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DEFINING RESISTANCE OF TOBACCO BUDWORM TO PYRETHROID INSECTICIDES WITH TOPICAL APPLICATION BIOASSAY IN TEXAS

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Abstract

Resistanceof third instar larvae of the tobacco budworm, *Heliothis virescens* (F.) (TBW) to cypermethrin (Ammo) was shown with topical applications to field collected strains from the Wintergarden (WG) cotton producing area in 1986, the Brazos Valley (BV) in 1987 and the Lower Rio Grande Valley, (LRGV) areas of cotton production in 1988. The first y LD50s were determined by topical application in TX was 1975 and the last was 1992. An LD50 of >0.2 µg/larva was used as a resistance threshold to separate resistant from susceptible strains. Susceptibility by the TBW to cypermethrin was shown in the WG areas in 1992. Resistance to permethrin (Pounce) was present in the WG area in 1985 and 1992. In 1986 and 1992 susceptibility was shown to bifenthrin (Capture) in the LRGV. In 1992 susceptibility was shown by cyfluthrin (Baythroid), deltamethrin (Decis), esfenvalerate (Asana), *lambda* cyhalothrin (Karate), tralomethrin (Scout) and zeta cypermethrin (Fury) by any field collected strains of TBW from the LRGV. Recessiveness of resistance factors were shown when cypermethrin and permethrin were topically applied to larvae. All the above pyrethroids were considered effective by the Texas Cooperative Agricultural Extension Service in the LRGV until they were removed in 2000.

Introduction

TBW has been and most certainly can be a major pest of cotton in TX. Since 1995 populations of this pest have been sporadic or not found in 98% of the fields in the BV, WG and LRGV cotton producing areas of TX.

The chemistry of the class of pyrethroid insecticides show many similarities and some dissimilarities (Sparks 1996). All the pyrethroid insecticides which are currently registered for use against the TBW and are esters because they are composed of an alcohol and a acid. All except esfenvalerate possess an unsaturated side-chain. All possess the same 3-phenoxy benzyl alcohol but two pyrethroids possess a substitution on the alcohol moiety. All except permethrin possess the cyano group on the alcohol. Esfenvalerate is composed of a different acid than shown for bifenthrin, cyfluthrin, cypermethrin, deltamethrin, permethrin, *lambda* cyhalothrin, tralomethrin and zeta cypermethrin. Zeta cypermethrin is composed of four of the eight isomers of cypermethrin. Structural requirements for toxicity of the pyrethroid insecticides to the TBW are difficult to define (Sparks 1996). All have one or more bromine, chlorine or fluorine atoms in their structure. Most of the changes in chemistry of pyrethroids in the 1980s were driven by the capability to synthesize the most toxic isomers to insects. Esfenvalerate (Asana) is the most toxic isomer of the four which comprise fenvalerate. (Wolfenbarger et al. 1989).

The most widely accepted hypothesis for initiation of resistance by TBW populations from the field to the pyrethroids is described (Miller 1996). The “classic” signs of resistance are exhibited by populations of TBW which are not killed in scattered fields in each of the cotton producing areas of Texas following frequent applications of selective doses of a pyrethroid (Miller 1996). These are called field control failures because there is a significant frequency and a high enough density of resistant larvae to cause damage (Roush and Luttrell 1987). Resistance will gradually “creep” into the agro-economic milieu as it builds and declines in repeated cycles (Miller 1996). The “ratchet up” effect of alternating build up and decline of resistance was seen in resistance monitoring data for TBW in the USA (Mullins 1991). In most cases resistance occurs in species that tend to be endemic because they are under more selection pressure for resistance (Miller 1996). Author suggests that resistance factors for TBW have to be consistent y after y in fields of the cotton producing areas across TX. Resistance cannot be declared in TX when the results from the laboratory bioassays are determined once or twice/y in one or two of the cotton producing areas.

Cypermethrin was selected by industry groups PEG-US and IRAQ-US and the Texas Agricultural Extension Service and Experiment Station as the standard pyrethroid to monitor for resistance by TBW. Here, there is information on field collected strains from the cotton producing areas from BV, LRGV and WG. LD50s by topical applications from field collected strains to eight pyrethroids were determined.

Removal of the suggested pyrethroids in TX was not justified because (1) mortalities by cypermethrin showed both resistance and susceptibility of field collected TBW in laboratory bioassays in one or more of the cotton producing areas of TX and (2) cross resistance was not determined for the other pyrethroids. Resistance in TX cannot be justified when one strain in one field in one cotton producing area is resistant but not from other fields in the same area and all the other areas. The pyrethroids were also removed from the list because other newer registered insecticides, i.e. transgenic Bt cotton (Bollgard), indoxicarb (Steward), spinosid (Tracer) and emamectin benzoate (Denim) as well as several anticholinesterase insecticides because they are effective and are suggested insecticides against this pest.

Published references are shown on toxicity of cypermethrin and the other pyrethroids based on the topical application to larvae of TBW from field collected strains collected from cotton. Resistance to the pyrethroids was monitored by the topical bioassay to larvae prior to and during the use of the AVT. None of the information on topical applications of pyrethroids was used to remove them as suggested insecticides against TBW from TX. Bifenthrin, cyfluthrin, cypermethrin, deltamethrin, esfenvalerate, *lambda* cyhalothrin, tralomethrin and zeta cypermethrin are all registered for use and were suggested for use on cotton. They were removed in 2000 as suggested insecticides from the LRGV (Norman and Sparks 2000). References on pyrethroids from TX used to justify their removal were identified by an asterisk in the citations. Other references on toxicity of TBW from LA showed LD50s based on topical application of strains collected from TX.

Resistance by a larval population to a pyrethroid is based on the topical application to larvae which are progeny from adults or larvae collected from fields in the BV, LRGV and WG cotton producing areas in TX from 1975 to 1992. The same or different threshold for resistance has to apply to each pyrethroid. Resistance by the TBW to cypermethrin by one strain in each area sampled may be shown each y, but this does not mean there is cross resistance to all the other pyrethroids in all the other cotton producing areas. In 1990 selection pressure on populations in the cotton producing areas by pyrethroids was low, but resistance levels were high (Mullins et al. 1991). Populations of TBW were generally low in all cotton producing areas of TX in 1990. Permethrin was the first registered pyrethroid in 1979 (Gage et al. 1991). *Gamma* cyhalothrin was the last registered pyrethroid and it has not been monitored for resistance in TX. Response levels of a pyrethroid insecticide can and do vary within and between each area because the TBW moths can disperse within and between these areas. Moths can also disperse both south and north from any given area.

Topical application bioassay is described by Anonymous (1970) for larvae of TBW. Larvae were collected from cotton are reared to adults. This collection is considered to be a strain and it is identified by location and y. Species was identified at this time. Adults were paired and the larvae were placed on artificial diet to time of treatment. It takes 30 to 40 d for collection to the d of first progeny. Three to seven d later the larvae are treated and the LD50 and 95% confidence interval, as mg/larva, and slope ± standard error were determined (SAS 1988). The length of time for the bioassay from collection to the last d of determining the mortalities would take about 50 d.

Literature on LD50s from the three cotton producing areas of TX .

In the LRGV LD50S of permethrin for a field collected strain of TBW were 0.0058 to 0.06 mg/larva from 1974 to 1976 (Davis et al. 1975, Davis et al. 1976 and Wolfenbarger et al. 1977). LD50s showed only susceptibility to different isomers of permethrin. Resistance and susceptibility for pyrethroids against TBW were can be separated by a resistance threshold of LD50 >0.2 mg/larva in TX (Martinez-Carrillo and Wolfenbarger 2003). Ratios which indicate the magnitude of differences in the LD50 for a susceptible strain and a field collected strain can also be used but the LD50 for the susceptible strain has to continually be monitored (two to three times/y). From 1978 to 1981 only susceptibility to permethrin by TBW was determined in the LRGV from LD50s which ranged from 0.03 to 0.19 mg/larva) (Wolfenbarger et al. 1984).

In 1976 only susceptibility to deltamethrin was determined in the LRGV (Davis et al. 1977); LD50 of 0.00086 mg/larva was determined. From 1978 to 1981 only susceptibility by field collected strains to cypermethrin, cyfluthrin and deltamethrin with LD50s of 0.025, 0.029 and 0.0026 mg/larva, respectively (based on 20 mg larvae as mg/g in Wolfenbarger et al. 1982). From 1979 to 1983 LD50s of cypermethrin against field collected TBW from

BV, WG and the LRGV were 0.12, 0.082, 0.1, 0.029 and 0.022 mg/larva and all were susceptible (Staetz 1985). LD50s to permethrin by TBW from LRGV were 0.029, 0.018, 0.02, 0.02, 0.05, 0.06 to 0.09 mg/larva in 1981 to 1983, 1985 and 1987 to 1988, respectively (Martinez-Carrillo and Wolfenbarger 2003). All strains were susceptible.

LD50s of permethrin for strains from the BV were 0.013, 0.26, 0.29 and 0.25 µg/larva in 1982 (Staetz 1987), 1987 (Staetz et al. 1989) 1988 and 1989 (Staetz et al. 1991), respectively. In BV resistance was indicated from 1987 to 1989. LD50s of permethrin for strains from WG were 0.011, 0.089, 0.08, 0.12 in 1985, 1986, 1987 and 1988, respectively (Staetz et al. 1989) and 0.35 in 1989 (Staetz et al. 1991). Resistance to cypermethrin was not indicated in any population in the LRGV as LD50s were all <0.2 mg/larva to 1988 but it was shown in 1989. This is variation in susceptibility.

In 1985, LD50s of 0.016 and 0.1 µg/larva for cypermethrin and permethrin were shown by a strain from the WG (Leonard et al. 1987). The strain was susceptible to both pyrethroids. Resistance by the TBW to cypermethrin in TX was first determined in 1986 by a strain from the WG area (Leonard et al. 1987). In 1987 in the same area resistance by TBW was shown to bifenthrin, cypermethrin and permethrin but susceptibility was shown by esfenvalerate, deltamethrin and *lambda* cyhalothrin (Sparks et al. 1988). Cross resistance was not shown for these pyrethroids in this area. This is why resistance cannot be declared for all the pyrethroids all the time.

LD50s of cypermethrin were stable in the WG from 1980 to 1986 (Staetz et al. 1988). In 1987, from the same location, another TBW strain was resistant (LD50 was >0.2 µg/larva) to cypermethrin (Staetz et al. 1988). In 1986, an LD50 for a field collected strain in the LRGV for esfenvalerate (0.034 µg/larva) showed susceptibility (Wolfenbarger et al. 1989). In 1992 resistance to permethrin was shown by an LD50 of 3.83 µg/larva while LD50s of bifenthrin and cyfluthrin from field collected strains were 0.068 and 0.19 µg/larva, respectively, showing susceptibility (Wolfenbarger and Vargas-Camplis 1997). Cross resistance was not shown with these results.

Staetz et al. (1988 and 1991) discussed advantages and disadvantages of the topical application bioassay to third instar larvae. LD50s of cypermethrin in LRGV were 0.028, 0.025, 0.018, 0.06, 0.044 and 0.082 and 0.0.056 µg/larva in 1980,1981,1982,1983,1985, 1987 and 1988, respectively. LD50s from WG of 0.01, 0.08, 0.068, 0.11 and 0.032 µg/larva were determined in 1983, 1985, 1986, 1987 and 1988, respectively (Staetz et al. 1988). Only susceptibility was determined for the strains from the LRGV and WG to 1988. LD50s of cypermethrin from the BV were 0.012, 0.26, 0.13, 0.29, 0.41 and 0.25 µg/larva in 1982, 1987, 1988, 1989, 1990 and 1991 (Staetz et al.1989 and 1991) In the BV susceptibility was determined in 1983 and 1986 while resistant populations were determined in the other y. Resistance cannot be declared in these areas because the variation in LD50s.

In 1979, a population of larvae were collected from a cotton field near Brownsville, TX and an LD50 of 0.060 µg permethrin/larva (3.01 µg/g for a 20 mg larva) in the first larval generation of communal mating of 30 pairs of moths (Wolfenbarger 1990). Ten to 30 pairs were paired in subsequent generations. In generation 13 an LD50 of 0.028 µg/larva (1.42 µg/g) was determined indicating a reversion of susceptibility. Each larva was treated each generation with a dose of permethrin and reared the survivors to the next generation.

Then in generation 11 a single pair showed an LD50 of >1,000 µg/larva Moths were obtained from the communal matings. In generation 12 one single pair was again established from the surviving progeny of larvae in generation 11. An LD50 of >1,000 µg/larva was again determined. Then in generations 13 and 14 LD50s were 0.14 and 0.067 µg/larva. These LD50 show a dramatic reversion to susceptibility. Author suggests that this loss of resistance was caused by “lack of fitness” of the highly resistant progeny of the single pair and recommend that future monitoring of responses to pyrethroids over widespread areas in a systematic manner so that any changes in response can be noted.

In 1992, in the LRGV, three strains of TBW were shown to be resistant (LD50s were >0.2 µg/larva) to cypermethrin in different fields near La Blanca and San Perlita, in the LRGV; LD50s ranged from 0.076 to 0.78 µg/larva, a 10 fold difference (Norman et al. 1993 and Wolfenbarger and Vargas-Camplis 1997). Strains from Brownsville and field two from La Blanca were susceptible to cypermethrin that same y. The La Blanca strain

(fields two and three) were susceptible to bifenthrin and cyfluthrin, respectively. Cross resistance to pyrethroids was not shown.

In the late 1980s two strains selected for resistance, i.e. PEG87 and Dupont, were more resistant to cypermethrin than field collected from BV (McCafferty et al. 1991). Insecticides were topically applied to third instar larvae in all comparisons. LD50s of 52 and 74 for the Dupont strain and 4,882 and 70,000 for the PEG87 strain were greater than the LD50 of 0.017 mg/larva for the susceptible strain. LC50s of field collected strains from Snook and Hearne of BV were six to 11 greater than other LD50s of 0.009 and 0.013 mg/larva of the susceptible strain.

In the WG from 1985 to 1990 (Staetz et al. 1991) and 1992 (Wolfenbarger and Vargas-Camplis 1997) LD50s of cypermethrin were 0.12, 0.42 and 0.67, 0.081 and 0.035 µg/larva, in 1985, 1986, 1987, 1990 and 1991, respectively. Resistance was determined in 1986 and 1987 while reversion to susceptibility was determined in 1990 and 1991. In 1991 LD50s of 0.13 and 0.085 µg/larva of TBW were determined in strains from Snook (BV) and Uvalde (WG), respectively (Martin et al. 1992). The LD50 against the strain from winter garden was resistant in 1986 and 1987 but susceptible in 1985, 1990 and 1991. The strain from Snook was susceptible. Resistance by cypermethrin cannot be declared when LD50s rise and fall. In 1992 populations of TBW were resistant to permethrin in the WG and cypermethrin in the LRGV (Wolfenbarger and Vargas-Camplis 1997). These were the last LD50s determined for these pyrethroids against TBW in TX.

Larvae, used in a single pair test in 1989 and 1990, were collected from different fields in the LRGV (Wolfenbarger et al. 2000 and Table 1). Adults were allowed to emerge and were paired singly. As much as possible we tried to pair moths from the same fields. Three (13%) of the 23 LD50s of cypermethrin against the larvae of the single pairs had LD50s >0.2 µg/larva. The greatest LD50 to larvae was from moths collected from fields located about 70 km apart but moths from the other two collections were from a field near Brownsville. Larvae of these three single pairs were resistant while the other 87% were susceptible. This means that response was variable and resistance is not consistent in all TBW populations across the LRGV. Larvae from single pairs of moths collected as larvae from different fields across the LRGV is an excellent methodology for evaluating resistance. There was a 64 fold difference in LD50s and great variation was determined.

A laboratory susceptible strain was maintained at the Brownsville and Weslaco laboratories and showed LD50s of <0.01 µg/larva for all eight of the suggested pyrethroids listed for TX (Wolfenbarger and Vargas-Camplis 1997). The strain had been maintained in the laboratory for two decades without the addition of any other populations. Bifenthrin and esfenvalerate showed LD50s of 0.017 and 0.018 µg/larva. Cyfluthrin, deltamethrin, *lambda* cyhalothrin, permethrin and zeta cypermethrin showed LD50s ranging from 0.001 to 0.006 µg/larva against this strain. LD50 of tralomethrin (as HAG 107), was 0.0018 µg/larva against this strain (Wolfenbarger and Harding 1982). LD50s were determined from 1976 to 1980 which allowed the 10 fold difference in the LD50s. As shown for these same pyrethroids tested against field collected strains there is variation in response.

In 1981 an LD50 of 0.0038 µg/larva (Table 2) was determined for permethrin using multiple pairs of the same susceptible strain (Wolfenbarger and Vargas 1997). The strain was susceptible. The author wanted to determine the amount of variation in two to five single pairs in four subsequent generations. LD50s of the single pairs ranged from 0.0058 to 0.015, 0.0026 to 0.14, 0.0086 to 0.018 and 0.0038 to 0.024 µg/larva in generations two, three, four and five, respectively. All single pairs were susceptible (LD50 <0.2 µg/larva). Differences in LD50s were 3, 54, 2 and 6 in generations two through four. This demonstrates the problem of using a discriminating dose of a susceptible strain to indicate whether a field collected strain is resistant or susceptible.

Mechanisms of resistance can play a large role in how response patterns can vary in larval populations in each field in each of the cotton producing areas of TX. Target site resistance is present in all development stages while the various factors exhibiting metabolic resistance are present in third stage and older larvae (Campanhola and Plapp (1989a). Most of the mechanisms for resistance may be known but it not know how levels of these mechanisms flow in populations in TX. Nerve insensitivity (or target site) resistance to cypermethrin present in field collected strains is described (Gladwell et al. 1990). It is unknown what the effect of each pyrethroid is on nerve insensitivity level for all the strains in TX. Target site resistance to cypermethrin was found in four field collected strains of TBW from the BV (McCaffery et al. 1991). The pyrethroids cause the sodium gate system present in the

central nervous system to remain open following exposure (Sparks 1996). This needs to be determined for larvae across TX. Field collected TBW strains from the BV do not possess monooxygenase activity by first instar larvae.

Of interest was the variation shown for nerve insensitivity and
penetration profile of only cypermethrin into larvae of larvae of strain of TBW (Clower et al. 1992). Strains from WG (Hondo) and the BV (College Station) showed about equal levels of nerve insensitivity but values ranged from 62% to 32% when the larvae were susceptible while 82% to 50% of the larvae were both susceptible and heterozygous while 18% to 50% were resistant. Penetration of cypermethrin through the larval cuticle showed completely different qualitative levels for >67% of insects from WG and BV.

Third instar larvae of TBW, collected from cotton leaves treated with cypermethrin, exhibited a incomplete recessive mode of inheritance (Roush and Luttrell 1990). From other references mode of inheritance for cypermethrin and permethrin has been summarized as autosomal and incompletely recessive when third instar larvae were treated (Wolfenbarger and Bartlett 2003). This does not mean that all populations of TBW are resistant to all pyrethroids with the same mode of inheritance (Hatfield et al. 1991). Further research is needed to understand the number of major or minor loci for pyrethroid resistance. They also stated that all mechanisms involved in pyrethroid resistance are not expressed in all populations of this species. It is clear that many issues remain with regard to the overall understanding of the genetic basis of pyrethroid resistance in the TBW. Additional research is essential to determine the frequency of genes responsible for resistance.

Conclusion

From 1975 to 1992 LD50s of eight pyrethroid insecticides to different strains of tobacco budworm larvae were determined by topical application for resistance and susceptibility from BV, WG and LRGV. A resistance threshold of LD50 >0.2 mg/larva for cypermethrin but other LD50s could be determined for other pyrethroids. Pyrethroids should be suggested for use by Texas Agricultural Cooperative Extension Service. None of the strains were consistently resistant to one of the pyrethroids. Both resistance and susceptibility of the strains was exhibited. Populations should be evaluated for resistance separately against populations in each of the cotton producing areas of TX.

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Table 1 . Toxicity of cypermethrin to larvae from single pairs by topical application. Lower Rio Grande Valley, TX. 1989-1990.

Cross	Larvae	Slope ± SE	LD_{50}	95%

	treated		(µg/larva)	Confidence Interval
Weslaco x Weslaco	79	1.13 ± 0.3	0.027	0.011-0.05
Brownsville x Los Indios	133	0.9 ± 0.1	0.053	0.0069- ∞
Weslaco x Weslaco	59	0.83 ± 0.3	0.071	0.025-0.71
La Feria x Brownsville	50	0.86 ± 0.4	0.074	0.028-$8.6x10^8$
Weslaco x Las Milpas	71	1.4 ± 0.3	0.077	0.042-0.14
Weslaco x Weslaco	191	1.7 ± 0.2	0.079	0.059-0.11
Weslaco x Weslaco	182	1.48 ± 0.3	0.089	0.046-0.18
Las Milpas x Edinburg	311	0.77 ± 0.1	0.096	0.049-0.17
Weslaco x Weslaco	96	1.84 ± 0.5	0.12	0.041-0.8
Weslaco x Weslaco	61	1.4 ± 0.3	0.14	0.069-0.28
Santa Rosa x Brownsville	92	0.6 ± 0.22	1.75	0.36-88.61

Table 2. Toxicity of permethrin against a laboratory susceptible strain of the tobacco budworm by topical application. 1981.

Larvae tested	Slope ± SE	LD_{50} (µg/larva)	95% Confidence Interval
Generation 1 (multiple brothers-sisters)			
205	1.26 ± 0.31	0.0038	0.0018-0.0063
Generation 2 (single pairs)			
290	2.18 ± 0.32	0.0058	0.0038-0.0086
56	2.94 ± 0.52	0.0071	∞-∞
150	1.42 ± 0.29	0.011	0.0066-0.024
160	2.49 ± 0.45	0.015	0.0095-0.059
Generation 3(single pairs)			
290	1.22 ± 0.19	0.0024	0-0.0075
75	0.58 ± 0.14	0.0026	∞-∞
192	1.37 ± 0.41	0.0054	0.004-0.016
103	0.68 ± 0.17	0.095	∞-∞
121	0.39 ± 0.14	0.14	0.0048-∞
Generation 4 (single pairs)			
312	2.09 ± 0.32	0.0086	0.0038-∞∞
175	0.16 ± 0.062	0.015	0.0038-∞∞
430	0.74 ± 0.21	0.018	0.014-0.025
Generation 5 (single pairs)			
176	0.54 ± 0.17	0.0038	0.0027-0.005
214	0.7 ± 0.27	0.024	0.013-0.057