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

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Abstract

Laboratory bioassays were carried out to determine susceptibility levels of the pink bollworm (PBW) to the Bt endotoxin CryIAc. PBW larvae survival was also evaluated in Bt and non-Bt cotton bolls, under field conditions. Survival of PBW larvae from the different generations was reduced as the toxin concentration increased. PBW larvae did not survive beyond the first instar at a concentration of 1.0 µg of CryIAc/ml of diet in laboratory bioassays. Weight and size of PBW larvae from the fourth generation were reduced with increasing concentrations of the endotoxin CryIAc. There were no large PBW larvae (third and fourth instars) in naturally or artificially infested Bollgard® cotton bolls. PBW larvae did not develop beyond second instar in Bollgard® cotton bolls. Therefore, current PBW population from the Comarca Lagunera is highly susceptible to the endotoxin CryIAc.

Introduction

Pink bollworm (PBW), *Pectinophora gossypiella* (Saunders) is the key pest of cotton in the Comarca Lagunera, Mexico. Cotton producers made 7.0, 1.3 y 2.5 insecticide applications to control this pest in 1996, 1997 and 1998, respectively. In 1997 and 1998, 18.0 and 47.5% 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 use, 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 use. 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 concentratios 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 F1 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.

Materials and Methods

PBW Collection

Rosetted blooms infested with first generation PBW larvae and bolls infested with second, third, and fourth generation larvae were collected from non-Bt cotton fields close to Bt cotton fields at Matamoros, Coahuila. Samples consisted of 550 rosetted blooms and from 200 to 400 bolls. 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. The lids of these containers lids had a 5.0 cm diameter circle with a nylon screen. Paper towel circles of 5.0 cm diameter were placed over the nylon screen for egg

Reprinted from the *Proceedings of the Beltwide Cotton Conference*
Volume 2:1246-1248 (1999)
National Cotton Council, Memphis TN

laying. The paper towel pieces with eggs were fixed to the walls of half-liter paper containers, which had artificial diet ("pink bollworm diet", Southland Products Inc., 201 Stuart Island Rd, Lake Village, AR 71653). Also, small larvae were placed individually in 25 ml plastic cups. Then, these paper containers and small plastic cups were placed in plastic boxes (30 x 20 x 17 cm) with nylon screened lids and paper towel on the bottom. Mature larvae burrowed out of the paper containers or small plastic cups 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 different concentration of the endotoxin CryIAc and evaluating their development (Bartlett et al. 1997; Simmons et al. 1998). MVP Bioinsecticide (Mycogen, San Diego, CA) was mixed into distilled water to produce stock solutions of 0.001 and 0.01%. Necessary amounts of these stocks solutions were added to one liter of hot liquid diet (not exceeding 60 °C) to produce final concentrations of 0.001, 0.01, 0.03, 0.1, 0.3 and 1.0 µg of CryIAc/ml of diet. Then diet with toxin was cut in small pieces and placed in 25 ml plastic cups. Finally, a neonate larvae was transferred into each cup. Ten to 20 larvae per concentration and four replications for each concentration were bioassayed. Larvae from first, second and third generation were maintained in a rearing room at a temperature of 28 ± 2°C and a L:D 14:10 photoperiod. Larvae from forth generation were divided in two groups, one of them was reared in the above rearing room and the other one was reared in an incubator at 29±2°C in darkness. After fifteen days of rearing in the rearing room and 21 days of rearing in the incubator, survival and developmental stage 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). Also, larval weight and size was measured for the fourth generation bioassay in the rearing room.

PBW Survival in Bt and Non-Bt Cotton Bolls

Four 50-m-long rows of the conventional variety Deltapine 5690 and the transgenic variety NuCOTN 353 of cotton were planted at La Laguna Experiment Station on May 23, 1998. Twenty five 15- or 16-days-old bolls of each variety were infested manually with two neonate larvae on July 31, August 8 and 29, and September 6, at 69, 77, 98 and 106 days after planting, respectively. Others 25 bolls of each variety were tagged as controls. After 15 days of infestation, 20 infested bolls and 20 control bolls were collected and checked to record numbers of small larvae (first and second instars) and large larvae (third and fourth instars).

Results and Discussion

PBW Susceptibility to CryIAc

Survival of PBW larvae in the rearing room (28±2°C and a 14:10 photoperiod) from the different generations was reduced as the toxin concentration increased. PBW survival in control treatments ranged from 65.0% to 91.2%. Survival at concentrations of 0.001 and 0.01 µg of CryIAc/ml of diet was high, ranging from 32.5% to 90.0%, whereas, at concentrations of 0.1 and 0.3 µg/ml was low, ranging from 0 to 16.2%. PBW larvae did not survive at a concentration of 1.0 µg/ml. PBW larvae from fourth generation, which were bioassayed in the incubator at 29±2°C in darkness, had higher survival than PBW larvae from the same generation but bioassayed in the rearing room, however, they did not survive at 1.0 µg/ml (Fig. 1) These results indicate that PBW population from the Comarca Lagunera in 1998 is highly susceptible to the endotoxin CryIAc during this first growing season of transgenic cotton. Simmons *et al*. (1997) reported 70 and 4% survival at the concentration of 3.2 µg/ml for Barlett-R and APHIS-S strains, respectively, and a few individuals from field populations surviving at the concentration of 1.0 µg/ml.

Weight and size of PBW larvae from the fourth generation were reduced with increasing concentrations of the endotoxin CryIAc (Fig.2). This means that PBW larvae surviving to moderately high concentrations (0.1 and 0.3 µg/ml) of CryIAc have their growth inhibited, which suggests that they do not represent an economic problem for transgenic cotton in commercial fields.

PBW Survival in Bt and Non-Bt Cotton Bolls

Numbers of small and large PBW larvae were high in conventional cotton bolls, both in infested and control bolls (Table 1). On the contrary, low infestations of small larvae were detected in transgenic cotton bolls, but there were not infestations of large larvae in any of the sampling dates (Table 2). These results suggest that CryIAc concentrations in transgenic cotton plants are higher than that one required (1.0 mg/ml) to inhibit completely PBW development, according to laboratory bioassays. With this respect, Greenplate et al. (1998) reported CryIAc concentrations in fruiting structures of transgenic cotton varied from 1-2 µg/g to 10-15 µg/g at 120 and 40 days after planting. Therefore, CryIAc levels in transgenic cotton plants are more than enough to control current PBW population of the Comarca Lagunera.

Conclusions

PBW larvae did not survive beyond the first instar at a concentration of 1.0 µg of CryIAc/ml of diet in laboratory bioassays. There were no large PBW larvae (third and fourth instar) in naturally or artificially infested Bollgard® cotton bolls, under field conditions. PBW larvae did not develop beyond second instar in Bollgard® cotton bolls. Therefore, current PBW population from the Comarca Lagunera is highly susceptible to the endotoxin CryIAc.

Acknowledgements

We thank R. Haces G. for his support to this study, R. Hernández L., J. D. Monsivais H., J. L. Palacios V. and L. Ramírez R. for their field and laboratory assistance.

References

Bartlett, A. C. and W. W. Wolf. 1985. Pectinophora gossypiella. *In*: Handbook of Insect Rearing Vol II. R. F. Moore and P. Singh (eds.). Elsevier. New York, NY. pp: 415-431.

Bartlett, A. C., T. J. Dennehy, and L. Antilla. 1997. An evaluation of resistance to B. t. toxins in native populations of the pink bollworm, pp. 885-887. *In*: P. Dugger and D. A. Richter (eds.). Proceedings, 1997 Beltwide Cotton Conferences. National Cotton Council, Memphis, TN.

Benedict, J. H. 1996. Bt cotton: opportunities and challenges, pp. 25-29. *In*: P. Dugger and D. A. Richter (eds.). Proceedings, 1996 Beltwide Cotton Conferences. National Cotton Council, Memphis, TN.

Greenplate, J. T., G. P. Head, S. R. Penn, and V. T. Kabuye. 1998. Factors potentially influencing the survival of *Helicoverpa zea* on Bollgard cotton, pp. 1030-1033. *In*: P. Dugger and D. A. Richter (eds.). Proceedings, 1998 Beltwide Cotton Conferences. National Cotton Council, Memphis, TN.

Simmons, A. L., T. J. Dennehy, B. E. Tabashnik, L. Antilla, A. Bartlett, D. Gouge, and R. Staten. 1998. Evaluation of B. t. cotton deployment strategies and efficacy against pink bollworm in Arizona, pp. 1025-1030. *In*: P. Dugger and D. A. Richter (eds.). Proceedings, 1998 Beltwide Cotton Conferences. National Cotton Council, Memphis, TN.

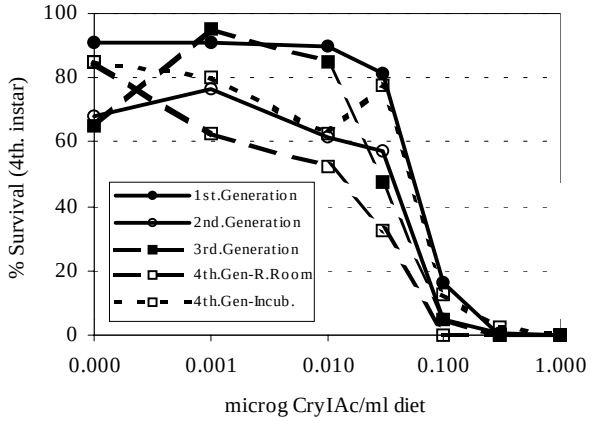

Figure 1. Response of pink bollworm from the Comarca Lagunera, Mexico, to Bt endotoxin CryIAc, in 1998.

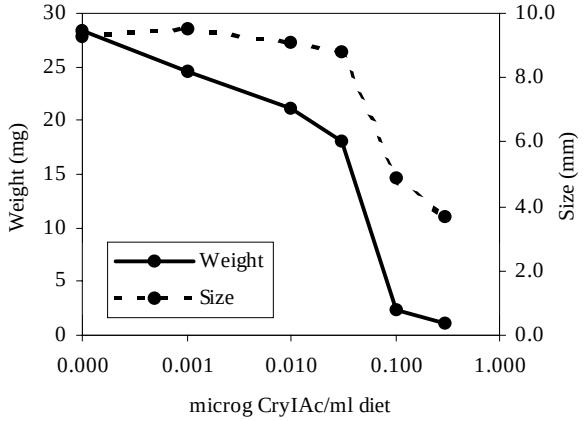

Figure 2. Weight and size of pink bollworm larvae from the fourth generation surviving to Bt endotoxin CryIAc, in 1998.

Table 1. Numbers of small and large PBW larvae in 20 cotton bolls, variety Deltapine 5690, after 15 days of infestation.

Date	Infested bolls		Control bolls	
	Small	Large	Small	Large
July 31	1.0	35.0	1.0	15.0
August 8	13.0	35.0	18.0	27.0
August 29	4.0	46.0	9.0	28.0
September 6	13.0	73.0	18.0	23.0

Table 2. Numbers of small and large PBW larvae in 20 cotton bolls, variety NuCOTN 35B, after 15 days of infestation.

Date	Infested bolls		Control bolls	
	Small	Large	Small	Large
July 31	2.0	0	3.0	0
August 8	0	0	1.0	0
August 29	21.0	0	4.0	0
September 6	19.0	0	18.0	0