MONITORING FOR TOLERANCE TO CRY IA(C) IN POPULATIONS OF HELIOTHIS VIRESCENS FROM MEXICO

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Abstract

Tobacco budworm (TBW) populations from different cotton producing areas of northwestern Mexico were monitored in 1998 and 1999 for response to the *Cry* IA (c) toxin, which is expressed in BOLLGARD® cotton. Bioassays were carried out on field-collected populations of TBW and compared to a laboratory susceptible population. Overlay concentrations of 0.05 m µg/ml of the toxin were applied on lepidoptera diet and data on mortality, larvae reaching 3rd instar and percent growth inhibition were obtained 5 days after treatment. Results indicated that this dosage prevented larvae from reaching the 3rd instar, indicating the susceptibility to the toxin in the populations evaluated. The comparison between weigh of treated versus untreated larvae produced a percentage of growth inhibition, and this ranged from 96% to 98% in 1998, and it was from 98% to 99% in 1999. These data indicate the high susceptibility of the CryIA(c) toxin to the treated larvae. Monitoring of resistance has not indicated any shift to resistance to this toxin in populations from northwestern Mexico.

Introduction

1999 was not a good year for cotton in Mexico, mainly because the low price of fiber. BOLLGARD® cotton was planted in 24,763 hectares, 32 % less than in 1998. The trend of Bt cotton in the last four years is shown in Figure 1. This technology, has been well accepted for pink bollworm control in some cotton areas of Mexico, but still is not totally accepted for control of TBW or Bollworm. As a preventative measure for resistance management, agricultural authorities in Mexico require to develop data on the response of populations under selection pressure from Bt cotton in order to detect any change that could indicate resistance problems. In this regard MONSANTO (line owner of this technology) has established a resistance management strategy based on leaving refuges close to planted areas of transgenic cotton. In order to evaluate the success of the strategy and detect any shift in response to the *Cry* IA (c) protein, base line information and technology for monitoring of resistance was developed in 1997 and 1998 (Martinez and Berdegue, 1999). This paper presents data obtained in 1998 and 1999, monitoring with a diagnostic dosage, TBW populations collected from different cotton growing areas of northwestern Mexico.

Materials and Methods

Insects

TBW larvae were collected from several commercial cotton-fields in the agricultural areas of Sonora, Sinaloa, and South Baja California during 1998 and in Sinaloa and Sonora in 1999. Field collected larvae were placed in 1 oz. plastic cups with a small piece of artificial lepidoptera diet (Sothland Products Inc, Lake Village AR.) and sent to the INIFAP Entomology laboratory, located in the Field Experimental Station, in Cd. Obregon, Sonora. Larvae were reared on artificial diet and maintained in walk in chambers at 26˚C, 70% R.H. and 14:10 (L:D) photoperiod, until used for bioassays. A colony maintained in the laboratory since 1982 has been used as a reference strain for susceptibility.

Insecticide

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).

Bioassays

Bioassays for monitoring of resistance were conducted using 0.05 µg/ml diet overlays. This concentration has demonstrated that prevents larvae from reaching 3rd instar and have been considered as a diagnostic dose for discrimination of resistant genotypes (Sims et al. 1996, Martínez and Berdegue, 1999) .Each bioassay included a concentration of 0.05 µg/ml of MVP II powder suspended in 0.2% agar, 200 µl of suspension were applied over the artificial diet placed on each well of a 64 well assay trays (Jarold Mfg. Co. St. Louis, MO). Each well had a 2.0 ml capacity and contained 1.0 ml of diet. Once the prepared diet had 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. Around 500 larvae were used for each site of collection which was referred as a colony. There were five or more replications, in different days until the total number of 500 larvae was reached. Mortality of larvae, body weights and number of larvae reaching 3rd instar, were recorded at 5 days. Percent inhibition (stunting) was estimated by dividing

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weight of treated larvae by weight of control multiplied by 100.

Results and Discussion

During 1998 in Sinaloa two colonies were evaluated one from Culiacan (Cul-98)) and another from Los Mochis (Moch-98). In the first colony 512 larvae were treated and in the second one 544, none of them reached third instar, percent inhibition was 98% and 97% respectively Larval weight was 0.47 mg in Cul-98 and 0.58 mg in Moch-98 whereas the weight in the check was 22.74 and 21.18 respectively. These results indicate 98% and 97% growth inhibition produced by the toxin (Table1). In 1999 six colonies from this state were evaluated two from Culiacan (Cul-99-1 and Cul-99-2), Two from La Cruz de Elota (Cruz-99-1 and Cruz-99-2) one from Guasave (Gua-99) and one from Los Mochis (Moch-99). Results from these colonies indicate that there were no third instar observed in any of the colonies at the diagnostic dosage applied. Larval weigh varied from 0.42 mg to 0.53 mg in treated material and from 23.93 mg to 30.62 mg in untreated material used as a check this yielded 98% percent inhibition for all colonies (Table2).

In Sonora three colonies were evaluated during 1998 all of them collected in the Yaqui Valley and identified as early (VY-98-E), middle (VY-98-M) and late (VY-98-L) according to date of collection during the cotton growing season. The results indicate that no third instars were obtained in any of the colonies evaluated. Larval weight varied from 0.67 mg to 0.79 mg in treated material and from 22.60 mg to 33.79 mg in untreated. These data show that there was a percentage inhibition from 96% to 98% in treated and untreated larvae (Table 1). In 1999 two colonies were established from the Yaqui valley (VY-99-1 and VY-99-2). No third instar larvae were obtained after the application of the diagnostic dosage and the mean larval weigh was 0.43 mg and 0.44 mg respectively. The larval weigh in untreated material was 29.18 mg and 24.34 mg which results in 99% and 98% inhibition for both colonies (Table 2).

In South Baja California, there were evaluated two colonies during 1998, both collected from Santo Domingo valley (STD-98-1 and STD-98-2). No third instar larvae were observed in 1056 larvae treated with the diagnostic dosage. The mean larval weigh in treated material ranged from 0.45 mg to 0.54 mg and it was 21.65 mg and 19.46 mg in untreated larvae this results in 98% and 97% inhibition of growth respectively (Table1). The cotton area was drastically reduced in 1999 and no collections were done this year.

During 1998 and 1999 there was used as a comparison a susceptible colony that has been maintained in laboratory since 1982. In both years 1056 larvae were treated with the diagnostic dosage and no third instars were observed. The mean larval weigh in treated material was 0.63 mg in 1998 and 0.29 in 1999. Whereas that in untrated material the mean larval weight was 25.45 mg and 32.11 respectively. Percent inhibition results were 98% and 99% for 1998 and 1999 evaluations.

Conclusions

Results from this study indicate that the diagnostic concentration used (0.05 μg/ml) has given a good indication of the response of TBW populations of northwestern Mexico to the toxin CryIA(c). Monitoring of resistance in two years have shown that there is not indication of any change in response of the populations evaluated as compared to a susceptible colony. Monitoring of resistance is important to detect any shift in response that could indicate selection of resistant populations in areas where Bt transgenic cotton is planted.

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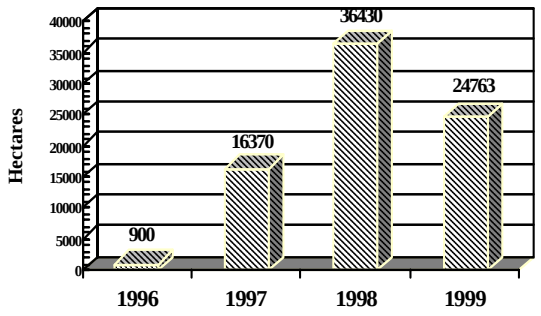

Figure 1: BOLLGARD® cotton planted in Mexico

Table 1. Data obtained in 1998 from different colonies evaluated for response to a diagnostic dosage (0.05 µg/ml) of the Cry IA (c) toxin of *Bacillus thringiensis* on Tobacco Budworm Populations from Mexico.

State	Colony	No. Treated	3erd instar	Mean Weight	Check Mean Weight	% Inhibi Tion
Sinaloa	Cul-98	512	0	0.47	22.74	98
	Moch-98	544	0	0.58	21.18	97
Sonora	VY-98-E	576	0	0.79	20.69	96
	VY-98-M	464	0	0.77	33.79	98
	VY-98-L	512	0	0.67	22.60	97
BCS	STD-98-1	528	0	0.54	19.46	97
	STD-98-2	528	0	0.45	21.65	98
	Susc.	480	0	0.63	25.45	98
Average		518	0	0.61	23.45	97

Table 2. Data obtained in 1999 from different colonies evaluated for response to a diagnostic dosage (0.05 µg/ml) of the Cry IA (c) toxin of *Bacillus thringiensis* on Tobacco Budworm Populations from Mexico.

State	Colony	No. Treated	3erd instar	Mean Weight	Check Mean Weight	% Inhibi tion
Sinaloa	Cul-99-1	512	0	0.42	23.93	98
	Cul99-2	592	0	0.51	27.80	98
	Cruz-99-1	656	0	0.46	26.52	98
	Cruz99-2	592	0	0.52	30.62	98
	Gua-99	544	0	0.54	26.41	98
	Moch-99	544	0	0.53	29.91	98
Sonora	VY-99-1	544	0	0.43	29.18	99
	VY-99-2	576	0	0.44	24.34	98
	Susc.	576	0	0.29	32.11	99
Average		571	0	0.46	27.87	98