Sustaining the Efficacy of Bt Toxins

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INTRODUCTION

In 1997 corn, cotton and potato cultivars that produce insecticidal proteins were grown on more than five million acres of US farmland. The acreage dedicated to these cultivars is likely to increase dramatically in the next two-to-five years. The use of these cultivars decreases the use of broad spectrum insecticides, especially in cotton and potato production. The toxin gene is derived from the bacterium, *Bacillus thuringiensis* (Bt), which has been naturally fermented and used as an organic pest control tool for decades. The toxin breaks down rapidly in the environment and is harmless to humans, vertebrates, and even most beneficial insects. In almost all ways this is the natural insecticide that you might expect environmentalists to dream about.

So why have Bt toxin-producing crops been met with so much concern from the environmental and academic community? The issue is sustainability. Until recently, all formulations of fermented Bt had incredibly short insecticidal half lives in the field. The toxic action of the bacteria all but disappeared within two days after exposure to sunlight. Organic and conventional farmers who relied on Bt had to carefully time their spraying of the spore/crystal formulations to make sure that the bacteria were in the right place at the right time. This was difficult, but the positive side to this was that the pest population was typically exposed to the toxin only at times of peak pest densities. (Farmers would be wasting money if they sprayed pests when densities were low.) From an evolutionary perspective, this meant that the majority of insects in the pest population were never exposed to the toxin. These unexposed insects served as an “evolutionary buffer” to the development of resistance in the pest population.

Currently available Bt toxin-producing cultivars have the potential to almost completely eliminate this evolutionary buffer because they typically produce the toxin in all plant parts throughout the growing season. This means that if the engineered cultivars were widely adopted, almost all of the insects in the targeted population would be exposed to the toxin. We have learned the lesson over and over again with persistent synthetic pesticides that when an insect population is put under relentless exposure to a pesticide, it typically responds with genetic changes that make it resistant to the pesticide. Rapid pest adaptation is not limited to synthetic insecticides. Insect pests have adapted to cultural controls and biological control agents when the selection pressure is intense (Gould, 1991). There is no reason to think that the situation will be dramatically different with engineered crops.

There are two general types of responses to the potential problem of pest adaptation to Bt crops:

1) We can search for novel Bt toxins and other insecticidal proteins. Gene coding for these toxins could be engineered into crop plants when the efficacy of the currently used Bt toxins is lost to pest resistance.
2) We can develop approaches for using Bt toxin-producing plants that maintain evolutionary buffers that slow the rate at which resistance evolves.

I think it would be economically and ecologically prudent to take both approaches. There would be economic benefits for certain groups if resistance to Bt evolves quickly, because they already own the replacements. These could either be producers of the next generation of transgenic insecticidal cultivars, or producers of the conventional insecticides that may replace Bt cultivars. For all the other stakeholders, a longer life for Bt crops would seem to be economically beneficial.

On the environmental side, Bt toxins appear to be exceptionally benign to non-target organisms. It is feasible that other proteins (or more novel resistance factors) will be found that are equally benign, but this is far from assured. It is likely that searches for environmentally friendly but pesticidal proteins will be of benefit to society at large. Even with the best resistance management program, some pest species are likely to adapt to Bt toxins, and there is always the problem that there are some pests for which no effective Bt toxins have yet been found. Perhaps the novel proteins found in broad surveys of microbial proteins will fill pest control niches that Bt toxins can’t fill. Basic studies of pest and plant biochemistry might also reveal some new approaches for developing insect resistant cultivars that don’t involve the use of toxins at all.

Unfortunately, today we have no clear replacements for Bt toxins. There has been a lot of talk about replacements but we lack human toxicology studies, environmental fate studies, and data on the impact of novel Bt replacements on crop productivity. I have and will continue to emphasize insecticidal cultivars in this paper because I am familiar with them and because they are currently the most widely used transgenic pest control tools (herbicide tolerant crops do not offer direct crop protection). Of course, there are other transgenic pest control tools such as virus resistant plants. Little is known about the potential for viruses to adapt to these plants, but it is certainly not outside the realm of possibilities. Viruses and other plant pathogens have always presented plant breeders with a formidable challenge because of their ability to adapt to resistant cultivars (Gould, 1991). There is no special reason to expect that these organisms will not be able to adapt to transgenic, pathogen-resistant cultivars.

For the rest of this paper I will focus on the potential of slowing down the evolution of pest resistance to Bt crops. Slowing the evolution of such resistance could be useful to society, and from a more pragmatic perspective it is useful to understand how and why the EPA is proceeding to use regulations to help enforce resistance management in Bt crops.

GENERAL RESISTANCE MANAGEMENT TECHNIQUES FOR TRANSGENIC INSECTICIDAL CULTIVARS

Resistance management is based on general principles of population genetics. A number of reviews are available that discuss details of applying these principles to engineered crops (Roush, 1996, 1997; Tabashnik, 1994; Gould, 1991, 1998). Below I will give a general overview of principles of resistance management techniques in engineered crops. I will sacrifice some precision in hopes of making the presentation more accessible. Readers can obtain more details from the references in the bibliography.

Resistance management techniques take advantage of two factors in population genetics that can impact the rate of evolution. The first factor is the difference in fitness between resistant and susceptible genotypes. Fitness is defined as the number of offspring contributed to the next generation by a single female. It is approximately equal to the probability of survival multiplied by the average number of offspring expected from each survivor. (We typically express fitness as a relative value, setting the fitness of the most fit genotype to 1.0. The fitness of the other genotype then become a proportion of the fitness of the most fit genotype.) Because it is very hard to estimate fecundity, many studies only measure survival and assume that all survivors have the same fecundity. Any approach to engineering or deploying toxic cultivars that decreases the difference in fitness between resistant and susceptible insects slows the rate of evolution.

The second factor used in resistance management is manipulation of the inheritance of fitness. When resistance is inherited as a dominant trait, the heterozygotes (RS) are just as fit as homozygous resistant insects (RR). Because the RS heterozygotes are initially much more common than resistant RR homozygous insects, dominant expression of the resistance in these RS heterozygotes speeds up evolution of resistance. Conversely, when resistance is inherited as a recessive trait the RS heterozygote is no more fit than the susceptible homozygote, so any change in the number of resistant individuals is based on high fitness of the rare RR resistant homozygotes. This typically slows the rate of evolution.

An example may help to clarify this point. If the initial frequency of resistance genes is one in one thousand (0.001), and each individual carries two genes, we expect there to be about 0.002 (or two in one thousand) RS heterozygotes, 0.998 SS susceptible homozygotes, and only 0.000001 RR homozygote resistant insects. If resistance was recessive and both the RS and SS insects had a fitness of 0.01 (1 percent) compared to the RR fitness of 1.0, the frequency of resistance genes would increase to about 0.002 in the next generation. This happens because the one in a million RR insects each produce 100 times more offspring than the other genotypes. In a rough approximation, the decimal point moves two places to the right (0.000001 to 0.0001) but that still is very few individuals. If resistance is dominant, then the fitness of the RR and RS insects would be 1.0, while the fitness of the SS insects would be 0.01. This causes the frequency of resistance genes to increase from 0.001 to 0.0835 in the next generation. This much faster rate of change is due to the fact that RS heterozygotes start at 0.002 and move to 0.2. The point is that RR homozygotes are so rare, initially, that they can't cause a rapid change in the overall proportion of resistance genes, even if they were a thousand times more fit than the other genotypes.

A resistance management strategy that can cause inheritance of resistance to be recessive will typically slow down resistance evolution in the population. How can this be done? One way is to have plants produce a very high concentration of the Bt toxin. The US Environmental Protection Agency's (EPA) Science Advisory Panel (EPA, 1998) recently defined this high dose as 25 times the amount of Bt toxin needed to kill 99 percent of the SS insects. They came up with this definition because genetic studies of insects with resistance genes have shown that RS insects can not survive when the concentration of the Bt is 25 times higher than the concentration that kills SS insects. This basically means that the high dose kills almost all SS and RS insects, making their fitness almost equal (i.e., almost recessive). The approach of building a plant with this high dose has also been the goal of industry (Fischoff, 1996).

The EPA Science Advisory Panel concurred with findings of other scientists in concluding that the high dose by itself wasn't sufficient because even with this recessive inheritance resistant pest populations would still evolve too quickly. They recommended that the first population genetics principle described above be added to any resistance-management strategy. This is the idea of decreasing the difference in fitness between RR, RS, and SS insects. They recommended providing refuges for susceptible insects to achieve this. How does this decrease the fitness difference? Again, an example is useful. If the RR fitness is 100 times that of SS insects when they are on Bt plants, but their fitness are equal on non-Bt plants, a small refuge of non-Bt plants can have a dramatic effect on the fitness difference. When 10 percent of the plants are non-Bt, the fitness of the RR insects is 1.0 times 0.9 (i. e., the frequency of Bt plants) plus 1.0 times 0.1 (i. e., the frequency of non-Bt plants). The total is 1.0, no surprise. For the SS insects fitness is 0.01 times 0.9 plus 1.0 times 0.1. The total is 0.109. So without a refuge the difference in fitness between the RR and SS insects is 100-fold. With the 10 percent refuge it is a little less than 10 fold. This small refuge slows the rate of resistance development dramatically.

The refuge serves another essential function. It ensures that the RR insects will likely mate with SS insects coming from the refuge. This will produce RS insects that will be killed by the high dose of Bt toxin. If the refuge is placed relatively far away from the Bt plants compared to the insects ability to move before mating, the refuge is less beneficial because RR insects will mate with each other instead of with the SS insects.

Combining the refuge and high dose is widely accepted as the most feasible way of slowing the rate of resistance development at this time. Other approaches have been discussed (Gould, 1998; Roush, 1996), but they have not gained acceptance or are not feasible with today’s technology. These other approaches should not be ignored, but in the next few years we will need to concentrate on the refuge/high dose approach. Most scientists agree that the refuge/high dose approach has one theoretical Achilles’ heel. This is the possibility that a resistance mechanism is present in some insects which confers more than 25 fold resistance on the RS insects. If this happens the effectiveness of the high dose decreases dramatically.

IMPLEMENTING THE REFUGE/HIGH DOSE APPROACH

Most applied entomologists regard the theoretical Achilles’ heel of the refuge/high dose approach as much less troublesome than the problems associated with implementing this approach.

A number of reports have recently been published that evaluate current attempts at implementing the refuge/high dose approach and make recommendations (Ostlie et al., 1997; Forrester and Pyke, 1997; EPA, 1998; Andow and Hutchison, 1998; Gould and Tabashnik, 1998; Whalon and Ferro, 1998). Most of these reports are crop specific because the implementation problems are highly dependent on the biology of the pests and the agricultural practices associated with the crop. I will try to summarize some of the issues that have arisen regarding Bt corn, cotton, and potato.

Corn

Of the three Bt crops, corn is grown on the largest acreage. The most often discussed target pest is the European corn borer (ECB), which can feed on many plants, but feeds primarily on corn in large agricultural areas. Relatively little insecticide is used to control the ECB because the larvae feed inside the plant where they are hard to reach with sprays and where they cause damage that is hard to notice. Bt corn can increase yields by around 10 percent in many areas. If the Bt genes were placed in cultivars that were also best in agronomic performance there could be incentive for farmers to plant wall to wall Bt cultivars and forget about refuges. The EPA did not initially mandate refuges because it was assumed that Bt cultivars would initially be limited. The EPA is now revisiting this issue (EPA, 1998). It was also expected that all Bt cultivars would provide a high dose for ECB throughout the summer. It is now clear that some cultivars do not provide such a dose (Ostlie et al., 1997; Andow and Hutchison, 1998).

In revisiting the refuge issue it has become apparent that the ECB moths don't typically move long distances before mating. Although there is certainly a need for more research in this area, we already know enough to recommend that refuges be placed adjacent to the Bt crop.

Because ECB mostly feeds on corn, the refuge must be composed of non-Bt corn. The current recommendations are between 20 and 50 percent of corn acreage in non-Bt corn depending at least in part on whether the farmer sprays the non-Bt corn for ECB control. Any time a farmer sprays a non-Bt field its refuge status is diminished. The more effective the spray the more the refuge is diminished.

Can farmers and society accept a 25 percent unsprayed refuge? The first year this is implemented there could certainly be economic damage to the refuge corn. But, consider the fact that with a 25 percent refuge only one out of four eggs lands on a non-Bt corn plant. We must ask if an ECB population whose fitness has been diminished from 1.0 to 0.25 will remain a major corn pest. An economic analysis (Hurley et al., 1998) has indicated that over a long period of years farmers may gain more by having a 20 percent refuge than by maintaining no refuge at all, even if resistance does not evolve.

ECB is only one major corn pest affected by Bt. In some areas, the Southwestern corn borer (SWCB) is a major pest. Because it is naturally more tolerant of Bt toxins there is some question about whether current corn cultivars provide a high dose (Ostlie et al., 1997; EPA 1998). There is definitely a need for more research in this area. The corn earworm (also known as the cotton bollworm) attacks corn but is not generally considered a major pest of corn. The impact of Bt corn on this pest will be discussed in the cotton section.

Cotton

Unlike corn, cotton is typically sprayed to control the target pests of Bt cultivars. Left unchecked, the cotton bollworm (also known as the corn earworm), the tobacco budworm, and the pink bollworm can each cause significant yield loss. The EPA and Monsanto, the producer of Bt cotton, developed a refuge/high dose plan before Bt cotton was commercialized. This plan gives farmers two refuge options. They can plant up to 96 percent Bt cotton if they leave a four percent refuge that is not treated with conventional insecticides that kill the target pests. Alternatively, they can plant up to 80 percent of their cotton acreage in Bt cultivars and manage insects in the 20 percent non-Bt cotton with all registered insecticides except Bt sprays. It is assumed that the conventional controls kill about 80 percent of the insects in the non-Bt cotton. This mortality reduces the 20 percent refuge to about four percent in terms of SS moths produced.

Gould and Tabashnik (1998) pointed out a number of problems with this plan, as did the EPA (1998). One striking problem was the assumption that there was a high dose. Although a high dose (by EPA 1998 standards) is achieved for the tobacco budworm, and may be achieved for the pink bollworm, it is certainly not achieved for the cotton bollworm. With this pest species 20 percent or more of the larvae survive the dose of Bt in plants with the Monsanto gene. This creates a real dilemma. Industry and the EPA have set the refuge/high dose approach as the standard, but the current plants don't produce a high dose. Population genetic models indicate that without a high dose the refuge needs to be much larger than four percent. Gould and Tabashnik (1998) argue for a refuge of about 50 percent or 17 percent, depending on whether farmers are or are not allowed to use conventional insecticides in the non-Bt cotton.

It has been proposed that there is less need for a non-Bt cotton refuge in the case of the cotton bollworm because a large proportion of the larvae feed on corn. There are two problems with this proposal. One is that the corn only has a large proportion of cotton bollworms in one of the three to five generations of bollworms over the summer. The second problem is that companies are trying to get the EPA to allow them to plant Bt corn in areas where Bt cotton is grown. The Bt corn also produces less than a high dose for this insect, so if the two crops with moderate doses are planted near each other the risk for resistance becomes very high.

While we assume that there is a high dose for tobacco budworm and pink bollworm, field data from Australia indicates that this assumption requires more testing. In Australia, it has been found the environmental factors can significantly decrease the production of Bt-toxin in cotton plants (Forrester and Pyke, 1997).

Another problem with the current resistance management plan is the lack of limits on the distance between the refuge and the Bt crop. The tobacco budworm moths appear to move long distances early in the spring, but in the summer they tend to move very little. The pink bollworm often stays in the same field for a number of generations. This has prompted recommendations for keeping the Bt and non-Bt cotton plants within 0.5 miles of each other whenever the tobacco budworm is a pest, and to interplant Bt and non-Bt cotton as blocks within fields when the pink bollworm is present.

It is not recommended to plant a seed mixture of Bt and non-Bt seeds, especially for the tobacco budworm because the larvae move from plant to plant. If Bt and non- Bt plants are within crawling distance, a RS larva might feed on a high dose plant for just long enough to get an intermediate dose and then could move onto a non-Bt plant. Lab and field studies have shown that larvae spend less time on Bt than non-Bt plants. This would ruin the high dose part of the resistance management plan.

Potato

The potato has only one target pest for Bt toxin in the US, the Colorado potato beetle (CPB). Fortunately, the plants produce a very high dose relative to the CPB's tolerance. The only real problems with Bt potatoes are the placement and maintenance of the refuge. Here, the CPB offers a real challenge. Unlike all the other pests mentioned above, the CPB is a beetle that feeds on plants as a larva and as an adult. Additionally, the adults often move short distances before mating. The problem is that seed piece mixes can't be recommended because the larvae and adults move between plants while feeding, and field to field mixtures are a problem because adults don't move far enough before they mate. Whalon and Ferro (1998) recommend that blocks of non-Bt potatoes be planted on the edges (or within) Bt potato fields.

Another problem is that potatoes are high value crops so farmers are reluctant to allow any CPB damage. A new insecticide, Imidicloprid, commonly used in potato, can kill almost 100 percent of the potato beetles. If this insecticide is used in a refuge, the refuge basically disappears. Whalon and Ferro (1998) recommend that farmers rotate fields to decrease CPB numbers and avoid use of this extremely toxic insecticide. In the appendix to the EPA (1998) document, a rough guide is given for how to determine if a refuge is producing enough insects to slow the development of resistance. The rule of thumb that emerges from this is that at least 500 insects should be produced in the refuge for every resistant insect produced in the Bt crop. This can be achieved with relatively small refuge size if the Bt crop, like Bt potato, produces a very high dose and insects in the refuge are not heavily sprayed.

CONCLUSIONS

Resistance management with Bt crops is far from simple. It has forced researchers to learn a lot more about the biology of the targeted insects. And, we still have a lot more to learn. It is pointed out in the EPA Science Advisory Panel Report (1998) that we should take a conservative approach in developing management plans until we know enough to make the plan requirements less stringent.

In the consensus statement of the EPA Science Advisory Panel it is recommended that:

1) A refuge/high dose strategy must be employed for target pests within the current understanding of the technology.
2) Regulatory strategies should serve to provide growers with a sustainable approach that encourages compliance for utilizing this valuable and environmentally friendly technology.
3) To the extent possible, feasibility should figure in the development of resistance management plans.
4) Needs of growers who rely on Bt sprays should be taken into consideration.

If the EPA follows the general guidance of the Science Advisory Panel, as well as more detailed recommendations by informed researchers (e.g., Andow and Hutchison, 1998), the use of Bt crops could probably be sustained until the next generation of environmentally benign transgenic cultivars are carefully tested and ready for commercialization.

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