SUSCEPTIBILITY STATUS OF BOLL WEEVIL TO MALATHION IN TEXAS Patricia V. Pietrantonio and Terry Junek Dept. of Entomology Texas A&M University College Station, TX

<u>Abstract</u>

Vial bioassays were conducted to estimate the susceptibility of boll weevils (*Anthonomus grandis grandis*) to malathion in Texas. Weevils tested in the year 2001 were from Burleson and Lubbock Counties. Susceptibility data previously obtained from the laboratory strain of boll weevils (USDA-ARS Mission, TX) were used as reference. Weevils from both counties were susceptible to malathion. This is significant because the boll weevil eradication program began in 2001 for Burleson County.

Introduction

The Boll Weevil Eradication Program is ongoing in Texas and ULV-malathion is the primary insecticide applied for this purpose. Continuous evaluation of the effectiveness of malathion for boll weevil populations is critical to ensure the success of the eradication effort and has been listed as one of the ten research priorities by the USDA. This need is justified because the numerous ULV applications used in eradication zones exert high selection pressure on weevils. In 2000, we had monitored weevils from four different locations in Texas: Burleson, Lubbock, Nueces and Hidalgo Counties. In 2001 we concentrated in Burleson County because of its incorporation to the boll weevil eradication program and the fact that this county has a history of high organophosphate (OP) treatment frequency against weevils. Further, increased tolerance to OP insecticides had been found in 1988 (Kanga et al, 1995) and in 1999 (Pietrantonio et al., 2000) for boll weevils in this area. Weevils from Lubbock were also tested although they had resulted highly susceptible in 2000.

Methods

Susceptible Weevil Strain

For comparisons with the field-collected weevils we used the malathion susceptibility data obtained in our laboratory in 2000 from susceptible laboratory weevils purchased from the Mission Plant Protection Center, USDA-APHIS-PPQ, in Mission, TX (Pietrantonio and Sronce, 2001). In 2000, weevils from Mission TX had a LC50 of 1.95 and a LC95 of 8.16 ug malathion/vial, non-significantly different from those obtained in 1999, respectively (Pietrantonio and Sronce, 2001).

Burleson County Weevils from Squares

Punctured squares were collected from a research field on the Texas A&M University farm, USDA Field 14. Squares were brought to the laboratory and stored for seven days, then manually opened to remove the pupae. Pupae were placed in 150 mm Petri dishes on vermiculite and kept at 27°C until adults emerged. Adults were removed each day and placed in containers with fresh cotton squares for food prior to the malathion vial bioassay. Squares collected in June and July were collected prior to Malathion treatment by the Boll Weevil Eradication Foundation Program. Six hundred and ninety weevils were tested from squares.

Burleson County Trapped Weevils

Ten pheromone traps were placed on the Texas A&M University Farm on the Brazos River Bottom. Adult weevils were collected from traps and fed organic apple for 11 to 17 days prior to the malathion vial bioassay. Boll weevil pheromone lures used were Hercon LURETAPE with Grandlure (active ingredient: Grandlure 1.2%) (10 mg. A.I./lure) from Great Lakes IPM, Vestaburg, MI. Four hundred and seven trapped weevils were tested.

Lubbock County Weevils from Bolls

Bolls collected in Lubbock were shipped overnight to College Station, some had adult weevils of unknown age and others had weevil pupae and larvae.

Infested cotton bolls that produced adult weevils of unknown age were collected in a cotton field from the USDA-ARS research farm located on the northeast corner of the intersection of Quaker Ave. and 4th Street, Lubbock, TX (Lubbock County, TX) on 9/27/01. These bolls were opened on 9/28 and weevils were fed a boll weevil synthetic diet for 3 days prior to the malathion vial bioassay.

Infested cotton bolls were also collected from Texas Tech University Campus Research Farm located on the northwest corner of the intersection of Indiana Ave. and 4th Street, Lubbock, TX (Lubbock County, TX) on 10/8/01. Adults found in these bolls were of unknown age and fed organic apple for 3 days prior to bioassay. In total, three hundred twenty-five adult weevils of unknown age emerging from bolls from both locations in Lubbock were tested.

Lubbock County 2-3 Day Old Weevils Emerging From Larvae and Pupae in Bolls

Most of the bolls collected on 10/8/01, however, contained pupae and larvae and were held for several days prior to opening. All of these emerging weevils were fed organic apple for 3 days prior to the malathion vial bioassay. Three hundred thirty-one 2-3 day-old weevils emerging from these larvae and pupae were tested.

Boll Weevil Bioassays

Malathion ULV was obtained from Cheminova, the same lot has been used for bioassays since 1999. Malathion concentrations were: 0.1, 0.3, 0.6, 1, 3, 6, 10, 30, 60 and 100 µg malathion/vial. Vials were kept in an insect incubator at 16L:8D photoperiodic cycle and at 27°C. Control vials were with acetone. Five weevils per vial were used, vials were kept loosely capped and inverted. Mortality was recorded both at 24 h and 48 h; values were corrected for control mortality. Numbers of live, knocked-down and dead weevils were recorded. Weevils that could stand up on their own were recorded as alive. Weevils that could not stand up but responded to a pinch on the snout were recorded as knocked down. All others were recorded as dead (Anonymous, 1968). Mortality values included numbers of dead and knocked down weevils. Resistance ratios were calculated both for LC50 and LC95. Data were analyzed for both 24 h and 48 h of exposure using the POLO PC program and graphs were with SigmaPlot, however, only data at 48 h are reported as recommended (Anonymous, 1968). Only bioassays where χ^2 values were non-significantly different at the 0.05 probability level (data fit the linear model) were used for pooled data analysis; cases in which χ^2 values exceed the table value are indicated. The vial bioassay methodology had been previously validated (Teague et al., 1983).

Results and Discussion

In 1999, weevils of 2-3 days of age that emerged from squares collected in Burleson County were about 8 times more resistant than the laboratory colony of the same age, for both the LC50 and LC95 after 48 h of exposure. After 48 h of exposure, trapped weevils were 3.3-6.8 times less tolerant than weevils emerging from squares and overall, trapped weevils are about 3 times more tolerant than the laboratory colony both, after 24- and 48-h of exposure (Pietrantonio et al., 2000). Combined, these data suggested that monitoring for malathion resistance should be expanded to include weevils from other areas of Texas.

Assays for Burleson County weevils from squares were from 3 different dates. For the 48 h exposure data (Table 1) one of the bioassays was not well represented by a line (August 8 2001). Subsequent parallelism and equality tests indicated that the other two dosage-mortality lines were equal and parallel, thus these were pooled to obtain a representative dosage-response line (Table 1, Fig. 1).

Assays for trapped weevils in Burleson County were from three different dates. At 48 h of exposure, the bioassay from 10/18 was not well represented by a line (Table 2, Fig. 2, dashed line). Parallelism and equality tests indicated that the other two lines were parallel and equal (Table 2, Fig. 2), thus, data from these two dates were pooled (Table 2).

Two assays were performed with adult weevils of unknown age collected in Lubbock County. After 48 h of exposure both tests were well represented by a line; both dosage-mortality lines were parallel but not equal (Table 3, Fig. 3).

Three assays were performed with 2-3 day-old weevils from bolls collected in Lubbock. After 48 h of exposure the three assays were well represented by a line; and the lines were equal and parallel, thus the data were pooled. The pooled data did not have high heterogeneity, thus it is a good representation of the susceptibility status of the population (Table 4, Fig. 4).

Figure 5 shows a comparison of the status of susceptibility to malathion of 2-3 day-old weevil populations for both counties for 2000 and 2001. Statistical analyses indicated that the dosage-mortality response of the Lubbock population is statistically significantly different between 2000 and 2001, and that the lines are indeed parallel. For Burleson County the dosage-mortality lines of 2-3 day-old weevils are neither equal or parallel between years.

A statistical comparison of the malathion susceptibility of trapped weevils between the years 1999 and 2001 for Burleson County indicated that the dosage-mortality response lines were not equal (weevils from 2001 being more susceptible) but parallel, indicating similar standard deviation within each population (Fig. 6).

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Acknowledgments

This research was supported by a grant from COTTON INC. We are indebted to Dr. Patricia O'Leary for contract management. From USDA ARS, College Station, TX, Dr. J. Lopez, allowed us to use Field 14. TAMU entomologists M. Parajulee, S. Carroll and M. Arnold, Lubbock TAES, are acknowledged for square collection. Student-workers L.-A. Lawton and C. Marsh helped with bioassays.

	Tab	le I. Malathio	n Resistance Vial M	lonitoring for th	ne Boll Wee	evil	
		Burleson (County, weevils fror	n squares, 2-3 o	lay old		
			July-August	2001			
		48 H	ours Exposure to M	alathion at 27°	2		
Date	LC50	Resist.	LC95	Resist.	χ²	df	# of Insects
	µg/vial	Ratio LC50	μg/vial	Ratio LC95	70		Tested
	(C.L.)		(C.L.)				
7/10/01	1.749	0.896	13.004	1.594	0.021	1	134
	(.186 - 5.150)		(4.469 - 218.475)				
8/8/01	1.415	0.726	3.951	0.484	8.605*	1	168
8/14/01	1.176	0.603	4.708	0.577	0.554	3	388
	(.907 - 1.464)		(3.507 - 7.318)				
Pooled	1.192	0.611	5.400	0.662	6.228	4	522
7/10 &	(.709 - 1.776)		(3.306 - 14.122)				
8/14							
<u> </u>	6 1 1 1						

Table 1 Malathian Resistance Vial Manitoring for the Ball Weevil

C.L. = confidence limits

RR compared to year 2000, 2-3 day old susceptible

* Indicates χ^2 value significant at P = 0.05

October 2001 48 Hours Exposure to Malathion at 27°C								
Date	LC50 µg/vial (C.L.)	Resist. Ratio LC50	LC95 μg/vial (C.L.)	Resist. Ratio LC95	χ²	df	# of Insects Tested	
10/1/01	1.898 (1.289 - 2.562)	0.973	6.665 (4.417 - 16.566)	0.817	0.131	1	190	
10/18/01	2.916	1.495	21.531	2.639	6.686*	2	113	
10/29/01	1.031	0.529	19.254	2.360	1.649	1	104	
Pooled 10/1 & 10/29	1.739 (.870 - 2.556)	0.892	8.965 (5.209 - 42.499)	1.099	0.917	2	294	

Table 2. Malathion Resistance Vial Monitoring for the Boll Weevil Burleson County, trapped adults, age unknown (>2 weeks)

C.L. = confidence limits

RR compared to year 2000, 2-3 day old susceptible

* Indicates γ^2 value significant at P = 0.05

Table 3. Malathion Resistance Vial Monitoring for the Boll Weevil
Lubbock, weevils from bolls, age unknown (>3 days)
October 2001

48 Hours Exposure to Malathion at 27°C							
Date	LC50 µg/vial (C.L.)	Resist. Ratio LC50	LC95 µg/vial (C.L.)	Resist. Ratio LC95	χ^2	df	# of Insects Tested
10/1/01	1.232	0.632	6.352	0.778	1.457	1	219
10/24/01	2.934 (1.704 - 5.009)	1.505	12.133 (6.500 - 77.433)	1.486	0.108	1	106
Pooled	1.682 (1.175 - 2.444)	0.863	11.271 (6.164 - 38.393)	1.381	0.464	2	325

C.L. = confidence limits

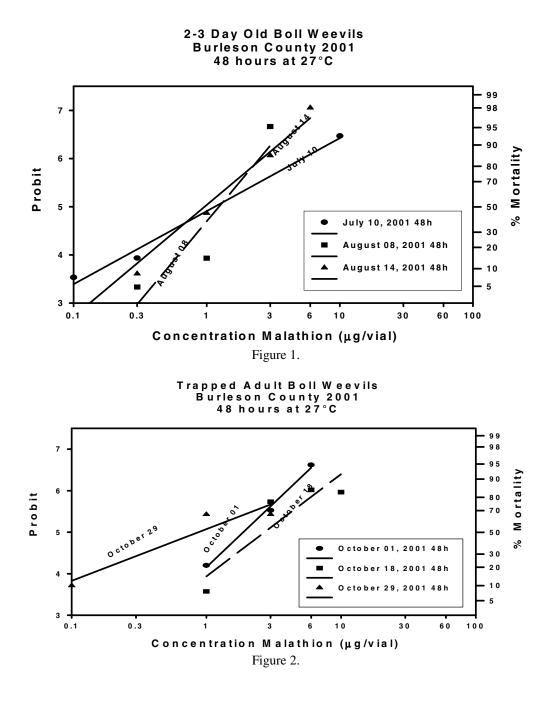
RR compared to year 2000, 2-3 day-old susceptible

Table 4. Malathion Resistance Vial Monitoring for the Boll Weevil									
Lubbock, weevils from bolls, 2-3 days old									
			October	2001					
48 Hours Exposure to Malathion at 27°C									
Date	LC50	Resist.	LC95	Resist.	χ²	df	# of Insects		
	µg/vial	Ratio LC50	µg/vial	Ratio LC95	<i>7</i> 0		Tested		
	(C.L.)		(C.L.)						
10/16/01	3.138	1.609	8.732	1.070	0.189	1	125		
	(1.838 -		(5.881 - 33.879)						
	4.253)								
10/18/01	2.867	1.470	3.567	0.437	0.498	1	150		
10/22/01	2.376	1.218	13.719	1.681	0.006	1	56		
Pooled	2.791	1.431	8.645	1.059	0.729	2	331		
	(1.994 -		(5.868 - 22.220)						
	3.647)		. ,						

Table 4 Malathion Resistance Vial Monitoring for the Boll Weevil

C.L. = confidence limits

RR compared to year 2000, 2-3 day old susceptible



>3 Day Old Boll Weevils Lubbock 2001 48 hours at 27°C

