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FIELD STUDIES TO SUPPORT INSECT RESISTANCE MANAGEMENT (IRM) PLAN FOR THE DOW AGROSCIENCES' *B. t.* COTTON

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Abstract

A method outlined by the Scientific Advisory Panel to the Environmental Protection Agency (US Environmental Protection Agency, 1998) was employed to investigate the high-dose expression of the Dow AgroSciences' cotton line MXB-13 (Cry1Ac/Cry1F proteins). Field-plots of cotton plants expressing and non-expressing the Bt proteins were inoculated with >270,000 tobacco budworm (*Heliothis virescens* [F.]) in three different locations over a period of 56 days. This inoculation regime, plus the natural occurrence of other Lepidoptera, resulted in recovery of only 3 tobacco budworm neonates, 5 cotton bollworms (*Helicoverpa zea* [Boddie]), 3 cabbage loopers (*Trichoplusia ni* [Hübner], and no soybean loopers (*Pseudoplusia includens* [Walker]) in the MXB-13 Bt-cotton plants. In non-Bt cotton (PSC-355 near-isoline variety) 616 tobacco budworms, 253 cotton bollworms, 1,721cabbage loopers and 157 soybean loopers were recovered utilizing the same sampling regime.

MXB-13 cotton represents an important tool to manage tobacco budworm and other lepidopteran pests, adding to the sustainability of other environmentally sound pest control alternatives.

Introduction

Cotton (*Gossypium hirsutum* L.) has been genetically modified to express two separate insecticidal crystal proteins from the bacterium *Bacillus thuringiensis* (Bt) for the control of key lepidopteran pests. Cotton genotype GC510 was transformed to contain the genes that express full-length synthetic protoxins (synpro) of Cry1F or Cry1Ac. Transgenic lines were backcrossed with a non-transgenic elite variety, PSC-355. Subsequently, Cry1F(synpro) and Cry1Ac(synpro) lines were crossed to produce the stacked product, MXB-13. Cotton line MXB-13 (a cross between Cry1F event 281-24-236 and Cry1Ac event 3006-210-23) also contains a herbicide-resistant selectable marker gene that expresses the phosphinothricin acetyltransferase (PAT) protein that imparts tolerance to glufosinate-ammonium herbicide.

A key step in devising an effective and practical product durability plan is investigating whether or not Bt-cotton expresses a high dose against the key target pests (US Environmental Protection Agency, 1998). For target insects against which the cotton is high dose, the high-dose plus a small-refuge IRM strategy is appropriate. For target pests against which the dose is not high, additional information may be needed to establish the optimal IRM strategy.

The 1998 and 2000 Scientific Advisory Panels (SAPs) defined high dose for lepidopteran-resistant plant incorporated protectants as 25 times the dose required to kill 99% of the susceptible insects (US Environmental Protection Agency, 1998, 2001). By implication from the suggested methods for demonstrating high dose, high dose can be defined as that sufficient to kill 99.99% of insects in the field, or sufficient to cause high mortality of instars that are around 25 times more tolerant of the protein than are neonates. The SAPs described five imperfect methods for providing indication that the high dose criteria are met, suggesting the use of at least two of the five methods to provide reasonable assurance of high dose. Described below is a field-based method to address this question for tobacco budworm, presenting also field observations of other key cotton pests for which this method was not intended to demonstrate high dose.

Materials and Methods

Survey for Survival of Large Numbers of Plants in the Field Infested with Tobacco Budworm

Plots of MXB-13 and near-isoline non-Bt (PSC-355) cotton were established in Wayside, Mississippi, Macon Ridge, Louisiana, and College Station, Texas. Cotton plants (seeded at 10 seeds per meter) were grown under locally accepted agronomic conditions, except for the omission of insecticides beyond 2 weeks after cotyledon stage, with the exception of Texas where the site was under boll weevil eradication program. This site had frequent malathion applications. Cotton was planted in 4 randomly arranged plots of 8 rows (1.0 m apart, 18.2 m long) per treatment in Wayside and Macon Ridge. At College Station the trial consisted of a single plot of 16 rows by 36.5 m per treatment, divided into 4 subplots of 4 rows by 36.5 m. The three trials were surrounded by at least 12 rows or 12 m of non-Bt cotton plants in all directions.

Eight weekly artificial infestations of *H. virescens* in Wayside and College Station and 9 in Macon Ridge, from the field-adapted Dow AgroSciences colony, began approximately at pinhead square in each location. Insects arrived at the research facilities as 2-day old eggs and were hatched and released in the field following the guidelines of Jenkins et al (1995). A total of 87,000 neonates were inoculated onto Bt-cotton plots at Wayside, 138,000 at Macon Ridge, and 45,000 at College Station throughout the entire trial. Plots were evaluated weekly by inspecting for larvae and damage produced in 40 squares and 40 bolls, choosing plants at random in rows infested with neonates the previous week. When this evaluation was concluded, 40 beat-cloth (1.0 m long) samples were immediately taken from the same rows, shaking cotton plants above the cloth. A final evaluation was made 3 weeks after the last inoculation by inspecting 250 first-position lower bolls per plot from 250 randomly chosen plants. All the *Helicoverpa* and *Heliothis* larvae found in all the evaluations were collected, placed in vials, and identified to species.

Data were analyzed by two sample t-test.

Results and Discussion

Evaluations of squares for the presence of tobacco budworm larvae, beginning one week after the first inoculation, are presented in Table 1. Weekly inspections of 160 squares per treatment revealed only 3 *Heliothis virescens* in MXB-13 plots. It should be noted that these insects were neonates, and do not indicate the ability to survive on Cry1Ac/Cry1F expressing plants. Square damage by heliothines throughout the season for the 3 locations averaged 2.4% on MXB-13 and 28.7% for PSC-355.

The beat-cloth method recovered more larvae from the control plots than from Bt-cotton. This was particularly useful because it allowed different species and larval stages (not only early stages [L1-L2]) that are commonly found in squares and terminals to be collected. Data from this method indicate no tobacco budworm survival on MXB-13 (Table 2) and very few lepidopteran pest as compared with non-Bt cotton (Tables 3 and 4).

Inspection of 160 bolls per location each week produced no live tobacco budworm larvae on MXB-13 (Table 5). Final inspection of 1,000 first-position lower bolls also produced no live *Heliothis virescens* or *Helicoverpa zea* in MXB-13 (Table 5).

Across all three collection methods, 3,840 squares and 6,040 bolls were examined, and beat-cloth samples of 9,900 plants were made from MXB-13 plants infested with $\geq$ of 270,000 neonates. Only 3 *H. virescens* neonates were found on MXB-13 cotton (compared with 679 different size larvae using the same sampling regime in the non-Bt control plots). This indicates that MXB-13 provides high dose against the tobacco budworm and greatly reduces damage caused by other important lepidopteran pests.

References

Jenkins, J. N., J. C. McCarthy, Jr., and M. S. Moghal. 1995. Rearing tobacco budworm and bollworm for host plant resistance research. Technical bulletin 208. Mississippi Agricultural and Forestry Experiment Station.

US Environmental Protection Agency. 1998. Final report of the Subpanel on *Bacillus thuringiensis* (Bt) Plant-Pesticides and Resistance Management. FIFRA Scientific Advisory Panel meeting, February 9-10, 1998.

US Environmental Protection Agency. 2001. Bt plant pesticides risk and benefits assessments. FIFRA Scientific Advisory Panel meeting, October 18-20, 2000. SAP report number 2000-07.

Table 1. Number of tobacco budworm (*Heliothis virescens*) larvae found per 160 squares on Bt-cotton (MXB-13) and non-Bt cotton (PSC-355) plots in 3 different locations in 2002.

	TRIAL LOCATED IN					
	LOUISIANA		MISSISSIPPI		TEXAS	
Evaluation	MXB-13	PSC-355	MXB-13	PSC-355	MXB-13	PSC-355
1st week	0	1	0 b	6 a	0	1
2nd week	0 b	4 a	0 b	14 a	ND	ND
3rd week	1b	5 a	0 b	8 a	ND	ND
4th week	0 b	5 a	0 b	8 a	0	2
5th week	ND	ND	0 b	16 a	1 b	12 a
6th week	0 b	3 a	0 b	6 a	0 b	4 a
7th week	0 b	3 a	0 b	16 a	0 b	3 a
8th week	1 b	9 a	0 b	4 a	0	0
9th week	0 b	11 a	0	0	0	0

ND= No data taken.
Means in columns by trial location followed by different letters, differ statistically at t$\geq$0.05 level.

Table 2. Number of tobacco budworm (*Heliothis virescens*) larvae found utilizing a beat-cloth method on 40 meters on Bt-cotton (MXB-13) and non-Bt cotton (PSC-355) plots in 3 different locations in 2002.

	TRIAL LOCATED IN					
	LOUISIANA		MISSISSIPPI		TEXAS	
Evaluation	MXB-13	PSC-355	MXB-13	PSC-355	MXB-13	PSC-355
1st week	0	2	0 b	3 a	0	1
2nd week	0	0	0 b	48 a	ND	ND
3rd week	0 b	5 a	0 b	8 a	ND	ND
4th week	0 b	20 a	0 b	11 a	0 b	10 a
5th week	0 b	28 a	0 b	13 a	0	1
6th week	0 b	42 a	0 b	13 a	0 b	9 a
7th week	0 b	16 a	0 b	20 a	0	1
8th week	0 b	6 a	0 b	4 a	0	1
9th week	0	2	0 b	6 a	0	1

ND= No data taken.
Means in columns by trial location followed by different letters, differ statistically at t≥0.05 level.

Table 3. Number of cotton bollworm (*Helicoverpa zea*) larvae found utilizing a beat-cloth method on 40 meters on Bt-cotton (MXB-13) and non-Bt cotton (PSC-355) plots in 3 different locations in 2002.

	TRIAL LOCATED IN					
	LOUISIANA		MISSISSIPPI		TEXAS	
Evaluation	MXB-13	PSC-355	MXB-13	PSC-355	MXB-13	PSC-355
1st week	0	0	0 b	76 a	0	1
2nd week	0	0	0 b	20 a	ND	ND
3rd week	0 b	4 a	1 b	30 a	ND	ND
4th week	0 b	12 a	0 b	17 a	0	1
5th week	1 b	14 a	0 b	13 a	0 b	12 a
6th week	0 b	14 a	0 b	10 a	0 b	9 a
7th week	1 b	8 a	0	1	0	1
8th week	2 b	7 a	0	2	0	0
9th week	0	1	0	0	0	0

ND= No data taken.
Means in columns by trial location followed by different letters, differ statistically at t≥0.05 level.

Table 4. Number of cabbage looper (*Trichoplusia ni*) and soybean looper (*Pseudoplusia includens*) larvae found utilizing a beat-cloth on 40 meters on Bt-cotton (MXB-13) and non-Bt cotton (PSC-355) plots in two different locations in 2002.

	TRIAL LOCATED IN					
	MISSISSIPPI		TEXAS			
	Cabbage Loopers		Cabbage Loopers		Soybean Loopers	
Evaluation	MXB-13	PSC-355	MXB-13	PSC-355	MXB-13	PSC-355
1st week	0 b	112 a	0	0	0	0
2nd week	1 b	311 a	ND	ND	ND	ND
3rd week	0 b	8 a	ND	ND	ND	ND
4th week	1 b	40 a	0	0	0	0
5th week	0 b	29 a	0 b	453 a	0 b	10 a
6th week	0 b	102 a	1 b	317 a	0 b	22 a
7th week	0 b	8 a	0 b	236 a	0 b	34 a
8th week	0 b	5 a	0 b	61 a	0 b	84 a
9th week	0 b	4 a	0 b	35 a	0 b	7 a

ND= No data taken.
Means in columns by trial location followed by different letters, differ statistically at t≥0.05 level.

Table 5. Number of tobacco budworm (*Heliothis virescens*) larvae found per 160 bolls and a final evaluation of 1,000 bolls on Bt-cotton (MXB-13) and non-Bt cotton (PSC-355) plots in 3 different locations in 2002.

	TRIAL LOCATED IN					
	LOUISIANA		MISSISSIPPI		TEXAS	
Evaluation	**MXB-13**	**PSC-355**	**MXB-13**	**PSC-355**	**MXB-13**	**PSC-355**
3rd week	0	1	0 b	4 a	ND	ND
4th week	0 b	9 a	0 b	8 a	0 b	3 a
5th week	ND	ND	0 b	15 a	0 b	16 a
6th week	0 b	7 a	0 b	6 a	0 b	14 a
7th week	0 b	5 a	0 b	10 a	0 b	8 a
8th week	0 b	28 a	0	2	0 b	6 a
9th week	0 b	19 a	0 b	9 a	0	0
Final eval.	ND	ND	0 b	23 a	0 b	11 a

ND= No data taken.

Means in columns by trial location followed by different letters, differ statistically at $t \geq 0.05$ level.