RESISTANCE TO MICROBIAL INSECTICIDES: THE SCALE OF THE PROBLEM AND HOW TO MANAGE IT

W D GELERNTER
Pace Consulting, 1267 Diamond Street, San Diego, California, 92109 USA

ABSTRACT

Confirmed cases of insect resistance to microbial insecticides in the field have been rare to date, primarily because the performance limitations of these products have inhibited their acceptance in mainstream agriculture. With the 1996 commercial introduction of transgenic crops that express *Bacillus thuringiensis* (*Bt*) toxins, this picture is about to change. Research conducted over the past 10 years indicates that insects are indeed capable of developing resistance to *Bt* toxins and *Bt* toxin/spore mixtures, suggesting that resistance to *Bt* crops is likely unless management plans to avoid it are implemented. A resistance management strategy that combines the use of high doses of *Bt* toxins with the use of non-treated crop refugia was implemented in *Bt* cotton in 1996, and will most likely be implemented for additional *Bt* crops in the near future.

INTRODUCTION

As microbial control researchers, we are faced with a harsh dilemma. As long as microbial insecticides remain a novelty, restricted to niche markets by their less than optimal field performance, the specter of the development of insect resistance is minimal. Yet if our research efforts finally result in real improvements in insecticidal activity, thus making microbial insecticides a necessity, rather than a novelty to growers, we end up in the uncomfortable position of wishing for the day that these products are so well accepted that resistance becomes a real problem.

That day has now arrived, with the 1996 commercial introduction of transgenic, *Bacillus thuringiensis* (*Bt*) - expressing crops whose performance equals, or even surpasses that of the most efficacious synthetic chemical insecticides. Because the dramatically improved performance of these engineered crops all but guarantees widespread adoption and ultimately, over-use of these products, we are dealing, for the first time, with the possibility of widespread resistance to a microbially-based insecticide. While few would turn back the clock in order to erase the potential risk of resistance, the loss of *Bt* as a pest management tool needs to be avoided, if at all possible. In this chapter, I will review the potential for development of resistance to *Bt*, our current understanding of the mechanisms that will produce *Bt* resistance, and the strategies that have been proposed to avoid it. Because of the paucity of experience with resistance to microbial insecticides other than *Bt,* and the limited potential for development of insect resistance to these products in the short-term, the focus here is exclusively on *Bt.* However, we can look forward to the day (with all of the conflicting emotions described above) when it will be necessary to extrapolate our knowledge about *Bt* resistance to that for other microbial control products.

RESISTANCE TO BT: THE SCALE OF THE PROBLEM

In 1985, McGaughey reported that laboratory populations of Indian meal moth larvae (*Plodia interpunctella*) had developed resistance to *Bt* var. *kurstaki*. Following the initial shock, for many believed that resistance to insect pathogens (including *Bt*) was unlikely, the rush was on among researchers and *Bt* producing companies to determine the degree to which *Bt* resistance could occur in other insect species. The results are impressive (Table 1). Almost all insect species investigated, including Lepidopterans, Coleopterans and Dipterans, have developed resistance to *Bt* in laboratory selection experiments, and one insect, the diamondback moth (*Plutella xylostella*) has developed serious resistance to *Bt* var. *kurstaki* and the Cry1C delta endotoxin under field conditions.

Table 1. Insect resistance to *Bacillus thuringiensis*: A chronological summary of field and laboratory-selection observations where LC_{50}s in selected populations were 20-fold or more higher than in unselected populations. * resistance was derived in the field.

Resistant Insect Species	Insect Common Name	Bt strains/toxins	Author
Plodia interpunctella	Indian meal moth	*Bt* var. *kurstaki* (HD-1)	McGaughey, 1985
Heliothis virescens	tobacco budworm	Cry1Ab	Stone et. al., 1989
*Plutella xylostella**	diamondback moth	*Bt* var. *kurstaki* (HD-1)	Tabashnik et. al., 1990
Heliothis virescens	tobacco budworm	Cry1Ac	Gould et. al., 1992
Culex quinquefasciatus	southern house mosquito	Cry11A (CryIVD)	Gill et. al., 1992
Leptinotarsa decemlineata	Colorado potato beetle	*Bt* var. *tenebrionis*	Whalon et. al., 1993
Trichoplusia ni	cabbage looper	Cry1Ab (activated)	Estada and Ferre, 1994
Spodoptera exigua	beet armyworm	Cry1C	Moar et. al., 1995
Chrysomela scripta	cottonwood leaf beetle	Cry3A	Bauer, 1995
Spodoptera littoralis	cotton leafworm	Cry1C	Muller-Cohn et. al., 1996
*Plutella xylostella**	diamondback moth	Cry1C	Liu et. al., 1996

On average, significant resistance (greater than a 20 fold increase in LC_{50} values) has occurred within 15 - 20 generations of laboratory selection. Interestingly, researchers have been unable to develop resistant lines of mosquitoes (*Culex quinquefasciatus* or *Aedes aegypti*) to *Bt* var. *israelensis*, a variety that contains a combination of toxins including the cytolytic, or cyt1A toxin. It is possible that the Cyt1A toxin, due to its more general mode of action, may be able to prevent development of resistance, or even reverse insect resistance to a variety of *Bt* delta endotoxins (Georghiou and Wirth, 1997). Some key points derived from the studies listed in Table 1 include:

- the data accumulated over the past 12 years indicates that insects have the ability to develop resistance to *Bt* delta endotoxins -- as single toxins and as mixtures with other delta endotoxins and/or *Bt* spores.

- almost all studies conducted were laboratory selection experiments, conducted under artificial conditions, and most of the data is based on only three insects -- *P. xylostella, P. interpunctella* and *H. virescens.* While these studies are important, they may not always accurately predict what will happen under field conditions
- resistance to *Bt* is not currently a serious problem under field conditions, with only one insect species, the diamondback moth (DBM), demonstrating resistance in cruciferous crops in the U.S. (Hawaii, Florida, New York), the Philippines, Thailand, Malaysia, Guatemala, Honduras, Costa Rica, and Japan (Perez et. al., 1997; Tabashnik, 1994)

It is instructive to explore why, with over 40 years of commercial use of *Bt* products, the DBM should be the only insect to develop resistance to *Bt.* First of all, resistant DBM populations are subjected to 50 or more treatments of *Bt* per year, which is more intense use of *Bt* than for any other insect management system that I am aware of. This is due to the fact that the DBM has developed resistance to all other major groups of insecticides, thus leaving growers few control options other than *Bt,* as well as to the fact that cruciferous crops are primarily grown in warm climates, where DBM populations are treated on a year-round basis. In addition, the DBM is not highly mobile, and therefore unlikely to mate with individuals from other locations, thus serving to reproductively isolate resistant DBM populations. While the DBM situation may be unique, it, along with McGaughey's earlier work on the Indian meal moth served the important task of convincing researchers, companies and regulators that resistance to *Bt* was possible, and therefore a real and a serious problem.

Mechanisms(s) and genetics of resistance

For *P. xylostella* and *P. interpunctella,* the primary mechanism for resistance appears to be reduced or no binding of the *Bt* toxin to receptors on the insect's midgut epithelial cells (Tabashnik, 1994). The picture is less clear for *H. virescens,* where insects resistant to all of the Cry1A toxins showed no binding of *Bt* toxins to the Cry1Aa receptor, but still showed binding to the Cry1Ab and Cry1Ac receptors (Lee et. al., 1995). With only a few insects studied and conflicting results reported, it is clear that we are just beginning to understand the mechanisms of resistance to *Bt.*

For the three most-studied insects (*P. interpunctella, P. xylostella, H. virescens*), inheritance of resistance appears to be partially or completely recessive. The resistant trait was not sex-linked, nor was it maternally inherited (Tabashnik, 1994; Gould et. al., 1995). For one colony of resistant DBM, a single autosomal recessive gene was found to confer resistance to four toxins (Cry1Aa, Cry1Ab, Cry1Ac and Cry1F). Although not yet reported for other insect species, the concern is that the presence of a multiple toxin resistance gene in insects will increase the rate of evolution to entire groups of toxins, used either in combination or rotation (Tabashnik et. al., 1997).

Cross-resistance

Cross-resistance, when selection with one insecticide causes resistance to a different insecticide, has been observed in at least six species of insects for various *Bts* (Table 2). Cross resistance among the Cry1A toxins (as seen in *P. interpunctella* exposed to *Bt* var. *aizawai* or *entomocidus* strains that contained Cry1Aa and Cry1Ab, or *H. virescens* strains exposed to Cry1Ac) was not surprising, due to the structural similarity of these proteins. Less expected was cross-resistance among toxins with less homology, such as between Cry1A toxins and Cry1F/Cry1J toxins in *P. xylostella* (Tabashnik et. al., 1996), between Cry1A and Cry1F/Cry2A for *H. virescens,* and between Cry3A and Cry1B for *C. scripta* (Bauer, 1995). Given our current state of knowledge concerning delta endotoxin structure and function, it appears that we cannot predict when, and to which *Bt* toxins cross-resistance will develop until more information is available.

Table 2. Cross -resistance to *Bacillus thuringiensis* toxins. Cross resistance to a given toxin was considered to have occurred only when the initial Bt strain did not include this toxin, and when LC_{50}s had increased by 20-fold or more, compared to susceptible populations.

Resistant Insect	**Initial resistance**	**Cross resistance**	**Author**
P. xylostella	*Bt* var. *kurstaki* (HD-1)	Cry1F, Cry1J	Tabashnik et. al., 1996
S. littoralis	Cry1C	Cry1E	Muller-Cohn et. al., 1996
S. exigua	Cry1C	Cry1Ab, Cry1H, Cry1E, Cry2A	Moar et. al., 1995
P. interpunctella	*Bt* var. *aizawai* (HD-112)	Cry1Ac	McGaughey & Johnson, 1994
P. interpunctella	*Bt* var. *aizawai* (HD-133)	Cry1Ac, Cry1B, Cry2A	McGaughey & Johnson, 1994
P. interpunctella	*Bt* var. entomodicus (HD-198)	Cry1Ac, Cry2A	McGaughey& Johnson, 1994
H. virescens	Cry1Ac	Cry1Ab,Cry1F, Cry1Aa, Cry2A,	Gould et. al., 1995
C. scripta	Cry3A	Cry1B	Bauer., 1995

Reversal of Resistance

To determine whether a moratorium in *Bt* applications could return an insect population to its original state of susceptibility to *Bt*, laboratory selection experiments have been carried out on a variety of Lepidopteran insects (*P. xylostella, P. interpunctella, H. virescens, and S. littoralis)* and on the Colorado potato beetle, *L. decemlineata.* When researchers stopped exposing resistant insects to *Bt,* they saw 10-fold or greater decreases in LC_{50}s in as few as four generations for *P. xylostella* (Tabashnik, 1994), in 8 generations for *S. littoralis* (Muller-Cohn et. al., 1996), in 12 generations for *L.* decemlineata (Whalon et. al., 1993) and in 25 or more generations for *H. virescens* and *P. interpunctella* (Tabashnik, 1994).

Reversion is believed to occur when there is a fitness cost (for *P. xylostella*, reduced fecundity, lower percent egg hatch, and lower survival rates have been observed [Tabashnik, 1994]) associated with *Bt* resistance. Under selection pressure from *Bt*, this fitness cost is not as important as the insect's newly evolved ability to survive exposure to *Bt.* However, when *Bt* applications stop, the resistant insects no longer have an advantage over their susceptible counterparts, and are in fact less able to compete. As a result, resistant individuals are slowly lost from the population, and reversion to susceptibility occurs.

There are some inconsistencies that the process of reversion does not explain, however. First, for reasons that are unclear, a small but constant proportion of highly resistant individuals are sometimes maintained in revertant colonies. For this reason, when populations of revertant *P. xylostella* were exposed to *Bt* var. *kurstaki,* a rapid increase in resistance was observed (Tabashnik, 1994). Another troubling and related observation is that total reversion to susceptibility has not been observed in any of the insect populations that have been evaluated. Finally, for at least two insect species, *P. interpunctella* (Tabashnik, 1994) and *P. xylostella* (Liu et. al., 1996), resistance has become stabilized after long periods of selection with *Bt* var. *kurstaki.* In other words, reversion to susceptibility did not occur, even when insects were no longer exposed to *Bt* var. *kurstaki* toxins for lengthy periods. Taken as a whole, the data on reversion suggests that halting *Bt* applications to resistant insects may have little or no effect on a decrease in resistance. This will also have a serious impact on the utility of rotations as a resistance management tool, as described below.

Assessing the risk of the development of resistance to *Bt*

As for other insecticides, development of insect resistance to *Bt* relies on high selection pressure, or constant exposure to *Bt*. Some of the conditions which increase high selection pressure, and therefore the risk of resistance to *Bt* include:

- frequent applications of *Bt,* with minimal use of other products, over a period of months or years
- use of *Bt* over a large area, with minimal use of other products targeted against the same pest
- reproductive isolation of the insect population due to low mobility and minimal mating among insects from different areas
- target insect species where all life stages are susceptible to the *Bt* product
- short insect generation times
- no crop rotation
- no fitness cost (no deleterious effects) associated with *Bt* resistance
- dominance of the *Bt* resistance gene

In most of the situations in which *Bt* products are currently used, almost none of the risk factors listed above are met; in short, *Bt* is not used on a broad enough scale for resistance to occur. The exception is the DBM's development of resistance to field applications of *Bt* var. *kurstaki* (Tabashnik, 1990), where all but the last two risk factors above have been amply met. The conditions that led to DBM resistance to *Bt* are fairly unique, and unlikely (though not impossible) to arise for other insects targeted with *Bt* foliar applications. Due to the commercial introduction of *Bt* expressing transgenic crops in 1996, however, our concerns regarding resistance to *Bt* have only just begun.

MANAGING RESISTANCE TO BT

In 1996, *Bt* expressing transgenic corn, cotton and potatoes (Table 3) were commercially introduced in the United States and were planted on over 2 million acres of farmland. Overall performance of the crops has been superlative. By overcoming most of the problems that have plagued microbial insecticides -- most importantly, delivery to the target insect -- *Bt* plants have taken the quantum leap necessary for the transformation of *Bt* from a novelty to a mainstream product. Adoption of *Bt* crops by growers appears to be limited only by the quantity of seed available; for cotton alone, it is projected that the number of planted acres will increase to 2.4 million in 1997 (Cline, 1997).

Table 3. *Bt* expressing transgenic crops commercially introduced in 1996.

Product Name	Company	*Bt* toxin	Target Insect Larvae
'Bollgard® Cotton'	Monsanto, St. Louis, MO	Cry1Ac	tobacco budworm
'Knock-Out® Corn'	Ciba Seeds, Greensboro, NC	Cry1Ab	European corn borer
'NatureGard® Corn'	Mycogen, San Diego, CA	Cry1Ab	European corn borer
'NewLeaf® Potatoes'	NatureMark, Boise, ID	Cry3A	Colorado potato beetle

Nothing is perfect, however, and *Bt* crops are no exception. For example, the single toxins encoded in each plant have a narrow host range. When several thousand acres of *Bt* cotton became infested with high numbers of a secondary pest, the cotton bollworm (*Helicoverpa zea*) in 1996, this more *Bt* tolerant insect caused significant levels of damage in fields where the target insect, the tobacco budworm (*H. virescens*) was well controlled (Cline, 1997). Other cotton pests such as the beet armyworm, *Spodoptera exigua*, are also not well controlled by the Cry1Ac containing cotton plants. Growers who experienced these problems must now consider whether the $32.00 per acre "technology fee" charged by Monsanto is worth the price, although for most growers, who in the past have spent well in excess of $32.00 per acre for control of tobacco budworm with synthetic chemistry, the answer will be easy to reach.

Another, and even a greater concern, is the widespread development of resistance to *Bt*. With *Bt* production occurring in almost all plant tissues, throughout the life of the crop, *Bt* crops represent the worst case scenario for increased selection pressure and development of insect resistance. With only one year on the market, there are no signs of Bt resistance in the field yet, but without plans to avoid resistance, it will most assuredly occur.

<u>Technical Approaches</u>

Recognizing the threat for development of *Bt* resistance, researchers began in the 1980s to study the mechanisms, genetics and likelihood of resistance, resulting in the work summarized above. Based on this work, as well as on resistance management research for other pesticides, a series of theories for resistance management have been proposed (Gould, 1997):

1. Refugia: Refuges are areas of non-treated crops that are set aside specifically to supply a source of susceptible insects. In theory, placement of a refuge field of non-*Bt* crops next to a field of *Bt* crops will allow susceptible individuals (who will far out-number the rare resistant individuals) to mate with resistant insects, thus diluting the resistant gene pool. The concept of refugia is broadly embraced by researchers, and makes the most obvious

common-sense of any of the proposals suggested. However, there are still unanswered questions: How big does the refuge have to be? How far from the field of *Bt* crops should it be? How will these parameters vary for different pest/crop systems? For insects with limited mobility (and therefore with less likelihood of different field populations mating) such as the Colorado potato beetle, these are particularly important questions. Finally, it has been questioned whether growers or companies will voluntarily advocate the planting of refuges (where yield loss is likely to occur), and if they do not, how compliance will be enforced, and by whom.

2. High doses of *Bt* toxin: Commercially available *Bt* crops have been engineered to produce toxin levels in excess of 25 times the LC_{99} for the target insect. This high dose strategy is based on the assumption that the gene for resistance is recessive and rare. Under these conditions, all susceptible homozygotes and heterozygotes would be killed by the high dose of *Bt*, allowing only the rare resistant homozygotes to survive. In combination with untreated refuges, this plan would allow any surviving resistant homozygotes to mate with susceptible insects from the refuge, thus diluting and therefore slowing the rate of resistance. The strategy of combining refugia with high toxin expression has been endorsed by most scientists in this area of research, and forms the basis for management plans mandated by the Environmental Protection Agency (EPA), the U.S. federal regulatory body (see below). Key issues with this strategy include our lack of knowledge to support the assumptions of heredity and gene frequency made above. In addition, the dose of a given *Bt* toxin may be high for one insect such as the tobacco budworm, but may be an intermediate or low dose for a more tolerant insect such as the cotton bollworm. Under these conditions, cotton bollworm resistance could advance more rapidly.

3. Seed mixtures: Within-field mixtures of toxin-containing plants and toxin-free plants are a variation on the theme of refugia. Under this scenario, susceptible insects would survive on toxin-free plants, thus allowing mating between susceptible and resistant insects within the same field. Favored by researchers for several years, this concept fell into disrepute following the publication of an article by Mallett and Porter (1992). They suggested that if insect larvae are able to move from one plant to another, the beneficial effects of seed mixtures would be negated. For example, it has been shown that both European corn borer larvae (Gould, 1997) and Colorado potato beetle larvae (Hoy and Head, 1995) are more likely to leave a *Bt* corn or potato plant, respectively, than they are to leave a susceptible plant. If these larvae are heterozygotes that normally would be killed by the *Bt* plant, but instead survive because they have moved onto a toxin-free plant, then the heterozygote, with its resistance gene, will survive and resistance will be hastened.

4. Toxin mixtures: Although all commercially available *Bt* crops contain only one toxin each, companies are actively involved in developing crops that express two or more insecticidal materials. In 1996, Mycogen Plant Sciences introduced corn hybrids containing a *Bt* toxin targeted against the European corn borer (ECB), as well as a group of genes that confer "native resistance" to first generation ECB. Monsanto is working on cotton plants that express a tobacco budworm active *Bt* toxin, as well as high terpenoid levels, which are known to decrease growth rate and survival (Sachs et. al., 1996). There is also a great deal of interest in the role of the cytolytic toxin, cyt1A, in preventing, or even reversing resistance to *Bt* var. *israelensis* (Georghiou and Wirth, 1997).

In theory, toxin mixtures will decrease the risk of resistance because insects with resistance to two or more toxins will be extremely rare, or even absent. The empirical data we have to support this concept is contradictory. There is no scientific support for the use of mixtures of conventional insecticides to delay resistance (Gould, 1997) and we already know that insects can develop resistance to the mixture of four toxins present in commercial formulations of *Bt* var. *kurstaki.* The possibility of cross-resistance among toxins in a mixture is also an undesirable possibility. On the other hand, the mixture of toxins present in *Bt* var. *israelensis* appears to be able to prevent development of resistance in mosquitoes; this may be due to the presence of the cyt1A toxin in this mixture (Georghiou and Wirth, 1997).

5. Rotation or alternation of *Bt* toxins: This strategy, which entails alternation in time of *Bt* toxins with other *Bt* toxins, or with other insect management methods (insecticides, beneficial insects, etc.) relies on a reversion to susceptibility when use of the *Bt* toxin is discontinued. As described above, this may be an unreliable assumption to make. In addition, for *Bt/Bt* rotations, the possibility of cross-resistance must be factored in.

<u>What we don't know</u>

It is important to stress that the proposals above, as well as the assessment of their advantages and disadvantages, are based primarily on theoretical models. A dilemma facing resistance management research is that the large acreages required for conducting field validation studies would themselves engender the risk of development of resistance during the course of the study! For this reason, we don't really know if any, or all of the approaches above will work at avoiding development of resistance. Our knowledge of the fundamentals required to build predictive models -- inheritance of resistance, frequency of resistance genes, mechanisms of resistance, understanding of cross-resistance, mobility and reproductive behavior of target insects, and more -- is extremely limited, and what we do know is based on only a very few insects.

It is also important to recognize that the tools we have available to detect *Bt* resistance in the field (primarily bioassays) are not sensitive enough to identify resistance in the early stages of development, making timely deployment of any mitigation factors that we do develop difficult, if not impossible. While a great deal of research conducted over the last 10 years has vastly improved our understanding of *Bt* resistance, a great deal more needs to be done for us to feel confident that resistance can be managed (i.e. slowed down, once it occurs), much less avoided. Yet at the same time that we recognize the need for more work, *Bt* crops are being sold, and are being widely adopted by growers who would never in their lives have considered applying a *Bt* foliar spray. Under these circumstances, what is the right course of action to take from a technical, and even from a moral standpoint? There is a full spectrum of opinion on this question, from organic growers and environmentalists, who want commercial sales of *Bt* crops immediately halted until more is known, to companies and growers who feel that the benefits of *Bt* crops far outweigh the risks, and that over-regulation will deprive agriculture and the environment of a useful tool. To highlight these arguments, two contrasting positions are presented below, each compatible with the technical information we have accumulated, as well as with the desire to best utilize the environmental benefits of *Bt,* but differing considerably.

Position 1: Avoid the "Tragedy of the Commons"

In 1968, Garrett Hardin published a classic essay which uses the metaphor of a communal pasture, or commons, to describe how easily natural resources can be destroyed. In his example, it is in the self-interest of each individual villager to graze as many cattle as possible on the commons, thus resulting in a healthier and larger herd. However, when each villager takes this same position, the commons soon becomes overgrazed, eventually leading to the destruction of each villager's herd. The problem? It was in no one's interest to preserve the commons, and thus the tragedy of the commons. The solution? In Hardin's view, technical solutions have no value in this situation. Instead, it is a social arrangement of "mutual coercion" that can force the villagers to make the right decisions for their long term future. If we view *Bt* in the same way Hardin views the commons -- as a public resource (as many organic farmers and environmentalists already do), there are parallels to the situation Hardin describes. For example, it doesn't appear to be in any one group's self-interest to invest in the implementation of long range plans for avoiding development of resistance to *Bt*. For growers, the financial demands of farming dictate short-term strategies targeted towards increased yields and profits on a year-to-year basis, a condition that would lead to over-use of high performance *Bt* crops. Crop advisers and seed salespeople, the key source of technical information for growers, are likewise driven to provide technical advice that will promote higher profits this year, and not some time in the future. Similarly, companies who have invested millions of dollars in bringing *Bt* crops to the marketplace are anxious to re-coup their investment, and even more importantly, to prove to investors that biotechnology is worth additional investment. While all of the groups involved have a sincere desire to avoid development of resistance to *Bt*, their short-term survival, just as that of the herdsman on the commons, will result in indiscriminate use of the resource of *Bt,* and the eventual development of resistance. Extrapolating from Hardin's model, the tragedy of the commons for *Bt* can be avoided only by bringing together representatives of all of the groups involved -- growers, crop advisers, industry, researchers and environmental groups -- to form a body that oversees development and implementation of efforts to avoid resistance. There is some evidence that an oversight committee of this sort can be successful in promoting self-enforcement through "mutual coercion" of resistance management plans. In Australia, government researchers, industry, growers and crop advisers came together when *Heliothis armigera* resistance to synthetic pyrethroids was first observed (Croft, 1990). This group has since implemented a voluntary plan that restricts the use of pyrethroids, thus significantly decreasing the rate at which development of resistance occurred. A similar Australian group has recently been formed to deal with the potential for development of *H. armigera* resistant to *Bt* cotton. It is less clear that such a group could be effective in a country the size of the United States, where crop acreages are much larger, much more dispersed, and growers are a much more diverse group. However, it is clear that if formation of such an oversight group is necessary for avoiding development of *Bt* resistance, then commercialization efforts must cease until such a group can be brought into existence.

Position 2: Bring the benefits of *Bt* to mainstream agriculture

It can be argued that although some groups regard *Bt* as a public resource, in reality its use has been severely restricted to niche markets where safety, or lack of other alternatives makes the limitations of *Bt* foliar sprays less significant. According to Wood/MacKenzie and Agrow marketing reports, 1995 sales of *Bt* foliar sprays worldwide were estimated at $92 million, or approximately 1% of the $8 billion worldwide insecticide market. Thus, the weaknesses of *Bt* foliar sprays -- lack of residual activity, poor delivery to the target insect, and narrow host range -- have restricted their market potential. The recent commercial introduction of new, low toxicity synthetic chemistries such as imidacloprid (Bayer), 20-hydroxy ecdysone (Rohm and Haas), pyrroles (American Cyanamid), spinosyn (Dow Elanco) and fipronil (Rhone-Poulenc) are actively competing with *Bt* foliar sprays, thus further reducing their market potential.

Given the small role that *Bt* sprays play in worldwide insect control, development of resistance to *Bt* and its concomitant loss of product status, devastating as it would be to certain growers and foresters (and insect pathologists!) would not have any significant impact on the environment, especially given the new, safer insecticides that might replace it. In contrast, *Bt* crops represent the first time that an insect pathogen derived product has performed well enough, and consistently enough to become a sought after insect control tool in mainstream agriculture. With the widespread use of *Bt* crops becoming a reality, the reductions in the use of more toxic pyrethroids, organophosphates and carbamates has been significant, particularly in cotton (Cline, 1997). But the environmental benefits of *Bt* crops are not restricted only to direct decreases in the use of conventional insecticides; benefits will also likely derive from the re-establishment of beneficial insect populations, which will also serve to keep insect populations under control. With these types of benefits, even the risk of eventual resistance, although not negligible, may seem worth taking. One could then reason that the environmental and food safety benefits of having *Bt* crops in the marketplace, even for only five years, would be worth the risk of resistance, and that minimal or no regulation regarding resistance management programs should stand in the way of commercialization of these products.

<u>The Reality: Technical solutions are not sufficient</u>

The current consensus on *Bt* resistance management is a compromise forged between the two positions above and is based on the concept that the high dose strategy, combined with the use of refuges, is the best technical approach for managing the development of resistance to *Bt*. Of the *Bt* crops commercialized in 1996, the cotton resistance management plan is the most explicit and most stringent. Based on input to the U.S.EPA from industry, academic researchers and environmental groups, cotton growers must agree to implement one of two resistance management plans:

1. for every 100 acres planted with *Bt* cotton, the grower must plant a refuge of 4 acres of non *Bt* cotton that is not to be treated with any insecticides for tobacco budworm, cotton boll worm, or pink bollworm (*Pectinophora gossypiella*).

2. for every 100 acres planted with *Bt* cotton, the grower must plant a refuge of 25 acres of non *Bt* cotton that can be treated with any products other than *Bt* based products

The above cotton plan is required by the U.S. EPA, and as such is the first pest control product for which the EPA has developed a mandatory resistance management plan. For *Bt* potato and corn products, resistance management plans are at this point voluntary. For example, NatureMark, the manufacturer of *Bt* potatoes, has requested that growers plant no more than 80% of their potato acreage with *Bt* potatoes; the remaining 20% of their acreages should be treated with non-*Bt* products. Refuge plans are currently under development for corn. However, all companies that received their conditional registrations from the EPA in 1995 and 1996 must develop and implement *Bt* resistance management plans by the year 2001 to maintain their registrations.

How well will these, or other plans work at avoiding development of resistance to *Bt*? Given our current state of knowledge, is it even feasible to hope that resistance can be avoided, or will we only be able to delay its inevitable occurrence? The answers to these questions are impossible to forecast, but I do believe that the plans currently in place represent the best technical solutions we have available. However, the level of compliance with the plans -- the "social arrangement" that Garrett Hardin wrote about -- is equally important, but much less easy to predict or quantify. As applied scientists, we have too frequently overlooked the critical role of the end-user in the success of management plans that although technically perfect (in our eyes), rarely seem to actually work. The increasingly large research area of *Bt* resistance provides a good opportunity for us to more successfully integrate input from growers and other end-users into the development of practical management programs that will insure the continued usage of *Bt* and other microbial insecticides.

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