MONITORING OF THE PINK BOLLWORM SUSCEPTIBILITY TO THE BT ENDOTOXIN (CryIAc) IN MEXICO

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Abstract

Laboratory bioassays were carried out to determine susceptibility levels of pink bollworm (PBW) populations from four cotton production regions of Mexico to the Bt endotoxin CryIAc. Bt cotton performance was evaluated by sampling bolls and quantifying PBW infestations in four locations of the Comarca Lagunera, Mexico. PBW populations from the Comarca Lagunera, Jiménez and Delicias, Chih., and Mexicali, B. C. are susceptible to the endotoxin CryIAc. The PBW population from Mexicali, B. C. was the most susceptible to the toxin. PBW larvae developed on bolls were more susceptible to the toxin than those developed on squares. Larvae growth (size and weight) was drastically reduced by toxin concentrations of 0.1 and 0.3 μg/ml. Bt cotton is highly effective against the PBW in the Comarca Lagunera, which has caused a significant reduction in the number of insecticide applications.

Introduction

Pink bollworm (PBW), *Pectinophora gossypiella* (Saunders) is the key pest of cotton in the Comarca Lagunera and the Mexicali Valley, Mexico. It is also a primary pest in Jiménez and Delicias, Chih., Mexico. In the Comarca Lagunera, cotton producers made 7.0, 1.3, 2.5, and 0.5 insecticide applications to control this pest in 1996, 1997, 1998, and 1999, respectively. During 1997, 1998, and 1999, 17, 47, and 88% of the cotton acreage in this región was planted with transgenic varieties of cotton, respectively. The main advantage obtained with the use of Bt cotton in this region is a reduction in the insecticide usage, which has the following positive effects: 1) an increase in the biolgical control of pests, 2) a reduction in the negative impacts from the direct exposure of humans and wilde life to insecticides, and 3) a reduction in the environmental contamination risks from insecticide usage. However, a possible disadvantage and biggest challenge to face with Bt cotton technology is how to delay the development of pest resistance to Bt endotoxins (Benedict 1996). Therefore, monitoring of main cotton pests susceptibility to Bt endotoxins is a key component in the development and establishment of a PBW resistance management program to Bt cotton. Watson and Kelly-Johnson (1995) determined susceptibility levels of PBW from Safford and Yuma, AR, to Bt endotoxin before the introduction of transgenic varieties of cotton into this región. They found that an increase in toxin concentration affected the developmental rates of PBW larvae and pupae. Larvae did not develop beyond third instar at concentrations higher than 0.047 μg of CryIAc/ml of diet, and a concentration of 0.375 μg of CryIAc/ml of diet completely nullified larval developement. Bartlett (1995) demonstrated that a laboratory strain of PBW could rapidly respond to selection pressure for resistance to Bt endotoxin present in the leaves of transgenic cotton plants. Bartlett *et al*. (1997) determined the baseline levels of susceptibility of PBW to Bt endotoxin from five locations of Arizona. They found that an increase in the endotoxin concentration caused a decrease in PBW survival and development. Larvae from native or APHIS-S strains did not pupate (at 21 days) at concentrations higher than 0.005 μg of CryIAc/ml of diet; whereas, larvae from the resistant strain SOOTY-BTX were able to mature at all concentrations evaluated. Simmons *et al*. (1998) determined that four field populations of PBW were more susceptible to the endotoxin CryIAc than the susceptible reference strains APHIS-S and Marana-S. They also reported that Bartlett-R and APHIS-S strains had 70 and 4 % survival, respectively, at a concentration of 3.2 μg of CryIAc/ml of diet, but only few F_1 offspring of the four field populations survived a concentration of 1.0 μg of CryIAc/ml of diet. Greenplate *et al*. (1998) determined that CryIAc concentration in cotton fruiting structures varied from 10-15 to 1-2 μg/g of fresh weight at 40 and 120 days after planting, respectively. Similarly, CryIAc levels in cotton plant terminals changed from 20 to 5 μg/g of fresh weight at 40 and 120 days after planting, respectively.

In this report we present the second year of studies conducted in the Comarca Lagunera and the first year of bioassays carried out in Jiménez and Delicias, Chih., and Mexicali, B. C., México to determine PBW susceptibility to the Bt endotoxin CryIAc and Bt cotton performance.

Materials and Methods

PBW Collection

Rosetted blooms and bolls infested with PBW larvae were collected early and late in the season during 1999, respectively, from non-Bt cotton fields close to Bt cotton fields from four locations of the Comarca Lagunera, Mexico. Rosetted blooms were collected at the end of the season

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during 1999 from one comercial non-Bt cotton field of Jiménez, Chih., Mexico. PBW infested bolls were collected at the end of the season during 1999 from one comercial non-Bt cotton field of Delicias, Chih. and Mexicali, B. C., Mexico. Bloom and boll samples were taken to the Entomology Laboratory of La Laguna Experiment Station. Then they were placed into plastic boxes (30 x 20 x 17 cm), which had a metal screen to suspend bolls about 3.0 cm high and sheets of paper towel on the bottom. Mature PBW larvae cut out infested bolls, drop down and pupate in the paper towel.

PBW Rearing

Rearing method reported by Bartlett and Wolf (1985) and Simmons et al. (1998) with some modifications, was followed. Pupae obtained from boll collections were placed in one-liter plastic containers for adult emergence and oviposition. Eggs were placed into half-liter plastic containers, which had artificial diet ("pink bollworm diet", Southland Products Inc., 201 Stuart Island Rd, Lake Village, AR 71653). Then, small larvae were placed individually in 25 ml plastic cups, which were placed in plastic boxes (30 x 20 x 17 cm) with nylon screened lids and paper towel on the bottom. Mature larvae pupated in the small plastic cups or burrowed out of them and pupated within the paper towel sheets. Pupae were collected and placed into one-liter plastic containers to obtain adults and initiate another cicle of oviposition.

Laboratory Bioassays for PBW Susceptibility to CryIAc

Bioassays consisted on feeding neonate larvae with wheat germ diet containing diagnostic concentrations of the endotoxin CryIAc and evaluating their development and weight (Bartlett et al. 1997; Simmons et al. 1998). MVP bioinsecticide (Mycogen, San Diego, CA) was mixed into distilled water to produce a stock solution of 0.01%. Ten and 100 ml of this stock solution were added to one liter of hot liquid diet (not exceeding 60 ˚C) to produce final diagnostic concentrations of 1.0 and 10.0 μg of CryIAc/ml of diet, respectively. These diagnostic concentrations were selected based on the results of bioassays carried out in 1998 (Fig. 1), and they correspond approximately to the toxin concentrations in cotton plant fruiting structures at 120 and 40 days after planting, respectively (Greenplate et al. 1998). Then diet with toxin was cut in small pieces and placed in 25 ml plastic cups. Finally, a neonate larva was transferred into each cup. Twenty larvae per concentration and four replications for each concentration were bioassayed. PBW larvae were maintained in a rearing room at a temperature of 28 ± 2˚C and a L:D 14:10 photoperiod. After 21 days of rearing, larval survival, developmental stage, and weight were evaluated. Survival percentages were estimated based on the initial number of larvae tested and the final number of larvae reaching maturity (4^{th} instar larvae + pupae + adults + exit holes). Weight reduction percentages were estimated based on average weights of larvae surviving to the diagnostic concentrations respect to average weights of control larvae.

Effects of CryIAc on Larval Size and Weight

PBW larvae obtained from non-Bt cotton bolls collected late in the season in the Comarca Lagunera were used for this study. Pupae obtained were placed in plastic containers for adult emergence and oviposition. Neonate larvae were exposed to several CryIAc concentrations and their size and weight were measured at the ages of 3, 6, 9, 12, 15, 18, and 21 days. Toxin concentrations evaluated were 0.01, 0.03, 0.1, and 0.3 μg of CryIAc/ml of diet and a control (diet without toxin). Twenty larvae per toxin concentration and larval age were initially tested, including the control, and from nine to 20 surviving larvae were finally evaluated.

Field Performance of Bt Cotton in the Comarca Lagunera

During 1999, medium and large bolls were collected from Bt and non-Bt cotton fields from four locations (Tlahualilo, Dgo., Matamoros, F. I. Madero, and San Pedro, Coah.) of the Comarca Lagunera and reviewed to determine PBW larval infestations. From 1146 to 4013 bolls were sampled during different periods of time, beginning in July 7 and ending in August 25. Bolls were dissected and the small (1^{st} + 2^{nd} instars) and large (3^{rd} + 4^{th} instars) larvae were quantified. Additionally, a sample of 500 medium and large bolls were collected from conventional and transgenic cotton fields from the same locations. This sample was divided in four groups of 125 bolls each and placed into small emergence cages. Emergence cages had a wood box (30 x 30 x 14 cm) on the bottom and a pyramidal top (29 cm high) made of wood and metallic screen, which has a half-liter glass jar on the top to trap emerging PBW adults. Emergence cages were subjected to room conditions and checked each two days for quantifying adults emerged.

Results and Discussion

PBW Susceptibility to CryIAc

For the concentration of 1.0 μg/ml, from 52.5 to 91.2% of PBW larvae reached the second instar or greater stages and 0 to 20% reached the third instar or greater stages, but none was able to reach maturity ($\geq 4^{th}$ instar) in the Comarca Lagunera, Jiménez and Delicias, Chih., except for one location (San Pedro, Coah.), where 2.5% larvae survived through the 4^{th} instar. All larvae from Mexicali, B. C. died or remained as first instar. In the Comarca Lagunera, significantly less larvae reached the third instar or greater stages when they were collected from bolls late in the season compared to those collected from rosetted blooms early in the season. Larval weight reduction ranged from 88.4 to 99.7% for the different locations (Table 1).

For the concentration of 10. 0 μg/ml very few larvae (maximum of 5.0%) reached the second instar. There were no larvae reaching the third instar. Larval weight reduction ranged from 96.0 to 99.9% for the different locations (Table 2).

These results show that there are not indication of PBW resistance to the toxin CryIAc in the different cotton production regions of Mexico. PBW population from Mexicali, B. C. was the most susceptible to the toxin. Results for the PBW population of the Comarca Lagunera suggest that larvae developed on bolls are more susceptible to the toxin CryIAc, compared to those developed on squares and forming rosetted blooms, which can be due to differences in nutritional properties of those fruiting structures. Similar results were found during 1998 in the Comarca Lagunera, since survival of four generation larvae, which developed on bolls, was the lowest (Fig. 1).

Effects of CryIAc on Larval Size and Weight

Size and weight of PBW larvae were reduced with increasing concentrations of the endotoxin CryIAc (Fig. 2 and 3). Very low rates of larval size and weight increase were observed at concentrations of 0.1 and 0.3 μg/ml. Maximum larval weight for 0.1 μg/ml (7.5 mg) and 0.3 μg/ml (5.0 mg) corresponded approximately to the average weight of second instar larvae (about seven days of age) in the control.

Field Performance of BT Cotton in the Comarca Lagunera

High levels of larval infestations were observed in conventional cotton bolls in the Comarca Lagunera. However, there were not detected any larva surviving beyond the second instar in transgenic cotton (Table 3). Similarly, high numbers of PBW moths emerged from conventional cotton bolls. On the contrary, only seven moths emerged from 2000 transgenic cotton bolls (Table 4). Since about 2% plants in Bt cotton do not express the toxin, it is highly probable that those moths were developed in non-Bt cotton bolls.

These results indicate the high efficacy of Bt cotton against the PBW in the Comarca Lagunera, which explains mainly the reduction of insecticide usage to control this pest in this region. With this respect, the acreage planted with Bt cotton has been 0, 17, 47, and 88% for 1996, 1997, 1998, and 1999 and the number of insecticide applications has been 7.0, 1.3, 2.5, and 0.5, respectively.

Conclusions

PBW populations from the Comarca Lagunera, Jiménez and Delicias, Chih., and Mexicali, B. C. are susceptible to the endotoxin CryIAc. The PBW population from Mexicali, B. C. was the most susceptible to the toxin. PBW larvae developed on bolls were more susceptible to the toxin than those developed on squares. Larvae growth (size and weight) was drastically reduced by toxin concentrations of 0.1 and 0.3 μg/ml. Bt cotton is highly effective against the PBW in the Comarca Lagunera, which has caused a significant reduction in the number of insecticide applications.

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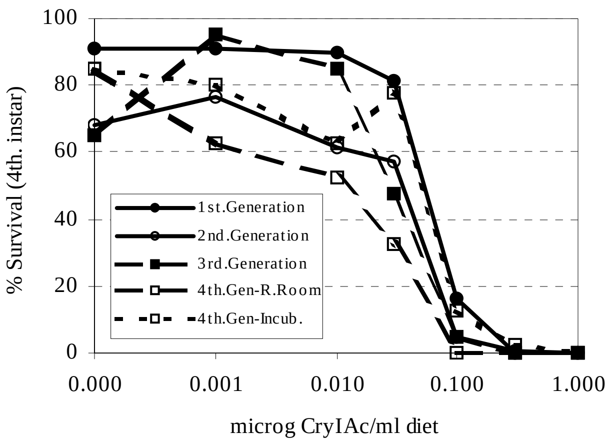

Figure 1. Response of pink bollworm from the Comarca Lagunera, Mexico, to Bt endotoxin CryIAc, in 1998.

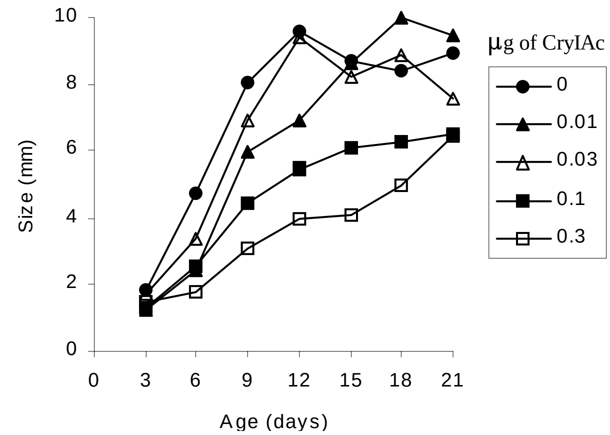

Figure 2. Size of pink bollworm larvae surviving to different concentrations of CryIAc, in 1999.

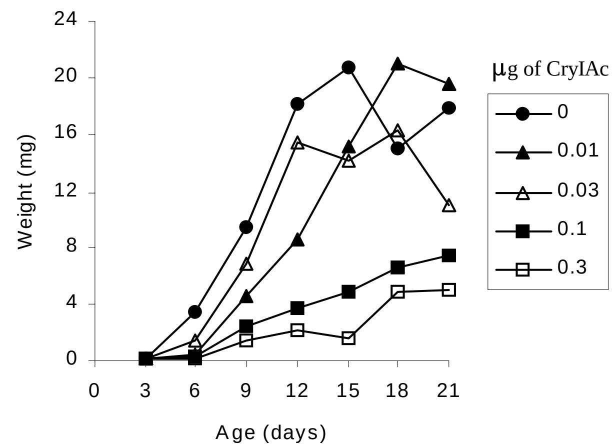

Figure 3. Weight of pink bollworm larvae surviving to different concentrations of CryIAc, in 1999.

Table 1. Evaluation of the diagnostic concentration of 1.0 µg of CryIAc/ml of diet on several PBW populations, in 1999.

Location	Collection date	% larvae ≥ (instars)			% weight reduction*
		2nd	3rd	4th	
Comarca Lagunera:					
Tlahualilo, Dgo.	Jun. 24	53.7	16.2	0	94.6
	Aug. 5	86.2	50	0	92.8
Matamoros, Coah.	Jun. 25	52.5	20	0	94.9
	Jul. 27	65	0	0	91.8
F.I.Madero, Coah.	Jul. 1	55	13.7	0	96.2
	Jul. 27	80	0	0	88.4
San Pedro, Coah.	Jul. 5	61.2	17.5	25	92
	Jul. 27	91.2	50	0	90.8
Jiménez, Chih.	Sep. 3	81.2	18.7	0	92
Delicias, Chih.	Ago. 10	86.2	25	0	93.7
Mexicali, B. C.	Ago. 3	0	0	0	99.7

Larval weight reduction estimated respect to average weights of control larvae.

Table 2. Evaluation of the diagnostic concentration of 10.0 µg of CryIAc/ml of diet on several PBW populations, in 1999.

Location	Collection date	% larvae ≥ (instars)			% weight reduction*
		2nd	3rd	4th	
Comarca Lagunera:					
Tlahualilo, Dgo.	Jun. 24	0	0	0	99.4
	Aug. 5	37	0	0	96.9
Matamoros, Coah.	Jun. 25	0	0	0	99.9
	Jul. 27	0	0	0	99.8
F.I.Madero, Coah.	Jul. 1	12	0	0	99.5
	Jul. 27	37	0	0	98.1
San Pedro, Coah.	Jul. 5	0	0	0	99.9
	Jul. 27	50	0	0	99.7
Jiménez, Chih.	Sep. 3	0	0	0	99.7
Delicias, Chih.	Ago. 10	25	0	0	99.5
Mexicali, B. C.	Ago. 3	0	0	0	99.8

* Larval weight reduction estimated respect to average weights of control larvae.

Table 3. Infestation of PBW on Bt and non-Bt cotton bolls in the Comarca Lagunera, in 1999.

Location	Cotton type	Date	Bolls	PBW larvae	
				Small	Large
San Pedro, Coah.	Non-Bt	Jul. 7-Aug. 22	2890	2301	1615
	Bt	Jul. 7-Aug. 22	4013	207	0
F.I.Madero, Coah.	Non-Bt	Aug. 7-25	1253	35	128
	Bt	Aug. 7-25	1146	0	0
Matamoros, Coah.	Non-Bt	Jul.21-Aug. 24	2259	559	440
	Bt	Jul.21-Aug. 24	2509	161	0
Tlahualilo, Dgo.	Non-Bt	Jul.23-Aug. 16	1259	295	106
	Bt	Jul.23-Aug. 16	1721	225	0

Small larvae = 1^{st} + 2^{nd} instars. Large larvae = 3^{rd} + 4^{th} instars.

Table 4. PBW adults emerged from Bt and non-Bt cotton bolls in the Comarca Lagunera, in 1999.

Location	Cotton type	Date	PBW adults
San Pedro, Coah.	Non-Bt	Aug. 25	351
	Bt	Aug. 25	2
F.I.Madero, Coah.	Non-Bt	Aug. 20	59
	Bt	Aug. 20	1
Matamoros, Coah.	Non-Bt	Sep. 9	471
	Bt	Sep. 9	0
Tlahualilo, Dgo.	Non-Bt	Sep. 9	561
	Bt	Sep. 9	4

Sample size was 500 bolls.