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BOLLGARD COTTON AND PYRETHROID RESISTANCE IN TOBACCO BUDWORM IN TAMAULIPAS, MEXICO

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Abstract

Insecticide susceptibility in *Heliothis virescens* F. was determined for the 8 years (1991 – 2001) with larvae sampled from cotton in southern Tamaulipas, Mexico. Before 1996, when Bollgard® cotton expressing the Cry1A(c) δ-endotoxin was introduced into the region, two important patterns were documented. The first was economically significant increases in resistance to pyrethroids. The second was occurrence of virtually complete control failures in the field during 1994-1995. The largest resistance changes were recorded for the type II pyrethroids, cypermethrin and deltamethrin. These are the most widely used products in the region. Resistance ratios for these products increased up to >100-fold from 1991 to 1995. After 1996, the resistance levels declined. This clear trend towards reversal of resistance to type II pyrethroids can be understood, in part, of the high adoption rate of transgenic cotton in the region, from 31.2% in the beginning (1996) to around of 100% in 1998. Now tobacco budworms in this region are susceptible to type II pyrethroids. Two effective and fundamentally different pest management tools are now available to cotton growers in southern Tamaulipas. Transgenic cotton, coupled with careful use of synthetic pyrethroids, offer the possibility of sustainable and profitable cotton production.

Introduction

Inadequate management of the tobacco budworm, *Heliothis virescens* (Fabricius), caused the ruin of cotton production in southern Tamaulipas, Mexico, during the 1970s (Adkisson 1972, Bottrell and Adkisson 1977). In the 1980s, encouraged by increased cotton prices and the introduction of pyrethroid insecticides, cotton boomed again. However, the irrational use of pyrethroids led to rapid resistance development. The loss of pyrethroid effectiveness in 1995 provoked another crisis in the control of *H. virescens* (Terán-Vargas 1996). This second disaster resulted in a 94.7% reduction in cotton cultivation the following year. In 1996 transgenic cotton (Bollgard®), which expresses the δ-endotoxin Cry1A(c) of *Bacillus thuringiensis* Berliner var. *kurstaki* (Bt), was introduced and it effectively controlled *H. virescens*. With the possibility of controlling the tobacco budworm, farmers again became interested in this crop.

A series of benefits could be expected from use of Bt crops. These include reduction in the use of conventional insecticides, reduced environmental pollution, greater protection of beneficial fauna, low impact on human health, and higher yields and profits (Roush and Shelton 1997, Betz et al. 2000, Edge et al. 2001, Traxler et al. 2002, Shelton et al. 2002, Bennet et al. 2003). However, reduction in the level of resistance to conventional insecticides as a collateral effect of Bollgard® cotton use has not been documented.

Use of Bollgard® cotton, between 1996 and 2001, allowed farmers in southern Tamaulipas, to substantially reduce use of conventional insecticides against the tobacco budworm (Monsanto 1996, 1997, 1999, 2000, 2001, 2002). Considering that resistance to pyrethroids is unstable in the absence of selection pressure (Curtis 1987, Roush and McKenzie 1987, Plapp et al. 1990, Graves et al. 1991, Kanga et al. 1995) and that no cross-resistance between *B. thuringiensis* δ-endotoxins and conventional insecticides has been documented (Tabashnik 1994, Wu and Gou 2004), it was hypothesized that the use of Bollgard®, contributes to reduction of resistance to type II pyrethroids.

Materials and Methods

Location. The study was conducted during 1991, 1994, 1995, 1997, 1998, 1999, 2000 and 2001 in the entomology laboratory at CESTAM (Southern Tamaulipas Experimental Station), a research center belonging to The Instituto

Nacional de Investigaciones Forestales, Agrícolas y Pecuarias, Mexico (INIFAP-National Institute of Forestry, Agriculture and Livestock Research).

Insects. During September and October, at least 200 *H. virescens* larvae of different instars were collected in commercial fields of conventional cotton in southern Tamaulipas area to establish that year's colony. After the introduction of Bollgard® in 1996, the collections were made in the transgenic cotton refuge areas. Collected larvae were placed individually in 30 mL plastic cups with 15 mL of an artificial diet (Southland Products Incorporated. Lake Village, Arkansas) and maintained in the laboratory until pupal stage. Pupae were placed in 2 liters plastic boxes (Rubbermaid No. 3) lined with paper towels. In these boxes, the adults emerged, copulated and oviposited. Adults were fed a solution of 10% sugar in distilled water and produced F1 or F2 generations for the bioassays. Every two days eggs were collected and neonate larvae placed individually in plastic cups with 10 mL of the artificial diet. Insects were kept at 25 ± 2 °C, 60 to 80% RH and a photoperiod of 12:12 h.

Insecticides. The following technical grade insecticides were used: permethrin (Canamex, S. A. de C. V.), cypermethrin (Canamex, S. A. de C. V.) and deltamethrin (Agrevo Mexicana, S. A. de C. V).

Bioassays. The bioassay method proposed by the Entomological Society of America (Anonymous 1970) was used to determine *H. virescens* resistance to insecticides with the modifications proposed by Staetz (1985), which include depositing a microliter of acetone with a known quantity of the toxic on the pronotum of third instar larvae (25 ± 3 mg) with the aid of an electric microapplicator (ISCO Model M; Instrumentation Specialties Company, Inc., Lincoln, NE) and a 500 µL microsyringe (Hamilton Company, Reno, NV). For each bioassay a range of biological response was determined. Subsequently, four to nine intermediate dosages were included to cover the range. Ten larvae per dosage per replication were used, plus a control to which only acetone was applied. A total of three to four replications per colony were performed on different days. Mortality was evaluated 72 h after application. When mortality in the control was equal to or less than 10%, it was corrected using Abbott's formula (Abbott 1925). As a basis of comparison, a susceptible strain of *H. virescens* collected in 1982 in Obregón, Sonora, Mexico (referred in table to as Obregón) (Martínez-Carrillo 1991) was used.

Statistical analysis. The log dosage-probit response line was obtained with the Probit analysis (Polo-PC 1987). At a given level of mortality, it was considered that there were no significant differences in the response between the field and susceptible strains when the fiducial limits overlapped. The resistance ratio (RR) was calculated by dividing the LDx of the field strain by the LDx of the susceptible strain.

Results and Discussion

Type II pyrethroids, such as cypermethrin and deltamethrin, constituted the most frequently used group of insecticides in the southern Tamaulipas to control *H. virescens* in cotton. The most important changes noted in the response of *H. virescens* were to this type of insecticide. From 1991 to 1995 the biological effectiveness of these insecticides progressively decreased. This, due to the intense type II pyrethroid selection pressure exerted on this pest and a high propensity toward resistance (Crowder et al. 1984; Forrester et al. 1993), and it led to serious control problems in the field. With the introduction of transgenic cotton expressing Cry1A(c) to this region in 1996, applications of insecticides to control tobacco budworm, where this technology was adopted, were no longer needed. Therefore, resistance to type II pyrethroids decreased significantly (Table 1). The RR_{50} of cypermethrin increased drastically from 1991 to 1995 (from 14.4 to 57.7-fold). An even more pronounced trend emerged in RR_{95} values (29.1 to 157-fold, respectively) with the highest value occurring in 1994 (227.6 fold) (Table 1). Throughout the studied years the field and susceptible strains were significantly different in resistance. In general, resistance increased from 1991 to 1995 and descended after 1997. Because of its high biological effectiveness (100 g of a.i. ha^{-1}) and its low price, cypermethrin was the most used type II pyrethroid for the control of *H. virescens* in conventional cotton during the study period.

Resistance to deltamethrin, another type II pyrethroid, followed similar trends. From 1991 to 1995, RR_{50} increased from 8.7 to 16.7-fold, and RR_{95} rose from 17.5 to 103.7-fold (Table 1). Like cypermethrin, the levels of resistance rose between 1991 and 1995 and decreased from 1997 to 1999. In 1999, RR_{95} (4.9-fold) was not significantly different from the susceptible strain (Table 1).

The intense use of these products was due primarily to two factors: a) the tobacco budworm is the key lepidopteran cotton pest of the region, whereas *Helicoverpa zea*, is nearly absent and b) when type II pyrethroids were initially introduced they were highly effective against *H. virescens*. In 1995 serious control problems were inferred from RR values (>100-fold) (Table 1). As a result, the area cultivated with cotton decreased by 94.7%, from 54,897 ha in 1995 to 2,868 ha in 1996 (Table 2).

The decrease in type II pyrethroid resistance after 1996 could be due to the following factors: reduction of insecticide use and instability of resistance, immigration of susceptible phenotypes into the cotton area, emergence of non selected individuals in areas planted with Bollgard® cotton, the huge reduction in cotton acreage resulted in a reduced tobacco budworm density, hence insecticide-selected individuals might be crossed more efficiently with those from areas which were not exposed to pyrethroids (wild host plants, refuge and Bt cotton).

They estimated that the use of Bollgard® cotton, from 1996 to 2001, reduced insecticide use by 115,610 liters. (Monsanto, 1996, 1997, 1999, 2000, 2001, and 2002). Of the total amount of insecticide applications against *H. virescens*, 70% to 80% were carried out with type II pyrethroids; among them cypermethrin, deltamethrin, β-cypermethrin, and λ-cyhalothrin had the highest use pattern (Monsanto 1997, 1998, 1999, 2000, 2001). In southern Tamaulipas, *H. virescens* is not exposed to type II pyrethroids in Bollgard® cotton nor in alternative crops.

Another factor operates in pyrethroid resistance. Pyrethroid resistance can be unstable due to the reproductive disadvantages of resistant insects (Campanhola and Plapp 1989, McCutchen et al. 1989). When this phenomenon occurs, resistance significantly declines in absence of selection pressure (Campanhola et al. 1991, Clarke and Ottea 1997). We suspect this may have occurred in southern Tamaulipas where a significant reduction in the use of type II pyrethroid against *H. virescens* was estimated (Table 2).

Because tobacco budworm is not a pest in sorghum, soybeans, maize and tomatoes in southern Tamaulipas (Ávila and Terán 1993), the use of type II pyrethroids in these crops does not influence the susceptibility to insecticides in *H. virescens.*

Similarly, the immigration of susceptible insects is an essential factor in the reduction of resistance levels over time (Georghiou 1972; Forrester et al. 1993; Leonard et al. 1995). It is likely that this factor contributed in the reversal of resistance to type II pyrethroids in southern Tamaulipas by providing susceptible individuals from wild plants.

Terán-Vargas (2005) studied *H. virescens* adult emergence from Bollgard® and conventional cotton areas in 80:20 refuge option fields. Cages for adult emergence (Fife and Graham, 1996) were set up in Bollgard® and conventional cotton areas during growing season. A mean emergence of 500 tobacco budworm adults in Bollgard® and 5,000 adults per hectare in conventional cotton was recorded. Based on this information and the relative proportion of Bollgard® cotton , an estimate of 0.05 to 0.77 tobacco budworm adults were produced in Bollgard® cotton for every adult produced in conventional cotton during 1996 to 2001 (Table 3). Recent local studies indicate that the tobacco budworm population in southern Tamaulipas is susceptible to the Cry1A(c) δ-endotoxin (Martínez-Carrillo and Berdegue 1999); however, these individuals were not selected with type II pyrethroids. It is highly probable that these individuals together with those from wild host plants contributed substantially to the observed reversal in resistance. It is also possible that individuals from Bollgard® cotton came from plants that do not express the Cry1A(c) δ-endotoxin (Gould 1998), or that express the toxin to a lesser degree (Gould and Tabashnik 1998, Greenplate 1999, Benedict and Altman 2001).

The RR_{50} to type I pyrethroid permethrin was low every year of the study, with values below 4.4-fold. The highest RR_{95} value was observed in 1995 (15.6-fold) and it decreased to 0.1-fold in 2001 (Table 1). In southern Tamaulipas, permethrin is not used to control *H. virescens* because of its low biological effectiveness, compared with the type II pyrethroids.

When it first became available, two factors influence use of transgenic cotton in southern Tamaulipas. First, farmers were unfamiliar with transgenic cotton. Second, the Mexican government restricted the planted area (to 896 ha in 1996) due to agronomic and environmental uncertainties. Later on, as cotton growers became aware of the advantages of this technology, cotton cultivation increased. In 1998, 16,460 ha of Bollgard® cotton were planted, constituting 88.5% of the total cotton growing area (Table 2). In spite of this, the total cotton area planted (conventional and Bollgard®) decreased considerably. In 2000, the world cotton price was estimated as low as $0.35 per pound (Aserca 2005). In this economic environment, southern Tamaulipas farmers significantly decreased cotton acreage during 2001. Taken together, the low price, low yield (1.4 ton cottonseed ha^{-1}) (SAGARPA 2001), and lack of adequate government support, cotton is no longer cultivated. Nonetheless, the availability of transgenic cotton and loss of resistance to type II pyrethroids create a very positive outlook for profitable cotton production in southern Tamaulipas.

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Table 1. Tobacco budworm (*Heliothis virescens*) resistance to insecticides pyrethrois in southern Tamaulipas, Mexico.

Insecticide	Strain	Year	n	Slope(±SE)	DL_{50} (µg/larvae) (95% CI)	RR_{50}	DL_{95} µg/larvae (95% CI)	RR_{95}	χ^2
Permethrin	Obregón	1995	280	2.79±0.27	0.18 (0.15-0.21)	1.0	0.69 (0.53-1.00)	1.0	1.67
	Sur de Tam.	1991	270	1.29±0.13	0.38 (0.23-0.63)	2.1	7.17 (3.20-27.64)	10.3	10.11
		1994	120	1.72±0.28	0.41 (0.27-0.58)	1.3	3.67 (2.07-10.20)	5.3	3.87
		1995	360	1.44±0.13	0.78 (0.61-1.01)	4.4	10.86 (6.70-21.21)	15.6	2.16
		1997	210	1.32±0.19	0.23 (0.05-0.48)	1.3	4.06 (1.53-81.54)	5.8	11.95
		1998	210	1.81±0.21	0.26 (0.11-0.72)	1.5	2.13 (0.76-91.01)	3.1	20.40
		2001	240	1.92±0.20	0.01 (0.01-0.02)	0.0	0.08 (0.05-0.17)	0.1	6.11
Cypermethrin	Obregón	1995	320	1.96±0.19	0.02 (0.01-0.02)	1.0	0.11 (0.06-0.30)	1.0	12.6
	Sur de Tam.	1991	240	1.45±0.16	0.23 (0.14-0.35)	14.4	3.14 (1.63-9.34)	29.1	6.49
		1994	300	0.96±0.10	0.48 (0.25-0.93)	30.0	24.58 (7.66-240.13)	227.6	14.9
		1995	320	1.30±0.13	0.92 (0.54-1.60)	57.7	16.95 (6.79-109.92)	157.0	12.9
		1997	210	1.47±0.20	0.30 (0.20-0.42)	18.7	3.93 (2.34-9.02)	36.4	1.2
		1998	180	1.82±0.24	0.10 (0.04-0.20)	6.0	0.77 (0.31-17.33)	7.1	11.4
		1999	150	1.95±0.29	0.13 (0.09-0.17)	8.0	0.89 (0.56-1.95)	8.2	0.7
		2001	180	3.27±0.41	0.31 (0.26-0.36)	19.3	0.98 (0.75-1.49)	9.1	3.2
Deltamethrin	Obregón	1995	240	3.15±0.35	0.004 (0.003-0.007)	1.0	0.015 (0.009-0.062)	1.0	10.8
	Sur de Tam.	1991	150	1.88±0.25	0.035 (0.018-0.071)	8.7	0.262 (0.112-2.332)	17.5	4.1
		1994	270	1.85±0.16	0.063 (0.048-0.084)	15.7	0.806 (0.479-1.714)	53.7	4.5
		1995	320	1.21±0.14	0.067 (0.045-0.099)	16.7	1.555 (0.734-5.592)	103.7	6.3
		1997	210	1.84±0.22	0.050 (0.020-0.104)	12.5	0.394 (0.165-5.595)	26.3	17.4
		1998	210	1.77±0.23	0.031 (0.018-0.065)	7.7	0.266 (0.106-3.858)	17.7	11.4
		1999	180	2.04±0.28	0.012 (0.009-0.015)	3.0	0.074 (0.048-0.152)	4.9	3.2

RR = Resistance ratio = LD of the respective year for the field strain/LD for the susceptible strain.

Table 2. Area planted with conventional and Bollgard® cotton and use of pyrethroid insecticides in southern Tamaulipas, Mexico.

Year	Area planted				Rounds[3]	Pyrethrois type II use	
	Convencional cotton[1]		Bollgard cotton[2]			Liters applied[3]	Liters Saved[4]
	ha	%	ha	%			
1990	19 648	100			2.0	19 648	
1991	33 443	100			2.0	33 442	
1992	20 294	100			2.0	20 294	
1993	22 685	100			2.5	28 356	
1994	61 223	100			4.0	122 446	
1995	54 897	100			5.0	137 243	
1996	1 972	68.8	896	31.2	5.0	4 930	2 240
1997	2 484	23.1	8 300	76.9	2.4	2 981	9 960
1998	840	11.5	6 460	88.5	2.1	882	17 230
1999	6 646	55.1	5 419	44.9	2.7	8 972	7 315
2000	6 084	58.4	4 332	41.6	2.4	7 301	5 198
2001	44	14.9	251	85.1	3.0	66	376

(1) Distrito de Desarrollo Rural 162 González y 161 Mante SAGARPA.
(2) Monsanto Comercial, S.A. de C. V.
(3) Conventional cotton (Average rate 0.5 L/ha of formulated product).
(4) Bollgard® cotton.

Table 3. Estimation of adult *Heliothis virescens* emergence in conventional and Bollgard® cotton in southern Tamaulipas, México.

Year	Cultivated area (ha).		Adults (thousands)		Bollgard/conventional proportion
	Conventional cotton[1]	Bollgard cotton[1]	Conventional cotton	Bollgard cotton	
1996	1 972	896	9 860	448	0.05
1997	2 484	8 300	12 420	4 150	0.33
1998	840	6 460	4 200	3 230	0.77
1999	6 646	5 419	33 230	2 709	0.08
2000	6 084	4 332	30 420	2 166	0.07
2001	44	251	220	125	0.57

(1) Average emergence of 500 tobacco budworm adults in Bollgard® and 5,000 adults in conventional cotton.