MONITORING RESISTANCE TO PYRETHROIDS AND *CryIA(c)* TOXIN IN TOBACCO BUDWORM POPULATIONS FROM THE YAQUI VALLEY, SONORA MEXICO

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Abstract

The tobacco budworm is an important insect pest of cotton in Mexico, This pest has developed resistance to a wide variety of insecticides commonly used for its control. Pyrethroids have achieved control of this pest, however, intensive use of these products has selected resistant populations. Transgenic Bt cotton is an alternative control method for this and other insect pests in cotton, the area planted with these materials has increased through time in Mexico. The high selection pressure imposed by Bt cotton is a concern, and resistance management strategies have been implemented using refugia with conventional cotton trying to delay resistance. Data obtained up to now have not shown any shift in response to these materials.

Introduction

The tobacco budworm (TBW) *Heliothis virescens* (F.) is an important pest of cotton and other crops in Mexico, this insect has been responsible for the abandonment of some cotton agricultural areas due to development of resistance (Adkisson 1970, Martinez-Carrillo et al. 1991, Perez 1983). Resistance management strategies have been implemented in some areas in Mexico, mainly to reduce selection pressure to pyrethroids (Martinez-Carrillo 1990). Bt Transgenic cotton was planted commercially since 1996 and has increased trough time reaching 27,000 has in the 2001 cotton season. Although the cotton area in Mexico has had a negative trend, Bt transgenic cotton has maintained the same area in the last three years. The resistance management strategy in Bt transgenic cotton is based in leaving refuges with conventional cotton close to areas planted with Bt cotton. In order to detect any shift in response of tobacco budworm populations to the *CryIA(c)* toxin present in the Bollgard varieties planted in Mexico, a resistance monitoring program has been implemented. In this paper data obtained in the Yaqui, Valley of Sonora, on resistance monitoring for pyrethroids and *Cry IA(c)* toxin are presented.

Materials and Methods

Insects

Tobacco budworm moths were collected in wire cone traps (Harstack 1979) baited with sex pheromone specific for this insect. The moths were selected, using only those that look healthy for pyrethroid resistance monitoring. Larvae and eggs of TBW were collected directly from the field and taken to the Entomology laboratory of the Yaqui Valley Experimental Station, where they were maintained in walk-inn chambers set at 27°C, 14:10 hr, photoperiod and 70% RH, until used in the *CryIA(c)* bioassays.

Bioassays

The glass vial technique (Plapp et al. 1987) was used for pyrethroid resistance monitoring; 20 ml scintillation vials were coated in the inner surface by introducing 1 ml of technical grade insecticide dissolved in acetone. Once the insecticide solution was deposited in the vials, they were rolled for 15 min. in a conventional hot-dog roller to evenly coat the inner surface as the acetone evaporated. Treated vials were then placed in front of an electric fan for 15 min. ventilation to eliminate all acetone residues. Each vial was capped with a plastic cap and stored in a dark cool place until used. One moth was placed in each vial treated with 10 µg/vial of cypermethrin and mortality was recorded 24 hr later. For the *Cry IA(c)* bioassay, a concentration of 0.05 µg/ml of MVP II powder suspended in 0.2% agar was used as diagnostic dosage, 200 µl of the suspension was applied over artificial diet placed on each well of a 64 well assay tray (Jarold Mfg. Co. St. Louis, MO). Each well had a 2.0 ml capacity and contained 1.0 ml of lepidopterous artificial diet. Once the diet dried, one neonate TBW larvae was placed in each well. The trays were then covered with plastic ventilated covers and incubated at 27° C, 70% R.H. and 14:10 photoperiod, for 5 days. More than 500 larvae were used for each set of observations. Percent mortality, larval weight and the number of larvae reaching 3rd instar. were recorded at 5 days (Sims et al. 1996). Percent inhibition (stunting) was estimated by dividing weight of treated larvae by weight of control multiplied by 100.

Insecticides

For Pryrethroid resistance monitoring Cypermethrin was used as a representative of this chemical group. The 10 µg/vial concentration was used as the diagnostic dosage. Lypophilized MVPII toxin (Mycogen Corp.), provided by Monsanto Comercial S.A. de C.V. was used as a standard for the *Cry IA (c)* protein. This biological insecticide is the closest in biological properties to the protein expressed in Bollgard cotton (Gould el al. 1995). The diagnostic dosage used was 0.05 µg/ml.

Results and Discussion

Pyrethroid Resistance Monitoring

Data from 1994 up to 1997 showed the same trend, starting from zero percent of moths surviving the diagnostic concentration up to 10% survivors (Figure 1). The increase in survivorship was typical of selection pressure through the season. Since 1998 an increase in percent of survivors was detected. In March 17% of the moths survived the diagnostic concentration. It was suspected that this population was selected in horticultural crops. The percent survival declined in May to 2%, and then started to increase again in June, July, August and September. In 1999 the same tendency was observed although the percent survival in March was lower than the year before, declined in May and increased during June July and August until it reached 14%, declining in September to 7%. In the 2000 year, the data were similar to the year before but with higher survivorship, and the same trend continued in the year 2001. As seen in Figure 1, the tendency in the last three years has been an increase in the percent of moths surviving the diagnostic concentration, this is an indication that these populations have been selected for Pyrethroid resistance. Although no control failures have been reported, it has been recommended to support the resistance management strategy suggested about ten years ago (Martinez-Carrillo 1990), which consisted in not spraying pyrethoids during a window between flowering and full boll development. This period, in the Yaqui Valley falls around the 20 of June and 20 of July. The highest survivorship has been in August and it is not higher than 15%, which, is much lower than data obtained in USA where control failures have been reported with this insecticide group (Graves et al. 1991, Mullins et al. 1991).

Bt Cotton Resistance Monitoring

The data obtained from 1998 to the 2001 year, in the Yaqui, Valley are presented in Table 1. In these years no third instars have been observed in the treated material, indicating that the toxin is retarding larval development.

In 1998, early, middle and late season collections were treated, from 1552 larvae treated, none reached third instar, larval weight varied from 0.67 to 0.79 mg, whereas in untreated larvae the median weight ranged from 20.69 mg to 33.79 mg. Percent inhibition was 96%, 98% and 97% for early, middle and late season collections. Data from a susceptible colony used as reference indicated that from 480 larvae treated, none reached third instar, larval weight was 0.63 mg in treated material and 25.45 in untreated, which gave 98% growth inhibition or stunting.

During the 1999 season, 1120 larvae were treated from collections made in middle and late season. Larval weight in treated larva was 0.43 mg and 0.44 mg, whereas in untreated material it was 29.18 mg and 24.34 mg respectively. This date showed that there was 99% and 98% growth inhibition for these colonies. In the susceptible colony from 576 larvae treated none reached third instar, larval weight was 0.29 mg in treated material and 32.11 mg in untreated yielding 99% of growth inhibition.

In the year 2000 three colonies were tested for resistance to the *CryIA(c)* toxin. From 1680 larvae tested none reached third instar, larval weight in treated larvae was 0.41 mg, 0.47 mg, and 0.54 mg respectively for early, middle and late season colonies. The weight of untreated material was 25.54 mg, 26.84 mg, 26.63 mg which yields 98% inhibition for each one of the colonies. The susceptible colony showed that from 576 larvae evaluated none reached third instar, weight of treated material was 0.25 mg and untreated was 41.32 mg which resulted in 99% growth inhibition.

During the year 2001, three collections were made in early middle and late season. 1568 larvae were treated none reached third instar, larval weight for treated larvae in the early season colony was 0.59 mg and 21.87 in the untreated, which resulted in 97% growth inhibition. In middle season the treated larvae gave 0.53 mg and 22.66 mg the untreated, this was 98% of growth inhibition. The late season colony resulted with 0.59 mg in treated larvae and 22.93 in untreated, yielding 97% growth inhibition. The susceptible colony presented 0.29 mg in treated larvae and 22.15 in untreated, which resulted in 99% of growth inhibition.

Conclusions

Monitoring of resistance in TBW moths, through the vial bioassays showed that there is an increasing trend of selection for resistance to Pyrethroids in populations from the Yaqui, Valley, Sonora. Resistance management strategies should be re-

implanted in order to avoid control failures with this group of insecticides. Tobacco budworm populations continue to be susceptible to the *CryIA(c)* toxin present in Bt cotton.

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References

Adkisson, P. L. 1970. Impact of Insecticide Resistance in Insects on Cotton Production in South Texas and North-Eastern Mexico. Food and Agriculture Organization, Panel of Experts on Integrated Pest Control. Roma. Italy.

Gould, F., A. Anderson, A. Reynolds, L. Bumgardner, and W. Moar. 1995. Selection and genetic analysis of a *Heliothis virescens* (Lepidoptera:Noctuidae) strain with high levels of resistance to *Bacillus thuringiensis* toxins. J. Econ. Entomol. 88: 1545-1559.

Graves, J. B., B.R. Leonard, S. Micinski, D.W. Long and E. Burris. 1991. Status of Pyrethroid resistance in tobacco budworm and bollworm in Louisiana. pp.638-641 *En* Procc. Beltwide Cotton Production and Research Conferences. National Cotton Council. Memphis TN

Harstack, A.W. 1979. Moth traps for the tobacco budworm. J. Econ.Entomol. 72:519-522.

Martínez Carrillo J. L. 1990. Implementing a resistance management strategy for cotton pests in northwest Mexico. P 209. *En* Procc. Beltwide Cotton Production and Research Conferences. National Cotton Council. Memphis TN.

Martínez-Carrillo, J. L. L. P. Schouest Jr., and T.A. Miller. 1991. Responses of Populations of the Tobacco Budworm (Lepidoptera:Noctuidae) from Northwest Mexico to Pyrethroids. J. Econ. Entomol. 84(2) 363-366.

Mullins, J.W., S. L. Riley, C. A. Statez, R. J. Marrese, B. Rogers and B. J: Monke. 1991. Status of *Heliothis* resistance to pyrethroids in U.S. Cotton. A Report form PEG-US pp. 634-637. *En* Procc. Beltwide Cotton Production and Research Conferences. National Cotton Council. Memphis TN

Perez, C. R. 1983. El valle de Apatzingan cuenta con una nueva alternativa de cultivo: La Soya. Noti-INIA –SARH. 18:15-17.

Plapp, F.W., G. M. WcWhorter, and W. H. Vance. 1987. Monitoring for pyrethroid resistance in tobacco budworm in Texas-1986. pp. 324-326. . *En* Procc. Beltwide Cotton Production and Research Conferences. National Cotton Council. Memphis TN

Sims, S, B., J.T. Greenplate, T.B. Stone, M. A. Caprio, and F.L. Gould. 1996. Monitoring strategies for early detection of lepidoptera resistance to *Bacillus thuringiensis* insecticidal proteins. Chapter 23 In Molecular Genetics and Evolution of Pesticide Resistance. Thomas M. Brown Editor. American Chemical Society.

Table 1. Bioassay results on Tobacco budworm populations from the Yaqui, Valley, Sonora. Mexico.

Year and Period of Collection	Insects Treated	Weight Treated	Weight Untreated	Percent Inhibition
VY-1998-Early	576	0.79	20.69	96
VY-1998-Middle	464	0.77	33.79	98
VY-1998-Late	512	0.67	22.60	97
SUSCEPTIBLE	480	0.63	25.45	98
VY-1999-Middle	544	0.43	29.18	99
VY-1999-Late	576	0.44	24.34	98
SUSCEPTIBLE	576	0.29	32.11	99
VY-2000-Early	544	0.41	25.54	98
VY-2000-Middle	576	0.47	26.84	98
VY-2000-Late	560	0.54	26.63	98
SUSCEPTIBLE	576	0.25	41.32	99
VY-2001-Early	528	0.59	21.87	97
VY-2001-Middle	512	0.53	22.66	98
VY-2001-Late	528	0.59	22.93	97
SUSCEPTIBLE	528	0.29	22.15	99

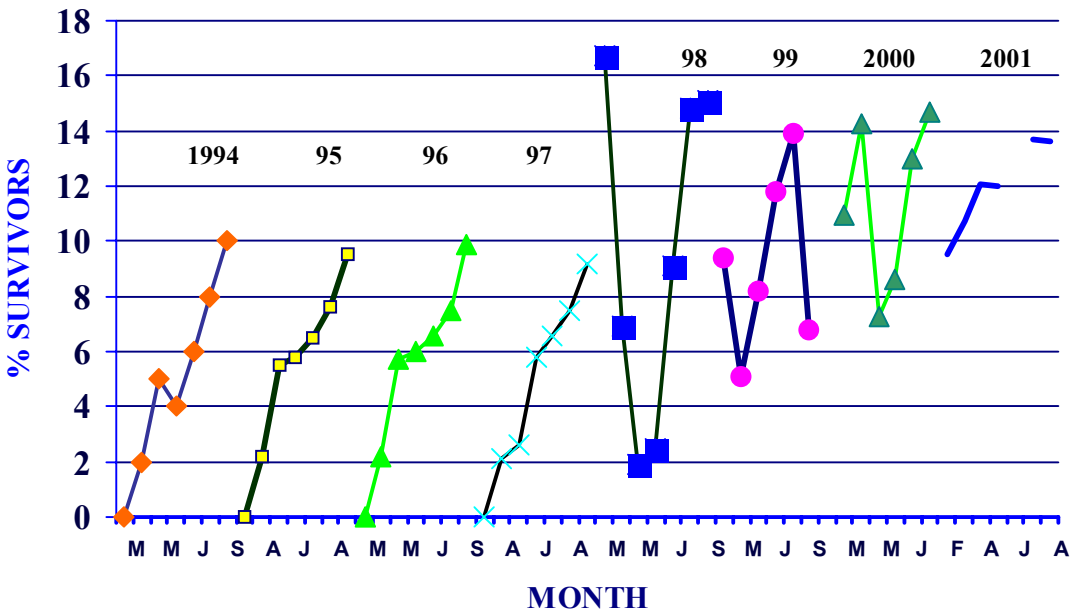

Figure 1.