TXGBE273 for two generations and then returned to TAM 105.

Estimates of R_0 and T for "returned" greenbugs indicated effects of TXGBE273 were stronger on greenbug reproduction than on lifespan. Where greenbugs reared exclusively on TXGBE273 averaged a 3-d increase (23%) in T vs. those reared exclusively on TAM 105, the "returned" greenbugs had only a 0.5-d (3%) increase in T vs. those reared exclusively on TAM 105. Moreover, while greenbugs reared exclusively on TXGBE273 had an average reduction in R_0 of 35 aphids/generation (60%) compared to those reared exclusively on TAM 105, the "returned" aphids had an average reduction in R_0 of 11.8 aphid/generation (20%). Therefore, the time to complete a new generation (T) recovered faster than the number of aphids produced per generation (R_0).

The results reported here suggest the kind of resistance exhibited by TXGBE273 would be very beneficial to integrated management of greenbugs in wheat. Such resistance has recently been incorporated into the first biotype E-resistant US wheat cultivar, 'TAM 110' (Lazar et al. 1997). Because the effects of the resistance we observed were not cumulative, greenbugs would persist in the field. However, the population would grow more slowly than greenbugs on TAM 105 due to an increase in T and a reduction in R_0. Theoretically, this would slow aphid damage to the plant because fewer individuals would be produced on TXGBE273 than on susceptible wheat cultivars in similar time periods. Also, natural enemies would have a better opportunity to keep greenbug abundance low on TXGBE273 than if the population was expanding at the rate allowed by TAM 105.

In regard to the role antibiosis plays in greenbug biotype development (see Porter et al. 1997 for a review of this topic), we do not believe our research encompassed sufficient generations to make conclusions confirming or rejecting the current theories.

Although our results are encouraging and clarify the effect of the *Gb3* locus on greenbug resistance in winter wheat, the research also brings into acute focus the need to determine how the resistance comes about, what process is initiated, compound produced, etc., that affects greenbugs. In addition, the research needs to be expanded beyond the laboratory stage to determine if these results hold up in the field and test the theoretical fit of TXGBE273 into an integrated management program.

ACKNOWLEDGMENTS

The authors thank C. M. Rush and S. R. Winter of the Texas Agricultural Experiment Station and C. D. Patrick of the Texas Agricultural Extension Service for their thoughtful reviews of the manuscript.

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COMPARATIVE EVALUATION OF A TRACTOR MOUNTED SAMPLER FOR SAMPLING ARTHROPODS IN COTTON

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ABSTRACT

Population estimates of selected arthropods including lepidopterous larvae, spiders, and adult and immature hemipterous and chewing predators in early season cotton, by sweep net, drop cloth, and a tractor-mounted sampler were compared. Population estimates by the tractor-mounted sampler were usually highly correlated to those provided by one or both of the other methods except for arthropods observed only at very low population levels. The tractor-mounted sampler most frequently detected presence of most arthropods at low population levels, and generally provided lower estimates of relative sample variation than sweep net or drop cloth methods. The need for modifications to the tractor-mounted sampler to improve the efficiency of collection of hemipterous nymphs is suggested. Our results indicate the tractor-mounted sampler provides an adequate means of sampling arthropods in early season cotton and may be particularly useful in monitoring arthropods occurring at low population levels.

INTRODUCTION

Recent experiences with secondary pest outbreaks associated with area-wide suppresion programs (Summy et al. 1996) have indicated a need to evaluate the impacts of early season insecticide applications on populations of beneficial arthropods in cotton. Important to such efforts is the ability to quickly and effectively sample relatively large areas; however, the sampling problem is further complicated by the low population densities of many arthropods encountered during the early season. Beerwinkle et al. (1997) have recently designed a tractor mounted sampler that was evaluated by Raulston et al. (1997) for efficiency of boll weevil collection from cotton. This same sampler may provide means for conducting studies of low population levels of other cotton arthropods. In 1996, we conducted an investigation of the effects of early season boll weevil insecticides on selected cotton arthropods (Sparks et al. 1997). To take advantage of the variation in arthropod population levels afforded by the various pesticide applications, the tractor mounted sampler was included along with the more conventional sweep net and drop cloth sampling techniques. The objective of this research was to assess relationships between the respective population estimates of selected arthropods obtained by sweep net, drop cloth, and the tractor mounted sampler.

MATERIALS AND METHODS

Samples were collected from four fields, ranging in size from 5.1 to 14.5 ha, located in the vicinity of Monte Alto, TX. Four or five plots of approximately equal size were established in each field and treated with different boll weevil insecticides to determine the effects of early season applications on beneficial arthropod populations (Sparks et al. 1997). Insecticides were applied aerially with the first application at about matchhead square stage. Two applications at 5- to 7-d intervals were originally planned, but excessive wind speed (>16 km/h) sometimes prevented completion of scheduled applications on a given day. All treatments were applied to field 1 on 30 April and again on 7 May 1996. First applications in fields 2 and 3 occurred on 8 and 11 May, and second applications were made on 14, 16, or 17 May 1996. First applications were made in field 4 on 7 May and second applications on 14 and 15 May 1996. Thus, treatments were applied at 5- to 8-d intervals. Our sampling evaluation sought to capitalize on the artificial manipulation of arthropod populations resulting from these applications.

Relative estimates of arthropod populations were obtained using a tractor-mounted sampler (Beerwinkle et al. 1997), sweep net, and drop cloth techniques. Four sample areas were designated within each plot, with plots arranged perpendicular to the rows. Sample areas for the tractor-mounted sampler were the center 10 of 20 outside rows on each end of each plot, and the center 10 of 20 rows in the center of each half of the remainder of each plot. Sample areas were 50 m long except when plots were too short, in which case sample areas were 25 m long and duplicate samples on each date were combined to yield a 50-m sample. On each sample date, one sample by the tractor-mounted sampler, one sweep net sample, and two drop cloth samples were collected from each designated sample area. Rows within each sample area that were sampled by the tractor-mounted sampler were selected randomly without replacement. Sweep net samples using a 38-cm diameter net (50 pendulum sweeps across the row per sample), and drop cloth samples using a 1-m^2 cloth, were collected on both sides and within a few rows of the tractor-mounted sampler areas. Sweep net and drop cloth samples were processed and recorded in the field. Tractor-collected samples were returned to the laboratory where larger pieces of plant debris were removed by a series of sieves (4-, 10-, and 100-mesh) and the contents of the 10- and 100-mesh sieves were examined under a dissecting microscope. Arthropods sampled included immatures of the bollworm/budworm complex (*Helicoverpa zea* [Boddie] or *Heliothis virescens* F.), loopers (*Trichoplusia ni* [Hübner]), and the beet armyworm (*Spodoptera exigua* [Hübner]); adults and nymphs of the minute pirate bug (*Orius* spp.), big-eyed bug (*Geocoris* spp.), and damsel bug (*Nabis* spp.); adults and larvae of the green lacewing (*Chrysoperla* spp.) and lady beetles (primarily *Hippodamia* spp.); and spiders.

Samples were collected before the first and second insecticide applications (usually on the day before application). Pretreatment samples were collected from all plots within a field on the same date regardless of the date of insecticide application. Samples within each field were also collected at 24 and 72 h after insecticide application, and additionally in field 1 at 7 d after the second application. Pretreatment sweep net samples were not obtained from field 1 because plants were too short for effective sampling. One sampling of field 4 by the tractor-mounted sampler was ommitted because of irrigation activities. Plant samples (20 plants per plot) were also collected weekly to determine plant height and numbers of fruit.

Repeated measures analyses of arthropod population levels among insecticide treatments failed to indicate significant differences among treatments (Sparks et al. 1997). Because population levels were often very low, samples were pooled to yield population estimates for each field on respective sample dates. When plots within a field were sprayed

on different dates, samples were pooled according to the time after treatment application rather than by calendar date. A total of 24, 24, and 25 pooled samples (tractor-mounted sampler, sweep net, and drop cloth, respectively) were obtained. Each pooled sample was composed of 16 or 20 samples for the tractor-mounted sampler and sweep net, and 32 or 40 samples for the drop cloth. Relationships between the sampling techniques for each taxon were assessed by correlating the mean number of arthropods per sample for each pair of methods on each sample date using the SAS procedure PROC REG (SAS Institute 1988). When the intercept was not significantly different from zero ($P>0.05$) and its omission did not inflate the mean square error, the correlation line was forced through the origin. The ranges of means of population estimates by each method as well as estimates of relative sample variation ($RV=[s_{\bar{x}}/\bar{x}]100$; Ruesink 1980) were calculated. Southwood (1978) suggested that an RV of 25 is sufficient for many sampling programs so we also determined the number of samples yielding an $RV \leq 25$ for each sample method.

RESULTS AND DISCUSSION

At the time of pre-treatment sampling, mean plant heights ranged from 18.8 (field 1) to 26.9 cm (field 3), and mean square populations (≥1/3-grown squares) ranged from 0.70 (field 3) to 0.98/plant (field 4). At the conclusion of sampling activities, mean plant heights ranged from 27.7 (field 2) to 36.3 cm (field 3), and mean square population levels ranged from 2.35 (field 2) to 6.98/plant (field 1). Blooms were present in all fields by the conclusion of sampling.

Relationships between population estimates of lepidopterous larvae by the different sampling methods were variable depending on the species and methods considered (Table 1). Relationships between population estimates of bollworm/budworm larvae by the different sampling techniques were statistically significant, but only samples by the tractor-mounted sampler and sweep net were strongly correlated. The relationship between population estimates of beet armyworm larvae by the tractor-mounted sampler and sweep net were significant but the correlation was relatively weak; estimates by neither of these methods were related to those by the drop cloth. Population estimates of looper larvae by all sampling methods appeared closely related. The correlation was strongest between estimates provided by the tractor-mounted sampler and drop cloth.

Population levels of bollworm/budworm and beet armyworm larvae were typically low, and only the tractor-mounted sampler provided RVs≤25 (Table 2). In contrast, the higher observed population levels of looper larvae resulted in some samples with RVs≤25 for all sampling methods, but this level of precision was obtained most often by the tractor-mounted sampler. The tractor-mounted sampler also tended to detect lepidopterous larvae more frequently (more samples with nonzero mean) than did the other methods.

Population estimates of hemipterous predators by the tractor-mounted sampler were strongly correlated to those by the drop cloth for adults but not for nymphs (Table 3). Population estimates by the sweep net method were statistically related to those by the tractor-mounted sampler for *Geocoris* and *Nabis* adults, but the correlation was strong only for *Geocoris* adults. Although the relationships between population estimates of *Orius*, *Geocoris*, and *Nabis* adults by the sweep net and drop cloth were significant, none of these correlations were very strong. Thus, the tractor-mounted sampler appeared more similar to the drop cloth than the sweep net in its ability to estimate populations of adult hemipterous predators. Failure to detect significant relationships between samples of nymphal *Orius* and *Nabis* may have been largely caused by the low population levels observed (Table 4). For these species, population levels of immatures were typically an order of magnitude less than for the

TABLE 1. Correlations of Population Estimates (Mean No./Sample) of Lepidopterous Larvae in Cotton from Tractor-Mounted (TM), Drop Cloth (DC), and Sweep Net (SN) Sampling Techniques.

Taxon	Comparison	F	df	P	Slope(±SE)	Intercept(±SE)	r^2
Bollworm/	TM vs DC	6.30	1, 22	0.02	4.99(1.99)	0.17(0.11)	0.223
Budworm	TM vs SN	80.86	1, 22	<0.01	4.05(0.45)	---	0.786
	SN vs DC	5.62	1, 22	0.03	1.03(0.43)	0.04(0.02)	0.203
Beet	TM vs DC	1.58	1, 22	0.22	4.91(3.90)	1.03(0.55)	0.067
Armyworm	TM vs SN	16.47	1, 22	<0.01	23.49(5.79)	---	0.428
	SN vs DC	1.34	1, 22	0.26	0.11(0.10)	0.04(0.01)	0.057
Looper	TM vs DC	367.65	1, 22	<0.01	21.17(1.10)	-0.77(0.42)	0.944
	TM vs SN	52.31	1, 22	<0.01	11.76(1.63)	---	0.704
	SN vs DC	51.59	1, 23	<0.01	1.22(0.17)	---	0.692

TABLE 2. Summary of Population Level (Mean No./Sample) and Relative Variation (*RV*) Estimates for Lepidopterous Larvae in Cotton Using Tractor-Mounted (TM), Drop Cloth (DC), and Sweep Net (SN) Sampling Techniques.

Taxon	Method	n[a]	Mean range[b]	*RV* range	No. *RV*≤25
Bollworm/	DC	9	0.025-0.219	33.94-100	0
Budworm	SN	8	0.050-0.375	38.30-100	0
	TM	11	0.050-1.688	18.52-100	1
Beet	DC	13	0.025-0.625	47.52-100	0
Armyworm	SN	13	0.050-0.150	53.75-100	0
	TM	21	0.050-9.562	19.45-100	2
Looper	DC	17	0.025-1.250	18.69-100	3
	SN	14	0.100-2.062	16.24-68.82	2
	TM	19	0.050-25.00	5.80-100	10

[a]Number of sample dates on which the mean > 0.
[b]Range of nonzero mean population levels.

respective adults. In addition, collection of soft-bodied insects by the tractor-mounted sampler often resulted in their dismemberment, and the collecting net (about 1 mm openings) may not have retained the smaller nymphs. Collection efficiency of hemipterous nymphs by this method may be improved by using a collecting net of finer mesh.

TABLE 3. Correlations of Population Estimates (Mean No./Sample) of Hemipterous Predators in Cotton from Tractor-Mounted (TM), Drop Cloth (DC), and Sweep Net (SN) Sampling Techniques.

Taxon	Comparison	F	df	P	Slope(±SE)	Intercept(±SE)	r^2
Orius Adult	TM vs DC	42.89	1, 20	<0.01	20.95(3.20)	-0.98(0.69)	0.682
	TM vs SN	0.51	1, 20	0.48	1.03(1.43)	1.81(1.04)	0.025
	SN vs DC	6.72	1, 22	0.02	0.35(0.14)	0.36(0.12)	0.234
Orius Nymph	TM vs DC	0.30	1, 20	0.59	-0.18(0.32)	0.02(0.02)	0.015
	TM vs SN	0.20	1, 20	0.66	-0.11(0.25)	0.02(0.02)	0.010
	SN vs DC	0.05	1, 22	0.82	-0.03(0.14)	0.03(0.01)	0.002
Geocoris Adult	TM vs DC	73.46	1, 23	<0.01	27.23(3.18)	---	0.762
	TM vs SN	101.52	1, 22	<0.01	7.68(0.76)	---	0.822
	SN vs DC	5.99	1, 22	0.02	0.68(0.28)	0.37(0.10)	0.214
Geocoris Nymph	TM vs DC	6.06	1, 22	0.02	4.32(1.76)	1.25(0.82)	0.216
	TM vs SN	0.74	1, 21	0.40	-9.90(11.51)	3.07(0.80)	0.034
	SN vs DC	1.49	1, 22	0.23	-0.04(0.03)	0.05(0.02)	0.064
Nabis Adult	TM vs DC	53.06	1, 23	<0.01	13.03(1.79)	---	0.698
	TM vs SN	12.43	1, 21	<0.01	1.69(0.48)	0.37(0.20)	0.372
	SN vs DC	8.34	1, 22	<0.01	2.02(0.70)	0.12(0.08)	0.275
Nabis Nymph	TM vs DC	1.25	1, 22	0.28	-0.54(0.49)	0.09(0.02)	0.054
	TM vs SN	0.01	1, 21	0.91	0.04(0.37)	0.08(0.02)	0.001
	SN vs DC	0.03	1, 22	0.86	0.05(0.28)	0.02(0.01)	0.001

The tractor-mounted sampler tended to detect adult hemipterous predators and nymphal *Geocoris* more frequently than other methods, and usually provided the largest proportion of samples with *RV*s≤25 (Table 4). None of the sampling methods provided this level of sampling precision for nymphal *Orius* or *Nabis*.

Population estimates by the tractor-mounted sampler and drop cloth, and the tractor-mounted sampler and sweep net, were closely related for chewing predators (Table 5). Relationships between samples by the tractor-mounted sampler and sweep net appeared somewhat stronger than those between the tractor-mounted sampler and drop cloth for adult chewing predators, and somewhat weaker for the respective larvae. Although samples of spiders collected by the drop cloth were significantly correlated to those collected by the tractor-mounted sampler and sweep net, the relationships were relatively weak. Population

estimates of lady beetle adults and larvae by the sweep net and drop cloth were less strongly related than either was to estimates by the tractor-mounted sampler. Samples of green lacewing larvae and spiders by the sweep net and drop cloth were signficantly correlated but the relationships were relatively weak, and a relationship between samples of green lacewing adults by these methods could not be demonstrated.

TABLE 4. Summary of Population Level (Mean No./Sample) and Relative Variation (*RV*) Estimates for Hemipterous Predators in Cotton Using Tractor-Mounted (TM), Drop Cloth (DC), and Sweep Net (SN) Sampling Techniques.

Taxon	Method	n[a]	Mean range[b]	*RV* range	No. $RV \leq 25$
Orius	DC	20	0.025-4.325	12.51-100	1
Adult	SN	20	0.050-2.550	14.22-100	3
	TM	21	0.050-14.750	16.72-100	5
Orius	DC	11	0.025-0.425	37.28-100	0
Nymph	SN	6	0.050-0.250	64.07-100	0
	TM	1	0.350	47.61	0
Geocoris	DC	21	0.025-1.550	14.42-100	1
Adult	SN	21	0.100-1.688	24.55-100	1
	TM	24	0.150-14.688	15.81-54.61	12
Geocoris	DC	19	0.031-1.350	23.53-100	2
Nymph	SN	8	0.050-0.250	49.20-100	0
	TM	23	0.100-12.600	23.05-72.95	3
Nabis	DC	15	0.025-0.375	22.77-100	1
Adult	SN	20	0.050-1.250	28.75-100	0
	TM	22	0.050-4.625	18.71-100	1
Nabis	DC	12	0.025-0.132	42.24-100	0
Nymph	SN	7	0.050-0.150	54.61-100	0
	TM	17	0.050-0.300	53.75-100	0

[a]Number of sample dates on which the mean > 0.
[b]Range of nonzero mean population levels.

The tractor-mounted sampler tended to detect green lacewing adults and larvae more frequently than did other methods, but the ability to detect spiders and adult and larval lady beetles was more similar between methods (Table 6). Except in the case of lady beetle larvae, the tractor-mounted sampler tended to provide population estimates with $RVs \leq 25$ more frequently than did other methods. Except for lady beetle adults, the sweep net least often provided this level of sampling precision.

TABLE 5. Correlations of Population Estimates (Mean No. per Sample) of Chewing Predators and Spiders in Cotton from Tractor-mounted (TM), Drop Cloth (DC), and Sweep Net (SN) Sampling Techniques.

Taxon	Comparison	F	df	P	Slope(±SE)	Intercept(±SE)	r^2
Green Lacewing Adult	TM vs DC	33.49	1, 22	<0.01	89.44(15.45)	0.56(0.46)	0.604
	TM vs SN	55.26	1, 22	<0.01	11.85(1.59)	---	0.715
	SN vs DC	3.23	1, 22	0.09	2.84(1.58)	0.10(0.05)	0.128
Green Lacewing Larva	TM vs DC	72.82	1, 22	<0.01	16.57(1.94)	0.54(0.30)	0.768
	TM vs SN	34.10	1, 22	<0.01	16.20(2.77)	---	0.608
	SN vs DC	8.59	1, 22	<0.01	0.44(0.15)	0.07(0.02)	0.281
Lady Beetle Adult	TM vs DC	67.07	1, 23	<0.01	23.93(2.92)	---	0.745
	TM vs SN	106.48	1, 22	<0.01	4.92(0.48)	---	0.829
	SN vs DC	27.78	1, 22	<0.01	2.59(0.49)	0.61(0.31)	0.558
Lady Beetle Larva	TM vs DC	87.80	1, 22	<0.01	32.37(3.45)	-5.60(3.59)	0.800
	TM vs SN	24.90	1, 21	<0.01	12.60(2.53)	6.82(4.76)	0.542
	SN vs DC	31.72	1, 23	<0.01	1.25(0.22)	---	0.580
Spider	TM vs DC	6.18	1, 22	0.02	3.24(1.30)	1.99(0.70)	0.219
	TM vs SN	3.04	1, 21	0.10	3.30(1.89)	2.52(0.72)	0.126
	SN vs DC	6.77	1, 22	0.02	0.30(0.12)	0.17(0.07)	0.235

In conclusion, population estimates by the tractor-mounted sampler appeared to be adequately related to those provided by one or both of the other methods except when population levels of the taxa being sampled were extremely low. When population levels were very low, detection of significant relationships between the tractor-mounted sampler and other methods was not only hampered by poor precision of the population estimates, but also by the frequency with which the tractor-mounted sampler detected certain taxa when the other methods did not. This pattern resulted in failure of many of the correlation lines to pass through the origin, and added considerable variability to some of the relationships.

The tractor-mounted sampler generally provided population estimates with lower *RV*s for most taxa than did the other sampling methods. The high frequency of detection and relatively high levels of apparent precision were likely facilitated by the large area sampled by the tractor-mounted sampler. We have observed that size of the area sampled by the tractor-mounted sampler can be manipulated with minimal impact on time and labor required to collect and process samples. Thus, it is possible that size of the area sampled can be adjusted to improve estimates of very low population levels of arthropods. This characteristic may offer a distinct advantage over other more commonly used sampling methods in studies where accurate estimates of spatial dispersion patterns are not required, especially if modifications to the sampler can improve its ability to collect immatures of hemipterous predators.

TABLE 6. Summary of Population Level (Mean No./Sample) and Relative Variation (*RV*) Estimates for Samples of Chewing Predators and Spiders in Cotton Using Tractor-Mounted (TM), Drop Cloth (DC), and Sweep Net (SN) Sampling Techniques.

Taxon	Method	n[a]	Mean range[b]	*RV* range	No. $RV \leq 25$
Green	DC	6	0.025-0.125	59.57-100	0
Lacewing	SN	13	0.050-1.000	25.82-100	0
Adult	TM	18	0.050-11.750	20.46-100	1
Green	DC	14	0.025-0.688	24.82-100	1
Lacewing	SN	14	0.050-0.438	35.95-100	0
Larva	TM	18	0.050-11.750	15.02-100	7
Lady	DC	24	0.100-2.275	12.19-59.91	4
Beetle	SN	24	0.125-4.150	12.12-68.31	9
Adult	TM	24	0.200-39.312	10.56-58.49	16
Lady	DC	23	0.025-2.700	13.47-100	6
Beetle	SN	16	0.050-8.350	29.10-100	0
Larva	TM	22	0.050-105.063	20.26-100	5
Spider	DC	25	0.125-1.300	14.30-58.62	8
	SN	23	0.050-0.700	24.59-100	1
	TM	24	0.800-7.625	9.73-34.03	17

[a]Number of sample dates on which the mean > 0.
[b]Range of nonzero mean population levels.

ACKNOWLEDGMENT

This article reports the results of research only. Mention of a proprietary product does not constitute an endorsment for its use by USDA.

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SUSCEPTIBILITY OF SOME VARIETIES OF *PHASEOLUS* SPP. TO *ZABROTES SUBFASCIATUS* (BOHEMAN)

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ABSTRACT

Fifteen varieties of bean, *Phaseolus* spp., were studied for relative susceptibility to the following effects of Mexican bean weevil, *Zabrotes subfasciatus* (Boheman): numbers of eggs laid, percentage of emergence (new progeny), damaged kernels, grain weight loss, and effect on germination. The sex ratio of progeny was also determined. The Tépari and Yorimuni varieties with the lowest numbers of laid eggs, new progeny, weight loss, and highest germination rates were the most resistant. Varieties Bolita Querétaro and Rio Grande were the most susceptible. *Z. subfasciatus* prefers small light-colored kernels such as Tépari and Yorimuni varieties.

INTRODUCTION

The Mexican bean weevil, *Zabrotes subfasciatus* (Boheman), has been reported to be one of the most important pests damaging stored beans, *Phaseolus* spp,. in the Sonora region of Mexico (Wong-Corral et al. 1992). Estimated losses have not been evaluated; however, farmers dislike storing beans due to the risk of attack by this pest. All known cultivated varieties of *P. vulgaris* are susceptible to attack by these weevils (Schoonhoven et al. 1982), but some wild varieties appear resistant to some species of bruchids (Schoonhoven et al.1983). Many species of beans, ranging from cultivated to wild varieties, occur in Sonora. The objective of this study was to determine the relative susceptibility of beans, *Phaseolus* spp., to attack by the Mexican bean weevil, *Zabrotes subfasciatus* (Boheman).

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MATERIAL AND METHODS

Bean varieties used in this study were collected in the southern locations of Navojoa and Ciudad Obregón, and in the central locality of Hermosillo in Sonora. The varieties were Pinto UI-111, Pinto UI-114, Flor de Mayo, Rio Grande, Negro Jamapa, Yorimuni, Tépari Más LCCG-30, Tépari Chihuahua 79, Canario 72, Ojo de Cabra, Peruano 87, Mayocoba, Bolita Quéretaro, Olate, and Pimono Azufrado. These varieties are commercially important and highly accepted by local consumers. All the samples were collected from the three locations mentioned above and were freshly harvested. Each sample was cleaned manually as well as in a Labofix, then stored at -5 to -10°C for one week to eliminate any possibility of internal infestation. Moisture contents of samples were determined using the AACC method 44-40 (American Association of Cereal Chemists 1995) and then adjusted to 12% either by adding water and/or by ambient drying. Samples were equilibrated under the experimental conditions for 14 days before the introduction of insects.

Mexican bean weevils used in this experiment were obtained as newly emerged adults from stock cultures maintained at the Entomology Laboratory of the University of Sonora. None of the insects were more than 2-days post-emergence when collected for use in these studies.

A 100-g sample of each bean variety was randomly placed in a circular tray (15 cm x 50 cm diameter) with 15 equally sized compartments. Fifty pairs (male and female) of 1-2 day old virgin adult weevils were released into the center of the tray. The tray was covered with muslin cloth and placed inside a controlled environmental chamber at 27±2°C and 70±5% relative humidity with alternating 12-hr illumination and 12-hr darkness. A 12-day period was allowed for the weevils to deposit their eggs on the grain; adults were then removed by sieving. Each bean variety was then transferred to a vial. Fifty randomly selected kernels were removed to determine the numbers of eggs laid on the grain surface. The vial with the remaining kernels was returned to the chamber until the first adult emerged. Newly emerged adults were removed by sieving, sexed and counted daily. After all adults had emerged, a random sample of 20 kernels was removed and the numbers of holes recorded to determine the percentage of damaged kernels. A tray with a sample of each variety and no insects was used as a control.

Grain weight loss was determined following the method described by Adams and Shulten (1978), which consisted of comparing the initial weight of sound grain and the final weight of infested samples.

A germination test was done on sound and infested kernels once the insect evaluations ended. Tests were conducted according to the rules of the International Seed Testing Association (ISTA 1966) in an automatic chamber with controlled conditions.

The experimental design consisted of a completely randomized block with three replications. Data were analyzed using the SAS general linear model; means were compared by the Tukey test to determine differences between varieties (SAS Institute 1985). A simple linear correlation was used to describe the relationship among variables evaluated.

RESULTS

The numbers of eggs deposited on each variety is reported in Table 1. The varieties were statistically different ($P \leq 0.05$). The highest numbers of eggs occurred on varieties Bolita Querétaro, Rio Grande, and Flor de Mayo; the fewest number of eggs were found in Yorimuni, Tépari MAS, and Tépari Chihuahua. This indicated that some varieties were more suitable, and thus more susceptible, for Mexican bean weevil oviposition than others. Varieties with low numbers of eggs were wild species; they were smaller than the others (Table 2) and also were lighter in color. Yetter et al. (1979), Gatehouse et al. (1987, 1989), Osborn et al. (1988), and Minney et al.(1990) have reported some of the resistance factors to be in the pericarp (color, smoothness, and chemical composition such the arcelins, amylase and trypsin inhibitors, and other secondary metabolites).

TABLE 1. Number of eggs laid on beans, emergence, damaged kernels, and weight loss caused by *Zabrotes subfasciatus* (Boheman)[a].

Variety	% Eggs laid	% Emergence	% Damaged kernels	% Weight loss
Pinto UI-111	22 abc	15 de	9.1 ab	14.0 cd
Pinto UI-114	84 abc	22 cde	31.0 cd	9.4 b
Olate	41 abc	36 bcde	19.1 bc	14.8 d
Flor de Mayo	105 abc	64 abcd	27.5 cd	13.2 cd
Rio Grande	134 ab	93 a	38.9 ab	18.7 cd
Jamapa	77 abc	76 abc	20.5 bcd	10.6 bc
Yorimuni	5 c	5 e	1.4 fg	6.6 a
Tépari MAS LCG-30	6 bc	3 e	0.9 ef	6.3 a
Tépari Chihuahua 79	11 bc	9 de	1.9 g	6.2 a
Canario 72	42 abcd	82 ab	21.1 de	17.8 cd
Ojo de cabra	42 abcd	52 abcde	31.9 ab	16.4 cd
Peruano 87	89 abc	71 abc	44.1 a	15.8 cd
Mayocoba	80 abc	65 abcd	31.0 cd	10.8 bc
Bolita Querétaro	149 a	73 abc	36.1 ab	16.2 cd
Pimono azufrado	77 abc	74 abc	29.4 cd	16.2 cd

[a]Within a column, means followed by the same letter do not differ significantly ($p>0.05$).

TABLE 2. Kernel size, sex ratio of the new adults emerged, and germination of *Phaseolus* sp. infested with *Zabrotes subfasciatus* (Boheman).

Variety	Kernel area (cm^2)	Sex rate ♀/♂	Germination[a]	
			Control	Infested
Pinto UI-111	9.4	2:1	87 a	77 ab
Pinto UI-114	9.2	1:1	83 a	78 a
Olate	9.1	1:1	85 a	79 a
Flor de Mayo	7.8	1:1	95 b	69 c
Rio Grande	6.2	2:1	81 a	64 b
Jamapa	7.2	1:1	87 ab	79 ab
Yorimuni	4.1	1:1	83 a	81 a
Tépari MAS LCG-30	4.5	1:1	84 a	83 a
Tépari Chihuahua 79	4.4	2:1	83 a	81 a
Canario 72	9.6	2:1	80 a	58 c
Ojo de cabra	9.3	2:1	95 b	48 d
Peruano 87	9.2	1:1	80 a	67 b
Mayocoba	9.0	1:1	80 a	76 a
Bolita Querétaro	8.1	3:1	81 a	71 ab
Pimono azufrado	6.1	2:1	80 a	61 c

[a]Within a column, means followed by the same letter do not differ significantly ($p>0.05$).

Results of emergence of new adult insects based on the numbers of eggs found on each variety after the infestation period are also presented in Table 1. Significant differences were found among the varieties ($P \leq 0.05$). Varieties such as Tépari MAS, Tépari Chihuahua, and Yorimuni, on which the lowest numbers of eggs were deposited also had the lowest progeny, whereas those varieties on which more eggs were laid (Bolita Querétaro, Rio Grande, and Flor de Mayo) had the highest percentages of emergence. A correlation coefficient of $r^2 = 0.86$ demonstrated a high relationship between numbers of eggs laid and percentage emergence. In the case of the small, white varieties (Tépari MAS, Tépari Chihuahua, and Yorimuni), we found many dead larvae which apparently were unable to penetrate the testa, an inability considered to be a resistance factor to bruchids. Tepari beans (*Phaseolus acutifolius*) were reported resistant to weevils by Cardona et al (1990), and this agrees with our results.

Results of numbers of kernels damaged are reported in Table 1. Variety Peruano, one characterized by an intermediate number of eggs (89) and emergence (71%) was highly damaged, followed by Rio Grande, Bolita Querétaro, and Flor de Mayo. Both Tépari varieties and Yorimuni were the least damaged varieties. The others varieties were intermediate. A correlation coefficient of $r^2 = 0.82$ indicated a strong relationship between percentage emergence and damaged kernels.

Table 2 also shows the sex ratio of progeny recovered from each variety. The highest ratio was three females for each male in variety Bolita Querétaro. Six varieties had a ratio of two females per each male, and the remainder of the varieties showed a one to one proportion. The varieties with a two females to one male ratio were the most severely damaged.

Percentage of weight loss is recorded in Table 1. Varieties Rio Grande, Bolita Querétaro, Peruano, along with Olate, Canario, Ojo de cabra, and Pimono azufrado had the greatest loss in weight. These results indicated that even though some varieties had fewer numbers of insects developing inside of them, the insects were able to feed more readily on them than in varieties with higher numbers of insects. This probably can be attributed to competition for food, repellency among the insects, or other factors affecting their behavior. The correlation coefficient between damaged kernels and weight loss was high ($r^2= 0.72$).

Germination values are given in Table 2. Variety Ojo de cabra, one of the most damaged had the highest reduction in germination (49.5%). Varieties Canario, Flor de Mayo, and Pimono azufrado also had low germination rates, indicating that the insect development affected the germ and thus its ability to germinate. Both Tépari varieties and Yorimuni had the highest germination rates and were least affected. Correlation coefficients between germination and percentage of emergence and germination with damaged kernels were $r^2= -0.47$ and $r^2= -0.60$, respectively. In the control tray, the weight loss, emergence, damaged kernels, and changes in germination were negligible throughout the duration of the experiment.

In conclusion, the larger seed varieties (Bolita Querétaro, Flor de Mayo, Pimono azufrado, Ojo de cabra and Rio Grande) appeared more susceptibility to the Mexican bean weevil. We found a clear relationship between size and color of the kernels with susceptibility to attack; small, white varieties were more resistant than large, colored ones. There was a high correlation between new progeny and damaged kernels and also between weight loss and damaged kernels. Germination was affected by insects, and there was a high negative correlation with damaged kernels. The highest sex ratio was three females for each male in variety Bolita Querétaro; the varieties characterized by a two to one sex ratio were those that were more damaged. A number of varieties can be considered resistant to the Mexican bean weevil; however, the mechanisms of resistance have not yet been studied. Biochemical tests currently are being developed in our laboratory to determine the factors responsible for the results presented herein.

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RESISTANCE IN A LOUISIANA STRAIN OF *CULEX QUINQUEFASCIATUS* TO SELECTED FYFANON® FORMULATIONS [1, 2, 3]

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ABSTRACT

Two Louisiana strains of *Culex quinquefasciatus* (Denham Springs and Lake Charles) were held in screened cages mounted on stakes and exposed to ground ULV applications of malathion from a truck-mounted cold fog generator. Three formulations of malathion were evaluated in the field tests: 1) Fyfanon® ULV (95% malathion); 2) Mix (95% malathion + 0.5% natural pyrethrum + 2.5% piperonyl butoxide); and 3) Ready To Use (RTU) 4606 (95% malathion + 1.0% unsynergized natural pyrethrum). Significantly greater mortality ($p<0.05$) was observed in the Denham Springs strain at 1, 12, and 24 hr post-treatment for all formulations as compared with the Lake Charles strain. With the exception of the 24 hrs post-treatment for the Denham Springs strain, the Mix formulation provided significantly greater ($p<0.05$) adult control at all post-treatment evaluation periods for both mosquito strains. At 24 hrs post-treatment for the Denham Springs strain, Fyfanon provided as effective control as the Mix formulation (93% and 95%, respectively). No test formulation provided effective control beyond 30.5 m from the spray route. Although initial knockdown was poor, malathion and the Mix formulations did provide excellent control at 24 hrs post-treatment.

INTRODUCTION

Aerial and ground-applied adulticides continue to be important components of mosquito management programs. Since organophosphates, in particular malathion, have long been used in mosquito abatement programs, continuous studies are necessary to evaluate product effectiveness and to monitor status of pesticide resistance in target pest and vector mosquito populations. In addition, studies also are warranted to determine ways in which to improve insecticide effectiveness. A field study in this regard was conducted during the summer of 1996 to determine the efficacy of adding natural pyrethrum and piperonyl butoxide to malathion to enhance this agents effectiveness in controlling *Culex quinquefasciatus* Say adults.

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[1] Diptera: Culicidae

[2] Manuscript approved by the Director of the Louisiana Agricultural Experiment Station as manuscript no. 97-17-0207.

[3] Mention of a commercial product does not constitute recommendation or endorsement by the State Agricultural Experiment Stations of Arkansas or Louisiana.

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MATERIALS AND METHODS

The three formulations of malathion were assessed as to which one provided the best initial knockdown and overall mortality for two strains of *Cx. quinquefasciatus* adults. One strain of *Cx. quinquefasciatus* evaluated in the field tests was collected from Lake Charles in Calcasieu Parish (southwest Louisiana), and the other strain was collected from Denham Springs in Livingston Parish (southeast Louisiana). The adult mosquitoes were collected using battery-powered, hand-held aspirators (Meek et al. 1985) and battery-powered, backpack aspirators equipped with screened collection cups as developed by the U.S. Department of Agriculture Center for Medical, Agricultural, and Veterinary Entomology in Gainesville, Florida.

Captured adult mosquitoes were lightly anesthetized with carbon dioxide, and groups of about 20 *Cx. quinquefasciatus* of both strains were transferred to individual 10.2 x 25.4 cm (4 x 10 in), cylindrical screened cages (Sandoski et al. 1983) used in the field tests. The caged adult mosquitoes were held at room temperature (22°C or 72°F) and at 40% Rh in the Calcasieu Parish Mosquito Abatement Department laboratory until being placed in the field and exposed to adulticide applications.

Ground ULV applications were conducted in three widely-spaced plots in a grass-covered air strip located near the Lake Charles airport. Each plot consisted of three rows of three 1.5 m (5 ft) high stakes (i.e., 3 x 3 pattern). Rows were situated perpendicular and downwind to the spray route and separated from one another by 30.5 m (100 ft). The 1.5 m (5 ft) high stakes within each row were set at 30.5, 61.0, and 91.4 m (100, 200, and 300 ft, respectively) downwind from the spray route. Selected environmental parameters were measured to ensure that appropriate conditions for pesticide spraying were present (e.g., wind speed and direction, air temperature and temperature inversion).

Prior to the adulticide evaluations, screened cages of both *Cx. quinquefasciatus* strains, used as the untreated adult controls, were placed on the stakes within the test plot for 10 min, removed, and transported to the laboratory. The untreated mosquitoes were anesthetized again with carbon dioxide and transferred to clean 237 ml paper cans with screened lids (= half pint ice cream containers). A cotton pad (2 cm^2) moistened with a 10% sucrose solution was placed on each screened lid for adult sustenance. The untreated mosquitoes were maintained in the nearby laboratory facilities of the Calcasieu Parish Mosquito Abatement Department (22 °C and 40% Rh). The mosquitoes were check for mortality at 1, 12, and 24 hrs.

Immediately prior to the each pesticide application, cages of the Denham Springs strain mosquitoes were attached to all stakes within the plots. Due to the limited number of mosquitoes available from Lake Charles at the time of the field tests, cages of this strain were paired with cages of the Denham Springs strain only along the center row of stakes.

Three insecticide formulations were evaluated for effectiveness against the two strains of *Cx. quinquefasciatus*: 1) Fyfanon® ULV (95% malathion); 2) Mix (95% malathion + 0.5% natural pyrethrum + 2.5% piperonyl butoxide); and 3) Ready To Use (RTU) 4606 (95% malathion + 1.0% unsynergized natural pyrethrum). A truck-mounted LECO 1600® ULV cold fog generator equipped with a 8663 spray head and nozzle was used to apply the insecticide to the cage test plots. All applications were made at a ground speed of 24.1 kph (15 mph), using a total flow rate of 177.4 ml/min (6.0 oz/min), and calibrated to deliver a 15μ mmd droplet. Ground ULV tests of the Mix and the RTU formulations were replicated four times each, while the Fyfanon tests were replicated twice.

Ten minutes after each pesticide treatment, the treated cages of mosquitoes were collected from the plot and transported to the laboratory. The treated mosquitoes were anesthetized and transferred to clean paper cans with sucrose pads as described previously for

control mosquito adults. Post-treatment mortality for untreated and treated adults were observed at 1, 12, and 24 hrs. Mean mortality data were corrected by Abbott's formula (Abbott 1925) and arcsine transformed for subsequent analysis of variance using GLM (SAS Institute 1985). Mean separation, where appropriate, was determined using LSD (SAS Institute 1985).

RESULTS AND DISCUSSION

Mortality at 1, 12, and 24 hrs post-treatment are presented in Table 1. Overall, significantly greater ($p<0.05$) mortality was observed in the for the Denham Springs strain as opposed to the Lake Charles strain of *Cx. quinquefasciatus* ($F=66.8$; $df=1, 359$; $p<0.05$), indicating organophosphate resistance in the latter population. In addition, statistical analysis by individual mosquito strain indicated a significant two-way interaction ($p<0.05$) between formulation and downwind distance for both the Denham Springs ($F=8$; $df=4, 269$) and Lake Charles strains ($F=10.2$; $df=4, 89$).

TABLE 1. Mean percent mortalities for two Louisiana strains of *Culex quinquefasciatus* adults exposed in cages to three ground ULV-applied formulations of malathion.

Post-treatment (hrs)	Formulation type	Mean Percent Mortality [a, b]					
		Lake Charles strain Distance downwind (m)			Denham Spring strain Distance downwind (m)		
		30.5	61.0	91.4	30.5	61.0	91.4
1	Fyfanon®	0 [b]	0 [b]	0 [b]	6.2 [bc]	3.1 [bc]	0.7 [c]
1	Mix	38.8 [a]	3.3 [b]	4.8 [b]	68.2 [a]	32.0 [b]	15.2 [bc]
1	RTU	9.9 [b]	1.9 [b]	2.8 [b]	24.3 [bc]	22.1 [bc]	17.5 [bc]
1	Untreated	0	0	0	0	0	0
12	Fyfanon®	21.4 [b]	16.6 [b]	6.8 [b]	78.2 [b]	54.2 [bcd]	44.7 [cde]
12	Mix	54.8 [a]	8.9 [b]	6.2 [b]	93.8 [a]	51.4 [bc]	26.2 [cde]
12	RTU	11.0 [b]	1.9 [b]	5.0 [b]	31.0 [cde]	22.1 [de]	17.5 [e]
12	Untreated	0	0	0	0	0	0
24	Fyfanon®	29.3 [b]	16.6 [bc]	6.8 [bc]	93.6 [a]	62.8 [b]	58.4 [bcd]
24	Mix	61.1 [a]	10.6 [bc]	8.0 [bc]	95.1 [a]	56.4 [bc]	28.4 [cde]
24	RTU	14.6 [bc]	1.9 [c]	6.4 [bc]	33.2 [bcde]	25.6 [de]	20.0 [de]
24	Untreated	0	0	0	0	1.3	1.4

[a] Mean separation conducted on Abbott corrected mortality data, with untreated mortality provided only for comparison.
[b] Means for time and strain followed by the same letter are not significantly different ($p<0.05$).

Mortality at 1 hr post-treatment was significantly greatest ($p<0.05$) for both the Denham Springs and Lake Charles strains exposed to the Mix formulation at a distance of 30.5 m downwind from the insecticide release point (68.2% and 38.8%, respectively). No other statistical differences in mortality were noted relative to formulation or distance for the Lake Charles strain. Furthermore, adult mortalities resulting from exposure of the Lake Charles strain to the Fyfanon treatment were not observed to differ from the untreated control mosquito mortalities at any exposure distance. Similarly, adult exposure to Fyfanon was significantly

less effective at 91.4 m against the Denham Springs strain (0.7%), as compared with all other treatments. Intermediate control occurred for all other formulation and distances and did not exceed 32 % (Table 1).

Mortality rates at 12 hrs post-treatment illustrate increased control by both Fyfanon and the Mix treatments against both mosquito strains. In contrast, mortality from exposure to the RTU treatment only slightly increased at a distance of 30.5 m over that observed at 1 hr post-treatment. Again, significantly greater mortality for the Lake Charles and Denham Springs strains was observed relative to exposure to the Mix treatment at a distance of 30.5 m (54.8% and 93.8%, respectively). Mortalities for the Lake Charles strain at other formulations and distances ranged from 1.9% to 21.4%, but were not significantly different ($p>0.05$). In contrast, significantly lower control of the Denham Springs strain resulted from exposure to the RTU formulation at a distance of 91.4 m. Again, all other formulation/distances provided intermediate control, ranging from 22.1% to 78.2%.

Mortalities for both species at 24 hrs post-treatment were greater over those observed at 12 hrs post-treatment. Significantly greater control of the Lake Charles strain was once again observed relative to the Mix treatment at 30.5 m. Mortalities for the other formulations and distances ranged from 1.9% to 29.3% but were not significantly different. In contrast to results at 1 and 12 hrs post-treatment, both Fyfanon and Mix treatments provided significantly greater control to the Denham Springs strain at a distance of 30.5 m with mortality rates of 93.6% and 95.1%, respectively, and these mortality rates did not significantly differ from each other. Mortality rates for the other formulations and distances ranged from 20.0% to 62.8% with no statistical difference among treatments.

Overall, results of this investigation indicate more effective control of adults exposed to Fyfanon or the Mix formulations containing malathion and natural pyrethrums as opposed to RTU regardless of the *Cx. quinquefasciatus* strain involved. Fyfanon and Mix formulations both provided excellent control at 24 hrs post-treatment, although initial 1 hr post-treatment control was poor. While the addition of natural pyrethrum and piperonyl butoxide had some effect on final mortality for the Denham Springs strain, it did not improve the effectiveness of malathion against the apparently resistant Lake Charles strain. Acceptable levels of adult control were only observed at a distance of 30.5 m downwind from the spray route irrespective of formulation or mosquito strain involved. These results indicate that formulations, mixtures, and other factors such as droplet size require additional investigation.

ACKNOWLEDGMENT

The authors express appreciation to Lucas Terracina (Calcasieu Parish Mosquito Abatement Department in Lake Charles, LA) and his staff for their cooperation in these tests. The authors also thank Kimsey Whitmore for her typing skills.

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INFLUENCE OF SOYBEAN PUBESCENCE ON ABUNDANCE OF BEET ARMYWORM, *SPODOPTERA EXIGUA*[1]

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Beet armyworm, *Spodoptera exigua* (Hübner), is a polyphagous insect of many wild and cultivated host plants throughout the world. Historically, beet armyworm larvae occurred on late planted seedling soybean plants in the Mississippi delta, resulting in little or no economic damage. However, during the last several years, beet armyworm larvae have been present during late season, feeding on foliage, flower buds, flowers, and young fruit, causing economic damage to soybeans (L. Lambert, unpublished data). Plant pubescence imparts to soybean resistance to some major defoliating insect pests (Lambert et al. 1992). This study was conducted to compare the abundance of beet armyworm larvae on glabrous, normal, and densely pubescent soybean isolines.

Glabrous, normal, and densely pubescence isolines developed from 'Davis' were used. Numbers of trichomes per unit area on the abaxial surface of 'Davis' leaves were previously reported by Lambert et al. (1992). Pubescence on the abaxial leaf surface of 'Davis' normal and dense isolines averaged 165 and 437 trichomes cm^{-2}, respectively; no trichomes occurred on leaves of the 'Davis' glabrous isoline. The isolines were planted in a field in 12-row by 53.34-m-long plots during the last week of May 1994. A randomized complete block design with three replications was used. Beet armyworms began ovipositing on soybeans within these plots during the last week of August, and larval abundance was assessed on 31 August. All plants in two, 0.61 m of row were collected from each replication for each isoline. Each plant collected was examined for beet armyworm larvae. Numbers of beet armyworm larvae were compared among soybean isolines. Data were analyzed by using PROC GLM (SAS 1988).

Significantly fewer beet armyworm larvae occurred on normal and densely pubescent isolines than on the glabrous isoline (Table 1); no significant differences ($P > 0.05$) occurred among replications and samples within replications. These results are in direct opposition to the behavioral responses of the corn earworm, *Helicoverpa zea* (Boddie) (Lambert and Kilen 1989). When corn earworm moths were given a choice among the three isolines, 57, 31, and 12 % of eggs were deposited on densely pubescent, normal, and glabrous isolines, respectively. Differences in infestations between these two moth species may have resulted from differences in the way females of these lepidopteran species

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oviposit eggs. Beet armyworm females, that oviposit eggs in a cluster, may have difficulty depositing eggs on leaves with trichomes. Corn earworm females deposit eggs singularly and may be able to deposit eggs on leaves with pubescence. In conclusion, this study showed that more beet armyworms infested the glabrous soybean isoline than the normal or densely pubescent isolines.

TABLE 1. Numbers of *Spodoptera exigua* Occurring on Glabrous, Normal, and Densely Pubescent 'Davis' Soybean Lines.[a]

Soybean Isoline	Mean number *Spodoptera exigua*/m of row
Glabrous	68.86 a
Normal	1.64 b
Densely pubescent	0.27 b

[a] Within a column, means not followed by the same letter are significantly different (GLM, [$P < 0.05$] by LSD).

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FIRST RECORD OF THE AMARANTH BORER FLY *AMAUROMYZA ABNORMALIS* (MALLOCH) (DIPTERA:AGROMYZIDAE) IN MEXICO

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The amaranth borer fly (ABF), *Amauromyza* (*Cephalomyza*) *abnormalis* (Malloch), was recorded for the first time in North America, the United States, in Washington, D.C. in 1903. It was described by Malloch in 1913 as *Agromyza abnormalis*. Subsequently, ABF was detected in California, Iowa, and Kansas in the United States as well as in Ontario and Quebec in Canada (Spencer 1981, Spencer and Steyskal 1986); thus, it has been considered a Nearctic species. The ABF collected from Montecillo, Edo. de Mexico, and San Miguel del Milagro, Tlaxcala, is the first record of this species in Mexico, extending its distribution into the Neotropical Region. Specimens collected in the localities above were determined by one of us (MvT) by dissecting the male genitalia. *Amauromyza abnormalis* is black and the length of the body is about 3 mm. Spencer (1981) and Spencer and Steyskal (1986) give complete morphological descriptions of the species.

In Mexico, *Amauromyza abnormalis* is an important pest of cultivated amaranth (*Amaranthus hypocondriacus* L. and *Amaranthus cruentus* L.), although its impact on these species has not been evaluated. Amaranth is an ancient crop harvested since Pre-Columbian times as one of the basic protein sources of the New World (Anonymous 1984). Althoug this crop is a nutritions dietary component, both as green vegetable and grain, its insect pest and diseases are poorly documented (Anonymous 1984, Espitia 1990, Gonzalez and Alejandre 1992, Vejar-Cota et al. 1994). Agromyzidae known to develop on cultivated *Amaranthus* species are the cosmopolitan and polyphagous leaf miners *Liriomyza bryoniae* (Kaltenbach), *Liriomyza brassicae* (Riley), and *Chromatomyia horticola* (Goureau), the North American oligophagous leaf miner *Haplopeodes minutus* (Frost), and the stem borer *Melanogromyza amaranthi* Spencer & Havranec in Venezuela (Sweetman 1928). Insect species from other families boring in amaranth stems in Mexico are *Herpetogramma bipunctalis* F. (Lepidoptera:Pyralidae) collected in Altepexi, Miacatlán, and Tehuacan in the state of Puebla and Huazulco in the state of Morelos (Vejar-Cota et al.1994); *Lixus truncatulus* F. (Coleoptera:Curculionidae); and a unidentified species collected in Chapingo, in the state of Mexico, and Ixtacuixtla and San Miguel del Milagro, in the state of Tlaxcala (Espitia 1990). This latter species could not be identified with certainly because specimens were not available, but the description and type of damage agree with ABF which we collected in Montecillo (5 Km from Chapingo) and San Miguel del Milagro.

The amaranth borer fly also has been found living in stems and roots of different species of *Amaranthus* and *Chenopodium* (Malloch 1913, Spencer 1981, Spencer and Steyskal 1986), although the status of the latter as a host is doubtful. A previous study (Bautista-Martínez et al. 1995) showed that in locations where ABF was collected in

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Mexico, *Scrobipalpa atriplicella* (Roeslerstamm) (Lepidoptera:Gelechiidae) was the only stem borer of *Chenopodium nuttalliae* Saff. and *Chenopodium album* L; no other species were found on these hosts.

At Montecillo and San Miguel del Milagro, we observed that ABF damaged both species of cultivated amaranth *Amaranthus hypochondriacus* and *A. cruentus*, both domesticated in Mexico and Guatemala (Anonymous 1984). *Amaranthus cruentus* was more heavity damaged than *A. hypochondriacus*. We also observed the widespread weed *A. hybridus* to be a wild host.

The behavior of ABF is similar to that of other borers like *S. atriplicella* as confirmed by Bautista-Martínez et al (1995). The larva penetrates the stems in the upper part of the plant, constructing galleries directed toward the base of the plant and damaging the vascular system. In addition to the direct damage done by insect, diseases can enter the plant via the entrance hole of the larvae. Espitia (1990) indicated that it is possible to find 20 or more larvae in a plant, without any apparent damage; however, under severe attack, a high percentage of plants can wither and subsequently die. Pupation occurs inside the stem (Spencer 1981), although we suspect that some pupate in the ground. *Amauromyza abnormalis* spends most of the winter in the pupal stage like other Diptera. Large numbers of ABF were collected from August to September 1996. During these collections we detected two parasitic wasp species, of the familiy Eucoilidae (Cynipoidea) and Braconidae (Ichneumonoidea); eucoilids emerged more frequently.

In view of the potential and growing importance of amaranth as a crop in Mexico and elsewhere, the importance of ABF as a pest should be investigated and monitoried. In addition, this biology, habits, distribution, and natural enemies of the insect should be studied.

AKNOWLEDGMENT

We thank M.C. Roberto Bernal for collecting amaranth plants in the field. We also are grateful to Drs. Jorge Luis Leyva Vazquez and Setephen D. Koch for an reviewing of this manuscript.

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SOUTHWESTERN ENTOMOLOGICAL SOCIETY

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BOLL WEEVIL, *ANTHONOMUS GRANDIS* BOHEMAN (COLEOPTERA: CURCULIONIDAE): A SUMMARY OF RESEARCH ON BEHAVIOR AS AFFECTED BY CHEMICAL COMMUNICATION[1]

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ABSTRACT

A summary of 30 years of extensive research on semiochemicals as they affect the boll weevil, *Anthonomus grandis* Boheman, in cotton is presented. Traps and the pheromone grandlure in particular have greatly extended our knowledge of the behavior of this economically important insect and will be key factors in eliminating this insect from the United States in the 21st century.

INTRODUCTION

Almost 30 years ago, the boll weevil, *Anthonomus grandis* Boheman (Coleoptera: Curculionidae), was termed the $10 billion insect (Dunn 1964). Assuming an annual loss of $300 million due to boll weevil and an estimated annual $70 million expenditure to prevent even greater losses (Knipling 1964), at least an additional $10 billion was lost due to the boll weevil in the ensuing 30 plus years. Assuming also that these figures have declined over the last ten years due to improvements in diapause control, pinhead square applications, use of pheromone traps, and boll weevil eradication programs, it would be safe to say that the boll weevil can now be accurately dubbed the "$20 billion insect." At one point it was estimated (Rainwater 1962) that about one-third of all insecticides used for agricultural purposes was applied for boll weevil control; this figure has now declined due to several factors which relegated the boll weevil to the third most important insect in U.S. cotton production in 1991 behind the cotton aphid, *Aphis gossypii* Glover, and the bollworm/budworm (*Helicoverpa/Heliothis*) complex (Hardee and Herzog 1992).

Over the years, however, it has been speculated that the extensive use of insecticides for boll weevil control has not only resulted in serious environmental contamination, but also caused a drastic reduction of natural enemies leading to an increase in populations of other pest species such as the bollworm/budworm complex, aphids, spider mites, and whiteflies. This, in turn, resulted in increased crop losses and intensive use of insecticides to protect the affected crops.

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

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Considerable research over the last quarter century has been directed toward the development of methods to reduce insecticide application for boll weevil control or to eliminate the pest from all infested cotton growing areas in the United States (National Cotton Council 1994). Semiochemicals, primarily pheromones, have shown much promise in manipulating populations of the boll weevil. General reviews of literature on the boll weevil are available (Dunn 1964, Mitlin and Mitlin 1968, Cross 1973, Parencia et al. 1985, Smith and Harris 1994). This discussion, however, is concerned only with reviewing reported findings on the behavior of the boll weevil as affected by chemical communication.

DETERRENTS AND REPELLENTS

There are contrasting views in the literature concerning the presence of a deterrent produced by female boll weevils after oviposition in a square. Early reports (Hunter and Pierce 1912) suggested that the number of offspring per female increased directly as the number of eggs laid one per square. Mitchell and Cross (1969) reported that females searched squares before oviposition and usually did not oviposit in a square already containing an oviposition puncture. Jenkins et al. (1975), however, suggested that the boll weevil cannot discriminate against egg-punctured squares. The fact remains, however, that most punctured squares in nature contain only one egg puncture but may have multiple feeding punctures (Mitchell and Cross 1969). More recently, McGovern et al. (1987) reported that female boll weevils failed to oviposit in 81% of squares that contained ovipositional punctures, and suggested that both physical and chemical signals were involved in this behavior.

Maxwell et al. (1963a) reported a boll weevil repellent from the volatile substances in cotton, but no additional testing or commercial value of the product has been reported. Hardee and Davich (1966) found a substance that deterred feeding of the boll weevil in the filtrate of a water extract of tung meal, a byproduct of the process of extracting oil from the seeds (nuts) of the tung tree, *Aleurites fordii* Hemsl. The active ingredient was later identified (Martin Jacobson, USDA-ARS, retired) and was commercialized as Bollex® which was recommended as a spray to deter the boll weevil, especially in the western cotton-growing areas of the United States. Tests in Mississippi (Hardee, unpublished) revealed no effectiveness of treatment of cotton with this product in reducing feeding or oviposition punctures.

FEEDING STIMULANTS

The possibility of utilizing baits for boll weevil control has been considered since Hunter and Hinds (1905) considered that boll weevils might be trapped with "sweets." However, not until reports by Keller et al. (1962), Maxwell et al. (1963b), and Jenkins et al. (1963) that extracts of parts of cotton and other plants contain an arrestant and feeding stimulant for the boll weevil did a series of reports appear on development of the bait principle for boll weevil control. There were reports that: (1) a feeding stimulant added to an insecticide more than doubled weevil mortality compared to an insecticide alone (Ridgway et al. 1966); (2) cottonseed oil was also a source of these materials (Daum et al. 1967); and (3) the addition of pathogens to the mixture (McLaughlin 1967, McLaughlin et al. 1968, 1969) showed promise in boll weevil control. McKibben et al. (1989) recently reported that extracts of squares of all sizes elicit greater feeding responses than all other plant parts. To date none of these has appeared in commercial use.

Subsequently, however, Wright and Chandler (1989a, 1990, 1991a, 1991b, 1992) and Wright (1993) reported development of a feeding stimulant, pheromone, and fungus (*Beauveria bassiana*) mixture (Naturalis®) for control of boll weevils. Frank and Slosser (1990) report that

this product had an insignificant effect on winter survival of boll weevils when applied in the fall. (Naturalis® is commercially available from Troy Biosciences, Phoenix, AZ.) In addition, McKibben, Smith, and co-workers (McKibben et al. 1990a, 1990b, 1991, 1992, 1993; Wolfenbarger and McKibben 1991; Smith et al. 1992; McGovern et al. 1993; Villavaso et al. 1993), report the use and commercial development of an attract-and-kill device for boll weevils, called "bait cone" or "bait stick" containing a feeding stimulant, pheromone, and toxicant, and which they recommend for management of overwintered boll weevils. (This product is currently available from Plato Industries, Houston, TX.) Later tests of this device by Fuchs and Minzenmayer (1992) and Parker et al. (1995) in Texas and Karner and Goodson (1993, 1995) in Oklahoma showed that bait sticks failed to reduce percentage punctured squares and numbers caught in monitoring traps, but more importantly, under the conditions of the test described by Fuchs and Minzenmayer (1992), fields with bait sticks had more boll weevil punctured squares than adjacent fields without bait sticks. The real commercial value of both of these bait devices remains to be demonstrated.

PLANT VOLATILES

Chemists at the USDA-ARS, Boll Weevil Research Unit at Mississippi State, MS, identified a multitude of volatiles associated with steam distillates of the cotton plant in a lengthy series of papers beginning in 1962 (e.g., Minyard et al. 1969, Gueldner et al. 1970, Thompson et al. 1970, others reviewed by Hedin et al. 1973). None of these reports, however, resolved the long-standing question of whether the boll weevil finds cotton through a sense of attraction and orientation to odors, since steam distillates of cotton plants may bear little or no resemblance to volatile profiles emitted by insect, plants, or plants fed upon by boll weevils (J. C. Dickens, pers. comm.). Indeed, Hunter and Pierce (1912) suggested that the boll weevil does not respond to plant odors but finds cotton through aimless flight of high numbers of individuals. Parencia et al. (1964) found no difference in numbers of emerging boll weevils found in 1, 5, or 20 cotton plants or in other crops. Mistric and Mitchell (1966) and Mitchell and Taft (1966) concluded that migrating boll weevils were attracted to caged and uncaged cotton plants. However, because the cotton had infestations of feeding boll weevils, the migrating weevils could have been attracted to pheromone produced by male boll weevils feeding on the cotton (Hardee et al. 1969b). Roach et al. (1971b) concluded that cotton trap crops were not as effective as wing traps baited with male boll weevils for survey or control of boll weevil populations and 1972 numbers of weevils that were captured around fields with wing traps were the same with or without cotton. Results of studies by Taft et al. (1969) confirmed the absence of long-range olfactory stimuli from the cotton plant, but Rummel et al. (1975) and White and Rummel (1978) maintain that overwintered and migratory weevil colonization in cotton appears initially to be a positive response to squaring cotton. Pieters (1976) reports positive response of boll weevils to fall trap crops, but this response was increased by the addition of pheromone. Hardee et al. (1971b) showed that addition of a cotton plant attractant to pheromone markedly increased attractiveness in the laboratory, but Hardee (unpublished) was never able to demonstrate this increased attractiveness in the field.

Unquestionably, cotton produces a short-range attractant for boll weevils. Keller et al. (1965) extracted a substance attractive to boll weevil from the thawed ice from a freeze-drying apparatus for lyophilizing cotton seedlings, squares, and bolls (Keller et al. 1963). Hardee et al. (1966c) developed a laboratory bioassay procedure for studying plant attractant substances which showed that (1) a water extract of cotton squares was highly attractive to laboratory-reared boll weevils, (2) several potential and real alternate host plants were attractive to boll weevils in the laboratory (Parrott et al. 1969, Maxwell et al. 1969), (3) unsexed

laboratory-reared weevils, 4-5 days old, fed on 10% sucrose-water solutions before testing gave the most reliable and reproducible response to plant attractant fractions (Hardee et al. 1966b), and (4) the organs of boll weevils that receive olfactory stimuli are located in the club of the antenna (Hardee et al. 1966a).

More recently, Chang et al. (1986) studied the attraction of cotton terpenes to boll weevils in the laboratory, but some evidence that boll weevils in nature respond to cotton plant volatiles was presented by McKibben et al. (1977) when they showed that volatile oil from steam distillates of cotton seedlings was attractive to emerging overwintered boll weevils and to late-season migrating weevils in the field. Eleven years later, Benedict and Chang (1988), Dickens (1989, 1990a, 1990b), and Dickens et al. (1990) reported the enhancement of insect pheromone response in the boll weevil by the addition of cotton volatiles (β-caryaphyllene, (E)-2-hexen-1-ol, and (E)-β-ocimene) to the pheromone mixture as measured by trap captures. To date, none of these volatiles have found their place in commercial usage.

PHEROMONES

Perhaps no field crop insect in the world has been studied more in the past 100 years than has the boll weevil. Much of what we know has been determined in the past 30 years since Cross and Mitchell (observed in 1962, published in 1966) and Keller et al. (1964) confirmed that the male boll weevil produces a wind-borne sex attractant (pheromone) that is attractive to females (Mitchell and Cross 1971). Subsequently, Cross and Hardee (1968) demonstrated for the first time, Bradley et al. (1968) confirmed, and Hardee et al. (1969b, 1970a) showed in detail that the male pheromone is not only a sex pheromone for females but also acts as an aggregating pheromone for both sexes, primarily in the spring and fall, and to a lesser degree in mid-season. Over the next 25 years scores of scientists studied the behavior, chemistry, management and elimination of the boll weevil in minute detail, all made possible by the pheromone and trap system developed during that time. These findings have culminated in the use of traps and pheromones as a very integral part of the National Boll Weevil Eradication Program in progress (National Cotton Council 1994). The following is a review of our findings with pheromones and traps since 1962.

In the earliest record of sexual attraction in the boll weevil, Hunter and Hinds (1905) concluded that females were not attractive to males and that "... instead of seeking widely for the females, the males are content to wait for them to come their way." After the reports of sex attraction by Cross and co-workers, Hardee et al. (1967a) developed a laboratory bioassay for use in pheromone isolation studies with chemists (Tumlinson et al. 1968). This bioassay procedure was used to: (1) confirm female attraction to males; (2) show that isolated males were more attractive and isolated females were more responsive than either sex caged together as groups of males or females; (3) demonstrate that sterile males were equally as attractive as untreated males when both were fed cotton squares (flower buds) as food (Bartlett et al. 1968); (4) reveal that peak sexual activity of both males and females occurred when weevils were 4-6 days old; (5) show that females responded to a single male, but response was significantly greater to 5, 10, or 25 males; (6) elucidate that virgin males were twice as attractive and virgin females were 3 times as responsive as mated males or females; and (7) reveal from comparisons between laboratory-reared and native weevils that food was of greater importance than culture in determining female response (Hardee et al. 1967b).

Additional diet studies in the laboratory and field in 1968-69 (Hardee 1970) showed that: (1) males fed cotton squares, bolls, and blooms were considerably more attractive than males fed terminals, cotyledons and leaves; (2) pheromone production by males was reduced by about 50% and 90% 1 hour and 24 hours, respectively, after food was removed; (3) males survived

well and produced pheromone in laboratory bioassays on a variety of foods (50-70% as much as on cotton squares) such as apples, bananas, okra, peaches, and string beans, but the most favorable diet was cotton squares; and (4) overwintered male boll weevils survived longer without food than laboratory-reared males, but both needed some food before pheromone production began. In field tests, however, Cross et al. (unpublished data) were not able to show response to male boll weevils fed on any diet except cotton. The results indicated that a constant supply of adequate food, preferably cotton squares or small cotton bolls, was essential to continued production of a high level of pheromone by males.

Simultaneously, the laboratory bioassay procedure facilitated the isolation (Tumlinson et al. 1968), identification, and synthesis (Tumlinson et al. 1969, 1971, Zurfluh et al. 1970) of the four components [(+)-*cis*-2-isopropenyl-1-methylcyclobutaneethanol, (1), Z-3,3-dimethyl-Δ^1-cyclohexaneethanol (2), Z-3,3-dimethyl-$\Delta^{1,\alpha}$-cyclohexaneacetaldehyde (3), and E-3,3-dimethyl-$\Delta^{1,\alpha}$-cyclohexaneacetaldehyde (4), were identified as the components of the male sex pheromone of the boll weevil] of grandlure, the name assigned to the pheromone of the male boll weevil (Hardee et al. 1971b). Subsequently, a great deal of effort was made to improve synthesis of the grandlure components (Billups et al. 1973, Gueldner et al. 1972, 1974), to study factors in the laboratory that influence activity of grandlure (Hardee et al. 1971b), to study the oxidative degradation of grandlure in storage (Henson et al. 1975), to develop a gas chromatographic procedure that would permit analysis of the four components with a single injection (Bull et al. 1971), and to develop a slow-release formulation of grandlure that would remain competitive with square-fed males in traps for at least one week (Hardee et al. 1972a). Hardee et al. (1971b) determined that: (1) inert firebrick was an effective carrier to use in laboratory bioassays; (2) grandlure was attractive to females at dosages as low as 5×10^{-6} but was most attractive at 2.5 to 50 nm; and (3) addition of a cotton plant extract to grandlure markedly increased its attractiveness. Bull et al. (1971) devised a gas chromatographic procedure that accurately detected concentrations as low as 20 ng of each of the four components of grandlure in a single injection. McKibben et al. (1971) developed a polyethylene glycol tablet formulation of grandlure that showed no decrease in activity after aging 128 hours under simulated field conditions. Hardee et al. (1972a) reported that a formulation of grandlure containing glycerol, water, polyethylene glycol, and methanol impregnated on cigarette filter was more than 80% as competitive as an attractant for seven days with caged, live males, fed cotton squares once or twice per week. They also showed that grandlure attracted boll weevils in sex ratios similar to live males, indicating that it evokes the aggregating response from both sexes as do live males. Subsequent to the development of this formulation, Moody et al. (1972) and McKibben (1972, 1974) developed devices for dispensing grandlure automatically to cigarette filters. Beginning in 1971, grandlure in this formulation was used with great success by 28 investigators representing eight agencies in seven states across the Cotton Belt (Hardee, unpublished) which made possible the beginning of widespread studies on the boll weevil utilizing traps and pheromones.

Subsequent to the above developments, Hedin et al. (1972) investigated all of the volatile constituents of the boll weevil to identify possible additional components of the pheromone, but the makeup of the commercial pheromone today remains as described by Tumlinson et al. (1969). Mitlin and Hedin (1974) were unable to find any specific precursor in the cotton plant for biosynthesis of the pheromone. Hedin et al. (1974) estimated the lifetime production of pheromone by a male boll weevil at 50 mg with no apparent decrease in production due to sterilization (Hedin et al. 1975, McGovern et al. 1975, 1976a) which Klassen and Earle (1970) had shown earlier. Huddleston et al. (1977) were able to inhibit communication in boll weevils in the laboratory with high concentrations of grandlure but failed to do so in the field. Gueldner and Wiygul (1978) found a rhythmic release of pheromone in male boll weevils held in a

continuous 16:8 light:dark 24-hr regime. In total darkness, pheromone release was decreased ten-fold. Hedin et al. (1979) reported compounds isolated from frass of female boll weevils showed some attraction for males in the laboratory but were unattractive in the field.

The effectiveness of males or extracts of males in different kinds of traps in capturing virgin, released females (Cross et al. 1967, 1969, Hardee et al. 1969a) was evaluated in 1965-66 in several field studies in Mississippi, Florida, and Mexico. It was concluded that a wing-type trap coated with an adhesive was the most effective trap for boll weevils. Hardee et al. (1969a) concluded from their field tests that: (1) males in close proximity to females were no more attractive to females than isolated males; (2) females responded to males as many as three times and from distances of as much as 250 feet; and (3) the high percentage of females captured in traps baited with males in the absence of competing males, and the low percentage captured with males in traps in an infested plot containing large numbers of competing males suggest, that the sex pheromone might have a major role in suppressing boll weevils in areas where populations are extremely low. This could occur in the spring after an effective fall diapause-control program has substantially reduced the number of competing overwintering male boll weevils. They also confirmed results from previous tests in showing that: (1) laboratory-reared males were as attractive and females were as responsive as native weevils if they had access to cotton squares (flower buds) as food; (2) isolated males were more attractive than grouped males; (3) the lack of response of recently mated females emphasized the need to capture females in traps before they mate with free, competing males; and (4) sterilization of males did not significantly decrease their attractiveness compared with untreated males. After the development (Cross and Hardee 1968) and use of the wing trap for boll weevils, several improvements in trap design were made in subsequent years including the Leggett trap (Leggett and Cross 1971), the in-field or Mitchell trap (Mitchell and Hardee 1974a, 1976b), and the Hardee (Story) trap (discussed by Mitchell and Hardee 1974a, Hardee 1976a). Additional designs or modifications of the above have been evaluated (Cross et al. 1971, Leggett et al. 1975, Leggett and Cross 1976, Harp and Turner 1976, White et al. 1977, McKibben 1977, Leggett 1979, Lopez 1980, Dickerson et al. 1981, 1987, Dickerson 1986a, 1986b, Rummel et al. 1987, Leggett and Dickerson 1989, Hendricks 1990), but the only two commercially available traps now in use in eradication, survey, or management programs are the Hardee traps (Hardee 1976a) (available from Consep Membranes, Bend, OR) or a modified in-field trap (Mitchell 1971; Mitchell and Hardee, 1974a, 1976b) (available from the Southeastern Boll Weevil Eradication Foundation, Inc., Montgomery, AL, or Hercon Environmental Co., Emigsville, PA). Recent studies by Hardee et al. (1996) showed that all three traps are equally effective in trapping boll weevils.

Subsequent to the identification and synthesis of the pheromone, grandlure (Tumlinson et al. 1969, 1970), and confirmation of its effectiveness in nature (Hardee et al. 1971b), considerable effort on the part of many people was devoted to the development of slow-release formulations. Beginning with the original cigarette-filter formulation (Hardee et al. 1971b, McKibben et al. 1971) and its 1-2 week activity, steps were undertaken to determine optimum ratios of grandlure components (Hardee et al. 1974a) and to find improved, less expensive, and longer-lasting formulations of grandlure (McKibben et al. 1971, 1972, 1974; Bull 1976; Bull et al. 1973a, 1973b; Coppedge et al. 1973; Hardee et al. 1974b, 1975a; Johnson et al. 1976b; Merkl 1977; Merkl et al. 1978; McKibben et al. 1980; Ridgway et al. 1987; Leonhardt et al. 1988, 1990; Leggett et al. 1989). In addition, compounds which are relatively stable mimics of the aldehyde components of grandlure may be used to provide longer-lasting lures to monitor boll weevil populations (Dickens et al. 1991). From a commercial standpoint, the laminated plastic dispenser described by Hardee et al. (1975a) remains to date the one used by most researchers, extension personnel, consultants, producers, and personnel with the Boll Weevil

Eradication Program and Mississippi Boll Weevil Management Program (available from Hercon Environmental Co., Emigsville, PA).

From their theoretical calculations on the effects of pheromones used for insect control, Knipling and McGuire (1966) concluded that under the right set of conditions, pheromones offer great potential in insect suppression. This potential of the pheromone and traps in survey, control, and eradication of the boll weevil (Hardee 1981, 1982) was studied in detail with wing traps (Cross and Hardee 1968) baited with males in west Texas in 1968 and 1969 (Hardee 1969; Hardee et al. 1970b, 1971a; Hardee 1974; Boyd et al. 1973a), in Mississippi in 1969 (Lloyd et al. 1972a) and in South Carolina in 1968 and 1969 (Taft et al. 1972). Conclusions from these studies were that: (1) live male boll weevils in traps afforded 60-80% suppression of boll weevils in the spring following an effective reproduction-diapause control program in the previous fall; and (2) one or two traps per acre were more effective than four or eight traps per acre in suppressing boll weevils. Subsequent studies of this type with more efficient traps such as the Leggett trap (Leggett and Cross 1971) or the smaller in-field trap (Mitchell and Hardee 1974a, 1976b) baited with grandlure (Hardee et al. 1975b; Mitchell et al. 1976, 1977; Rogers et al. 1976; Lloyd et al. 1980, 1981, 1983, 1985; Dickerson 1986b; Leggett et al. 1988a) proved the traps to be extremely effective at reducing numbers of boll weevils in fields, especially where beginning populations of overwintered weevils were 25/acre or less. Mitchell et al. (1976) captured 76% of a population of boll weevils with ten in-field traps/acre. Taft and Hopkins (1978) were able to suppress low or high populations of boll weevils with 10-80 traps/ha and suggested that traps be combined with other techniques in an integrated control program. These results collectively formed the basis for the current use of grandlure-baited Mitchell traps in boll weevil eradication (Coppedge and Ridgway 1973, Eden 1973, Frisbie 1976).

Several studies in the 1970's showed the potential effectiveness of grandlure in conjunction with the systemic insecticide, aldicarb, in a trap crop system for suppressing low density populations of overwintered boll weevils (Lloyd et al. 1972b, Boyd et al. 1973b, Scott et al. 1974, Gilliland et al. 1974a, 1974b, Rummel et al. 1976, Weaver 1982, Burris et al. 1984, Moore and Watson 1988, Chiles and Chiles 1990), but this system proved too costly and cumbersome for use in boll weevil eradication. Trap crops continue to be recommended for boll weevil control by the Louisiana Cooperative Extension Service.

For several years (beginning in 1967), males or grandlure in traps made possible the investigation of various behavioral activities (Hardee 1972) of the boll weevil which have led to a much clearer understanding of boll weevil ecology (Cherry 1974, Rummel 1984). From 1967 to 1972 pheromone traps were used: (1) in survey and ecological studies (Cross 1976; Bottrell et al. 1970, Carroll et al. 1993, Walker and Bottrell 1970, Roach et al. 1971b, Roach and Ray 1972, Beerwinkle et al. 1996, Mitchell et al. 1972, Moody et al. 1993); (2) in capture-mark-release studies (Price and Slosser1983); (3) in demonstrating that boll weevils will disperse up to 45 miles in search of cotton or other boll weevils (Davich et al. 1970; Ridgway et al. 1971; Johnson et al. 1975, 1976a); (4) in showing that treatment of male boll weevils with a chemosterilant does not reduce pheromone production (Klassen and Earle 1970, Earle et al. 1978); (5) in obtaining a positive correlation between the number of overwintering weevils captured and the number observed in the field (Roach et al. 1971a); (6) in demonstrating the effective substitution of traps for woods trash examinations for surveying potential populations of boll weevils (Hollingsworth et al. 1977); and (7) in determining that a metal wing trap (about 4 x 6 inch wings x 9 inch base) painted daylight fluorescent yellow over a white undercoat, coated with an adhesive, containing live male boll weevils or synthetic pheromone, and placed around a cotton field adjacent to overwintering sites at distances of 1-3 feet above ground was the most effective trapping procedure to date (Hardee et al. 1972b).

Considerable effort has been put forth in attempts to determine the relationship between time of entry of boll weevils into diapause, and presumably overwintering sites, and time of emergence from overwintering. Taft et al. (1973) suggested no relationship between the time boll weevils enter hibernation sites, their winter survival, or their subsequent emergence the following spring. In Mississippi, however, Mitchell et al. (1973) captured, marked, and re-released over 35,750 boll weevils from August to December with a different color scheme every two weeks. Their results indicated a tendency for weevils entering diapause early to emerge late next year and those entering diapause late in season to emerge early next year. Their results also indicated, and equally as important, that most marked weevils were recaptured in May, regardless of when they were marked, and the highest percentage re-captured were marked in October. Wade and Rummel (1978) and Rummel and Carroll (1983) obtained a different trend in showing that diapausing weevils which enter overwintering habitat in west Texas during the latter half of the season tend to emerge later the following growing season than boll weevils which enter overwintering sites early. All studies, however, show the importance of fall diapause programs in reducing September/November populations of boll weevils for lower emerging populations in the next growing season.

One of the most obvious potential uses of the pheromone trap has always been in predicting the need for overwintered and in-season boll weevil control (i.e., determining the need for pinhead square or in-season applications based on numbers of weevils per trap per unit area per unit of time). McClendon et al. (1976) produced a computer simulation model for estimating the efficiency of a boll weevil pheromone field trapping system. In Mississippi, Mitchell (1978) proposed the use of pheromone traps to provide an index for control decisions. Rummel et al. (1980) developed a pheromone trap index system for predicting need for overwintered boll weevil control in west Texas which was more accurate than field scouting in flagging overwintered weevil infestations before oviposition size squares were present. Seven states (AR, AZ, GA, MS, OK, SC, TX) use pheromone traps to recommend treatment thresholds and identify problem fields (Ridgway et al. 1985, Johnson and Gilreath 1982, Henneberry et al. 1988). Benedict et al. (1985) developed trap index thresholds in the lower Gulf Coast of Texas, and suggested a method for their use in the management of overwintered boll weevils and damage to commercial cotton. The Mississippi Cooperative Extension Service (Anonymous 1997) recommends treatment of cotton at first square if "... pheromone traps placed 1 per 20 acres accumulate 4 weevils per trap over the 4 weeks prior to squaring..." USDA-APHIS, The Southeastern Boll Weevil Eradication Foundation, and the Texas Boll Weevil Eradication Foundation utilize a series of trap indices for determining the need for diapause, pinhead square, and in-season control for boll weevils in the Boll Weevil Eradication Program. Witz et al. (1981) used a computer simulation model to show that probability of catch in traps may be increased 10 to 20% by adjusting in-field traps away from the usual uniform spacing throughout a field to field borders where weevils are more likely to land first. Carroll and Rummel (1985) in west Texas showed that pheromone traps are reliable indicators of relative abundance and activity of recently emerged overwintered boll weevils. Leggett et al. (1988b), in South Carolina, reported equal reliability but showed considerable variability of emergence and response among years and among fields within a year. They were also unable to show that degree-days are an appropriate time scale for predicting boll weevil emergence. On the other hand, Stone et al. (1990) were able to develop a degree-day model of boll weevil spring emergence and survivorship in the Rolling Plains of Texas, and suggested that Leggett et al. (1988b) failed to do so because they did not measure the influence of overwintering mortality on timing.

As the usage of pheromone traps in the late 1960's and early 1970's began to spread and increase in importance, the question of efficiency became increasingly important. Known

factors influencing trap efficiency are height above ground level, size, color, location, density, design of traps, grandlure concentration and ratio of the four grandlure components, and weevil diet (Hardee et al. 1972b, Lopez 1978, Lopez et al. 1978, Leggett 1980, Johnson et al. 1982, Leggett and Cross 1978, Leggett and Moore 1982). Ridgway et al. (1976) report efficiencies ranging from 12 to 93%, depending mostly on boll weevil density and trap design (Bottrell and Rummel 1976b, Rummel et al. 1977b). Walker (1984) determined that at population levels of 100 overwintered boll weevils per acre at least 400 row-ft of cotton must be sampled for 95% certainty of detection. Hardee (1976b) and Hardee and Boyd (1976) calculated that traps baited with grandlure were eight times as effective as whole-plant examinations in detecting very low density boll weevil populations. Because of their efficiency in detecting populations of boll weevils, Whitcomb and Marengo (1986) used traps to suggest that no weevils were found in Paraguay until 1990 (P. L. Morel, Cadelpa, Asuncion, Paraguay, pers. comm.). Snodgrass et al. (1979) determined that Leggett traps (Leggett and Cross 1971) captured 51 and 59% of responding boll weevils in two separate tests. Leggett et al. (1981) predicted that 10 in-field traps/ha would detect at least one of the progeny of one overwintered female per 2-ha field at least 99% of the time. McKibben and Cross (1984) developed an equation to estimate probability of zero populations in North Carolina in 1979-1980 based on trap usage. Several other studies (Leggett and Cross 1976, Leggett 1979, Lopez 1980) compare trap designs without determination of percentage capture efficiency.

Mitchell et al. (1972) found that spring temperatures affected emergence of overwintered weevils, but rainfall had more influence as temperatures increased. In their study of male-baited wing traps to evaluate overwintering habitats of boll weevils in the Rolling Plains of Texas, Bottrell et al. (1972) elucidated preferred overwintering sites and found most of them within 0.5 mile of cotton fields. Villavaso and Earle (1974) showed that reproducing male boll weevils and their frass were two to four times as attractive as diapausing males and their frass. Mitchell and Hardee (1974b) found that weevils captured in traps during most of the year do not accurately represent the physiological state of the total population at that time, but they are most representative during the months of April, May, and June. Mitchell et al. (1975b) successfully sterilized a small percentage of emerging overwintered weevils with pheromone and chemosterilant-laced diet with bait stations. Mitchell et al. (1975a) provided considerable evidence that attractiveness of male boll weevils is closely related to passage of food through the midgut and the resulting frass. Mitchell and Hardee (1976a) showed that long range movement of boll weevils was primarily with the wind, and that they are unable to detect odors from a distance greater than 600 ft. They also found that grandlure will attract weevils over a greater distance than a particular color, but in close range, color becomes more important. Bottrell and Rummel (1976a) were able to manipulate with pheromone the distribution of hibernating boll weevils in the fall in two out of three different sites. McGovern et al. (1976b) determined that overwintered male boll weevils produced less than 20% as much pheromone as laboratory males (McKibben et al. 1976). Merkl and McCoy (1978) captured boll weevils in Mississippi every month for five years except one; the largest numbers were captured in October and the smallest in March. Boll weevils respond to colors in the 500-525-nm (yellow-green) region of the spectrum (Hardee et al. 1972b, Roach et al. 1972, Leggett and Cross 1976, Cross et al. 1976), and the greatest response was obtained with highly reflective daylight fluorescent pigments that had this spectral characteristic.

In the lower Rio Grande Valley of Texas, Wolfenbarger et al. (1976a, 1976b) captured more boll weevils in March, the time of plant emergence, than at any other time of year except for the August-September period. In the same area, Guerra (1983, 1986) and Guerra and Garcia (1982) captured 90% of the total weevils captured between July and November. In addition, Guerra (1988) determined that weevils flew over 160 miles in seven weeks in September and

October. Wright and Chandler (1989b) found the greatest numbers of boll weevils in traps in south Texas occur in the brushy non-cotton habitat north of the Lower Rio Grande Valley, with highest activity occurring prior to square production in the spring. In the Lower Gulf Coast of Texas, Segers et al. (1987) caught reproducing boll weevils in traps all year, and diapausing insects almost every month, but the latter were highest from July to February; the numbers and state of diapause were more closely related to the availability of fruiting cotton than to weather. However, Jones and Sterling (1979) found a strong relationship between temperature and emergence of boll weevils from overwintering sites; the temperature threshold for flight of 95-98% of newly emerging weevils was 20°C. Fuchs and England (1989), and Fuchs and Minzenmayer (1990) showed a significant relationship between winter severity and number of weevils captured in traps. Rummel and Bottrell (1976a, 1976b) found that a seasonal decline in response to pheromone was not due to presence of pheromone-producing males in cotton but was regulated by unknown factors. Rummel et al. (1977a) found that emerging overwintered weevils fly at low levels (ground level - 4.6 m) in the spring, but late season, migratory weevils fly at higher levels (near ground level - 100 m), indicating the latter's tendency for long-range migratory flights toward new breeding and overwintering areas. Pieters and Urban (1977) detected boll weevils in traps on an isolated island 3.5 miles from cotton.

Hopkins et al. (1977) and Hollingsworth et al. (1977) found a positive correlation between surface woods trash examinations and pheromone traps in measuring overwintered weevil numbers, and they suggested that traps be used instead of woods trash examinations (Pfrimmer and Merkl 1981) as an easier and more effective sampling tool for boll weevils. Leggett and Taft (1979) and Leggett (1982) showed that traps baited with higher doses of grandlure remained effective for up to six weeks without rebaiting and captured more boll weevils than traps rebaited weekly with a lesser dosage. In cotton fields not planted to cotton the previous year, Leggett and Roach (1981) detected boll weevils with traps 35 days before infestations were found by the square sampling technique. Their data also indicated that weevils migrate mainly in the fall. Villavaso et al. (1983) showed that irradiated, diflubenzuron-fed males produced less pheromone than untreated and other sterilized males, but these same authors (1989) also showed that sterile boll weevils were 82% as competitive as native Arizona males. Hopkins et al. (1983) collected weevils in South Carolina that were sufficiently in diapause from August through November to survive the winter, but color of trap had no influence on percentage diapause. Bariola et al. (1984) demonstrated that infested bolls buried under 15 cm of soil in cages allowed no boll weevils to emerge, and that decline in numbers of weevils caught in traps occurred at about the same time that emergence from bolls was complete. Villavaso and McGovern (1986) reported that males of a laboratory-reared strain of boll weevils were more attractive than native males, suggesting that if less rigorous sterilizing methods were found, a highly competitive sterile weevil could be produced. Rummel et al. (1978) used pheromone traps to detect boll weevils feeding on *Hymenapappus* sp. Casteraceae) in Texas. Pheromone traps allowed Benedict et al. (1991) to show that boll weevils captured in traps in south Texas and northeastern Mexico contained pollen from 12 families of plants, suggesting potential feeding on a wide range of plant species. Jones et al. (1992) utilized pheromone traps in studying dispersal, host plants, and seasonal activity in northeastern Mexico. They also used pheromone traps to determine that captured weevils in northeastern Mexico contained pollen grains from 14 plant families (Jones et al. 1993). Sobrinko et al. (1992) recently reported the use of pheromone traps to study the distribution and behavior of boll weevils in Brazil. Entomologists from Texas recently produced a series of studies describing in detail the use of trap data in developing prediction methods and geographic information systems in the High and Rolling Plains of Texas (Fuchs 1993, Haldenby 1993, Rummel 1993, Slosser 1993, Stone et al. 1993, Trichillo 1993, Wilson et al. 1993).

CONCLUSIONS

From over 220 published reports of over 30 years research on semiochemicals affecting boll weevils by over 200 scientists representing 30 agencies, we can extract the following major findings which have strongly affected our knowledge of the boll weevil, the direction of our research efforts, and today's attempts to manage and even eliminate this pest entirely from the United States:

(1) Repellents and deterrents are known for the boll weevil but to date have not found commercial entry.

(2) Feeding stimulants and the pheromone, grandlure, are currently being combined with a pathogen and an insecticide to produce Naturalis® and the bait stick, respectively, as potential control measures for the boll weevil, but neither is being widely used on a commercial basis.

(3) The identification, synthesis, and formulation of the male-produced pheromone, grandlure, for both sexes, and the development of effective, mass-producible, and inexpensive traps made possible acquisition of the following important information: (a) emerging overwintered males must feed on cotton for 3-5 days to produce the pheromone; both overwintering males and females respond to the pheromone without feeding; and the same pheromone is attractive to boll weevils wherever they occur in the world - all important factors in detecting, managing, and eliminating the insect; (b) an individual female will make multiple responses to the pheromone, responses occur from insects up to 600 ft downwind from the pheromone source, boll weevils will move at least 45 miles from their origin, and most weevils enter hibernation in September and October in the fall and emerge in May and June; (c) such strong positive correlation occurs between numbers captured in traps and numbers infesting cotton that a prediction method of the need for pinhead-square applications is now available for Arizona, Arkansas, Georgia, Mississippi, Oklahoma, South Carolina, and Texas; (d) because traps baited with grandlure are at least eight times more efficient in detecting low level populations of boll weevils than hand sampling, grandlure-baited traps are the key element in determining success or failure of the current boll weevil eradication program; and (e) finally, the development of the boll weevil trapping system has not only made it possible to monitor, detect, and predict boll weevil populations, but traps and lures are now being used to manage the boll weevil with less insecticide usage and to eliminate the insect from parts, and eventually all, of the United States - all of which represents a prime example of the goal of research - recognize a problem, conduct research to find a solution, and transfer the technology for ultimate usage by American agriculture.

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AUTHOR INDEX TO VOLUME 22

UNITED STATES POSTAL SERVICE™

Statement of Ownership, Management, and Circulation

(Required by 39 USC 3685)

1. Publication Title	2. Publication Number	3. Filing Date
SOUTHWESTERN ENTOMOLOGIST	3 2 4 - 0 5 0	Sept. 17, 1997
4. Issue Frequency	**5. Number of Issues Published Annually**	**6. Annual Subscription Price**
Quarterly(March, June, Sept., October)	4	$10.00 individual $20.00 Institution

7. Complete Mailing Address of Known Office of Publication *(Not printer) (Street, city, county, state, and ZIP+4)*

Southwestern Entomological Society c/o Allen Knutson
Texas A&M University, College Station, TX 77843

Contact Person: Allen Knutson
Telephone: 972-952-9222

8. Complete Mailing Address of Headquarters or General Business Office of Publisher *(Not printer)*

Southwestern Entomological Society, c/o Allen Knutson
17360 Coit Road, Dallas, TX 75252

9. Full Names and Complete Mailing Addresses of Publisher, Editor, and Managing Editor *(Do not leave blank)*

Publisher *(Name and complete mailing address)*

Southwestern Entomological Society, c/o Darrell Bay
Department of Entomology, Texas A&M University, College Station, TX 77843

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Southwestern Entomologist		June	
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
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Percent Paid and/or Requested Circulation *(15c / 15g x 100)*		95%	95%

16. Publication of Statement of Ownership
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☐ Publication not required.

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