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DEFINITION OF RESISTANCE TO INSECTICIDES BY BOLLWORM FROM CORN, COTTON AND SORGHUM IN TEXAS

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Abstract

In Texas (TX), from 1967 to 2004, field collected bollworms, *Heliocoverpa zea* (Boddie) were not resistant to methomyl, thiodicarb, profenofos, methyl parathion nor any pyrethroid in laboratory bioassays based on LD50s or LC50s. They showed variation in susceptibility. Third instar larvae were bioassayed by topical application for LD50s. LD50s for methyl parathion and permethrin were <4.0 and <0.1 µg/larva, respectively from 1967 to 1993 in the LRGV. Male moths captured in traps were bioassayed by adult vial test (AVT) for LC50s; LC50s were <4.0 mg cypermethrin/vial from 1988 to 2004. Mortalities of moths treated with 10 µg/vial of cypermethrin ranged from 70% to 100%. Ninety-three to 100% mortality of neonate, first, second, third, fourth and fifth instar field collected larvae were killed with 0.5 to 10 mg cypermethrin/vial. These results indicate variation in susceptibility. Field control experiments conducted in 1984 and 1986 with pyrethroids and anticholinesterase inhibitors indicated great variation. Control >80% of bollworm larvae or larval feeding damage of squares and bolls in treated cotton compared to untreated cotton can be used as a threshold to indicate control. All pyrethroids and the indicated anticholinesterase insecticides could be used for control of larval populations of the bollworm in TX.

Introduction

Bollworm populations are found in cotton production areas of TX which also produce corn and grain sorghum. The three crops probably comprise 80% of planted fields in TX each year (y). Populations of the bollworm will be different sizes each y in each field. Corn is the favorite host of this pest. Sorghum is probably the least favorite, but it is an important host because it is planted extensively across TX. The timing of fruiting of corn and sorghum and the size of the populations in these crops is important for maximum populations to develop in cotton.

Traditionally, insecticides are applied to larvae of the bollworm because they are the damaging stage (Brattsten et al. 1986). The adult does not damage the corn, cotton or grain sorghum. Resistance has to be determined for larvae by each insecticide separately. The response to any pyrethroid or anticholinesterase insecticides by larvae from fields of corn, cotton and sorghum cannot be directly associated to response by adults in traps. Yet, adults have been the only stage monitored for the past decade across TX. The size of bollworm populations across TX each y in each field of each crop will and does vary considerably. In general there will be a positive correlation with the size of populations on cotton and the size of corn acreage in each crop production area across TX.

Methyl parathion has been used since the 1950s and profenofos since the 1970s against larvae of the bollworm in TX. The carbamate insecticides, methomyl and thiodicarb have been used since the 1970s. Since 1975, permethrin and other insecticides of the pyrethroid class with the cyano on the alcohol moiety have been available to control larval populations of this pest on the three crops in TX.

Populations of male adults of the bollworm from the Lower Coastal Plain and the Brazos Valley were designated as highly resistant to cypermethrin (Pietrantonio et al. 2004). LD50s of bifenthrin, cyfluthrin, cypermethrin, deltamethrin, esfenvalerate, *lambda* cyhalothrin, permethrin and tralomethrin were determined to larvae in 1990 in the LRGV and they indicated only susceptibility to larvae (Wolfenbarger et al. 1998).

LC50s, as mg/vial, of pyrethroids, organophosphorus and carbamate insecticides have been determined for adults of the bollworm across TX from 1988 to 2004. LD50s, as mg/larva, of pyrethroids, the carbamate methomyl and the organophosphorus insecticides methyl parathion and profenofos have been determined for the same insect from corn and cotton in the Lower Rio Grande Valley (LRGV) from 1967 to 1993. LD50s are

determined by methods of Anonymous (1970). LC50s are determined by methods of Kanga et al. (1996). LD50s and LC50s and their 95% Confidence Intervals (C.I.), as mg/larva and mg/vial, were determined (SAS 1988). Overlapping CIs indicate equal LD50s and LC50s.

Literature to 2004

In 1966 (Wolfenbarger and McGarr 1970), 1974 (Davis et al. 1975), 1981 to 1993 (Wolfenbarger et al. 1998 and Wolfenbarger 2000) showed only susceptibility to methyl parathion by field collected populations with topical application to bollworm larvae (Table 1). LD50s of methyl parathion were <7.5 µg/larva for the tobacco budworm, *Heliothis virescens* (F.) (Wolfenbarger 2000). LD50s of 7.5 mg/larva also should be a proposed resistance threshold for profenofos, methomyl and methyl parathion against the bollworm.

LD50s of permethrin were shown in 1974 (Davis et al. 1975) and 1981 -1983 and 1990 (Wolfenbarger et al. 1998 and Wolfenbarger 2000). LD50s <0.1 µg/larva indicate susceptibility (Table 1). A resistance threshold of LD50 >0.2 µg/larva for permethrin, some or all of the pyrethroids against the bollworm is suggested; this proposed resistance threshold was first suggested for permethrin against the tobacco budworm (Martinez-Carrillo and Wolfenbarger 2003). Other LD50s need to be determined for each of the other pyrethroids for field collections across TX because their are differences in toxicity.

In the LRGV in 1990 LD50s of esfenvalerate, deltamethrin, tralomethrin, *lambda* cyhalothrin, bifenthrin and cyfluthrin to field collections of bollworms were $7.6 \times 10^{-5,}$ 1.5×10^{-4}, 0.0018, 0.0036, 0.0074 and 0.083 µg/larva, respectively (Wolfenbarger et al. 1998). LD50 for cypermethrin was 0.0051 µg/larva. All bollworms were susceptible to these pyrethroids including the standard cypermethrin.

Even if a field has populations which are resistant to cypermethrin it is unknown what the response levels are to the other pyrethroids. This needs to be determined because most of the pyrethroids were more toxic than cypermethrin; esfenvalerate has an LC50 which was 67 times more toxic than cypermethrin. Even if LC50s of adults of all pyrethroids were equally toxic toxicity of the larvae still needs to be evaluated. The AVT can be used as a bioassay for larvae but it has to be evaluated as described for the topical application (Anonymous 1970). Two pyrethroids, i.e. zeta cypermethrin and gamma cyhalothrin, are registered for use in TX but their toxicity against the bollworm has not been determined.

In 1990 and 1991 profenofos showed LD50s of 0.24 and 0.47 µg/larva. In 1990 and 1993 methomyl showed LD50s of 1.67 and 0.04 µg/larva, respectively. The bollworm was susceptible to both of these anticholinesterase insecticides.

In 1975-6, LD50s, as µg/larva, for a laboratory susceptible strain treated with deltamethrin (NRDC 161) and permethrin in two ratios (40:60 and 25:75) of cis:trans mixtures were 0.00086, 0.017 and 0.013, respectively (Davis et al. 1977). All LD50s indicated susceptibility. The susceptible strain was obtained from USDA-ARS, Tifton, GA, in the 1970s and maintained in the USDA-ARS laboratory without additions of field collected or other laboratory strains. This is essential for the maintenance of susceptibility in a strain.

In 1990 LD50s of the same laboratory susceptible strain described above ranged from 0.001 to 0.0001 µg/larva for bifenthrin, cyfluthrin, deltamethrin, esfenvalerate, *lambda* cyhalothrin, permethrin and tralomethrin, respectively (Wolfenbarger et al. 1998). LD50 of cypermethrin was 0.0013 µg/larva. LD50s for methomyl, profenofos, methyl parathion and emamectin hydrochloride were 0.35, 0.28, 0.12 and 0.12 µg/larva, against the same strain, respectively. All the populations were susceptible.

In 1988, 1989 and 1993 LC50s of cypermethrin by AVT were 0.17, 0.44 and 0.09 µg/vial for field collected adult moths from the Brazos Valley, respectively (Kanga et al. 1996). LC50s in 1988 and 1993 were equal because confidence interval overlapped. In 1988 an LC50 of 0.05 µg/vial for another field collected strain was deemed susceptible (Kanga et al. 1996). Response levels in 1993 indicate a reversion to susceptibility. The

LC50 for cypermethrin in 1993 was significantly less than the greatest value determined in 2003 (Pietrantonio et al. 2004).

In 2003, LC50s ranged from 0.47 to 2.75 µg cypermethrin/vial in the Brazos Valley and the Lower Coastal Plain (Pietrantonio et al. 2004). In May, June, July, August and September in the Lower Coastal Plain LC*50s* were 0.47, 0.94, 2.52, 2.0 and 0.071 µg/vial, respectively; LC50s at both locations indicated a reversion to susceptibility. The mechanism(s) for this reversion to susceptibility should be determined. Presumably the moths which are collected in August and September are from another location than those collected in July and they are more susceptible. Maybe they are from sorghum or other wild hosts and were never selected by the insecticides applied to the cotton. Maybe the most likely mechanism for resistance by the adults, target site, changes due to temperatures in the endemic populations in the two locations. LC*50* to 2.52 µg/vial was significantly greater than all the other LC50s determined in the Brazos Valley. This LC50 was equal to the LC50s of 2.75 and 1.59 µg/vial from July and August determined in 2003 the Lower Coastal Plain. Again the LC50 indicate a reversion to susceptibility.

In 2004 LC50s of moths from the Lower Coastal Plain in June were 4.01, 4.0 and 2.0 mg/vial; these results showed a reversion to susceptibility (Pietrantonio et al. 2005) The LC50 from the Lower Coastal Plain in April was equal to the last LC50 in June. In the Brazos Valley, High Plains, Blacklands and Wintergarden areas LC50s were <3.0 mg/vial. All of these LC50s showed susceptibility.

It is unknown what LC50 of cypermethrin for bollworm indicates susceptibility and which indicate resistance. If the LC50s were 100 to 200 mg/vial (shown for the tobacco budworm from Brazos Valley) instead of the 1- 4 mg/vial it is unknown what doses would show resistance for moths that were heterozygous or homozygous. LC50s, determined each y, do not automatically indicate resistance when LC50s revert to susceptibility following the high value.

Percentage mortalities by cypermethrin of male bollworms collected from traps and bioassayed at the discriminating dose of 10 µg/vial were 99% to 100% in 1998 (Martin et al. 1999), 96% in 1999 (Martin et al. 2000); 97% to 100% in 2000 (Payne et al. 2001), 97% in 2000 (Pietrantonio et a. 2001), 84% to 100% in 2003 (Pietrantonio et al. 2004) and 70% to 100% in 2004 (Pietrantonio et al. 2005). These response levels only indicate susceptibility. It is unknown if the 0% to 30% of the moths bioassayed which survive the 10 mg/vial are resistant to pyrethroids. The mechanisms of resistance for moth of each population needs to be determined. Some of these moths will die in the bioassay. They may be alive at the 24 h reading for mortality but they will die because their age is unknown. It is unknown what the response levels will be for the progeny following a mating with females which are untreated or have survived the application of a pyrethroid or anticholinesterase insecticide across TX.

The AVT bioassay of male moths does not indicate resistance by larvae. The response level of the males on one d has not been shown to indicate resistance by bollworm larvae in fields <1 or >10 km away from the trap(s). The LC50s of females which may be adjacent to the trap(s) are not known.

The testing of larvae by AVT or topical applications from field populations is required to indicate resistance. It is easier and faster to monitor the response of adults when collected from traps, but larvae also need to be monitored for their response.

Field control trials with pyrethroids, a carbamate and an organophosphorus insecticide were conducted against bollworm from trans Pecos in 1984 and 1986 (Allen et al. 1987). Larval populations in both 1984 and 1986 were confirmed to be >60% bollworms. Field control by each insecticide was indicated when there were 80% fewer larvae or damaged fruit compared to the untreated.

In 1984, *lambda* cyhalothrin, fenvalerate and cyfluthrin showed 86% control and were effective. Esfenvalerate and permethrin, with 76% and 71% control, were not effective. Two formulations of cypermethrin, at the same rate, showed 86% and 71% control, respectively. Both resistant and susceptible strains were present in the populations. One formulation showed control, the other did not. The cholinesterase inhibitors thiodicarb and profenofos were effective against this insect with 86% and 81% control, respectively.

In 1986 cypermethrin showed 53% control while thiodicarb and profenofos showed 61% and 39% control, respectively; none were effective. One y thiodicarb and profenofos were effective against the bollworm but were not effective the second y. Confirmation of resistance has not been determined for thiodicarb and profenofos against this pest.

More field tests need to be conducted with pyrethroid or anticholinesterase insecticides for efficacy against known bollworm populations in the cotton producing areas across TX. Populations or damaged squares and bolls from treated have to be compared to populations or damaged squares and bolls from untreated cotton.

In 1988 doses of 0.5, 1.0, 2.5, 5.0, 10.0 and 5.0 mg cypermethrin/vial killed 93% to 100% of neonate, first, second, third, fourth and fifth instar larvae of field collected bollworm from BV, respectively (McCutchen et al. 1989). All populations were susceptible and these results indicate that cypermethrin can control larvae of this insect.

The only study on inheritance of response to a pyrethroid by the bollworm was conducted in South Carolina (SC) (Brown et al. 1997). The LC50 of the field collected strain was 3.06 µg/vial and the laboratory susceptible strain was 0.62 µg/vial. The cross showed 50% mortality at 2.5 µg/vial indicating that the inheritance pattern shows incomplete dominance. No LC50 was shown for the cross. The LC50 of the strain in 1996 (Brown et al. 1997) was equal to LC50s of adults from TX collected in 2003 shown by overlapping confidence intervals (Pietrantonio et al. 2004). Neither the TX or SC bollworms are considered to be resistant. Inheritance studies need to be determined for bollworm across TX. Females of a susceptible strain could be crossed with the males collected from the traps in the field.

An experiment was conducted to determine the continuity of resistance to methyl parathion by larvae of the bollworm collected as larvae from cotton in Nicaragua (Wolfenbarger 1996). LD50 of first generation larvae were 50 µg/larva. This LD50 indicates resistance by the bollworm. In the second generation larvae from single pairs were treated with 100 µg/larva. In generations three, four and five 54, 63 and 27 single pairs, respectively, all larvae were treated with 50 µg/larva. Populations of third instar larvae/single pair, available to treat, were 65%, 67%, and 77% less than generation two than in generations three, four and five, respectively, than generation two. Larval mortalities of single pairs increased each generation from 69%, in generation two, to 76% in generation three, 85% in generation four and 96% in generation five. Strain showed ever increasing susceptibility in generations three, four and five (Wolfenbarger 1996). Variation in mortalities of the single pairs ranged from 24% to 100% during the generations from two to five. No single pair showed a decrease in mortality nor a resulting increase in survivorship during the five generations the test was conducted. Results clearly show a reversion to susceptibility.

In 1982 LD50s of methomyl for field collected bollworms from the LRGV were 0.54 µg/larva while LC50s from the Coastal Bend ranged from 0.09 to 0.16 µg/larva (Wolfenbarger et al. 1987). The greatest LD50 was significantly different from the other LD50s but all were considered to be susceptible. LD50s of 0.071 to 0.34 µg/larva determined in 1983 were equal because their CIs overlapped. In 1994 the LC50 was 0.3 µg profenofos/vial of the field collection of adult males only indicated susceptibility (Kanga et al. 1996). LC50s of methomyl and profenofos were equal for a susceptible strain and field collected strain; resistance was not indicated.

Discussion

Resistance of bollworm can only be demonstrated for all the above insecticides each y in each field of corn, cotton or grain sorghum in TX by constant monitoring across TX. The LC50 or LD50 of larvae from field collected bollworms coupled with field control tests conducted in tandem can indicate resistance or susceptibility.

Size of field populations of bollworm larvae before and after each application must be determined. Populations sizes of eggs and larvae must be counted as mean/plant or number/hectare. Damage to squares and bolls on each of the same plants must also be determined. Two to three applications can describe the resistance or

susceptibility of populations in cotton. Treatments have to include an untreated check and least one rate of cypermethrin. Resistance levels can be defined if larval population sizes are determined one to two d before and one, three to five d following each application of an insecticide. Resistance would be expressed if control was <80%. Huffman, et al. (1995) is a good source of information on problems associated with conducting field tests and the kinds of information to be collected.

Larval populations in sorghum and corn are not always treated when larval populations of bollworm are present in 20% to 40% of the plants. For every 100,000 plants/ha 20,000 to 40,000 larvae could be present in each ha of each field. These are high populations. The next generation of larval populations in cotton could be high but they would be susceptible. Those that survive the pyrethroid applications in cotton for one or two generations would be selected and disperse to corn or wild hosts which may or may not have been treated with more pyrethroids. Populations which survive cotton will disperse to corn or wild hosts in the fall and winter; these populations are not always treated. Selection pressure is minimal so the populations will remain susceptible. In the spring the overwintering generations will attack the sorghum and corn.

Ratios are a poor index to suggest that resistance to any insecticide is present in TX (Brattsten et al. 1986). LC50s of field populations compared to LC50s of a susceptible strain can provide a "resistance ratio". Laboratory testing methods rarely simulate natural conditions because they do not take insect behavior into consideration. However, laboratory bioassays do allow for selection of resistance.

For a discriminating dose to be established the susceptible strain has to remain stable. Author suggests that an LC50 of any such strain will change every two or three y. The change could be dramatic.

LC50s of males by ATV ranged from 1 to 3 µg cypermethrin /vial (Pietrantonio et al. 2004). These LC50s of adults do not indicate resistance in TX. They ranged from <1 to 4 mg/vial (Pietrantonio et al. 2005). If LC50s of adults were to indicate resistance it does not mean that LC50s for larvae found in adjacent or nearby fields of corn, cotton or sorghum will be resistant. Author considers that each field is the location where resistance or susceptibility to an insecticide(s) could be present. Resistance determined in one cotton production area does not mean that resistance can be declared in all the other areas across TX.

Insecticide resistance for the bollworm is a dynamic, multidimensional phenomenon, dependent on biochemical, physiological, genetic and ecological factors (Brattsten, et al. 1986). Factors will vary with composition of populations for resistance or susceptibility, time of y and geographic location. Resistance will develop by the reproduction of individuals carrying genes altered by one or more of many possible mechanisms that allow survival after exposure to an insecticide. As the selective pressure caused by the insecticide becomes more prevalent genetic mutation(s) which express resistance will be determined within the exposed population.

It is possible that laboratory and field selection responses by the bollworm may develop in different ways (Brattsten et al. 1986). The genetic composition of moths is usually limited in laboratory selection experiments because biological and environmental stress are minimized. This tends to promote development of resistance due to contributions from more than one mechanism. In a field population genetic diversity is considerable, but environmental stresses may limit survival. Field populations might be based on a single major mechanism.

In the past, new insecticides were available to replace those to which resistance had developed (Brattsten et al. 1986). The new insecticides which are toxic to the bollworm include emamectin benzoate, indoxacarb, *gamma* cyhalothrin, spinosad and zeta cypermethrin. A new insecticide need not be more toxic than the old insecticides against the bollworm, but its evaluation should include consideration of its tendency to function as a selecting agent.

Old insecticides are those which have been used for 20 to 50 y against this pest. They should be managed to insure their continued usefulness. The old insecticides include bifenthrin, cypermethrin, deltamethrin, cyfluthrin, esfenvalerate, *lambda* cyhalothrin, methomyl, methyl parathion, profenofos, permethrin, tralomethrin, and thiodicarb. All could be used for control of the bollworm on these three crops across TX. Growers could then select a different insecticide for each application in an effort to prevent selection for resistance to one insecticide.

Large-scale experimentation is needed to establish the validity of current theories of resistance management, most of which are based on isolated successes and laboratory experiments (Brattsten et al. 1986). Large scale experiments should be focused on maintaining a susceptible gene pool of bollworm populations which would allow economical and convenient control by chemical insecticides.

Definition of resistance by bollworm has to be measured with both laboratory bioassays and field tests. Larvae for topical application or vial bioassay have to be collected from the fields and reared to the next generation for species identification and subsequent larval treatment. Comparison of consistency of laboratory and field tests will lead to justification of resistance. Resistance can be indicated if LD50s of >0.2 µg/larva of pyrethroid insecticides and >7.5 mg/larva for anticholinesterase insecticides by topical application are determined and >80% control is determined from two to four field tests for one cotton producing areas across TX. Other thresholds will be developed for vial bioassay of larvae.

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Table l. LD50s, as µg/larva, of methyl parathion and permethrin to field collected strains of bollworm from the Lower Rio Grande Valley of TX.

Year	Methyl Parathion	Permethrin
1967 a	0.5	
1970 b	0.024 - 0.0022	
1974 c	1.45	0.055
1981 d	0.22	
1982 d	0.38-0.58	0.004-0.017
1983 d	0.1-3.65	0.0023-0.014
1990 d e	0.1	0.0022
1991 e		0.014
1993 e	0.04	0.00023

a Wolfenbarger and McGarr (1970)
b Wolfenbarger et al. [1973]
c Davis, et al. (1974)
d Wolfenbarger (2000).
e Wolfenbarger et al. (1998).