INSECTICIDE RESISTANCE STATUS OF BOLL WEEVIL TO MALATHION AND OF BOLLWORM TO PYRETHROIDS IN TEXAS

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Abstract

Vial bioassays were conducted to estimate the susceptibility of boll weevils (*Anthonomus grandis grandis*) to malathion and of bollworm to cypermethrin in Texas. Weevils tested in the year 2000 were from Burleson, Lubbock, Hidalgo and Nueces Counties. The laboratory strain of boll weevils obtained from the USDA-ARS Mission, TX was used as an internal control. Weevils from Nueces County (South East Texas) and Hidalgo County (Lower Rio Grande Valley) were less susceptible than those from Lubbock (north Texas), that were highly susceptible to malathion. The increase in tolerance to malathion in boll weevils detected by this method indicates that monitoring for resistance should continue, with special attention to young weevils emerging from squares in areas under the eradication program. The current status of susceptibility of bollworm (*Helicoverpa zea*) to cypermethrin is reported.

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. We monitored weevils from four different locations in Texas: Burleson, Lubbock, Nueces and Hidalgo Counties. Only Nueces County has been under active eradication for 3 years. Burleson County, in the Brazos River Bottom near College Station, has not been included yet in the boll weevil eradication program but has a history of high organophosphate (OP) treatment frequency against weevils. Increased tolerance to OP insecticides had been found in 1988 for boll weevils in this area (Kanga et al, 1995).

Methods

Susceptible Weevil Strain

In order to establish a baseline for susceptibility to malathion and to be used in comparisons with the field-collected weevils we obtained the laboratory boll weevil strain from the USDA/ARS Biological Control and Mass Rearing Research Unit, Stoneville Research Quarantine Facility, in Stoneville, Mississippi. These weevils were used in bioassays for 1999 (Pietrantonio et al., 2000). In 2000 this susceptible colony was transferred to the Mission Plant Protection Center, USDA-APHIS-PPQ, in Mission, TX. The weevils were fed the same laboratory diet plugs both years, which were also obtained from the Stoneville laboratory. Laboratory weevils obtained in 2000 were noticeably bigger than those obtained in 1999, thus the weights of weevils were recorded previous to bioassays. Information from the rearing facility indicated that susceptible weevils were about or less than 18 mg in 1997 while up to 24 mg in the year 2000 (Leeda Wood, USDA Mission, TX, pers. comm.). The increase in size and weight of weevils from the susceptible laboratory colony was apparently due to minor changes in diet formulation and rearing technique after the colony was moved to Mission, TX. These changes included a slight increase in vitamins, ascorbic acid and Wesson's salt and the deletion of the Calco red dye from the diet; weevils were also kept on diet in Petri dishes, that resulted in less crowding and better moisture content. However, this fact did not affect the LC50 or LC95 values after 48 h of exposure. In 1999 the laboratory colony LC50 for weevils 2-3 days-old was 1.43 (1.1-1.8) ug malathion/vial and the LC95 8.73 (.68-12.4) ug malathion/vial (Pietrantonio et al., 2000). In 2000, weevils from Mission TX had a LC50 of 1.95 and a LC95 of 8.16 ug malathion/vial (Table 2), non-significantly different from those obtained in 1999, respectively.

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Weevils from Squares

Punctured squares were collected in various cotton fields across the State of Texas and were brought to the laboratory, stored for seven days, then manually opened to remove the pupae. Pupae were placed in plastic ventilated 150 mm Petri dishes on damp vermiculite and kept at 27˚C until adult eclosion. Adults were removed each day and placed in cages. Weevils were fed fresh pesticide-free cotton squares from variety HS-26 and Paymaster 1218 BG/RR and Paymaster 2280 BG/RR.

Square Collection Sites

In Burleson County squares were collected from a research field on the Texas A&M University farm (Field 14), near College Station, TX. This field did not receive any pesticide applications this year. Squares from Corpus Christi were collected in June and July at the TAMU Research and Extension Center from an ULV-malathion-treated field (3 acres) that had received 9-10 applications at 12 fl. oz./acre. This field had been under "fall diapause-treatment program" since 1996, and under active "full-season" Boll Weevil Eradication Program beginning in 1998 (http://txbollweevil.org/Zones/STWGtrapping.htm.). Squares from Lubbock County, TX, were collected at the TAMU Research and Extension Center Farm. These irrigated fields (Plots 101-103) had not received any pesticide treatments in 2000. Lubbock and Hale Counties had heavy weevil infestation in 2000 and were not under eradication (http://www.plainscotton.org/rkh/gtsumm00/WK39summ.html).

Squares from the Lower Rio Grande Valley area were collected from three different fields in Hidalgo County, TX. Squares from Hargill-1 field, which is located in the northern part of the county, were collected on May 28th. This field had been treated with Guthion™ at 0.25 lbs ai/acre five to six days before square collection. The second field was located in the Experiment Station Annex Farm, north of Mercedes, and it had been treated with Vydate™ at 0.35 lbs ai/acre two days prior to square collection, which occurred on July 13th. The third field was located in a commercial farm in north-eastern Hidalgo County. The only insecticides applied to the field were Bathroid™ at the rate of 0.03 lbs a.i./acre and then 5 days later Guthion™ at the rate of 0.25 lbs a.i./ acre. The Guthion™ treatment occurred approximately 10 days before square collection. None of the counties in the Lower Rio Grande Valley, including Hidalgo County, are under boll weevil eradication program.

Boll Weevil Bioassays

Vials were kept in an insect incubator at 16L:8D photoperiodic cycle and at 27°C. Malathion ULV was obtained from Cheminova and the same lot was used for bioassays in 1999 and 2000. Malathion concentrations were: 0.1, 0.3, 0.6, 1, 1.5, 3, 6, 10, 30, 60 and 100 µg malathion/vial. Control vials were with acetone. Five weevils per vial were used, vials were kept loosely capped and inverted. In each individual bioassay normally 30 weevils per concentration were used. For 2000, data from typically 3 bioassays were pooled. Mortality was recorded both at 24h and 48h; values were corrected for control mortality with the Abbott's formula. 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 using the

Basica Probit Analysis program (Raymond, 1985) and graphed using SigmaPlot. Only bioassays where χ^2 values were non-significantly different at the 0.05 probability level (data fit the linear model) are reported. The suitability of the vial method versus the topical bioassay method recommended for boll weevils (Anonymous, 1968) was demonstrated for two other organophosphate insecticides (Teague et al., 1983).

***Helicoverpa Zea* Bioassays**

Trapping of males and vial bioassays were conducted as described previously (Kanga et al., 1996; Martin et al., 1999; Pietrantonio et al., 2000). A total of 620 insects were used for calculations of LC50 and LC95 (Table 2) while about 500 insects were tested with IRAC vials.

Results and Discussion

We had previously determined that age and temperature influenced susceptibility to malathion in adult weevils (Pietrantonio et al., 2000). 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 for 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.

In the year 2000 weevils reared from squares collected in Lubbock, Lower Rio Grande Valley and Corpus Christi were included. These areas were chosen to reflect the diversity in weevil populations with different insecticide treatments. Corpus Christi was in the third year of the Full Season Eradication Program while the other areas were not under eradication. The laboratory colony was again used as an internal control. Weevils 2-3 days of age collected in C. Christi had the highest LC50 after 24 h of exposure, exhibiting a 7-fold higher tolerance than the susceptible strain (Fig. 1). Weevils from the Lower Rio Grande Valley were the next most tolerant with a tolerance ratio of about 3.8 for the LC50. Despite the high number of weevils tested, there was a significant variability around the LC95 for both, C. Christi and Rio Grande Valley, with tolerance ratios of 11.4 and 25, respectively, after 24 h of exposure. These results indicated the presence of heterogeneous populations in these two areas. After 48 h of exposure, weevils from Corpus Christi continued to be the most tolerant at the LC50 level with tolerance ratios of 2.5 (Table 1). Differences in LC95 observed at 24 h of exposure were not evident between C. Christi and Rio Grande weevils after 48 h, since the confidence limits for the LC95 overlapped (Table 1, Fig. 2). The weevils collected from Lubbock were highly susceptible, with both LC50 and LC95 lower than the laboratory reference strain after 24 h (Fig. 1) and 48 h of exposure (Tables 1; Figs. 2).

The year 2000 results of cypermethrin bioassays with bollworm (*H. zea*) males conducted with glass vials prepared in our laboratory are presented in Table 2. These data are in agreement with those we obtained during the same period using vials with two discriminatory concentrations sent to our laboratory through the Insecticide Resistance Action Committee, and prepared by G. Payne (see elsewhere in these proceedings). On July 7, bioassays in our laboratory with IRAC vials containing the discriminatory concentration of 10 ug/vial (which should have killed all heterozygous insects) showed 3.3 % survival; the same percentage survival was observed for the 5 ug/vial concentration. On July 13, vials with 10 ug/vial showed 4.4% survival while 5 ug-vials showed 15 %. Tests in August and September with IRAC vials did now show survivors at any of these two concentrations. The results in Table 2 and those of IRAC vials combined showed that as the season progressed there was an increase in the susceptibility of these insects to cypermethrin, contrarily to what could have been expected. Notice a higher LC50 in June and beginning of July (Table 2) and a decrease in the LC95 in September. One factor to consider is that early season *H. zea* may be emerging from corn and moving into cotton fields, they may be bigger and this may somewhat affect their response to cypermethrin in this assay (Fred Gould, personal communication). Regardless of this, data by Kanga et al., 1996, already indicated increased tolerance to cypermethrin for this area of Texas in 1988 and 1989, and similarly they observed an increase in susceptibility towards the end of the season. The susceptible laboratory colony collected between June-September of 1988 had a LC50 of 0.05 (0.01-0.09) ug/vial with LC90 of 0.57 (0.29-1.02) ug/vial. Our laboratory colony collected in 1998 and tested in September of 1999 had LC50 2.32 (0.6-2.9) and LC90 of 3.95 (3.3-7.4). For June 1999 the LC50 of field collected males (3 bioassays pooled) was 0.9 (0.06-1.87) ug/vial and LC90 8.84 (5.66-28.05) ug/vial. These last results are not statistically different from those obtained in June 2000 (Table 2). In conclusion, cypermethrin resistance is present in *H. zea* from Texas, however its level, as estimated by LC50 and LC95 has not significantly worsen during the last season.

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Table 1. Probit analysis of malathion vial bioassays for the boll weevil, data pooled from June - October 2000. Results after 48 h of exposure of 2-3 day-old weevils collected from squares. Nº = total number of weevils tested.

Weevil Origin	LC50 ug/ vial (Conf. Inter.)	Res. Ratio LC50	LC95 ug/ vial (Conf. Inter.)	Res. Ratio LC95	χ^2	Nº
Corpus Christi Squares	4.97 (3.62-6.51)	2.55	43.64 (26.20-108.0)	5.35	1.95	318
Rio Grande Squares	2.88 (2.11-3.71)	1.48	33.36 (23.06 - 56.20)	4.09	0.55	498
Lubbock Squares	0.91 (0.78-1.04)	0.47	2.80 (2.23-3.99)	0.34	1.68	560
Burleson County	0.77 (0.48-1.15)	0.39	12.9 (6.72-38.04)	1.5	6.15	229
Mission TX 2000	1.95 (1.76-2.16)	1	8.16 (6.92 - 9.99)	1	2.27	1320

All non-significant x²: Data are well represented by a line. The LC50 of Lower Rio Grande Valley weevils is not statistically different from that of Corpus Christi weevils. Note slight overlap of confidence intervals. Lubbock weevils are more susceptible than the susceptible laboratory colony. Weevils from Corpus Christi and Rio Grande Valley are statistically significantly more tolerant than those from Lubbock.

Table 2. Cypermethrin resistance monitoring bollworm males (*H.zea*), 2000. Data are in micrograms of cypermethrin/vial

Date	LC50	Conf. Intervals	LC95	Conf. Intervals	$\chi 2$
6/30/00	3.69	1.19-5.87	11.42	6.92-98.18	1.16
7/7/00	3.01	1.78-4.79	27.76	14.15-102.75	0.195
7/13/00	1.17	0.77-1.88	7.05	3.58-37.66	1.49
7/17/00	0.71	0.33-1.17	5.2	2.52-48.58	1.64
7/19/00	0.58	0.14-1.31	16.9	5.94-409.9	0.85
9/7/00	0.43	0.24-0.63	1.33	0.85-4.44	0.004

Data are well represented by a line, all $\chi 2$ are non-significant (probit analysis).

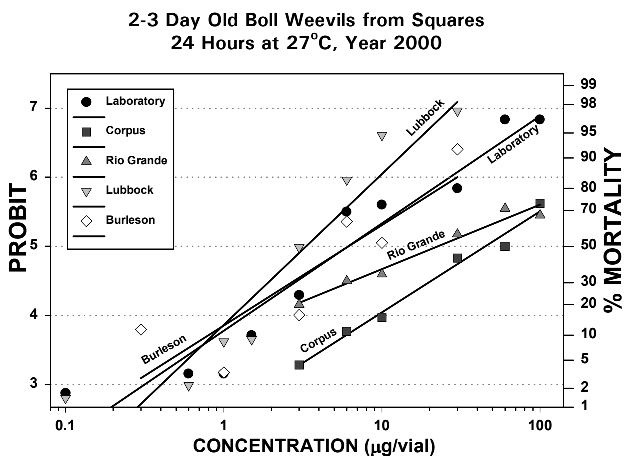

Figure 1. Probit analysis of weevils exposed for 24 h to malathion.

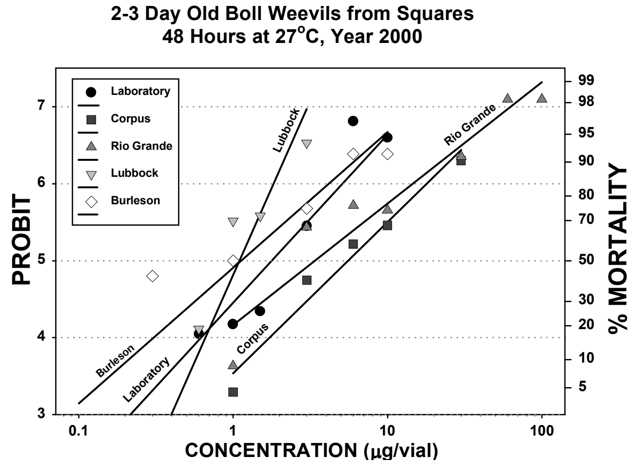

Figure 2. Probit analysis of weevils exposed for 48 h to malathion. See details in Table 1.