EPA UPDATE ON INSECT RESISTANCE MANAGEMENT FOR BOLLGARD™ COTTON

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Abstract

The United States Environmental Protection Agency (EPA) has made specific insect resistance management recommendations and requirements as part of the Bollgard™ cotton registration since 1995. Refuge size, structure, and deployment are important to the success of the high dose/structured refuge strategy. New insect resistance management requirements that focus on deployment and structure of the refuge for Bollgard™ cotton go into effect for the 2001 growing season. These requirements are: 95:5 external unsprayed refuge (at least 150 feet wide and planted within ½ mile), 95:5 embedded refuge (at least 150 feet wide), and 80:20 external sprayed refuge (planted within 1 mile, but preferably within ½ mile). Other refuge strategies are also under consideration. EPA will continue to examine the insect resistance management requirements as part of its current reassessment of the human health and environmental risks associated with Bollgard™ cotton. The current registration for Bollgard™ cotton expires September 2001.

Introduction

Insect resistance management (IRM) is the term used to describe practices aimed at reducing the potential for insect pests to become resistant to a pesticide. IRM is of great importance because of the threat insect resistance poses to the future use of *Bacillus thuringiensis* (Bt) plant-pesticides and Bt technology as a whole. Specific IRM strategies, such as the high dose/structured refuge strategy, will mitigate insect resistance to specific Bt delta-endotoxins produced in cotton, corn, and potatoes. Academic scientists, public interest groups, organic and other farmers have expressed concern that the widespread planting of these genetically transformed plants will hasten the development of resistance to pesticidal Bt delta-endotoxins. EPA shares these concerns and believes that effective insect resistance management can reduce the risk of resistance development.

The *Bacillus thuringiensis* subsp. *kurstaki* Cry1Ac delta-endotoxin insect control protein (and the necessary genetic material for its production) was registered for full commercial use in Bollgard™ cotton in October 1995 (EPA, 2000a). The Cry1Ac delta-endotoxin is toxic to certain lepidopteran insect pests, in particular, *Helicoverpa zea* (Boddie) (cotton bollworm, CBW), *Heliothis virescens* (Fabricius) (tobacco budworm, TBW), and *Gossypiella pectinophora* (Saunders) (pink bollworm, PBW).

Resistance management plans and resistance monitoring have been requirements of Bollgard™ cotton registration since 1995. The basic elements of an IRM strategy were discussed and confirmed at the March 1995 Federal Insecticide, fungicide, and Rodenticide Act (FIFRA) Scientific Advisory Panel (SAP) meeting (SAP, 1995). These elements were: knowledge of pest biology and ecology, appropriate dose expression strategy, appropriate structured refuge, resistance monitoring, remedial action plan, employment of integrated pest management (IPM), communication and education strategies for use of the product, and development of alternate modes of action. These elements have been used as the basis for formulating IRM strategies for Bt crops since this time.

Reprinted from the *Proceedings of the Beltwide Cotton Conference*
Volume 2:840-844 (2001)
National Cotton Council, Memphis TN

It is widely acknowledged that the two most critical elements of the existing IRM strategies for Bt crops are a high dose and use of a structured refuge (EPA, 1998; SAP, 1998). The high dose/structured refuge strategy assumes that there will be a single, recessive, and rare resistance gene in the population. The February 1998 SAP defined a high dose as 25 times the amount of Bt delta-endotoxin necessary to kill susceptible insects (SAP, 1998). A structured refuge is the non-Bt portion of a grower's field or set of fields that provides for the products of susceptible insects that may randomly mate with resistant insects that may emerge from Bt fields and dilute resistance.

The February 1998 SAP concluded that Bollgard™ cotton produced a high dose of the Cry1Ac delta-endotoxin to control tobacco budworm and pink bollworm, but produced only a moderate dose of the Cry1Ac delta-endotoxin to control cotton bollworm (SAP, 1998). The February 1998 SAP also suggested that production of 500 susceptible adults in the refuge that move into the transgenic fields for every adult in the transgenic crop areas (assuming a resistance allele frequency of 5×10^{-2}) would be a suitable goal. The size, placement, and management of the refuge are critical to the success of the high dose/structured refuge strategy to mitigate insect resistance to the Cry1Ac delta-endotoxin produced in Bollgard™ cotton.

This paper discusses the IRM requirements for Bollgard™ cotton for the 2001 growing season and various Bollgard™ cotton IRM strategies that are proposed beyond the 2001 growing season.

Discussion

2001 Refuge Requirements

There are three new structured refuge requirements that have specific structure and deployment improvements. Adding "structure" and mandatory ½ mile or 1 mile distance requirements for the 2001 growing season will improve the efficiency of the refuge. Seed production acreage is also included in the revised 2001 refuge requirements.

Growers must chose from these three structured refuge options for the 2001 growing season (EPA, 2000b):

" 1. 95:5 external structured unsprayed refuge. Ensure that at least 5 acres of non-Bt cotton (refuge cotton) must be planted for every 95 acres of Bt cotton. This refuge may not be treated with any insecticide labeled for the control of tobacco budworm, cotton bollworm, or pink bollworm. The size of the refuge must be at least 150 feet wide. The refuge must be managed (fertility, weed control and management of other pests) similarly to Bt cotton. The refuge must be planted within ½ linear mile from the edge of the Bollgard™ cotton field.
2. 80:20 external sprayed refuge. Ensure that at least 25 acres of non-Bt cotton must be planted for every 100 acres of Bt cotton. All cotton may be treated with insecticides (excluding foliar Bt products) labeled for control of the tobacco budworm, cotton bollworm, or pink bollworm. Ensure that a refuge is maintained within 1 linear mile (preferably within ½ mile) from the edge of the Bt cotton.
3. 95:5 embedded refuge. Plant at least 5 acres of non-Bt cotton (refuge cotton) must be planted for every 95 acres of Bt cotton. The refuge cotton must be embedded as a contiguous block within the Bt cotton field. For very large fields, multiple blocks across the field may be used. For small or irregularly shaped fields, neighboring fields farmed by the same grower can be grouped into blocks to represent a larger field unit, provided the block exists within one mile squared of the Bt cotton and the block is at least 150 feet wide.

> Within the larger field unit, one of the smaller fields planted to non-Bt cotton may be utilized as the embedded refuge. This refuge may be treated with any insecticide (excluding foliar Btk products) labeled for the control of tobacco budworm, cotton bollworm, and pink bollworm whenever the entire field is treated. The refuge may not be treated independently of the Bt cotton field.

For areas affected by pink bollworm only, the refuge cotton may be planted as single rows within the Bt cotton field.

In cases where placement of the refuge within one mile of the Bt cotton would be in conflict with state seed production regulations, the grower must plant the refuge as close to the Bt cotton as allowed."

Resistance Monitoring
Annual resistance monitoring is a mandatory requirement of registration. Monsanto has provided EPA annual resistance monitoring reports. After four years, there is no evidence of TBW, CBW, or PBW resistance to the Cry1Ac delta-endotoxin produced by Bollgard™ cotton cultivars under field situations.

TBW and CBW. Results from the Cry1Ac diet overlay bioassays indicated that TBW showed no change in susceptibility to the Cry1Ac delta-endotoxin from 1997 to 1999 (Sumerford et al., 1999 and Sumerford and Hardee, 2000). However, CBW showed some degree of increased tolerance (not resistance) to the Cry1Ac endotoxin found in Bollgard™ cotton in populations from South Alabama, the Mississippi Delta, Georgia, the Florida Panhandle, and South Carolina.

Preliminary results from the 2000 growing season indicate that the trend toward increased CBW tolerance to the Cry1Ac delta-endotoxin may have ended (Hardee, 2001). However, Hardee argues against making any definitive conclusions about whether the trend in increased tolerance has ended because CBW populations were extremely low in the Mid-South and Southeastern U.S. and sampling size was inadequate. In 2001, the resistance monitoring program will have explicit sampling locations and increased sample size (i.e., at least 50-100 individuals from each location) to reduce the variability associated with the data (Hardee, 2001).

These results indicate that factors selecting for CBW resistance may already exist in the field and that continued monitoring and further analysis is necessary to substantiate whether the trend for increased CBW "tolerance" will continue or has halted. The Agency will continue its close scrutiny regarding the susceptibility of CBW to the Cry1Ac delta-endotoxin.

PBW. Arizona has conducted a statewide monitoring of PBW susceptibility to Cry1Ac. Patin et al. (1999) reported that there were no major decreases in susceptibility of field populations to Cry1Ac in 1997 and 1998. The LC_{50} values differed <5-fold between the seven populations evaluated and ranged from 0.35 to 1.7 μg Cry1Ac/ml. The susceptible reference population, APHIS-S, had an LC_{50} of 0.53 μg Cry1Ac/ml.

Preliminary results from ten populations evaluated from the 1999 growing season indicate that susceptibility levels were similar to 1998 and that there is no evidence of reduced susceptibility of field populations of PBW to Cry1Ac (Dennehy et al., 2000). However, a 3.3-increase in larvae per boll surviving to ≥third instar in *Bt* cotton in 1999 was observed relative to 1998 (Dennehy et al., 2000).

Based on the results of extensive field monitoring for resistance in Arizona, the susceptibility of PBW to Cry1Ac in the field remains unchanged. However, there are resistant genes in Arizona PBW populations that confer high levels of resistance to Cry1Ac. In addition, the frequency of alleles for resistance to Cry1Ac in 1997 was higher than expected in Arizona. New PBW refuge options may prove to be more effective reducing the risk of resistance development. Such new options should be tested and, where proven effective, be implemented to reduce the risk of PBW resistance development to the Cry1Ac protein produced in Bollgard™ cotton. In addition, the PBW resistance monitoring program would be more effective at finding resistance before it became widespread if the entire geographic area in which PBW is an economic pest (e.g., parts of New Mexico, California, and Texas) was part of the program.

Proposed Refuge Options
In addition to the three refuge options required as terms and conditions for registration in 2001, there are several other refuge options that are under evaluation by EPA. These are briefly summarized in Table 1 below.
Efforts to determine the appropriate size of refuges have relied in part on models, most of which assume that random mating occurs between adults emerging from refuges and Bt cotton (Tabashnik, 1994 a, b; Gould, 1998; Gould and Tabashnik, 1998). If refuges are too far from Bt cotton, the chance for random mating is reduced which tends to accelerate the evolution of resistance (Capric, 1998).

The February 1998 SAP recommended that the Agency reexamine the current Bt cotton refuge options with regard to the distance between refuges and transgenic crops and the expected production of susceptible insects from different types of refuges. Without appropriate deployment, a refuge's efficacy could be minimized.

The greatest concern with the existing options is that they may be inadequate to mitigate CBW resistance development because Bollgard™ cotton only produces a moderate dose to control this insect. With CBW, 20% or more of the individuals may survive exposure to the Cry1Ac delta-endotoxin produced in Bollgard™ cotton (EPA, 1998).

Current models discussed in EPA's draft reassessment document (Section D) predict that the current refuge scenarios may inadequately mitigate CBW resistance development (EPA, 2000b). This concern appears to be greatest for the 95:5 external unsprayed structured refuge option, but exists also for the 80:20 external sprayed refuge option. However, there are many uncertainties associated with these models and the IRM plans must take into account growers ability and willingness to implement these plans.

CBW presents a challenge to Bt resistance management in that there is the potential for double exposure to the Bt delta-endotoxins in both Bt corn and Bt cotton (Bollgard™ cotton), potentially up to five or more generations of exposure in some regions. Cross-resistance to one or multiple Bt delta-endotoxins in Bt corn and Bt cotton becomes a concern not only for insects exposed to Bt crops, but insects that move to other crops in which Bt microbial pesticides are used. Given that different refuge strategies may be developed based upon where CBW is a resistance threat, accurate sampling data will be needed to predict suitable CBW overwintering areas.

The importance of movement at a localized level is important for the design of a refuge because of the need for random mating and oviposition. The 1998 SAP noted that research has shown substantial local population substructure can develop during the summer as a result of restricted movement of TBW and therefore deployment of a refuge is important (SAP, 1998). Based on ovipositional patterns for CBW, Capric (2000a) has indicated that untreated embedded refuges should be at least 100 meters (0.06 miles or approximately 100 rows) wide to minimize the risk of rapid resistance evolution associated with source-sink dynamics (i.e., the refuge must be wide enough so that all females do not lay all of their eggs in the Bt portion of a field and close enough to the Bt portion of the field so that there can be random mating and random oviposition of adults). Gould and Tabashnik (1998)) have recommended that the maximum distance between

Bt cotton fields and the non-Bt cotton refuge should be less than or equal to one mile. Capric (2000b) has recommended that this distance should be approximately ½ mile for CBW based on his movement studies.

There are also concerns with current PBW refuge options. Extensive and intensive recapture studies with PBW indicate that the adults move less than one kilometer (0.6 miles), particularly when suitable cotton is available (Tabashnik et al., 1999). Limited PBW larval and adult dispersal suggests that refuges should be placed very close to Bt fields. Preliminary data discussed by Carrière et al. (2001, in press) indicate that 4-5% in-field refuges may be inadequate to maintain susceptible PBW populations on non-Bt cotton. These authors recommend in-field refuges of more than 10% should be used for PBW resistance management in Bollgard™ cotton. Seed mixes are also a possible options that might be used to mitigate PBW resistance (see Patin et al., 1999). Tabashnik et al. (2000) show that genes conferring resistance to Cry1Ac and significantly increased ability to survive on Bollgard™ cotton were not rare in some Arizona field populations of PBW during 1997. However, these researchers also demonstrate that there were no increases in resistance allele frequency in 1998 and 1999 even when Bollgard™ cotton occupies large areas in Arizona.

Area-wide suppression/eradication programs using 100% Bollgard™ cotton for a two to three-year period may also be a consideration for those isolated areas in which PBW is a major economic concern. These areas include parts of California (San Joaquin, Imperial, Palo Verde Valleys), Arizona (Palo Verde Valley), and western Texas (El-Paso-Trans Pecos). However, since PBW is also a major pest in the Mexicali and Juarez areas in Mexico, then any large-scale area-wide suppression/eradication program should also involve these areas.

Most critical to the success of an IRM strategy is communication and education efforts with growers. The importance of grower education has been emphasized by EPA, USDA, Monsanto, academicians, and growers. With the new structure and deployment requirements to be implemented in 2001, educational efforts will have to address not only refuge size, but refuge deployment to achieve success. The possibility of two or more growers participating in some form of a community refuge is a concept under development. A community refuge may be beneficial to growers with numerous small fields that may impose difficulty in deploying the refuge. There are extensive logistical and compliance issues associated with implementation of a community refuge if it is to be an effective, sustainable option.

Based on the published research, additional information is needed to address larval and adult movement, mating behavior, ovipositional preferences, population dynamics, gene flow, survival and fecundity, fitness costs, and the use of alternate cultivated or wild hosts as refuges. Until there is further evidence that other hosts are proved to be suitable, only non-Bt cotton should be relied upon as refuge. The varied cropping systems for cotton, including local and regional differences, should also be considered. Improved models should be constructed as more useful predictive tools to compare likely resistance outcomes. Additional research and improved models will help strengthen the reliability of an IRM plan to effectively reduce the likelihood that TBW, CBW, or PBW will become resistant to the Cry1Ac delta-endotoxin.

The report from the October 18-20, 2000 SAP meeting, public comments, and additional research data will be used to evaluate the 2001 IRM requirements and to decide whether the Agency should modify these requirements. The current registration for Bollgard™ cotton expires September 2001.

Summary

Bollgard™ cotton expressing the Cry1Ac delta-endotoxin produces a high dose to control TBW, PBW, but only a moderate dose to control CBW. This conclusion was confirmed by the 1998 SAP. The 1998 SAP concluded that a refuge should produce a 500:1 susceptible to resistant ratio of susceptible to resistant individuals in the Bollgard™ cotton fields. There are data and predictive computer models that suggest that suggest that 95:5 external unsprayed and 80:20 external sprayed refuge options may not produce enough susceptible individuals to mate with putatively resistant individuals coming from the Bollgard™ cotton fields. This concern is greatest for CBW. That is, the current refuge options may be too small and not in close enough proximity to Bollgard™ cotton fields to produce enough susceptible individuals at the right time to mate with putative resistant individuals. Based on TBW and CBW movement and mating information, the threat of resistance is reduced if the refuge is placed within ½ mile or one mile[2] of the Bollgard™ cotton fields. Based on PBW movement information, the threat of resistance is reduced if the refuge is placed as close to the Bollgard™ cotton fields as possible, preferably within the field, or immediately adjacent to the field. Based on all of the scientific data available including computer models, long-term resistance management options should be developed and implemented to reduce the risk of TBW, CBW, and PBW resistance development.

Based on resistance monitoring information collected from 1996-2000, no effects outside the normal ranges of susceptibility to Cry1Ac have been reported for the TBW or PBW. Some degree of increased tolerance (not resistance) to the Cry1Ac endotoxin was observed in some CBW populations from the Mid-south and Southeastern U.S. from 1997-1999, but that trend was not continued in 2000. There is no evidence of field failure of Bollgard™ cotton due to TBW, CBW, or PBW resistance. The Agency will continue its close scrutiny regarding the susceptibility of TBW, CBS, and PBW to the Cry1Ac delta-endotoxin produced in Bollgard™ cotton.

Disclaimer
The opinions discussed in this article are those of the author and do not necessarily represent those of the United States government.

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Table 1. Refuge Scenarios for TBW, CBW, PBW Resistance Management.

Refuge Scenarios	External Unsprayed (Structured)	Embedded	External Sprayed
TBW, CBW, and PBW (2001 requirements) * Seed growers must plant the refuge as close as possible to Bt cotton fields when there is a conflict with seed production regulations.	5% external unsprayed (150 ft. wide); planted within ½ mile	5% embedded - at least 150 ft. wide (approx. 50 rows); For small or irregularly shaped fields, neighboring fields farmed by the same grower can be grouped into blocks to represent a larger field unit, provided the block exists within one mile squared of the Bollgard™ cotton and is at least 150 ft. wide. The refuge may treated as long as the whole field(s) (Bt and non-Bt) is treated. For PBW only, the refuge cotton may be planted as single rows within the Bollgard™ field.	20% planted within 1 linear mile, ½ mile preferred
TBW and CBW only: Cotton Pest Insect Management Forum	None	10% embedded refuge that is at least 300 ft. wide (approx. 80-100 rows); For small or irregularly shaped fields, neighboring fields farmed by the same grower can be grouped into blocks to represent a larger field unit, provided the block exists within one mile squared of the Bollgard™ cotton and is at least 300 ft. wide. The refuge may treated as long as the whole field(s) (Bt and non-Bt) is treated.	30% planted within 1 square mile area of the Bt cotton (at no point should a Bt cotton field be >1 linear mile from a non-Bt cotton refuge field)
TBW, CBW, and PBW: Gould and Tabashnik (1998)	None	16.7% embedded refuge (eight rows non-Bt cotton for every 48 rows of Bt cotton) – The non-Bt cotton should be planted in at least sets of two or more adjacent rows. The refuge may treated as long as the whole field(s) (Bt and non-Bt) is treated.	50% within 1 square mile area of the Bt cotton for TBW and CBWor immediately adjacent for PBW
PBW only:	None	10% embedded refuge in which at least one row of non-Bt cotton must be planted within every six to ten rows of Bt cotton. The refuge may treated as long as the whole field(s) (Bt and non-Bt) is treated.	20% within each square mile of land (one section), non-Bt cotton should be no more than one mile from the leading edge of each Bt cotton field
PBW eradication/suppression in California	0% non-Bt cotton:100% Bt Cotton -San Joaquin Valley; include Imperial and Palo Verde	None	None