RESPONSE OF TOBACCO BUDWORM POPULATIONS OF THE YAQUI, VALLEY, SONORA TO CRY2AB TOXIN OF BACILLUS THURINGIENSIS

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Abstract

Since 1996 Bollgard cotton which contains Cry1Ac protein for control of tobacco budworm, pink bollworm cotton bollworm has been planted in Mexico. In 2008 it is intended to introduce Bollgard II a Biotech cotton which expresses both Cry1Ac and Cry2Ab *Bacillus thuringiensis* proteins. In order to generate base data on the response of various lepidopteran insect pest to this toxin a series of bioassays have been performed. In this article results of the data obtained on tobacco budworm (TBW) *Heliothis virescens* populations from the Yaqui, Valley in Sonora Mexico are presented. TBW larvae were collected from commercial cotton fields taken to the Entomology Laboratory of the Experimental Field Station of INIFAP and reared on artificial diet until bioassayed. They were kept in a walk-inn chamber set at 27°C, 14:10 hr, photoperiod and 70% RH, A colony of *H. virescens* that has been maintained in our laboratory since 1982 was used as a reference standard for susceptibility. The Cry2Ab toxin used in these bioassays was provided by Monsanto Comercial S.A de C.V. Dose-response bioassays were performed using the diet overlay methodology. Larval mortality including those larvae that do not molted to second instar was recorded five days after treatment. Mortality data were analyzed by the Probit analysis. Results showed LC_{50} values ranging from 1.564 µg/ml to 0.850 µg/ml in the three susceptible colonies evaluated in 2003, 2004 and 2005. Mean LC_{50} value of these observations was 1.138 µg/ml which corresponds with observations reported in USA. The LC_{50} data from the Yaqui Valley field colonies, ranged from 0.768 µg/ml up to 2.054 µg/ml. These values also correspond to those reported in USA for Cry2Ab on tobacco budworm populations. Due to the fact that some variation has been reported in dose-mortality bioassays, more data on dose-mortality, growth inhibition and larval development are required to integrate and generate information to follow up the response of field populations exposed to Cry2Ab toxins.

Introduction

In Mexico biotech *Bacillus thuringiensis* (Berliner) cotton, that expresses the Cry1Ac protein has been planted since 1996. This technology has been well accepted by Mexican growers and it has reached up to 60.6% of the area planted to this crop (Martinez-Carrillo and Diaz-Lopez 2005). The impact on tobacco budworm (TBW) *Heliothis virescens*, cotton bollworm *Helicoverpa zea* and pink bollworm *Pectinophora gossypiella*, populations has been acceptable, but other lepidopteran insects have not been controlled such as *Spodoptera exigua*, *Spodoptera frugiperda* and *Trichoplusia ni*. In order to increase the spectrum of control of the biotech cotton, Bollgard II which expresses both Cry1Ac and Cry2Ab *Bacillus thuringiensis* proteins, is been planned to be introduced in Mexico next year. Bollgard II was granted registration in the United States since December 23, 2002 and it has been used commercially since 2003. It has provided higher control of all target species than Bollgard cotton (Matten and Reynolds 2003). The introduction of any new material to control insect pest increases the possibility for resistance development. In Mexico it is required the establishment of a strategy for resistance management for Bollgard cotton, and a resistance monitoring program to follow up the response of populations subjected to selection pressure with the Cry1Ac protein. In order to generate information on the response of these populations to the Cry2Ab toxin contained in Bollgard II a series of bioassays have been performed. In this article results obtained on TBW *Heliothis virescens* population from the Yaqui, Valley in Sonora will be discussed.

Materials and Methods

Insects.- Tobacco budworm larvae were collected from commercial cotton fields and taken to the Entomology Laboratory of the Yaqui Valley Experimental Field Station of INIFAP, where they were reared in artificial Lepidoptera diet (Southland Products Inc. Lake Village AR) until used in the Cry2Ab bioassays. Colonies were kept in a walk-inn chamber set at 27°C, 14:10 hr, photoperiod and 70% RH, A TBW colony that has been maintained in our laboratory since 1982 was used as a reference standard for susceptibility.

Insecticide.- The Cry2Ab toxin used in these bioassays was provided by Monsanto Comercial S.A de C.V. in Mexico. It was obtained from lyophilized tissue of transgenic corn containing the δ-endotoxin at a concentration of 5.7 mg/g. This material was maintained at -14 ºC, until used.

Bioassays.- Dose-response bioassays were performed using the diet overlay methodology (Sims et al. 1996). Each bioassay included seven to eight concentrations of the Cry2Ab toxin, which was suspended in 0.2% agar, 200 µl of the suspension were applied over the artificial diet placed on each well of a 64 well assay tray (Jarold Mfg Co. St. Louis, MO.)**.** Each well had a 2.0 ml capacity and contained 1.0 ml of Lepidoptera artificial diet (Southland Products Inc. Lake Village AR). Once the diet dried, one neonate TBW larvae was placed in each well. The trays were then covered with plastic self-adhering (Pull N´Peel Tab Bio-Cv-16; C-D International, Inc.) ventilated covers and incubated at 27° C, 70% R.H. and 14:10 photoperiod, for 5 days. 48 to 112 larvae were used for each concentration and a control treated only with 200 ul of agar solution at 0.2%. . There were four to five replications in different days. Larval mortality including those larvae that not molted to second instar was recorded five days after treatment. Mortality data were analyzed by the Probit analysis using the Polo Program (LeOra Software 1987) to estimate LC_{50}, 95% fiducial limits, LC_{95} and slope of the dose-response lines. The criterion for assessing significant differences ($P < 0.05$) between regression lines was that no overlap existed on the 95% confidence limits. Resistance Ratio was obtained by dividing LC_{50} of the field colony on the LC_{50} of the reference colony.

Results and Discussion

Data obtained in the susceptible population indicate LC_{50} values ranging from 1.564 µg/ml to 0.850 µg/ml in the three colonies evaluated in 2003, 2004 and 2005 (Table 1). The mean LC_{50} value of these observations is 1.138 µg/ml. Previous reports have shown LC_{50} values of 1,1 µg/ml of Cry2Ab in a susceptible colony of *Heliothis virescens* (Ali et al. 2007), which corresponds with our observations. The LC_{50} data from the Yaqui Valley field colonies, evaluated in 2003, 2004 2005 and 2006 (Table 1), ranged from 0.768 µg/ml in the observation performed in 2006 up to 2.054 µg/ml in 2005. Ali et al. (2007) report LC_{50} values ranging from 1.2 to 3.0 µg/ml for field populations collected in seven U.S. states during 2006. These values are similar to those obtained in our bioassays performed on populations from the Yaqui Valley, Sonora Mexico.

Table 1.- Concentration-Response data obtained by Probit analysis of the Cry2Ab toxin on *Heliothis virescens* populations from the Yaqui, Valle, Sonora. Mexico.

Colony	CL_{50} µg/ml	95% Fiducial Limits	CL_{95} µg/ml	Slope	Resistance Ratio
Susceptible 03	1.564	1.220 – 1.991	28.81	1.30	1.00
Susceptible 05	0.999	0.789 – 1.239	10.03	1.63	1.00
Susceptible 06	0.850	0.674 – 1.055	8.95	1.61	1.00
Yaqui Valley 03	1.630	1.252 – 2.095	45.79	1.14	1.04
Yaqui Valley 04	1.006	0.786 – 1.272	17.20	1.33	0.64
Yaqui Valley 05	2.054	1.672 – 2.510	20.73	1.64	2.06
Yaqui Valley 06	0.768	0.647 – 0.905	3.40	2.55	0.90

Considering fiducial limits as the criterion to separate response of these populations, we have that in the evaluations performed on the susceptible colony the colony assayed in 2003 is significantly different from the 2006 colony but not from the evaluated in 2005. The same results were obtained with the field population indicating that the 2006 colony was significantly more susceptible to the Cry2Ab toxin than the other colonies assayed.

Data at the LC_{95} show that the lowest value was obtained in the 2006 bioassays performed on the susceptible and field colonies. The highest LC_{95} value (45.79 µg/ml) was observed in the population collected in 2003.

The slope values ranged from 1.14 in the 2003 field colony up to 2.55 in the 2006 colony. Finally the highest resistance ratio was observed in the 2005 colony being 2.06 fold the LC_{50} value of the susceptible colony.

More data are needed in Mexico to establish a diagnostic dose for extensive monitoring of resistance in *H. virescens* populations to Cry2Ab toxin. It has been reported (Ali et al. 2007) and it was observed in these bioassays that there is some variation in the dose-mortality data. Even though less variation has been detected in *H. virescens* field populations than on *Helicoverpa zea* (Hardee et al. 2001, Ali et al. 2005, 2006, and 2007) more data on dose-mortality, growth inhibition and larvae development are required to integrate and generate information to follow up the response of field populations exposed to the Cry 2Ab toxins.

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