2006 Beltwide Cotton Conferences, San Antonio, Texas - January 3 - 6, 2006

DEFINING RESISTANCE TO PYRETHROIDS BY LARVAE OF TOBACCO BUDWORM WITH VIAL BIOASSAY IN TEXAS

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Abstract

The vial test (VT) for larvae of the tobacco budworm, *Heliothis virescens* (F.) (TBW), is superior to that for adults. Few LC50s of cypermethrin were determined for field collected larvae from TX. Most LC50s for larvae were determined for a selected resistant strain (PEG87), but this strain is not representative of responses to cypermethrin by those collected from cotton fields across TX. LC50s by VT of a susceptible strain of larvae by tralomethrin, lambda cyhalothrin, bifenthrin, cyfluthrin, cypermethrin, fenvalerate and permethrin ranged from a low of 0.025 to a high of 0.51 mg/vial, respectively, a 20 fold difference. Neonate larvae from one field in the Brazos Valley (BV) showed 10.3% (pretreatment), 25.6%, 19.5%, 44.5% and 26.6% resistance from field collected eggs 1-3 d following four applications of cypermethrin based on a discriminating dose of LC81. Neonates did not show high levels of resistance. Average levels of resistance to cypermethrin were 37.0%, 63.5%, 72.0% and 89.0% from 31 July to 20 August for first through the fifth instar larvae collected from cotton in the BV. Field collected larval populations increased in resistance as the season progressed.

Introduction

Field control failures by pyrethroids against larvae and the feeding damage by TBW were indicated in the WG (Uvalde and Garden City) and trans Pecos areas of cotton production in Texas in 1985-1986 (Plapp 1986, Plapp and Campanhola 1986, Plapp 1987, Campanhola and Plapp et al. 1987b and 1988 and Plapp, et al. 1989). Data was never shown to prove which pyrethroid failed to control the TBW populations in fields of these two areas of cotton production. Then, LC50s by VT were developed to monitor the levels of response of field collected larvae by cypermethrin and *lambda* cyhalothrin in TX, respectively (McCutchen and Plapp 1989a and Kostroun et al. 1992 and 1993). There is data by topical application on mortality of pyrethroids against larvae previously suggested for use across TX (Wolfenbarger 2006). VT bioassays were developed for *Heliothis/Heliocoverpa* larvae collected directly from the field (McCutchen and Plapp 1989a). These bioassays were easier and much faster than the topical bioassay used by the author where the larvae were collected and reared to larvae and treated one generation later (Wolfenbarger 2006). The percentage of *Heliothis/Heliocoverpa* neonate through fourth instar larvae present in each field on each d of sampling is difficult to discern. Author prefers to maintain the larvae on artificial diet and allow the adult to eclose for proof of species. Species has to be known for the larvae treated and it is most difficult to collect enough larvae of each instar on each d of collection from one field to conduct any bioassay.

To 1987, the consequences of resistance by field collected strains of this insect are described (Riley 1988). To 1990 the methods used to evaluate resistance to TBW by VT are described (Gage, et al. 1991a). To 1991, Clower et al. (1992) described TBW as a consistent pest of cotton in TX. Since 1995, populations of this insect have been low in most fields of the cotton producing areas of TX.

VT methodology for larval stages of tobacco budworm

Toxicity of cypermethrin against larvae of TBW in the cotton producing areas of TX was determined following foliar sprays and VT for neonate and each larval instar. Toxicity of cypermethrin by VT to a susceptible strain of TBW were determined by discriminating doses of each instar. LC50s and 95% confidence intervals (CI), as mg/vial, and slope ± standard error were determined by calculations (SAS 1988). CIs were used to determine significant differences between LC50s. Overlapping CIs indicate equal responses.

PEG-U.S. and IRAQ-U.S. summarized results of various AVT bioassays with only cypermethrin against this insect beginning in 1985 (Plapp 1986). The bioassay of larvae (Simonet et al. 1988) and first instar larvae following foliar sprays (Collins et al. 1988) were described. Industry groups summarized their results of bioassays from 1988

to 1991 (Riley et al. 1988, Rogers et al. 1990, Mullins et al. 1991 and Clower et al. 1992), respectively. LC50s for neonate larvae were determined by vial bioassay (Campanhola and Plapp 1989b). Foliar sprays of cypermethrin to cotton plant terminals were used to determine toxicity of TBW larvae (Roush and Luttrell 1987). A proposed testing sequence for monitoring resistance to pyrethroids against TBW was outlined in 1987 (Riley 1988). Other references on response of field collected strains and susceptible strains by VT with laboratory bioassays from 1986 to 1999 in TX are summarized here (Gage et al. 1991a). No resistance threshold has been proposed for VT against neonate and third instar and larvae.

Results of VT against the TBW

In 1980, cypermethrin, fenvalerate and permethrin indicated LC50s of 2.0 and 1.7, 4.4 and 3.9 and 39 and 36 µg/vial to larvae in 1978 and 1979, respectively, from the BV (Plapp 1981). LC50s for fenvalerate and permethrin were 2.7 and 5.7 mg/vial for a susceptible strain, respectively. Resistance to permethrin and susceptibility to cypermethrin and fenvalerate were determined. Cross resistance was not determined. But Campanhola and Plapp (1987a) stated that there was cross resistance by the pyrethroids because the toxicity for cypermethrin was the same for all pyrethroids. Author suggests that data is not available to confirm cross resistance for pyrethroids against TBW larvae for one or all cotton producing areas of TX. (Campanhola and Plapp 1987, 1988 and 1989ab) only used a selected resistant strain (PEG87). Great differences in toxicity to this selected strain of larvae compared to larvae of field collected strains from TX were determined (McCafferty et al. 1991). Only McCutchen and Plapp (1989 and McCutchen et al. (1989ab) and Kostroun and Plapp (1992 and 1993) bioassayed field collected strains of TBW by VT. In the future only toxicity of field collected strains should be used to compare to toxicity of a susceptible strain.

LC50s of a susceptible strain by VT using tralomethrin, *lambda* cyhalothrin, bifenthrin, cyfluthrin, cypermethrin, fenvalerate and permethrin were 0.025, 0.045, 0.068, 0.078, 0.19, 0.38 and 0.51 mg/vial, respectively showed a 20 fold difference (Campanhola and Plapp 1987a). CIs between LC50s of the seven pyrethroids were not shown so differences between LC50s cannot be determined. Slopes ranged from 0.83 to 1.5 and none were steep. If any field collected strain from TX were bioassayed the comparison would be valid. Four of the pyrethroids were more toxic to this susceptible strain than cypermethrin.

In 1989 LC50s of 122 and 257 ppm cypermethrin were determined for neonate and third instar larvae from BV and 74 and 32 ppm for the same stages of larvae from the WG (Rogers et al. 1990). These LC50s were 35 to 150 fold greater than LC50s of 1-2 ppm for the laboratory susceptible strain. Larvae of both stages were more susceptible in WG than BV. The dose of 10 µg/vial cannot be considered equivalent to expected field performance (Rogers et al. 1990).

LC50s for cypermethrin by VT bioassays of larvae from a susceptible strain from Stoneville, MS were 0.17 (neonate larvae), 0.28 (first instar larvae), 0.75 (second instar larvae), 1.8 (third instar larvae), 3.36 (fourth instar larvae) and 1.65 (fifth instar larvae) mg/vial (McCutchen, et al. 1989bc). In another bioassay of the same susceptible strain LC50s of 0.5, 0.75, 1.5, 2.7 and 4.2 mg/vial for first through fifth instar were determined (McCutchen and Plapp 1989). LC50s by VT for first and third instar larvae of the same susceptible strain of TBW were 0.004 and 0.054 (1992) and 0.085 (1993) mg *lambda* cyhalothrin/vial (Kostroun and Plapp 1992 and 1993). *Lambda* cyhalothrin was more toxic than cypermethrin against this susceptible strain.

LC50s of cypermethrin and the other pyrethroids need to be determined against larvae in fields in all cotton producing areas in TX. Larvae should be collected from the fields and reared to the next larval generation for identification of species. If both species are found in a collection from the field they can be reared separately so that the first generation larvae are bioassayed.

A bioassay with neonate larvae was evaluated in 1987 (Collins et al. 1988). Eggs were collected from the field, allowed to eclose and develop for species. Adults were allowed to oviposit. Following eclosion larvae were placed on leaves sprayed with ppm doses of cypermethrin. LC50s ranged from 9.0 (Midland in the northern rolling plains) to >71.0 ppm in the BV. In LRGV (McAllen) and the WG (Uvalde) LC50s ranged from 32.0 to 36.0 ppm. In LRGV (Progreso) LD50s ranged from 19.0 to 21.0 ppm. In the BV (Hearne and College Station) LC50s for neonate larvae ranged from 40 to 80 ppm for first instar larvae and 256 to 512 ppm for third instar larvae (Collins et

al. 1988). Eight criteria were developed for suitability of this resistance monitoring technique with foliar sprays against neonate larvae. This technique can distinguish between resistant and susceptible populations and reflect field performance. A resistance threshold has not been proposed for this bioassay method.

Toxicity of neonate larvae of a laboratory susceptible strain of TBW was evaluated by the VT bioassay in 1988. LC50s of <0.1 µg/vial indicate susceptibility. LC50s for bifenthrin, cyfluthrin, cypermethrin, deltamethrin, esfenvalerate, *lambda* cyhalothrin, permethrin and tralomethrin were 0.015, 0.022, 0.013, 0.012, 0.04, 0.04, 0.084 and 0.035 µg/vial, respectively, (Campanhola and Plapp 1989b).

Derived resistance of INSTAR procedure for first through the fifth instar larvae collected in the field and immediately bioassayed and the EGG-NEONATE procedure for neonate larvae collected as eggs from the field with VT are shown (McCutchen and Plapp 1988 and McCutchen et al.1989bc). Resistance by INSTARS procedure was determined by calculating percentage mortality with discriminating dose of a susceptible strain for neonate larvae and each larval instar of a field collected strain from BV.

The discriminating dose of the susceptible strain for neonate larvae was determined when regression of sigmoidal curve peaked at LC81 (McCutchen and Plapp 1989). Mortalities of this susceptible strain above LC81 did not increase. Mortalities of neonate larvae by EGG-NEONATE procedure were 10% (3 July), 26% (16 July), 45% (15 August) and 27% (19 August) (McCutchen et al. 1989 and McCutchen et al. 1989). Egg samples were taken 1-3 d following each application. Mortalities in cotton showed a 30% difference in resistance. They were far greater in this test than the previous test on cotton.

INSTAR procedure showed derived resistance of 37.0%, 63.5%, 72.0% and 89.0% on four sample d from 31 July to 20 August for neonate and first through fifth instar larvae collected directly from cotton in the BV (McCutchen et al. 1989c). Procedure showed increasing resistance by all stages of the larvae as the season progressed. The procedure would be valuable if used in fields in all areas. Mortalities of susceptible strain were 81%, 89.2%, 87%, 83.9%, 78.9% and 84.8% of neonate, first, second, third, fourth and fifth instar larvae at discriminating doses of 0.5, 1.0, 2.5, 5.0, 10.0 and 5.0 µg cypermethrin /vial, respectively (McCutchen et al. 1989bc). Derived resistance by EGG-NEONATE procedure was 86.3%, 71%, and 47% for TBW from tobacco and 10.5%, 5.7%, 0% and 15.3% from cotton. Resistance by TBW in tobacco was far greater than shown in cotton. In other bioassays derived resistance by INSTARS procedure for field collected larvae showed resistance of 65.5% from tobacco on one sample d and 80.8% and 27.8% from cotton on two sample d. Resistance was extremely variable for these bioassays.

Moths were bioassayed in the field at 10 mg cypermethrin/vial and mortalities were 86%, 80%, 85%, 96%, 79%, 79%, 66% and 48% on 3, 10, 17, 24 and 31 July and 7, 14 and 21 August, 1988, respectively (McCutchen et al. 1989 and McCutchen et al. 1989b). Moths were susceptible at 10 mg/vial except for the last two sample dates. Overall, the correlation between percent resistance in neonates collected as eggs in the field and control with cypermethrin was quite good.

A field test was conducted at 0.067 kg(A.I.) cypermethrin/ha in BV, TX; in the pretreatment count (20 June) taken four d before the first application egg counts/plant were 1.23, larvae were 0.08 and damaged squares were 2% (McCutchen et al. 1989a). The egg counts were exceedingly high while the larval counts and damage levels were low. One d after the applications (24 June, 1 July and 28 July) eggs and larvae/plant were 1.05, 2.1 and 0.9 and 0.14, 0.12 and 0.07, respectively. Damaged square counts were 4%, 2% and 2% on these same d and did not exceed damage threshold of 5% needed to initiate treatment. Egg populations were high throughout the test, but egg hatch in the untreated cotton was minimal at 12%, 5% and 6%. Larvae/ plant were 0.61 and 0.8 in the untreated, which indicate high populations. Only 24% control of the larvae in the treated cotton was determined. Yields were equal in treated and untreated.

Comparison of toxicity by different bioassay techniques.

In 1986 compared mortalities of larvae following sprays to cotton terminal bud foliar sprays in the BV and VT bioassay of larvae vs. toxicity of moths to TBW were compared (Roush and Luttrell 1987). Mortalities of third instar larvae were collected from WG and BV when sprays of fenvalerate and cypermethrin were 20%, 24%, 40% and 71% and compared to mortalities conducted with the adult vial test at 5 mg/vial. These foliar sprays and AVT mortalities showed a high correlation coefficient. If AVT mortalities are always related to mortalities of third instar larvae on cotton bud terminals at 0.011 to 0.067 kg (AI)/ha resistance could be accurately assessed. These results need to be further explored. Each bioassay method used in monitoring for resistance or susceptibility of larvae has particular advantages and disadvantages (Hatfield et al. 1991).

In 1987 VT, leaf spray and topical application bioassays were compared against field collected strains of TBW (Riley et al. 1988). In the BV LC50, LC50 and LD50 values were 40.1 µg/vial, 72.9 ppm and 0.31 µg/larva, respectively. In the WG the same values were 6.8 µg/vial, 34.2 ppm and 0.15 µg/larva, respectively. In the LRGV, the same values were 5.3 µg/vial, 22.6 ppm and 0.086 µg/larva, respectively. All three methods show resistance in the BV, but show susceptibility in the WG and LRGV. A susceptible strain shows LC50s of 0.7 µg/vial, 0.9 ppm and an LD50 of 0.0071 µg/larva for cypermethrin.

There was a significant correlation coefficient (r=0.99, P=0.01) between mortalities for moths and neonate larvae by VT following applications of cypermethrin by ground and aerial applications (Gage and Hatfield 1989, Gage et al. 1990 and Gage et al. 1991b). Moralities of moths ranged from 90% to 96% at field use rates of cypermethrin at 0.043 to 0.087 kg (AI)/ha with petri dish bioassays. Older larvae required 2.5 to 2.6 greater rate to produce mortalities equal to those determined for neonate larvae or moths. This important result also refutes the 10 µg/vial as a discriminating dose for separating populations which are resistant from those which are susceptible. It also relates vial bioassay to field results.

Discussion

It probably makes no difference whether larval vial bioassay or topical application of larvae by any pyrethroid is used to determine resistance. The values will be different, but they can be used to determine resistance across TX. The use of a resistance threshold is the best method to separate resistance from susceptibility but differences in LC50s or LD50s, based on overlapping confidence intervals of field collected and susceptible strains, can also be used. Toxicity determined for single pairs could be used to make this determination. Bioassay should be made in each area each y. Different resistance factors may be present in each of the areas of TX (Roush and Luttrell 1997). Resistance cannot be defined when most of the populations of TBW across TX are susceptible. Sampling for eggs and larvae in each field before and after each application gives the grower important information.

Mechanisms of resistance play a large role in how response patterns can vary in larval and adult populations in each field in 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 to pyrethroids may be known, but it is not known how activity levels of these mechanisms flow in populations. Nerve insensitivity (or target site) resistance to cypermethrin is described by field collected strains from TX (Gladwell et al. 1990). Target site resistance to cypermethrin was found in larvae of four field collected strains of TBW in the BV (McCaffery et al. 1991). Enzyme activity levels of field collected TBW strains were not extensive; strains from the BV did not posses monooxygenase activity in first instar larvae (McCaffery et al. 1991).

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

The Hardy-Weinberg calculation for percentage resistant larvae of each pyrethroid should be determined every y for five y from each of the areas in TX. Modification of calculation from a single dominant gene to a single recessive gene should be used for these pyrethroids.

In order to suggest that there is resistance to all pyrethroids one has to accept cross resistance among the insecticides in the pyrethroid class. Multiple resistance could be determined if larvae of each species from one collection are bioassayed with both pyrethroid and anticholinesterase insecticides. This has not been done by VT. Toxicity of cypermethrin across TX has been shown but toxicity by the other pyrethroids in the other areas of TX has not.

Conclusion

Cypermethrin can be used in TX for control of larvae of the TBW. The majority of the populations were susceptible but a few populations are resistant to cypermethrin. Resistance to each of the remaining pyrethroids has to be determined for populations across TX before resistance to them can be declared.

Acknowledgments

Thanks are extended to J. W. Norman, Jr. and A. N. Sparks for the larvae collections used in the single pair test from LRGV in 1989 and 1990. Thanks are extended to C. G. Sansone for his comments on resistance as they apply to the suggested insecticides for cotton in TX.

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