Monitoring of the Pink Bollworm[1] Susceptibility to the *Bacillus thuringiensis* Endotoxins Cry1Ac and Cry2Ab in México

Monitoreo de la Susceptibilidad del Gusano Rosado[1] a las Endotoxinas Cry1Ac y Cry2Ab del *Bacillus thuringiensis* en México

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Abstract. The pink bollworm, *Pectinophora gossypiella* (Saunders), is a primary pest of cotton, *Gossypium hirsutum* L., in Mexico. Baseline and diagnostic concentration bioassays were used to determine the susceptibility of this pest to *Bacillus thuringiensis* (Berliner) toxins Cry1Ac and Cry2Ab in different cotton-growing regions of Mexico, during 1998-2006. The LC_{50} values varied depending on the region and collection date or generation of insect. Pink bollworm populations from the Mexicali Valley, Baja California, were the most susceptible. In general, results obtained showed no indication of resistance by this insect in Mexico.

Resumen. El gusano rosado del algodonero, *Pectinophora gossypiella* (Saunders), es una plaga primaria del algodonero en México. Bioensayos de línea base y concentración diagnóstica se efectuaron para determinar la susceptibilidad de esta plaga a las toxinas de *Bacillus thuringiensis* (Berliner) Cry1Ac y Cry2Ab en diferentes regiones productoras de algodonero en México, durante 1998-2006. Los valores de CL_{50} variaron en función de la región y la fecha de colecta o generación del insecto. Las poblaciones del gusano rosado del Valle de Mexicali, Baja California, fueron las más susceptibles a las toxinas. Los resultados obtenidos muestran que no hubo indicios de resistencia de este insecto en México.

The pink bollworm, *Pectinophora gossypiella* (Saunders), is the key pest of cotton, *Gossypium hirsutum* L., in La Laguna region of the states of Durango and Coahuila, and the Mexicali Valley of Baja California State in Mexico. It is also a primary pest in Jiménez, Delicias, and Valle de Juárez, in the State of Chihuahua, Mexico. In La Laguna, about 88% of the cotton acreage (20,009 ha) was planted with *Bacillus thuringiensis* (Berliner) (Bt)-expressing cotton, during 2009. In this region, cotton producers applied insecticide 4.0 times to control the cotton pest complex, mainly against the boll weevil, *Anthonomus grandis grandis* Boheman, and the conchuela, *Chlorochroa ligata* (Say), in 2009. None of these applications

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has been directed against the pink bollworm since 1999. Therefore, the main advantage obtained with the use of Bt cotton in the region is a reduction in 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 susceptibility of main cotton pests to Bt endotoxins is a key component in the development and establishment of a pink bollworm resistance management program for Bt cotton. Watson and Kelly-Johnson (1995) determined susceptibility levels of pink bollworm from Safford and Yuma, AR, to Bt endotoxin before the introduction of transgenic varieties of cotton into the region. They found that pink bollworm larvae did not develop beyond the third instar at concentrations greater than 0.047 µg of Cry1Ac/ml of diet, and a concentration of 0.375 µg of CrylAc/ml of diet completely nullified larval development. Bartlett et al. (1997) determined the baseline levels of susceptibility of pink bollworm to Bt endotoxin from five locations in Arizona. Larvae from native or APHIS-S laboratory strains did not pupate (at 21 days) at concentrations greater than 0.005 µg of Cry1Ac/ml of diet; but, larvae from the resistant strain SOOTY-BTX were able to mature at all concentrations evaluated.

Rosetted blooms and bolls infested with pink bollworm larvae were collected from non-Bt cotton fields close to Bt cotton fields from several cotton-producing regions of Mexico. In La Laguna, pink bollworm larvae were collected during 1998-2002, 2004, and 2006. In Chihuahua State, Pink bollworm larvae were collected during 1999 (Jiménez and Delicias), 2000 (Delicias, Ascención and Valle de Juárez), 2001 (Ascención and Valle de Juárez), and 2002 (Valle de Juárez). In the Mexicali Valley, collections were made in 1999, 2001, 2004, and 2006. The method used to rear pink bollworms followed what was reported by Bartlett and Wolf (1985) and Simmons et al. (1998) with the following modifications: bioassays to determine the baseline susceptibility of the pink bollworms to Bt toxins consisted of feeding neonate larvae with wheat germ diet ("pink bollworm diet", Southland Products, Inc., Lake Village, AR) containing seven to 10 concentrations of the endotoxins Cry1Ac and Cry2Ab ranging from 0.001 to 10.0 µg. MVP II containing 19.1% Cry1Ac and ground corn leaf powder containing 0.6% Cry2Ab were provided by Monsanto. Survival and development (Bartlett et al. 1997, Simmons et al. 1998) at 28 ± 2°C and complete darkness were evaluated 21 days later. Ten to 30 neonate larvae for each concentration in four replications were bioassayed; thus, a minimum of 40 larvae and a maximum of 120 larvae were tested per concentration.

Additionally, a diagnostic concentration of 1.0 µg of Cry1Ac and Cry2Ab/ml of diet was evaluated for the different pink bollworm populations. Ten to 60 larvae for the diagnostic concentration and the control and four to eight replications for each of them were bioassayed; thus, a minimum of 40 larvae and a maximum of 300 larvae were tested. Percentages of survival when the larvae were reared in the environmental conditions described were estimated based on the initial number of larvae tested and the number of larvae reaching the second, third, or fourth instar.

In relation to the baseline susceptibility of the pink bollworm to the Bt endotoxins, the results obtained fluctuated depending on the region and collection date or insect generation. Thus, the LC_{50} values varied from 0.008 (0.006-0.012) to 0.056 (0.049-0.065) µg of Cry1Ac/ml of diet for the different pink bollworm generations during 1998 from La Laguna. For the same region, LC_{50} values were 0.033 (0.026-0.039) µg of Cry1Ac/ml of diet and 0.107 (0.092-0.129) µg of Cry2Ab/ml of diet, during 2001. For the Mexicali Valley, the LC_{50} values were 0.026 (0.019-0.036) µg of Cry1Ac/ml of diet and 0.044 (0.032-0.053) µg of Cry2Ab/ml of

diet, during 2001. For Valle de Juárez, the LC_{50} value was 0.090 (0.078-0.106) µg of Cry1Ac/ml of diet in 2002.

In general, pink bollworm larvae from La Laguna were not able to reach maturity (≥4th instar) at the diagnostic concentration of 1.0 µg/ml of Cry1Ac and Cry2Ab, except for at one location (San Pedro, Coahuila) in 1999 where 2.5% of the larvae survived through the 4th instar. Data obtained indicated an increased susceptibility of the pink bollworm to Cry1Ac during the last years (Table 1). All larvae from populations of pink bollworm from the Mexicali Valley died or remained first instar. Therefore, pink bollworm populations from this region were very susceptible to Bt endotoxins. Pink bollworm populations from cotton-growing areas of Chihuahua showed an important degree of tolerance to Cry1Ac during the 1999 and 2000 seasons. Particularly, a population collected from the Valle de Juarez at the end of the cotton-growing season in 2000 had 11.8% survival in the diagnostic concentration. However, during the following two seasons, pink bollworm larvae from Chihuahua exposed to diet containing Cry1Ac did not develop beyond the second instar (Table 1). These results showed there was no indication of pink bollworm resistance to the toxins Cry1Ac and Cry2Ab in the different cotton-production regions of Mexico, during recent seasons. Similarly, in Arizona the pink bollworm did not evolve resistance during 9 years of planting Bt cotton, which could be explained by refuges of non-Bt cotton, recessive inheritance of resistance, incomplete resistance, and fitness costs associated with resistance (Tabashnik et al. 2005). Particularly, in La Laguna, where Bt cotton has been planted since 2007, there has been a significant reduction in insecticide use to control the pest complex. Thus, in this region, no insecticide application has been directed against the pink bollworm since 1999.

Patterns of infestation levels of cotton pests vary widely throughout cotton-growing regions in Mexico, which has determined important differences in pest-management strategies and, consequently in the levels of Bt cotton adoption (Traxler et al. 2003). Thus, in La Laguna, pink bollworm is the key pest, followed by the bollworm, *Helicoverpa zea* (Boddie), and the conchuela, where a high adoption of Bt cotton (80-88%) has been implemented since 2006. In the Mexicali Valley, the main pests are pink bollworm; the silverleaf whitefly, *Bemisia argentifolii* (Bellows & Perring); and the cotton bollworm, where Bt cotton adoption was low until 2006 (26.0%), which seems to explain the large amount of susceptibility of pink bollworm to Bt toxins. Subsequently, a significant increase in Bt cotton adoption has been observed (78.3% in 2009). In Chihuahua State, the dominant pest is boll weevil, followed by cotton bollworm and pink bollworm, where an intermediate and stable Bt cotton adoption has been established (43.8% in 2006 and 46.7% in 2009). The implementation of the Pink Bollworm Eradication Program in Mexicali Valley and Chihuahua State could delay development of resistance by this insect to Bt toxins, because of the use of several control methods such as sterile males and mating disruption. On the other hand, in La Laguna, where there is great dependence on the use of a large proportion of transgenic cotton, the risk of resistance seems to be high (Tabashnik et al. 2009). Therefore, for the purpose of managing resistance by pink bollworm, greater effort is needed for the following aspects: monitoring of insect susceptibility to Bt toxins, implementing an effective refuge strategy, continuing the eradication program in the regions where it already is implemented and its expansion to La Laguna, as well as evaluating new control options, such as using several Bt toxins (pyramiding genes) and non-Bt toxins.

Table 1. Percentage of Pink Bollworm Larvae Developing Through a Given Instar on 1.0 µg of Cry1Ac and Cry2Ab/ml of Diet from Samples Collected in Different Cotton-growing Regions of Mexico

		Cry1Ac toxin			Cry2Ab toxin		
Region	Year	2nd instar	3rd instar	4th instar	2nd instar	3rd instar	4th instar
La Laguna	1998	0 - 7.5	0	0	---[a]	---	---
	1999	52.5 - 91.2	0 - 20.0	0 - 2.5	---	---	---
	2000	7.5 - 66.7	0 - 40.0	0	---	---	---
	2001	8.0	0	0	13.7	2.5	0
	2002	0.25	0	0	0.65	0	0
	2004	0	0	0	0	0	0
	2006	0	0	0	0	0	0
Mexicali	1999	0	0	0	---	---	---
	2001	15.0	0	0	21.2	0	0
	2004	0	0	0	0	0	0
	2006	0	0	0	0	0	0
Chihuahua	1999	81.2 - 86.2	2.5 - 18.7	0	---	---	---
	2000	0 - 77.5	0 - 66.2	0 - 11.8	---	---	---
	2001	0 - 2.8	0	0	---	---	---
	2002	7.5	0	0	---	---	---

[a]No bioassays performed.

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