LD_{50}-MORTALITY RESPONSE OF COTTON BOLLWORM *HELICOVERPA ARMIGERA* (HUBNER) (LEPIDOPTERA: NOCTUIDAE) TO THE PYRETHROID INSECTICIDE

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ABSTRACT

LD_{50}-mortality studies were conducted to determine the susceptibility of two strains of cotton bollworm, *Helicoverpa armigera*, collected from Marathwada region in 2011 to the pyrethroid insecticide cypermethrin. LD_{50} values were 5 and 8 times greater for cotton bollworm collected in September and October respectively than for a susceptible laboratory strain. Results in these investigations are similar to those observed where control failures due to pyrethroid resistance have been reported. Implications for future resistance management are discussed.

Key words: LD_{50}, Helicoverpa armigera, Marathwada, cypermethrin

INTRODUCTION

Since its introduction in the late 1970's, the pyrethroid class of insecticides has generally provided an efficacious and cost effective tool for the management of numerous insect pests in a wide range of cropping systems. Cotton producers in the 1980’s came to rely on pyrethroids as a main line of defense against the cotton bollworm, *Helicoverpa armigera*, and tobacco budworm, *Heliothis virescens*, two of the most serious pests of that crop. The development of resistance by the tobacco budworm to pyrethroids in the mid to-late 1980’s had a major impact on cotton insect pest management in many parts of the United States. Prior to the commercial release of transgenic *Bt* cotton varieties, growers in much of the U.S. Cotton Belt faced the daunting challenge of managing pyrethroid resistant tobacco budworm populations with a dwindling arsenal of effective materials, and pyrethroid resistance management became one of the most important issues facing entomologists in cotton.

As resistance to pyrethroids by the tobacco budworm spread across the Cotton Belt in the 1980’s and early 90’s, no published reports existed of resistance or decreased efficacy in North Carolina (Sparks *et al.,* 1993). At the turn of the twenty-first century, pyrethroids still played a significant role in bollworm management in Indian cotton. The importance of this class of chemistry continues today with the need to overspray *Bt* cotton varieties for control of *Helicoverpa armigera* and secondary bug pests and the recent labeling of pyrethroids for use in tobacco. Even before their commercial release, resistance to pyrethroids (linked to cross resistance to methyl parathion) was seen in California populations of tobacco budworm (Twine and Reynolds, 1980), and elevated LD_{50}’s were detected in the Mid-south before control failures were observed in the field (Luttrel *et al.,* 1987). There have been sporadic reports of control failures associated with the use of pyrethroids in cotton since their introduction (Bradley J.R., personal communication); nevertheless, because the insect is a minor pest in cotton in the area, there have been no empirical studies to test the susceptibility of cotton bollworm populations.

Indian especially in Marathwada region of the Maharashtra State’s agro-ecosystem as it pertains to cotton bollworm habitat has generally been characterized by a diversity of both cultivated and wild host species, and this diversity may have contributed to the delayed resistance evolution in the pest. However, the structure of the state’s agro-ecosystem has changed considerably in recent decades. Cotton production area has increased dramatically since the early 1980’s; however, the recent introduction and widespread adoption by growers of transgenic *Bt* cotton varieties have turned much cotton into an effective trap crop for cotton bollworm. Changes in farm policies have and will continue to result in adjustments to peanut and pigen pea plantings takes place.

The implications of these changes for cotton bollworm population dynamics, pyrethroid susceptibility, and insecticide resistance management in general are unknown. The efficacy of *Bt* δ endotoxin produced by the cotton plant (*Gossypium hirsutum* L.) against the cotton bollworm has in large measure alleviated much of the concern and taken a great deal of focus away from pyrethroid resistance management; nevertheless, this class of chemistry may continue to fill an important pest management niche Maharashtra. Monitoring the level of susceptibility of a target organism is a key component of an effective resistance management strategy. In the case of cotton bollworm in Maharashtra, there are no historical data regarding pyrethroid susceptibility nor is there currently a system for monitoring pyrethroid resistance. This study was conducted to determine the pyrethroid susceptibility of two cotton bollworm strains established from cotton planted fields in Marathwada region in 2011.

MATERIALS AND METHODS

Insects: Two laboratory strains of cotton bollworm were established from insects collected in Marathwada region in 2011. Eggs were obtained from cotton planted fields of different part of Marathwada regions on 11th September 2011 (strain ABD04) and from Parbhani on 22th August 2011 (strain PBN04). On each collection date, blooms and leaves of cotton plants containing eggs were removed from the field and transported to the lab; eggs were removed by soaking the cotton tissue in a 0.05% sodium hypochlorite solution for 10-15 minutes. Eggs were held in a rearing chamber at 28^{0}C and 70% relative humidity until eclosion at which time neonate larvae were transferred to 50 ml plastic diet cups (3 larvae per cup) containing approximately 10 ml of artificial diet (n=200 for ABD04 and n= 300 for PBN04). Larvae were reared at 28^{0}C and 70% relative humidity and a photoperiod of L:D 14:10. Fourth instar larvae from field collected eggs were examined to verify species; all species other than *Helicoverpa armigera* were discarded (>99% of developing larvae from both collection dates were cotton bollworm). Pupae were removed from diet cups and placed in 2 liter plastic ice cream buckets for adult eclosion, mating, and oviposition; adults were fed a 10% sucrose solution. A pyrethroid susceptible reference strain (NRA06) was obtained from Green Gold Seed Co. Aurangabad and it has been maintained without exposure to insecticides.

Bioassay Procedure. Pyrethroid dose-mortality studies were conducted according to guidelines established by the ESA's standard test method for determining resistance to insecticides in *Heliothis* (Anonymous 1970). Neonate larvae from each strain were placed individually into 50ml plastic diet cups containing approximately 15ml of artificial diet, and held in a growth chamber at 28^{0}C, 70-75% relative humidity and a photoperiod of L:D 14:10. Assays were initiated when larvae reached third instar (approximately 9 days after being placed on diet) and mean larval weight was 30mg. A single trial was conducted for each strain evaluated with six doses per trial and a minimum of 12 insects per dose. Tests were conducted using the F2 generation of strain ABD04 (n= 216) and the F3 generation of the PBN04 strain (n=360). Cypermethrin (98% pure) was obtained from Chemists and Druggist's Agency at Aurangabad and was dissolved in acetone to create a new stock solution for each replicate of each trial. Without removing them from the diet, insects were treated on the dorsal thorax with 1µl aliquots of acetone alone (control) or one of five concentrations of cypermethrin dissolved in acetone. After treatment, larvae were returned to the growth chamber and mortality was assessed at 24, 48 and 72 hrs. Larvae were considered dead if they failed to move any part of their bodies within 10 seconds of being prodded with a blunt probe. Results were subjected to probit analysis using SAS version 8.0 Software. Differences in pyrethroid susceptibility among strains were considered significant if the 95% confidence intervals for the LD_{50}'s did not overlap.

RESULTS AND DISCUSSION

While control failures have not occurred with great frequency in Marathwada region, researchers in other part of the world identified reduced pyrethroid susceptibility in tobacco budworm *Helicoverpa virescens* before control failures were reported in the field (Davis *et al.,* 1977; Crowder *et al.,* 1979, Brown *et al.,* 1982).

The LD_{50} estimates obtained from dose-mortality regressions in this study indicate that cotton bollworm populations in Marathwada region may be less susceptible to pyrethroids than previously thought. The LD_{50} for cypermethrin of strain ABD04 was 4 fold greater than the susceptible laboratory strain, and strain PBN04 was found to have a 9 fold increase in LD_{50} compared to the susceptible strain **(Table)**. Additionally, cotton bollworm resistance to pyrethroids has been shown to decrease after the first laboratory generation (Martinez-Carillo and Reynolds, 1983; Staetz, 1985); thus our results, based on bioassays of the F_2 and F_3 generations, may overestimate the actual susceptibility of the insects in the field. Luttrel *et al.,* (1987) reported LD_{50} values for tobacco budworm strains established from fields in Mississippi where control failures were observed that were lower than those seen in North Carolina in 2004. The LD_{50} value for cypermethrin was highest, during October in Aurangabad and lowest during August in Parbhani districts of Marathwada region (Nimbalkar *et al.,* 2009). Resistance to cypermethrin increasedThough reported here for the first time for Marathwada Region, the observed increase in the LD_{50} of cotton bollworms collected later in the season is similar to the phenomenon reported in other regions of the cotton belt where resistance to pyrethroids has been identified, and susceptibility levels decrease as the season progressed.

Table 1: LD_{50}-Dose-mortality response of two strains of *Helicoverpa armegira* larvae[a] collected from Marathwada Region in 2011 and a susceptible laboratory strain to cypermethrin in bioassays.

Strain	n^b	Slope ± SEM	LD_{50} (95% CL) ^{c}	LD_{90} (95% CL)^c
ABD04	360	1.274 ± 0.186	0.433 (0.220-0.619)	2.157 (1.770-2.826)
PBN04	216	1.445 ± 0.283	0.897 (0.684-1.231)	2.417 (1.869-3.613)
NRA06	294	30.900 ± 3.713	0.097 (0.084-0.113)	0.168 (0.145-0.203)

[a] **Third instar (30 mg)**
[b] **Number of larvae tested**
[c] **Dosages are expressed as micrograms of insecticide per larva.**

Because historical data are not available for Marathwada Region, it is unclear whether the high LD_{50} values obtained in this study are indicative of an area wide increase in resistance level, a spatially and/or temporally restricted decrease in susceptibility, or simply a consistently high tolerance to pyrethroids. If these results represent an overall increase in resistance in the population, the source of the resistance is unclear. It is currently believed that only a small portion of the total cotton bollworm population develops annually on other hosts plants in this region, and the selection pressure of pyrethroids in cotton prior to 2011 was probably low given that any application in that crop would have been illegal. Research has shown that Heliothis moths are capable of sustained flights of at least 300 km per night when conditions are favorable (Westbrook *et al.* 1990). Migration has been implicated in the rapid spread of resistance observed in the Midsouth in the 1980's (Plapp *et al.* 1990); however, no data exist that would document annual migration patterns of the cotton bollworm in Marathwada region. Cross resistance between pyrethroids and the organophosphate insecticide methyl parathion has been demonstrated (Crowder *et al.* 1979, Twine and Reynolds, 1980). While methyl parathion has not been used on cotton in over five years, the possibility that a similar mechanism of cross resistance is responsible for the elevated LD_{50}'s observed in this study cannot be dismissed.

Regardless of the mechanism(s) responsible for the low susceptibility to pyrethroids observed in the Marathwada Region cotton bollworm strains, the presence of resistance or tolerance may foreshadow future control failures if this class of insecticides is not used wisely.

The widespread use of pyrethroids in cotton (*Helicoverpa armigera* preferred host) could amplify the selection pressure placed on cotton bollworm populations considerably. While it is believed that *Helicoverpa armigera* has historically been exposed to pyrethroids in Marathwada only in a single generation annually, the use of the insecticide in cotton could increase the number of generations selected to two or three per season. Nevertheless, caution should be taken to ensure that selection of multiple generations within a single growing season, a key driver of resistance evolution, is minimized. The identification of heliothis larvae in non *Bt* cotton, and the use of alternative chemistries when cotton bollworm infestations occur would limit the selection pressure placed on the population and help guard against costly control failures. Our findings show that susceptibility to pyrethroids in the cotton bollworm strains tested in Marathwada Region is similar to that observed in pyrethroid resistant populations. The uncommon occurrence of control failures in Marathwada Region's cotton may reflect a generally low annual frequency of cotton bollworm infestation in that crop rather than an appreciable level of susceptibility.

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