MONITORING FOR RESISTANCE TO CYPERMETHRIN IN BUDWORM (*H. VIRESCENS*) AND BOLLWORM (*H. ZEA*) AND TO MALATHION IN BOLL WEEVIL IN THE BRAZOS RIVER BOTTOM, TEXAS

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Abstract

Vial bioassays were conducted during 1999 with adults of budworm, bollworm and boll weevil. Field collected moths were tested for resistance to cypermethrin and boll weevils for resistance to malathion. The frequency and level of resistance to cypermethrin continues to be very high in budworm and the frequency of this resistance is increasing in bollworm. The vial assay was used to monitor for resistance to malathion in boll weevil populations in Burleson County, TX. Differences in boll weevil susceptibility to malathion depending on weevil age or origin (traps or cotton squares) and bioassay temperature were determined. The susceptible strain of boll weevils obtained from the USDA-ARS in Stoneville, Mississippi, was used as an internal control. Mortality caused by malathion was found to be lower in 2-3 day-old weevils emerging from squares when compared with older weevils collected from squares and trapped weevils of unknown age.

Altogether, these data suggest that the frequency of pyrethroid use against bollworm should decrease. The increase in tolerance to malathion in boll weevils observed in the laboratory indicates that monitoring for resistance should continue, with special attention to young weevils emerging from squares. These findings need to be confirmed with malathion field efficacy studies.

Introduction

The introduction of transgenic *Bt* cotton in Texas has significantly reduced the amount of pesticides applied for the control of bollworm (*Helicoverpa zea*). However, pyrethroids are still used in Texas for this purpose in conventional cotton, in *Bt* cotton when levels of bollworms are unacceptable and for the control of *Lygus* bug in both cotton types. Budworm (*Heliothis virescens*), on the other hand, is highly resistant to pyrethroids and pyrethroid applications are not intended for its control. Despite this fact, it was of biological interest to determine the current levels of resistance in these populations.

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The boll weevil eradication program is ongoing in Texas and ULV malathion is the primary insecticide applied. Continuous evaluation of the effectiveness of malathion on boll weevil populations is critical to ensure the success of the eradication effort. This need is justified because the numerous ULV applications used in eradication zones exert high selection pressure on weevils and because increased tolerance to organophosphates was found in 1988 for boll weevils in the Brazos River Bottom, TX (Kanga et al, 1995). We monitored weevils from the Brazos River Bottom, area that is not currently under the boll weevil eradication program.

Methods

Insects

For bollworm (*H. zea*) trapping, pheromone Luretape ® (cat. # Z0484) was purchased from Hercon (Emigsville, PA) and was replaced biweekly. Pheromone for trapping budworm (*H. virescens*) was obtained from Scentry, Inc. (# L210). Males were collected using eight wire cone (Harstack et al., 1979) pheromone traps that were distributed approximately a quarter of a mile apart from each other in Porter's farm. The traps were located 1 mile west of the Brazos River on FM 60 and were surrounded by cotton fields. Males were collected overnight, twice a week. Early in the morning males were brought to the laboratory; assays were performed the same day. Only vigorous males with intact wing scale pattern were used for the bioassays.

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 susceptible boll weevil strain from the USDA/ARS Biological Control and Mass Rearing Research Unit, Stoneville Research Quarantine Facility, in Stoneville, Mississippi. The weevils were fed laboratory diet plugs.

Trapped Weevils. Eight traps were placed on the Texas A&M Farm on the Brazos River Bottom, near a pecan orchard (Burleson County, TX). Adult weevils were collected and fed cotton squares for 4-5 days prior to the malathion vial bioassay. About one thousand three hundred weevils were used in different bioassays. Pheromone was "Boll weevil pheromone dispensers" from Hercon LURETAPE with Grandlure/10 (active ingredient, Grandlure 1.2 %) (Hercon Environmental Co., Emigsville, PA).

Weevils from Squares. Punctured squares were collected from a research field on the Texas A&M University farm (Field 14). Squares were brought to the laboratory and 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 with organic apple for food.

Moth Bioassays

Cypermethrin technical grade (96.6%) was generously provided by FMC (Princeton, NJ). Acetone (GR) for control vials and for cypermethrin solutions was from EM Science (Gibbstown, NJ). Acetone was dried for at least 48 h on 4 Å molecular sieves (Sigma) or "t.h.e. desiccant" (EM Science) before insecticide stock preparation. Vials were prepared with only acetone for controls, and with 3, 10, 30 or 100 µg cypermethrin/vial. Vials were prepared by dispensing 0.5 ml of acetone or cypermethrin solutions and dried on a cold "hot-dog" roller under the hood for at least 1 h. Vials were prepared as needed and stored at 2 ˚C for no more than a month before use. Mortality was recorded at 24 h. Moths that could not fly were considered "knocked-down" and these were included as dead for calculations of % mortality. The percentage of survivorship was calculated with moths that could fly after 24 h of exposure. Bioassays for bollworm were with a cypermethrin discriminatory dosage of 3 µg/vial for resistant heterozygotes (Kanga et al., 1996) and 10 µg/vial for resistant homozygotes as proposed, although these discriminatory concentrations have not been precisely determined for bollworm (Martin et al., 1999). For budworm the discriminating dosages were as previously reported (Bagwell et al., 1997).

Boll Weevil Bioassays

Vials were kept in an insect incubator at 16L:8D photoperiodic cycle and at 25˚C, 27˚C or 29˚C. Malathion ULV was obtained from Cheminova. Malathion concentrations were: 1, 3, 6, 10, 30, 60 and 100 µg malathion/vial. Control vials were with acetone. Five weevils per vial were used. 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. The suitability of this method versus the topical bioassay method recommended for boll weevils (Anonymous, 1968) was demonstrated for two other organophosphate insecticides (Teague et al., 1983).

Results and Discussion

Bioassays with budworm indicated continuing high frequency and level of resistance to the pyrethroid cypermethrin in populations from the Brazos River Bottom, TX (Fig. 1). About 64.2 % of the moths survived the discriminatory concentration of 10 µg/vial, indicating that 64.2 % of the individuals are homozygote pyrethroid resistant (Fig. 1).

A high percentage of bollworms survived the proposed discriminatory dosage of 3 µg/vial, indicating that about 5.7-19.2 % of the individuals may be heterozygote resistant (Table 1). Up to 3.7 % of individuals survived at 10 µg/vial; this represents an increase from 1998 for Burleson Co., when no bollworms survived this dosage. This indicates that the frequency of bollworm homozygote resistant individuals is probably higher than this detected percentage since it is likely that the true discriminatory dosage for bollworm is below 10 µg/vial. The increase in the frequency of pyrethroid resistant bollworms is of concern in Texas. Reducing the frequency of pyrethroid use will help in delaying the increase in the frequency of pyrethroid resistant bollworms.

Age influences susceptibility to malathion in the boll weevil. Susceptible weevils of 2- 3 days of age are 1.3- 2.5 times more tolerant, at the LC50 and LC95 levels, respectively, than 6-17 day old weevils after 24 hours of exposure (Table 2). Susceptible weevils of 2- 3 days of age are 2.2 times more tolerant at the LC95 level than 6-17 day old weevils after 48 hours of exposure (Table 3).

Laboratory temperature during exposure to malathion has a strong effect on the mortality of trapped weevils after 48 hours of exposure (Table 4).
Weevils kept at 25˚C were 2.1- 4.5 times more tolerant than those kept at 29˚C and 1.5- 3.2 times more tolerant than those kept at 27 ˚C. Weevils kept at 27˚C were 1.4 times more tolerant than those kept at 29˚C.

Weevils emerging from squares at 2-3 days of age are 21- 15 times more resistant, for LC50 and LC95, respectively, than the laboratory susceptible colony of the same age after 24 hours of exposure (Table 5, Fig. 2). Weevils emerging from squares at 2-3 days of age are about 8 times more resistant than the laboratory susceptible colony of the same age after 48 hours of exposure (Table 6, Fig. 3).

Weevils emerging from squares are 20-32 times more resistant than the laboratory susceptible colony, for LC50 and LC95, respectively, after 24 h of exposure (Table 7). Weevils emerging from squares are 6-15 times more resistant than field trapped weevils (Table 7, in parenthesis). Trapped weevils are 3-2 times more tolerant than susceptible weevils for LC50 and LC95, respectively (Table 7).

Weevils emerging from squares are 8.4- 16.5 times more resistant than the laboratory susceptible colony, at the LC50 and LC95 levels, respectively, after 48 hours of exposure (Table 8, Fig. 4). Weevils emerging from squares are and 3.3- 6.2 times more resistant than field trapped weevils (Table 8, in parenthesis). Overall, trapped weevils are about 3 times more tolerant than the laboratory susceptible colony both for 24 and 48 h of exposure (Table 8, Fig. 4).

These data suggest that monitoring for malathion resistance should continue in Burleson County and should be expanded to include weevils from other areas of Texas where malathion use is intensive.

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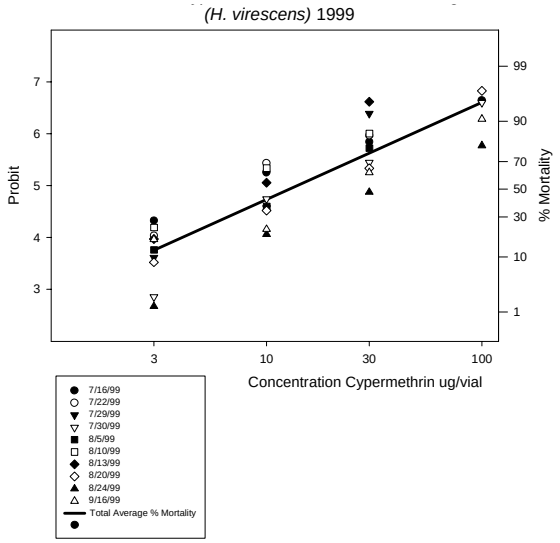

Figure 1. Budworm cypermethrin resistance monitoring, 1999.

Table 1. Cypermethrin resistance monitoring for bollworm 1999.

Month	# bollworm tested	% Corrected Survival 3 µg/vial	% Corrected Survival 10 µg/vial
June	385	16.9	2.8
July	360	19.2	1.5
August	190	9.1	0
September	299	5.7	1.9
October	140	0	3.7

Table 2. Malathion bioassay for the boll weevil September-December 1999, 24 h exposure, 27°C. C. L. = confidence limits.

Origin of Weevils	LC50 µg/vial (C. L.)	Resist. Ratio LC50	LC95 µg/vial (C. L.)	Resist. Ratio LC95	# of Insects Tested
Suscep.USDA (6-17 days old)	2.61 (2.0-3.2)	1	27.63 (21.4-38.5)	1	1440
Suscep. USDA (2-3 days old)	3.48 (2.3-4.8)	1.3	69.05 (44.8- 130.9)	2.5	680

Table 3. Malathion bioassay for the boll weevil September-December 1999, 48 h exposure, 27 ˚C. C. L. = confidence limits.

Origin of Weevils	LC50 (C. L.)	Resist. Ratio LC50	LC95 (C. L.)	Resist. Ratio LC95	# of Insects Tested
Suscep. USDA (6-17 days old)	1.09 (0.9- 1.2)	1	3.94 (3.4- 4.8)	1	1440
Suscep. USDA (2-3 days old)	1.43 (1.1- 1.8)	1.3	8.70 (6.8-12.3)	2.2	680

Table 4. Malathion resistance monitoring for the boll weevil September-October 1999, 48 h exposure. C. L. = confidence limits.

Trapped Burleson Co., age unknown	LC50 µg/vial (C. L.)	Resist. Ratio LC50	LC95 µg/vial (C. L.)	Resist. Ratio LC95	# of Insects Tested
25°C	4.17 (2.6- 6.1)	2.1	33.25 (19-97)	4.5	125
27 °C	2.75 (2.5- 3)	1.4	10.53 (9- 12.7)	1.4	1256
29 °C	2 (1.5- 2.5)	1	7.4 (5.6-11.5)	1	320

Table 5. Malathion resistance monitoring for the boll weevil. Collections September-December 1999, 24 h exposure, 27 °C. C. L. = confidence limits.

Origin of Weevils	LC50 µg/vial (C. L.)	Resist. Ratio LC50	LC95 µg/vial (C. L.)	Resist. Ratio LC95	# of Insects Tested
Suscep. USDA (2-3 days old)	3.48 (2.3-4.8)	1	69.05 (44.8-130.9)	1	680
Squares Burleson Co., (2-3 days old)	71.57 (56.2- 97.2)	21	1027.22 (576.3- 2,302.8)	15	862

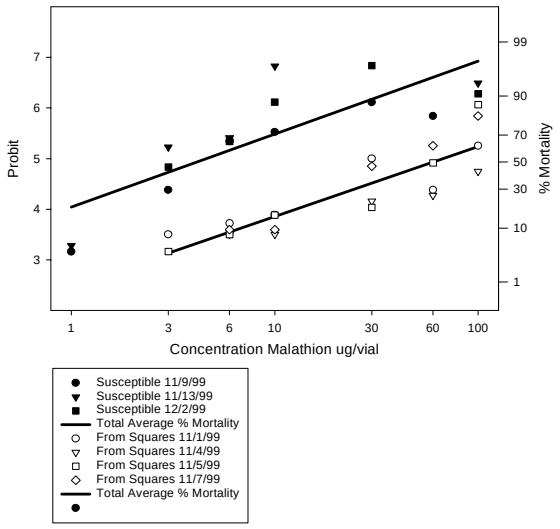

Figure 2. Malathion resistance monitoring for boll weevil 1999. Susceptible strain vs. weevils collected from squares, both of 2-3 days of age, 24 h at 27˚C.

Table 6. Malathion resistance monitoring for the boll weevil. Collections September-December 1999, 48 h exposure, 27 °C. C. L. = confidence limits.

Origin of Weevils	LC50 (C. L.)	Resist. Ratio LC50	LC95 (C. L.)	Resist. Ratio LC95	# of Insects Tested
Suscep. USDA (2-3 days old)	1.43 (1.1- 1.8)	1	8.70 (6.8-12.3)	1	680
Squares Burleson Co., (2-3 days old)	11.81 (10.3- 13.5)	8.3	77.73 (60.9- 105.1)	8.9	862

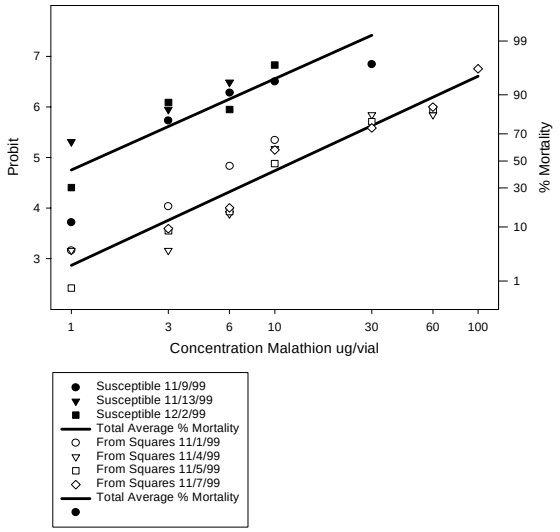

Figure 3. Malathion resistance monitoring for boll weevil 1999. Susceptible strain vs. weevils collected from squares, both of 2-3 days of age, 48 h at 27˚C.

Table 7. Malathion resistance monitoring for the boll weevil. Collections September-December 1999, 24 h exposure, 27 °C. C. L. = confidence limits.

Origin of Weevils	LC50 µg/vial (C. L.)	Resist. Ratio LC50	LC95 µg/vial (C. L.)	Resist. Ratio LC95	# of Insects Tested
Suscep. USDA (6-17 days old)	2.61 (2.0- 3.2)	1	27.63 (21.4-38.5)	1	1440
Trapped Burleson Co, age unknown	8.53 (7.6- 9.6)	3	60.23 (48.1-79)	2	1265
Squares Burleson Co., (1-7 days old)	52.50 (44.1-64.5)	20 (6)	883.82 (572.1- 1,531.4)	32 (15)	1523

Table 8. Malathion resistance monitoring for the boll weevil. Collections September-December 1999, 48 h exposure, 27 ˚C. C. L. = confidence limits.

Origin of Weevils	LC50 (C. L.)	Resist. Ratio LC50	LC95 (C. L.)	Resist. Ratio LC95	# of Insects Tested
Suscep. USDA (6-17 days old)	1.09 (0.9-1.2)	1	3.94 (3.4-4.8)	1	1440
Trapped Burleson Co., age unknown	2.75 (2.5- 3)	2.5	10.53 (9-12.7)	2.7	1265
Squares Burleson Co., (1-7 days old)	9.11 (8.2-10.1)	8.4 (3.3)	65.01 (53.7- 81.4)	16.5 (6.2)	1523

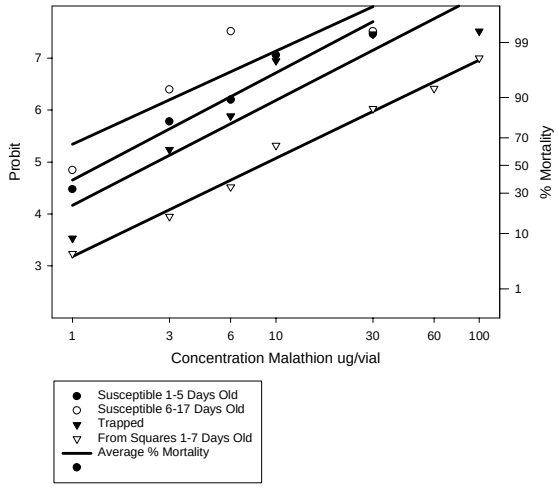

Figure 4. Malathion resistance monitoring for the boll weevil 1999, 48 h exposure, 27˚C.