TOBACCO BUDWORM RESPONSE TO CryIAc AND Cry2Ab TOXINS OF *Bacillus thuringiensis*

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Abstract

Bollgard® transgenic cotton which contains CryIAc toxin of *Bacillus thuringiensis* (Bt) is being commercialized in Mexico and resistance monitoring programs have been implemented to detect any shift in response of key lepidopterous pests affected by the toxin. Bollgard II® cotton with both CryIAc and Cry2Ab toxins is going to be available for growers soon and the high selection pressure imposed by Bt cotton is a concern. Tobacco budworm (TBW) is a key cotton pest that has been reduced by the CryIAc toxin, but now it is required to have data of both toxins, that may help to understand resistance processes in populations selected by these toxins. Bioassays were performed in field and laboratory TBW populations using the toxin overlay methodology. Results indicated that at the LC_{50} level Cry2Ab was 21.72 X less toxic than CryIAc in a susceptible colony and 25.51X less toxic in a TBW field population collected in the Yaqui, Valley, but it was 44.55X less toxic in a field population from the Mexicali Valley. Monitoring of resistance to CryIAc is required to allow planting of transgenic cotton in Mexico. With the introduction of Bollgard II® it will be necessary to have data on the response of both toxins to this and other target insect pests of transgenic Bt cotton. Monitoring of resistance should include besides diagnostic dosage bioassays, complete dosage mortality data which generates more information on the response of population under selection pressure at various concentrations.

Introduction

Transgenic plants with insecticide properties represent a new tool for insect pest management. Its use in Mexico has increased since they were available commercially in 1996. Due to the mode of action of the toxins responsible for insect control and its high expression in plants, there is a concern that resistance could evolve in those insect populations subjected to selection pressure by these toxins (Gould et al. 1995). In Mexico, as in other parts of the world, where transgenic Bollgard® cotton is used, resistance management strategies have been implemented. The resistance management strategy in Mexico for Bt transgenic cotton is based in leaving refuges of conventional cotton close to areas planted with Bollgard® cotton. In order to detect any shift in response of tobacco budworm (TBW) *Heliothis virescens*, Cotton Bollworm *Helioverpa zea* and pink bollworm *Pectinophora gossypiella* populations, to the CryIAc toxin present in the Bollgard® varieties, a resistance monitoring program has been implemented. Results up to now have not shown any change in response of these populations to the CryIAc toxin (Martinez-Carrillo et al. 2003). However, Bollgard II®, which contains both CryIAc and Cry2Ab toxins of *Bacillus thuringiensis* is going to be available soon for growers in Mexico. Considering that variety, environment and location may play a significant role in Cry2Ab expression (Akin et al. 2002), it becomes an important issue the generation of data bases that may help to understand the resistance processes in populations selected by these dual-toxins Bt cotton varieties. Data generated for both toxins on tobacco budworm are presented in this paper.

Materials and Methods

Insects

Tobacco budworm larvae and eggs were collected directly from commercial cotton fields in The Yaqui, Valley, Caborca, and Sonoyta, Sonora, and the Mexicali Valley, in Baja California Mexico. The biological material was sent to the Entomology Laboratory of the Yaqui Valley Experimental Station, where it was maintained in walk-inn chambers set at 27°C, 14:10 h, photoperiod and 70% RH, until used in the CryIAc and Cry2Ab bioassays. A susceptible colony maintained in this Laboratory was used for comparison of responses against field populations.

Bioassays

Cry IAc and Cry2Ab toxins were provided by Monsanto Co. Each toxin was suspended in 0.2% agar and 200 µl of the suspension was deposited over 1 ml artificial diet contained on each of 2 ml wells of a 64 well assay tray (Jarold Mfg. Co. St. Louis, MO). Once the diet dried, one neonate TBW larvae was placed in each well. Trays were then covered with plastic ventilated covers and incubated at 27° C, 70% R.H. and 14:10 h photoperiod, for 5 days.

Eight and 10 concentrations per bioassay were used for CryIAc and Cry2Ab toxins respectively, and 64 neonate larvae in each concentration. Larvae placed on uncontaminated diet were used in each bioassay as a control. Percent mortality, larval weight, and number of larvae reaching 3rd instar, were recorded 5 days later (Sims et al. 1996). Probit analysis was performed on mortality data. Percent inhibition (stunting) was estimated by dividing weight of treated larvae by weight of control multiplied by 100.

Results and Discussion

Cry IAc

Data for the CryIAc toxin, bioassayed on TBW populations from Sonoyta, Caborca and Mexicali Valleys are presented in Tables 1 to 3.

In the Sonoyta population, third instar larvae were present in dosages of 0.025 µg/ml and lower. Besides the presence of third instars, weight inhibition is used as a criterion to measure susceptibility of TBW to Cry toxins. In this case, the lowest inhibition was 79.86% in the 0.010 µg/ml dosage (Table 1). Probit analysis of mortality data indicated an LC_{50} of 0.036 µg/ml, fiducial limits 0.023 to 0.052 µg/ml, LC_{95} 1.60 and slope 1.00 (Table 7).

The Caborca population showed third instar larvae in dosages of 0.025 µg/ml and lower The lowest percent inhibition was 85.91% at the 0.010 µg/ml dosage (Table 2). Mortality data analyzed by probits indicated a LC_{50} of 0.037 µg/ml, fiducial limits ranged from 0.025 to 0.051 µg/ml, the LC_{95} was 1.12 µg/ml and slope 1.10 (Table 7).

TBW population collected in the Mexicali Valley as with the other populations showed third instars only with dosages of 0.025 µg/ml and lower. The lowest percent inhibition was 91.10% at the 0.010 µg/ml dosage (Table 3). Dosage mortality data indicated a LC_{50} of 0.064 µg/ml, fiducial limits ranging from 0.044 to 0.087 µg/ml, LC_{95} 2.55 µg/ml and slope 1.00 (Table 7).

In all these colonies, dosages of 0.05 µg/ml prevented third instar larvae formation. This dosage is considered a diagnostic dosage for resistance monitoring programs in Mexico (Martinez-Carrillo et al. 2003). As measured by weight inhibition (stunting) at the lowest dosage used, the colony from Sonoyta was the less susceptible to CryIAc. However based in mortality data the LC_{50} data from Caborca and Sonoyta are more susceptible than all other populations evaluated in our Labotarory (Table 7).

Cry2Ab

Data for Cry2Ab toxin of *Bacillus thuringiensis* on TBW is presented in Tables 4-6., for a susceptible, Yaqui Valley and Mexicali Valley populations

Results for the susceptible colony indicate that third instar larvae were observed at dosages of 0.5 µg/ml and lower, percent inhibition was lower than 90% at dosages of 0.25 µg/ml. Probit analysis of dosage mortality data showed a LC_{50} of 1.564 µg/ml and LC_{95} of 28.81 µg/ml, this is 21.72X, and 16.95X respectively, as compared to the susceptible colony treated with CryIAc (Table 7).

The population from the Yaqui Valley presented third instar larvae at 0.5 µg/ml and lower dosages. As before at 0.25 µg/ml percent inhibition was lower than 90%. Mortaliy data analysis indicated an LC_{50} of 1.607 µg/ml and LC_{95} 31.52 µg/ml which are 25.51X and 14.80X higher as compared to a population from the same area but, treated with CryIAc (Table 7).

In the population reared from the Mexicali Valley, third instar larvae were observed at 0.5 µg/ml and lower dosages. Percent inhibition less than 90% was obtained with dosages of 0.5 µg/ml, being different from the other two populations evaluated. Probit analysis indicated a LC_{50} of 2.851 µg/ml and LC_{95} 48.27 µg/ml, this is 44.55X and 18.93X higher, respectively, as compared to a population treated with CryIAc (Table 7).

TBW populations evaluated were less sensitive to Cry2ab than to CryIAc. Comparison of populations to Cry2ab indicate that the Mexicali TBW population was significantly more tolerant than populations from the Yaqui Valley and the susceptible colony considering fiducial limits overlap as the criterion for separation of response (Table 7).

Conclusions

Data showed that TBW is less sensitive to Cry2Ab toxin of *Bacillus thuringiensis* than to CryIAc. Base line data have been generated to evaluate response of this insect to both toxins through time as Bollgard II is used in Mexico, Monitoring resistance should include besides percent growth inhibition and prevention of third instar larvae formation at the diagnostic dosage, complete dosage mortality data which generates more information on the response of population under selection pressure at various concentrations.

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References

Akin, D. S., S. D. Steward, and M. J. Knighten. 2002. Quantification of toxin levels in cotton expressing one and two insecticidal proteins of Bacillus turingiensis. Procc. Belwide Cotton Conf. 2002.

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.

Martinez Carrillo, J. L., and M. Berdegue. 1999. Responses of tobacco budworm populations from Mexico to Bt CryIA© toxin. Procc. Beltwide Cotton Conf. Vol. 2: 965-967.

Martinez-Carrillo, J. L., U. Nava-Camberos and C. Rodriguez M. 2003. Monitoring Resistance in Mexican Populations of TBW, CBW and PBW for response to CryIA (c) ttoxin of *Bacillus thuringiensis*. Resistance Pest Management Newsletter. Vol 13 No. 1 (Fall 2003).

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 of *Bacillus thuringiensis* CryIAc toxin on tobacco budworm populations from Sonoyta, Sonora. Mexico.

Concen tration	**Insects treated**	**Insects killed**	**Third instars**	**Weight mg**	**Percent inhibition**
5.0	48	48	0	0	100
1.0	64	60	0	0.03	99.89
0.5	64	56	0	0.12	99.59
0.25	64	50	0	0.36	99.72
0.10	64	47	0	0.91	96.86
0.05	64	41	0	1.16	96.00
0.025	64	36	10	2.85	90.17
0.010	64	22	22	5.84	79.86
Check	128	14	102	28.99	

Table 2. Bioassay results of *Bacillus thuringiensis* CryIAc toxin on tobacco budworm populations from Caborca, Sonora. Mexico.

Concen tration	**Insects treated**	**Insects killed**	**Third instars**	**Weight mg**	**Percent inhibition**
5.0	48	48	0	0	100
1.0	64	62	0	0.02	99.90
0.5	64	57	0	0.09	99.56
0.25	64	52	0	0.21	98.98
0.10	64	44	0	0.44	97.87
0.05	64	38	0	0.61	97.05
0.025	64	33	8	1.08	94.77
0.010	64	21	10	2.91	85.91
Check	112	10	96	20.66	

Table 3. Bioassay results of *Bacillus thuringiensis* CryIAc toxin on tobacco budworm populations from Mexicali, Baja California. Mexico.

Concen tration	Insects treated	Insects killed	Third instars	Weight mg	Percent inhibition
5.0	48	48	0	0	100
1.0	64	58	0	0.07	99.72
0.5	64	51	0	0.16	99.36
0.25	64	47	0	0.20	99.21
0.10	64	42	0	0.42	98.33
0.05	64	33	0	0.66	97.38
0.025	64	24	8	1.44	94.27
0.010	64	20	12	2.24	91.10
Check	144	14	128	25.17	

Table 4. Bioassay results of *Bacillus thuringiensis* Cry2Ab toxin on tobacco budworm populations from a susceptible colony.

Concen tration	Insects treated	Insects killed	Third instars	Weight mg	Percent inhibition
20	64	61	0	0.10	99.57
10	64	59	0	0.27	98.83
5	64	46	0	0.36	98.44
2.5	64	39	0	0.67	97.11
1	64	23	0	1.06	95.43
0.5	64	20	5	1.66	92.85
0.25	64	14	10	2.96	87.25
0.1	64	11	14	4.82	79.24
0.05	64	9	20	6.97	69.98
0.01	64	5	34	11.84	49.01
Check	160	8	141	23.22	

Table 5. Bioassay results of *Bacillus thuringiensis* Cry2Ab toxin on tobacco budworm populations from the Yaqui, Valley, Sonora. Mexico.

Concen tration	Insects treated	Insects killed	Third instars	Weight mg	Percent inhibition
40	64	60	0	0.14	99.34
20	64	58	0	0.18	99.15
10	64	56	0	0.25	98.83
5	64	53	0	0.43	97.98
2.5	64	38	0	0.84	96.05
1	64	27	0	1.05	95.07
0.5	64	20	4	2.12	90.04
0.25	64	15	8	4.18	80.36
0.1	64	4	19	9.10	57.24
.0.05	64	2	31	12.97	39.05
Check	144	10	135	21.28	

Table 6. Bioassay results of *Bacillus thuringiensis* Cry2Ab toxin on tobacco budworm populations from the Mexicali Valley in Baja California. Mexico.

Concen tration	Insects treated	Insects killed	Third instars	Weight mg	Percent inhibition
40	64	60	0	0.15	99.33
20	64	59	0	0.40	98.21
10	64	48	0	0.61	97.27
5	64	38	0	0.89	96.02
2.5	64	34	0	1.19	94.68
1	64	18	0	1.73	92.26
0.5	64	12	9	3.79	83.05
0.25	64	9	12	7.55	66.23
0.1	64	5	20	10.51	53.00
.0.05	64	3	30	14.87	33.50
Check	96	4	90	22.36	

Table 7. Probit analysis data for response of tobacco budworm populations from Mexico, to *Bacillus thuringiensis* CryIAc and Cry2Ab toxins.

Colony/ Toxin	LC_{50} µg/ml	Fiducial Limits	LC_{95} µg/ml	Slope
CryIAc				
Susceptible*	0.072	0.055 -- 0.092	1.70	1.20
Yaqui, Valley*	0.063	0.044 – 0.086	2.13	1.08
Sonoyta	0.036	0.023 – 0.052	1.60	1.00
Caborca	0.037	0.025 – 0.051	1.12	1.10
Mexicali	0.064	0.044 – 0.087	2.55	1.00
Cry2Ab				
Susceptible	1.564	1.220 – 1.991	28.81	1.30
Yaqui Valley	1.607	1.232 – 2.057	31.52	1.27
Mexicali	2.851	2.248 – 3.562	48.27	1.34

*Taken from Martinez-Carrillo and Berdegue 1999. BWCC Vol. (2) 965-967.