RESPONSES OF TOBACCO BUDWORM POPULATIONS FROM MEXICO TO Bt Cry IA(c) TOXIN

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Abstract

Base line information was generated on tobacco budworm populations from Mexico to the *Cry* IA (c) toxin, expressed in the BOLLGARD cotton. This *Bacillus thuringiensis* transgenic cotton is growing in importance in Mexico, and it is important to have basic information on the response of populations selected by this toxin. Bioassays were carried out on field-collected populations of TBW from two agricultural areas in northeastern and one from northwestern Mexico. They were compared to a laboratory susceptible population. Overlay concentrations of the toxin were applied on lepidoptera diet and data on mortality, larvae reaching 3rd instar and percent growth inhibition were obtained 7 days after treatment. Treated larvae were maintained under laboratory controlled conditions with 26°C, 70% R.H. and 14:10 photoperiod. Results indicated that there was not significant differences in LC_{50} values, and percent growth inhibition in field collected versus the susceptible population. Dosages of 0.05 µg/ml prevented larvae from reaching the 3rd instar and are suggested as diagnostic for discriminating susceptible and resistant strains.

Introduction

B. thuringiensis transgenic BOLLGARD cotton has been planted in Mexico since 1996 and has increased in importance through time, going from 900 hectares planted in 1996 up to 36,430 in 1998 (Figure 1). This technology, that expresses the *Cry* IA(c) protein is highly toxic to insect pests such as the tobacco budworm (TBW*) Heliothis virescens*, the cotton bollworm *Helicoverpa zea* and other lepidoptera.

The use of transgenic cotton represents many advantages for insect pest management, unfortunately insect resistance is a concern to the success of this technology. Resistance to this protein in transgenic cotton, could reduce the advantages of reducing conventional use of insecticides, with the respective increase in cost of control, and other problems associated such as contamination and elimination of beneficial insects and other species. In this regard MONSANTO 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, it is important to develop base line information. This paper presents data obtained on TBW populations collected in different cotton growing areas of Mexico.

Materials and Methods

Insects

During 1997 larvae of TBW were collected from several commercial cotton-fields in the agricultural areas of south of Tamaulipas, and Altamira, Tamaulipas, in northeastern Mexico and in the Yaqui Valley, Sonora, in the northwestern cotton region. The biological material was placed in 1 oz. plastic cups with a small piece of artificial lepidoptera diet and sent to the Entomology laboratory in the Field Experimental Station, located in Cd. Obregon, Sonora. Larvae were reared on artificial diet and maintained in 26°C temperature chambers with 70% R.H. and 14:10 photoperiod, until used for bioassays. A colony maintained in the laboratory since 1982 was used as a reference strain for susceptibility.

Insecticide

Lypophilized MVPII (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

Dose-response bioassays were conducted using diet overlays. Each bioassay included seven or eight concentrations 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 tray. Each well had a 2.0 ml capacity and contained 1.0 ml of diet. Once the prepared diet had surface dried, one neonate TBW larvae was placed in each well. The trays were then covered with plastic ventilated covers and incubated at 26° C, 70% R.H. and 14:10 photoperiod, for 7 days. From 48 to 112 larvae were used for each concentration. There were four to five replications, in different days. Mortality was determined at 7 days, and at the same time there was recorded the larval weight and the number of larvae reaching 3rd instar.

Mortality data was analyzed by probits to estimate LC_{50}, fiducial limits, LC_{95} and slope, based in concentration-mortality relationships. Percent inhibition (stunting) was estimated by dividing weight of treated larvae by weight of control multiplied by 100. Concentrations preventing larvae from reaching 3rd instar were considered as diagnostic dose for discrimination of resistant genotypes (Sims et al. 1996) .

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Results and Discussion

Data obtained on the susceptible colony are presented in table 1, at the dosage of 0.025 μg/ml, eight larvae reached 3rd instar, and at higher dosages no one was detected. Larval median weight varied from 0 mg in the 5 μg/ml, up to 40.14 mg in the control. Larval stunting was 87.12% in the lowest dosage (0.01 μg/ml) up to 99.88%, in the 1 μg/ml concentration. The 5 μg/ml eliminated completely all larvae tested.

Results on the population collected in the Yaqui Valley are presented in table 2. It was detected that the dosage of 0.025 µg/ml presented six 3rd instar larvae and higher dosages did not presented larvae in this instar. The larval median weight varied from 0 mg in the dosage of 5 µg/ml up to 29.02 mg in the control. With respect to growth inhibition it was detected that the dosage of 0.01 µg/ml inhibited only 64.89% whereas the dosage of 1.0 µg/ml reached an inhibition of 99.87%. The dosage of 5 µg/ml eliminated all tested larvae. As compared to the susceptible colony it was detected less inhibition in the lower dosages but not in the higher ones.

In table 3 are presented the data for the population from Altamira, Tamauipas. In this population the dosage of 0.025 μg/ml presented ten 3rd instar larvae but at higher dosages the larvae did not reached this instar. The larval median weight varied from 0 mg in the 1 µg/ml dosage up to 25 mg in the control. In regard to growth inhibition the dosage of 0.01 µg/ml had an inhibition of 52.20% whereas the dosage of 0.05 µg/ml and higher, obtained a growth inhibition higher than 93%, dosages of 1.0 µg/ml eliminated all tested larvae.

Data from south of Tamaulipas are presented in Table 4, results show that dosages of 0.025 µg/ml obtained nine 3rd instar larvae, but higher dosages prevented the larvae from reaching this instar. The larval median weight varied from 0 mg in dosages of 1.0 µg/ml up to 29.02 mg in the control. Dosages of 0.01 µg/ml presented an inhibition of growth of 64%. Whereas dosages of 0.025 µg/ml had 90.24%. The 1.0 µg/ml dosage eliminated all tested larvae

Mortality data of these colonies analyzed by probits is presented in Table 5. The LC_{50} values varied from 0.061 µg/ml up to 0.088 in the populations from Tamaulipas, based on the overlap of fiducial limits there was not significant differences between the susceptible and the field-collected colonies. This is an indication that there has not been enough selection pressure with the *Cry* IA (c) protein, on the TBW population from Mexico. LC_{95}´s and slopes were also similar in all populations evaluated including the susceptible colony.

Sims et. al. 1996, observed that larval growth inhibition by sublethal dosages of the protein *Cry* IA (c), was a bioassay more sensitive than dosage-mortality responses. Bioassays based on larval growth inhibition are easy to carry out, in such a way that a diagnostic dosage may be determined that allows discrimination between susceptible and resistant genotypes. In this study 0.05 µg/ml was considered as the diagnostic dosage and is similar to that reported by Sims et al. (1996) First and second instar larvae are easily separated from those of third instar, and supplemented by percent weight inhibition may give a better analysis than dosage-mortality data.

Based on results from this study, dosages of 0.05 µg/ml and higher prevented treated larvae from reaching third instar (Tables 1, 4), Thus this dosage should be used as diagnostic for resistance monitoring in TBW populations from Mexico, These populations will be under selection pressure from this toxin as the use of Bt transgenic cotton increases and it will be important to detect any shift in response through a sensitive bioassay.

Conclusions

The bioassays performed in this study showed that there was not significant difference in dosage-mortality response to the *Cry* IA (c) toxin in all the populations evaluated, indicating that there has not been selection of resistant populations to this insecticide. Data obtained in this study will be basic to detect any change in susceptibility that may occur due to the extensive use of Bt transgenic cotton in Mexico. Dosages of 0.05 µg/ml, preventing larvae from reaching the third instar, together with larval growth inhibition, are a good option for resistance monitoring, as indicated by Sims et.al.(1996).

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References

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.

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.

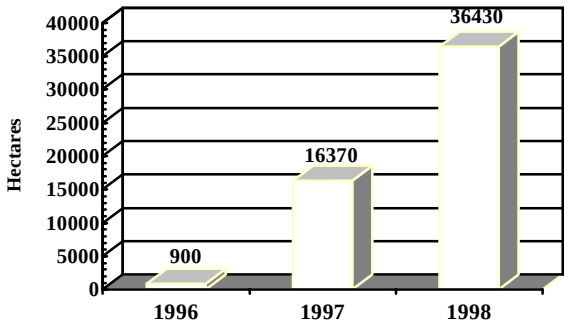

Figure 1: Transgenic BOLLGARD cotton planted in Mexico

Table 1. Bioassay results on a laboratory susceptible colony of tobacco budworm *Heliothis virescens* F.

Dosage μg/ml	Insects treated	Insects Dead	3rd Instar	Weight	Percent inhibition
5	48	48	0	0	100
1	48	46	0	0.05	99.88
0.5	112	98	0	0.18	99.55
0.25	96	67	0	1.41	96.49
0.1	80	48	0	0.62	98.46
0.05	95	45	0	1.49	96.29
0.025	79	30	8	2.26	94.37
0.01	63	14	25	5.17	87.12
Control	183	15	165	40.14	0

Table 2. Bioassay results on a population of tobacco budworm *Heliothis virescens* from the Yaqui valley, Sonora, Mexico. 1997.

Dosage μg/ml	Insects treated	Insects Dead	3rd Instar	Weight	Percent inhibition
5	16	16	0	0	100
1	48	44	0	0.04	99.87
0.5	56	45	0	0.11	99.62
0.25	52	39	0	0.18	99.38
0.1	52	33	0	1.43	95.07
0.05	52	26	0	1.72	94.07
0.025	52	17	6	3.22	88.9
0.01	52	11	22	10.19	64.89
Control	128	4	122	29.02	0

Table 3. Bioassay results on a population of tobacco budworm *Heliothis virescens* from Altamira, Tamaulipas, Mexico. 1997.

Dosage μg/ml	Insects treated	Insects Dead	3rd Instar	Weight	Percent inhibition
1	48	46	0	0	100.00
0.5	48	38	0	0.12	98.80
0.25	48	34	0	0.30	98.80
0.1	48	24	0	1.39	94.44
0.05	48	19	0	1.77	92.92
0.025	48	17	10	3.39	86.44
0.01	48	7	17	11.95	52.20
Control	60	3	57	25	0

Table 4. Bioassay results on a population of tobacco budworm *Heliothis virescens* from south of Tamaulipas. Mexico. 1997.

Dosage μg/ml	Insects treated	Insects Dead	3rd Instar	Weight	Percent inhibition
1	48	46	0	0.00	100.00
0.5	48	41	0	0.08	99.72
0.25	48	35	0	0.29	99.02
0.1	48	29	0	1.31	95.49
0.05	48	23	0	1.87	93.57
0.025	48	17	9	2.83	90.24
0.01	48	13	12	10.45	64.00
Control	60	3	57	29.02	0

Table 5. Probit analysis on TBW *Heliothis virescens* F., populations from Mexico.

Colony	LC_{50} μg/ml	Fiducial Limits 95%	LC_{95} μg/ml	Slope
Susceptible	0.072	0.055 -- 0.092	1.70	1.20
South Tamps-97	0.061	0.042 – 0.084	1.71	1.13
Yaqui-Valley-97	0.063	0.044 -- 0.086	2.13	1.08
Altamira Tamps-97	0.088	0.064 – 0.118	1.76	1.26