AGRICULTURAL BIOTECHNOLOGY

Assessing the odds: The emergence of resistance to *Bt* transgenic plants

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Proteins produced by a common bacterium *Bacillus thuringiensis (Bt)* and expressed in plants to protect them from insect attack have become the focus of debate among farmers, agricultural product manufacturers, policy makers, and the scientific community. Such debate is appropriate because *Bt* is the first of a long series of new products that are arriving in the marketplace, and the stakes for this technology are high. Of the $8.1 billion spent annually on insecticides worldwide, nearly $2.7 billion could be replaced with *Bt* biotechnology applications[1]. At least 16 companies are presently involved in developing transgenic crops with *Bt* genes. In the United States, in 1996, *Bt* transgenic crops were already grown on >3 million acres, a figure that includes the 1.7 million acres of Monsanto's (St. Louis, MO) transgenic cotton. The area for 1997 is expected to be >20 million acres for all crops[1].

Bt has been used for decades to spray foliage, but has had limited use outside of "organic" agriculture and Canadian forestry, accounting in total for less than 1% of the insecticide market. When incorporated into plants, however, *Bt* proteins are effectively made much more persistent and active, even against insects that feed at sites difficult, or impossible, to reach with sprays[2]. The current *Bt* cotton cultivars provide excellent control of two of the major cotton insect pests in the United States. Unfortunately, cotton is also attacked by *Helicoverpa* bollworms, which showed some tolerance to *Bt* cotton crops in experimental trials carried out from 1993 to 1995. Last year, reports of surviving bollworms on *Bt* cotton on commercial farms in Texas (and other states) promulgated the misconception that the cotton was "a failure" (e.g., ref. 3). However, the initial disappointment seems to have been ephemeral, and plantings of *Bt* cotton reportedly increased to about 2.4 million acres in 1997[4].

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The introduction of *Bt* transgenic crops allows marked reductions in the use of pesticides. Compared with historical averages of 5–12 insecticide sprays per year in the United States, *Bt* cotton required three or less insecticide treatments[4]. In field tests in Australia, *Bt* cotton was sprayed an average of six fewer times than conventional cotton (Bruce Pyke, Cotton Research and Development Corporation of Australia, personal communication).

Despite these considerable advantages of *Bt* crops, both to the environment and to farm worker safety, concern is widespread that these gains will be short-lived because of evolution of resistance in the pests. In particular, some public interest groups argue that the threat of resistance is so great, especially to organic growers, that the introduction of *Bt* transgenic crops should be stopped[3]. Field resistance to *Bt* has already developed in at least one insect, the diamondback moth, in several tropical countries (for review, see ref. 5).

Against this background, recent papers by two of the major contributors to our understanding of *Bt* resistance have attracted considerable attention. In March, Bruce Tabashnik and colleagues[6] elegantly confirmed, through an elaborate series of genetic crosses, that just one gene in the diamondback moth confers resistance to four different toxins from Bt. In April, Fred Gould and colleagues[7] reported that the frequency of the major *Bt* resistance genes in one of the most common cotton pests, *Heliothis virescens*, collected from four states in 1993—predating the widespread introduction of *Bt* transgenic cotton—was about 1.5×10^{-3}. This estimate, and its ramifications for the future of *Bt* transgenic cotton, was deemed sufficiently high to justify a commentary by Tabashnik[8] in the same issue. However, for both papers, it is some of the "minor" points, including some odd features of frequency estimates, that warrant particular attention.

With regard to Tabashnik's report[6], previous work in several laboratories had shown that strains from the field showed resistance to three Cry 1A toxins and to Cry 1F, but not to Cry 1B, Cry 1D, or Cry 1C (the principal toxin of *Bt* strains now widely used to control otherwise resistant diamondback moths). Resistance was also found to be due to one or a few genes[9,10]. Using single pair crosses, Tabashnik et al.[6] proved that resistance to all of these toxins was truly due to cross-resistance (one gene conferring resistance to more than one toxin) and not multiple resistance (several coexisting genes). Contrary to the impression given by some commentaries (e.g., *New Scientist*, March 8, p. 3), this was not a shock, but expected by most researchers in the field.

A far more perplexing finding from the Tabashnik paper was that the frequency of the *Bt* resistance allele in one laboratory "susceptible" strain was about 10%. Although resistance to *Bt* sprays is becoming more frequent in the tropics, we are unaware of any area in which control failures have occurred in less than 4–8 years of intensive *Bt* use. For instance, in a population of diamondback moths from Hawaii identified as "NO," it took at least 8 years of spraying as often as two to four times monthly to develop resistance ratios of 36-fold, a level that indicates from our experience only moderate resistance[11]

In our glasshouse experiments (Roush, R.T. and Shelton, A.M., unpublished data) with initial resistance allele frequencies at about 1%, where 20% of the population escaped exposure and only one *Bt* spray was made each generation, control failures occurred in 10 generations (i.e., less than a year in the tropics). Thus, we find it difficult to conceive that many (if any) field populations of diamondback moth had initial resistance frequencies at even 1%, much less 10%.

The second paper by Gould et al. may be most controversial with regard to the current introduction of transgenic *Bt* plants; there are some odd points here too. First, one of the most important facts revealed in the paper is merely an aside to some unpublished data: "The Bt-cotton cultivars currently deployed produce concentrations of toxin that are high enough to kill 100% of heterozygous larvae with low levels of *Bt* toxin resistance." Although it is noted in the introduction of the paper that "the rate at which resistance develops is tightly linked to initial allele frequency," simulations show that the rate at which resistance evolves is much more strongly influenced with mortality of larvae heterozygous for resistance[2] (Fig. 1). A 10,000-fold difference in the initial allele frequency does not generally cause even so much as a fivefold delay in the emergence of resistance, but an increase in mortality of the heterozygotes from 80% to 99% (not to mention the 100% observed by Gould and colleagues in their unpublished trials) can cause at least a 10-fold delay of resistance, even in the range of the 10^{-3} frequency estimated by Gould et al.

In this context, it is perhaps only of secondary importance to question whether Gould et al. have actually measured the frequency of a single major gene, as claimed, or instead, have measured the frequency of a resistance phenotype that may be under control of more than one locus. Not all specialists will agree that the data fit expectations for major single genes.

A decision about whether to support the use of *Bt* transgenic crops is not simple. Projections indicate that *Bt* transgenic plants have the potential to capture one-third of the insecticide market and to substantially reduce environmental concerns about pest control. Resistance is a harrowing prospect, yet the speed at which resistance might evolve is unknown. As Gould et al.[7] note, if the current resistance management mandates are followed, it should take at least 10 years before resistance becomes a problem in *Heliothis virescens*, because of the high mortality of heterozygotes. This would not only outlast most intensive use of *Bt* sprays on the diamondback moth, but also the historical average for chemical sprays against cotton bollworms. However, there is little question that the implementation of resistance management in the field lags far behind what would be considered best practice on the basis of past experience and theory, and a 10- or even 20 year target hardly seems appropriate enough for the benefits provided by this technology. We suggest that the best course is to get on with the implementation of resistance management practices. If these are not adopted, the odds for resistance look pretty good.

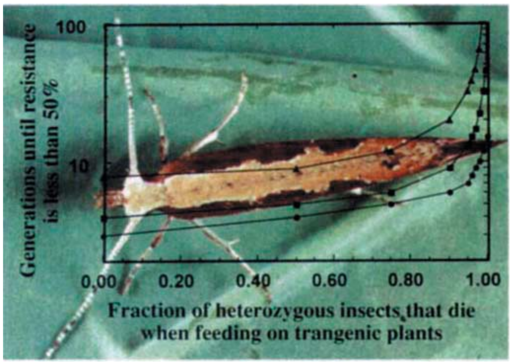

Figure 1. Time (in generations) until the frequency of a resistance allele (R) exceeds 50% for a range of initial frequencies for the resistance allele. Assumes that 10% of the population escapes exposure each generation, 100% of exposed susceptible homozygotes fail to develop, and that resistant homozygotes are unaffected.

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