IMPLEMENTING RESISTANCE MANAGEMENT

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Abstract

Resistance management systems for transgenic cottons expressing endotoxin protein (Bt cotton) are practical, needed components of contemporary cotton production. This is especially true for production areas of the Midsouth where tobacco budworm (*Heliothis virescens*) is threatening profitable cotton production. Although some changes in farming practices are required, the potential benefits of establishing refuges for susceptible genotypes are large. Distribution of refuge areas within the Bt cotton and grower support of the refuge concept are critical components of implementing resistance management programs for Bt cotton. These refuges should be widely distributed to insure that resistant genotypes emerging from the Bt cotton mate with susceptible genotypes produced in the refuge. The precise design and size of refuge areas are difficult to define because local scale movement and oviposition patterns of tobacco budworm are poorly understood. However, the optimum size seems to be between multiple planter widths and small fields. Most importantly the refuge areas must be practical components of normal production practices. Grower commitment to resistance management is probably the single most important factor governing the rate at which resistance to Bt cotton will develop in tobacco budworm populations. Grower are encouraged to cooperate and implement area-wide management systems that will increase the useful life of this very valuable technology.

Resistance Management

Resistance management is a continuum of ideas ranging from the very theoretical, academic aspects of evolutionary biology to the very practical, technical decisions made by cotton farmers on a daily basis. Development of resistant pest populations is inevitable when most of the individual pests are exposed to highly efficacious control agents, like the transgenic cottons which express endotoxin proteins of *Bacillus thuringiensis* (Bt cotton). These unique insecticidal plants are almost immune to damage from tobacco budworm (*Heliothis virescens*) and cotton bollworm (*Helicoverpa zea*) because the insecticidal proteins are expressed continuously in all plant tissues. Feeding by susceptible insect herbivores on Bt cotton results in death and removal of that individual's genetic material from the total pool of reproductive insects. Most the insects surviving to reproduce in Bt cotton will be resistant individuals. With each generation of exposure to Bt cotton this preferential selection for resistant types of insects will continue and the total population will be composed of higher and higher percentages of Bt resistant insects. Factors that influence the speed that this process occurs are intensity of the selection process and the fraction of the total pest population that is exposed to the selection process. The continuous expression of endotoxin in all plant parts of Bt cotton creates an ideal environment for the development of resistance in tobacco budworm, a species that primarily utilizes cotton as a host plant during some periods of the growing season. The speed at which resistance will develop will increase as the insecticidal plants are deployed over large contiguous areas. Most of the tobacco budworm population will be exposed to the selection process. Resistance management does not eliminate the selection process, and resistance is inevitable. Resistance management simply involves a manipulation of management options that will slow the selection process and increase the effective life of Bt cotton. While the theoretical concepts of evolutionary biology form the basis for understanding this process, the most critical components of effective resistance management are the management decisions made by individual farmers. This paper examines a few of the important questions farmers may have regarding resistance management programs for Bt cotton.

Why manage resistance to Bt cotton?

Bt cotton is the only efficacious alternative to traditional insecticides. Efficacy of most of the traditional insecticides is rapidly eroding because of resistance. This is especially true for insecticides used to control the tobacco budworm, a species that caused significant damage to the 1995 cotton crop in Mississippi even though treatments were being applied. Bt cottons expressing the Bollgard gene will be commercially available in 1996 and growers are anxious to adopt this highly effective alternative control measure. Experiments conducted by a group of researchers in Mississippi during 1995 (Reed et al. 1996) report yields of 145, 745 and 1274 lb of lint per acre for small plots not treated with insecticide, those treated with current recommended insecticides, and those planted to a Bt cotton (NuCotn 33). In large plot studies by the same group, Bt cotton production was compared to current insect control practices at 5 locations in the state. Yields of the Bt cotton were 120 lb of lint per acre higher than those of grower selected varieties protected with typical insect control programs. Insect control costs, excluding the cost of the Bt seed, were ca. $60 per acre less on the Bt cotton ($35.37 per acre) than on the cotton treated with traditional insecticides ($93.78 per acre). Assuming the Bt cotton seed will cost ca. $30 per acre, Bt cotton management systems cost growers ca. $30 less than the current systems based on traditional insecticides and may increase net returns by increasing yield. Obviously, Bt cotton represents a mechanism to increase grower profits in areas where insecticide-resistant tobacco budworm is a problem. Given

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the declining efficacy of traditional insecticides and the high efficacy of Bt cotton, growers must efficiently utilize the insecticidal plants and preserve effective control measures for the tobacco budworm.

What is really known about resistance to Bt?

Prior to 1985, resistance to Bt endotoxin in insects was not known nor considered to be of much importance. Following McGaughey's report of Bt resistance in the Indian meal moth (*Plodia interpunctella*) in 1985 (McGaughey and Johnson 1985), numerous reports of Bt resistance were recorded in the literature. Tabashnik and colleagues (Tabashnik et al. 1990) found field populations of the diamondback moth (*Plutella xylostella*) resistant to foliar sprays of Bt, and resistance to Bt has since been documented in at least four other species of insects: Colorado potato beetle (*Leptinotarsa decemlineata*), tobacco budworm (*H. virescens*), almond moth (*Cadra cautella*), and sunflower moth (*Homoeosoma electellum*). Interestingly, one of the first reports of resistance was a laboratory selection experiment with tobacco budworm (Stone et al. 1989). This experiment was conducted by Monsanto scientists and illustrated the genetic capacity of tobacco budworm to adapt to Bt endotoxin. In more recent studies, Gould and colleagues (Gould et al. 1995) have selected tobacco budworm colonies for high levels of resistance to Bt. Some of these colonies survive exposure to Bt cotton. Given the number of insects collected to establish these populations, resistant genes may be as common as 1 in 1000. Studies by Gould and others have also provided evidence that Bt resistance is probably inherited as a single recessive gene. The mechanism of resistance is most likely reduced binding to receptors in the insects midgut (Van Rie et al. 1990). Additional studies have been conducted by Monsanto scientists (Stone and Sims 1993) and researchers at Mississippi State University (Wan 1995) to establish base-line levels of susceptibility of tobacco budworm and cotton bollworm to endotoxin protein. These base-line data serve as a reference point for confirming suspected resistance in field populations. Continuing efforts to find Bt resistance in field populations of tobacco budworm have not yet been successful. Because of the importance of Bt cotton and the rather high frequency of Bt resistant genes (1 in 1000) reported in the North Carolina study, effective resistance management is critical to the cotton industry.

Why is a refuge strategy important?

Numerous approaches to managing Bt resistance have been proposed and studied to varying degrees. Some the more popular approaches include mixtures of toxins, limited expression of insecticidal protein, and mixing seed for insecticidal plants with seed for non-expressing, non-insecticidal plants. All effective resistance management strategies require a source of susceptible insects to dilute the reproductive potential of the resistant insects. The seed mixture strategy was an attempt to create a refuge for susceptible insects within the Bt cotton field. It was intensively studied over the past few years (Parker et al. 1995) because of the high appeal from an implementation perspective. Recent research has shown that tobacco budworm and cotton bollworm larvae move from plant to plant. This movement may actually accelerate selection for resistant insects under some conditions (Mallet and Porter 1992). As a result, the seed mixture strategy has been largely abandoned and other approaches to creating a refuge for susceptible insects are being pursued. Without a refuge for susceptibility and a resistant gene frequency as high as 1 in 1000, severe resistance problems can develop in only a few generations (Figure 2). When refuges as small as 5 to 10% are incorporated into the management system, dramatic increases in the effective life of Bt cotton are projected (Figure 3). Although creation of these refuges for susceptible genotypes will require some management changes for farmers, the potential gains are great.

What is the most practical way to implement the refuge strategy?

The industry groups marketing Bt cotton, state and federal regulatory agencies, and cooperating public sector scientists support the concept of a mandatory refuge. The license agreement a farmer must sign to grow Bt cotton requires that the farmer provide a refuge for susceptible insects. The two options in the agreement are : (1) for every 100 acres of cotton plant 25 acres of non-Bt cotton that can be treated with traditional insecticides to control Lepidoptera, and (2) for every 100 acres of Bt cotton plant 4 acres of non-Bt cotton than cannot be treated for Lepidoptera. Given these operational constraints, farmers must determine what percent of their total cotton acreage will be devoted to Bt cotton and how the refuge areas will be distributed within the Bt cotton. Economic factors and grower perspectives regarding the value of Bt cotton will determine what percent of each farm is planted to Bt cotton. The distribution of refuge areas within the Bt cotton is an issue more directly affected by insect biology. Table 1 shows the acres required for a single RR genotype (homozygous for resistance) at different pest densities. At gene frequencies as low as 1 in 1,000,000 it would be extremely rare for resistant insects to mate with each other. However, the probability of a RR mating with a RR or RS (heterozygous for resistance) is much higher at gene frequencies expected from the North Carolina study. At frequencies of 1 in 1000, a RR may exist every few hundred acres and RS's would be much more common. The primary goal of the refuge strategy is to provide SS genotypes (homozygous for susceptibility) to mate with the RS and RR genotypes. By widely distributing the refuge areas throughout the Bt cotton, the chance of a SS individual produced in the refuge mating with a RS or RR individual produced in the crop are increased. The optimum size of the refuge is not precisely known.

Possibilities range from “plant within rows”, to “rows within fields”, to “planter widths of several rows”, to “multiple planter widths”, to “fields within farms”, to “farms with counties”, etc. Previous studies indicate that plants within rows and single rows within a field are probably not good refuge designs because of movement of larvae between plants and possible across rows. Refuges the size of large fields and total farms would likely be poorly distributed among the Bt acreage. The most practical and useful scale of the refuge seems to be between the size of multiple planter widths and small fields. Biological factors influencing the effectiveness of the refuges are distances that moths travel prior to mating and the oviposition pattern relative to site of emergence. In refuge areas too small, the ovipositing moths may deposit large numbers of eggs on Bt cotton. These susceptible insects will die when they feed as larvae on the Bt cotton and the effective size of the refuge will be reduced. Therefore, the size of the refuge and the distribution of refuge areas throughout the Bt cotton acreage must both be considered in developing an optimum design. Research is underway at Mississippi State University to study the influence of moth movement on refuge size, but more data are needed. A preliminary study conducted in 1995 indicated that tobacco budworm moths tend to oviposit most of their eggs within an area of several to tens of rows from a site of release. This suggests that refuges the size of multiple planter widths would be most effective. More research is needed to confirm these preliminary observations.

Summary

With the commercialization of Bt cotton, resistance management becomes a very practical, individual issue. Resistance to Bt cotton will develop. How long this selection process takes will be largely determined by how effective the refuge strategy is. Grower cooperation and commitment to resistance management are keys to effective implementation of resistance management strategies for Bt cotton.

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Table 1. Abundance of RR genotypes at different gene frequencies.

Gene Frequency	RR Frequency	Acres for a Singe RR at Different Infestation Densities		
		5%	10%	25%
1/100	1/10,000	5	1	0.5
1/1,000	1/1,000,000	500	100	50
1/10,000	1/1000,000,000	50,000	10,000	5,000
1/100,000	1/10,000,000,000	5,000,000	1,000,000 500,000	
1/1,000,000	1/1,000,000,000,000	500,000,000	1,000,000,000	500,000,000

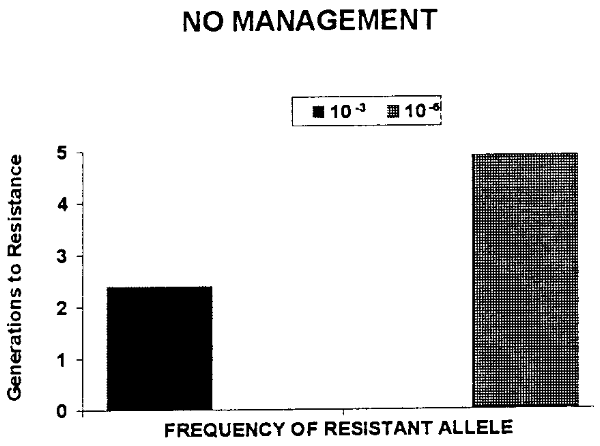

Figure 2. Generations to resistance with no refuge.

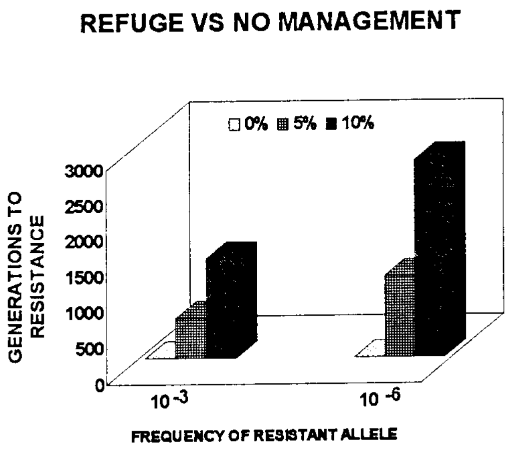

Figure 3. Generations to resistance at 0, 5 and 10% refuges.