Potential for effectively managing evolution of resistance to *Bacillus thuringiensis* toxins expressed in insect resistant Bt crops.

J. Concepción Rodríguez Maciel
Programa de Entomología y Acarología
Colegio de Postgraduados
concho@colpos.mx

Introduction

Bacillus thuringiensis Berliner is a gram-positive soil bacterium that produces insecticide toxins highly active toward economically important Lepidopteran, Coleopteran and Dipteran pests (Höfte and Whitely 1989, Knowles 1994). Bt-formulations have been used for more than 50 years, mainly in organic crop production (Cannon 1993). Unfortunately the first commercial formulations were not widely used due to their poor and unpredictable field efficacy, very short persistence and high cost (Roush 1994). Nowadays, Bt-toxins use is increasing due to the development of more potent formulations, widening spectrum of controlled pests, predictable field efficacy, little or no effect on humans, friendly to the environment and lack of negative impact on beneficial and non target organisms. However, their actual market price is too high to be used on less profitable crops as maize.

Recent advances in genetic engineering have been made possible to insert the genes responsible for expressing Bt-insecticidal crystal proteins in important crops (Gasser and Fraley 1989, Koziel *et al* 1993). Insecticidal transgenic crops are able to effectively defend themselves against susceptible target pests without further input of conventional insecticides (Meeusen and Warrn 1989, Koziel *et al.* 1993, Perlak *et al.*1993, Feldman and Stone 1997) and thus enhancing compatibility to biological control, (Gould *et al.*1991, Gill *et al.* 1992, Starnes *et al.* 1993, Fitt *et al.* 1994, Roush 1994, Tabashnik 1994). In Bt-cotton, the overall use of conventional insecticides can be reduced in the range of 50-60% (Roush and Shelton 1997).

Pest susceptibility to Bt-toxins is a valuable resource that must be preserved; otherwise these benefits are at risk due to the potential for insect resistance (Hokkanen and Wearing 1994, 100, Tabashnik *et al.* 1999). This concern is based on the following facts:

a) Transgenic insecticidal crops (TIC) tend to maintain a constant titer of toxin versus foliar sprays; and hence select for resistance for longer time as happens with highly residual insecticides.

b) The use of TIC is more likely to select a large percentage of the target insect population.

c) The best recipe to develop resistance consists on “using the same insecticide always and a high dose”. This condition in perfectly met by TCI.

Continuous and intense selection pressure exerted by Bt-crops provides ideal conditions for resistance to develop (Comins 1977a, b, Gould 1986, McKensie 1996), as documented in recent reviews on evolution and management of resistance to Bt (Ferré *et al.* 1995, Gould 1998, McGaughey and Whalon 1992, Tabashnik 1994). In consequence, it is of high priority to deploy preventive resistance measures (RM) in order to delay the onset of resistance and mitigate its evolution once it shows up. According to U. S. EPA (1998), the challenge is to maintain population susceptibility for at least 10 years. To achieve that goal is highly desirable to deploy RM as an integral part of IPM programs (Ostlie *et al* 1997).

Factors influencing evolution of resistance to Bt-crops

Before attempting to design and implement a RM, is of great importance to identify those factors influencing, positively or negatively, the evolution of resistance. In this document we identify five main factors: pest, Bt-crop, refuge, Bt-genes/toxins and social/economic (Table 1). A through understanding of them will allow identifying the resistance risk level and wisely implementing efficient RM.

Initial gene frequency: alleles for resistance exist in the population before exerting insecticide selection pressure. It is considered that R-alleles in non selected populations ranges between 10^{-2} (Georghiou and Taylor 1977) and 10^{-13} (Whitten and McKensie 1982). Recent studies suggest that initial R-alleles frequency toward Bt-toxins may be as common as 10^{-3} in some species (Gould *et al.* 1995, 1997; Gould and Tabashnik 1998). Unfortunately, it is difficult to detect these alleles before they reach frequencies of 10^{-2} to 10^{-1} (Roush and Miller 1986).

In Comarca Lagunera Region, México, the pink bollworm, *Pectinophora gossypiella* (Saunders), used to be the most important cotton pest before 1996. Bollgard® cotton expressing the Cry1A(c) toxin has been extensively grown in that area and nowadays is extremely difficult to find a single larva. Weinzierl *et al.*(1997) surveyed 325 acres of Bt-corn looking for European corn borers, *Ostrinia nubilalis* (Hübner), and found only two

larvae, out of 4,500,000 expected from non Bt-corn. These results are consistent with the estimated initial frequency of R-alleles conferring resistance to Bt-toxins.

Table 1. Known or suggested factors influencing the evolution of resistance to Bt-crops.

A. Pest

- Genetic
  - Initial frequency of R alleles
  - Number of R alleles
  - Dominance of R alleles
  - Fitness of Bt-selected individuals
- Biological/Ecological
  - Random mating
  - Offspring per generation
  - Generation turnover
  - Diferential overwintering survival
  - Migration (short and long)

B. Bt–crop

- Level of toxin expression
- Duration of Bt–toxin expression
- Tissue – specific expression
- Bt–crop market penetration

C. Refuge

- Size
- Structured/non structured
- Sprayed/unsprayed
- Synchrony with Bt–selected individuals
- Amount of S individuals produced

D. Bt–genes/Toxins

- Number of commercial Bt–genes available
- Degree of cross resistance among Bt–toxins used
- Use of mixture or alternating Bt–crops
- Single/Stack genes
- Bt–toxin efficacy

E. Social/Economic

- Cultural attitude toward transgenic crops
- Profitability of Bt–crops
- Level of social organization
- Complexity of resistance management strategies
- Small/large scale farming.

RM must be implemented well before resistance level reaches detectable levels (McGaughey and Whalon 1992). Once these alleles are common, it could be too late to

manage resistance. Unfortunately is difficult to convince farmers about the importance to deploy RM when resistance is not a problem.

In some cases, a single gene is enough to express the necessary resistance level to allow the individual to survive to the applied selection pressure. In other cases, it is necessary to accumulate more that one gene to become resistance. The time required to develop resistance depends on the amount of required genes. As this number increases, the population will take longer time to achieve the status of "resistant" (Georghiou and Taylor 1986). Resistance to Bt-toxins appears to be inherited as a single gene and high levels of resistance has been observed after 15 or more generations under laboratory Bt-selection (Tabashnik 1994, Gould *et al.* 1995, Gould 1998).

Laboratory controlled studies are important to determine the genetics of insecticide resistance. In order to know the inheritability of an R-trait, group homozygous resistance individuals are crossed to similar number of homozygous susceptible ones. Full bioassays are carried out with the parental strains as well as will the F1 progeny. If the F1 ldp-line is close to the homozygous susceptible parental strains, resistance is recessive; otherwise is dominant. When that line is in the middle, resistance is considered co-dominant. Resistance management strategies are more efficient when resistance is recessive (Tabashnik *et al.* 1999) because a smaller selection pressure is required to eliminate heterozygous individuals, thus preventing the onset of homozygous resistant ones. In most of the cases, resistance to Bt-toxins varies from partially to completely recessive (Tabashnik 1994, Ferre *et al.* 1995*, Gould* et al. 1992, Tabashnik *et al.* 1997 a, b).

In general, resistant individuals are less fit than susceptible ones (Georghiou and Taylor 1986) in absence of selection pressure; otherwise resistance would be a common phenomenon regardless or insecticide use. Recent studies suggest that Bt-resistant genotypes are less fit in relation to their resistant counterparts (Tang *et al.* 1997); suggesting that resistance is unstable.

Random mating between resistant and susceptible individuals is crucial to slow the onset of resistance. Non random mating occurs when a susceptible individual looks for a similar genotype; apparently, to mate, a susceptible individual is unable to recognize a resistant genotype. To minimize non random mating between Bt-selected individuals and those unselected ones, the refuge should be deployed close to the transgenic insecticidal crop. Unfortunately, local pest movement and time to mate is poorly understood under field conditions.

Higher sexual reproductive potential increases the probability to develop resistance (Georghiou and Taylor 1986). A sexual species with high fertility produces more genetic variation among offspring, thus increasing the possibility of selecting for resistance. The same trend is observed with the number of generations per year (Georghiou 1981). Most Bt-susceptible pests affecting maize have from one to three generations per year.

In many growing areas, the winter is characterized by very low temperatures. This phenomenon influences the life expectancy of over wintering individuals. Considering that Bt-resistant individuals are less fit, cold temperatures may reduce their relative frequency; thus mitigating the rate at which resistance evolves. However, more studies are needed to clarify the importance of differential overwinter survival.

Migration plays a critical role in delaying resistance. Its influence is so great that in absence of migration, resistance develop at similar rate regardless if the character is recessive or dominant (Georghiou and Taylor 1977a, 1986). The amount of arriving susceptible individuals is critical to dilute resistance by allowing the cross with the RR insects emerging from the Bt-crop, thus leading to a SS offspring that could be eliminated by the insecticidal transgenic crop.

Long and short migration is considered important. Long migration involves the movement of insect pest across growing areas. This phenomenon is particularly important because RM may be overpowered by the income of significant amount of resistant individuals from careless managed areas.

Short distance migration involves the movement of insects from the refuge to the Bt-crop and vice versa, as well as the movement from one plant to another (Mallet and Porter 1992). More ecological studies are needed to understand this factor and hence improve deployment of refuge to increase the interaction between Bt-selected and unselected individuals.

The percentage of insect mortality is positively correlated with the level of Bt-toxin expression. Resistance will not evolve if cero or 100% of mortality is imposed on treated individuals. High Bt-toxin expression together with a refuge is considered the main RM strategies (Roush 1994, 1996, 1997a, Gould 1998). In this context, a high dose is defined as 25x the Bt-toxin concentration necessary to kill fully susceptible larvae (U.S. EPA 1998). The purpose consists on expressing enough concentration to kill all heterozygous genotypes, thus preventing the formation of homozygous resistant individuals (Roush and Daly 1990, Gould 1994). Bt-crops have been able to kill 100% of heterozygous genotypes

in *Heliothis virescens* (Fabricius) (Gould *et al.* 1997) and *Plutella xylostella* (Linnaeus) (Roush 1994, Metz *et al.* 1995). Cry1A(b), Cry1A(c), and Cry9c genes provide highly effective and selective control of the major Lepidopteran pests of maize (Koziel *et al.* 1993, Fischoff 1996, Jansens *et al.* 1997). The Cry1A(c) gene is particularly effective against Spodoptera exigua (Hübner) (Moar *et al.* 1995), *P. gossypiella* (Saunders), and *H. virescens* (Fabricius).

In maize, Bt-toxin expression declines as the plant mature after anthesis, thus creating a window for heterozygous survivorship. This decline is not important when insect pest does not affect the crop at this stage, as is the case of *Spodoptera frugiperda* (J. E. Smith) which cause maize damage on early development.

Bt-toxin expression on specific maize tissue will influence life expectancy of RS individuals. If the entire plant expresses the Bt-toxin, the selection pressure is exerted in all susceptible insects feeding there. Otherwise, the larvae have the choice to move toward tissues with low Bt-toxin expression and thus create the conditions for RS genotypes to survive.

Bt market penetration influences the overall Bt selection pressure by defining the percentage of the total target population that is affected. Growing areas with less than 50% of the acreage planted with the Bt-crop, have small risk for resistance. As Companies increases its efforts to introduce Bt-crops and farmers know the benefits, the acreage will increase in size and then the necessity to deploy more effective RM.

In Mexico, well in advance, the Ministry of Plant Protection estimate the total acreage for cotton crop in all permitted areas, and after revising companies' application, allow them to market certain percentage of Bt-cotton. However, availability of water or economic resources make important changes in the actual acreage of cotton. In consequence, the initial planned market penetration frequently increases, for example from 50 to 95%; thus, increasing the risk for resistance development.

The main function of the refuge is to provide enough SS individuals to mate with the Bt-selected ones (Alstad and Andow 1995, Roush 1996, Andow and Hutchison 1998, Gould 1998). This role could change as the refuge is contaminated by Bt-resistant insects; in consequence the actual role should also be monitored.

The estimate refuge size ranges from 5 to 60% (Ostlie *et al.* 1997), indicating the burden of defining the ideal balance between the economic burden of deploying a refuge and the effectiveness in delaying the onset of resistance.

It is difficult to convince farmers about the importance of using certain amount of their land to rear insect pests, arguing that this is an effective way to face a problem that does not exist at that time (Pilcher and Rice 1998). A number of computer models have been explored to evaluate the impact of refuge size on the potential of Bt-resistance (Tabashnik 1990) and achieve a consensus between risk perception and scientific data. The situation becomes more complicated when more than one Bt-crop sharing the same pest, coexist in the same growing area.

In Bt-cotton growing areas of Mexico, two Bt-cotton:refuge ratio are used: 80:20 and 96:4. In the first option, for each 80 acres of Bt-cotton, farmers must deploy 20 acres of non Bt-cotton; target pests in the refuge can be chemically controlled except with Bt-toxins. When choosing the 96:4 option, four acres of non Bt-cotton must be planted for 96 acres of Bt-cotton and the refuge is kept unsprayed. Each farmer decides the best option and the 96:4 option is becoming more popular.

Depending on its size, the refuge can be sprayed or remain unsprayed. Since the population density is abated when the refuge is treated, its size should be bigger enough to produce the same amount of SS-individuals as the untreated one. However, number of SS-individuals is not the only important factor involved. In consequence, is of paramount importance to consider the impact of conventional insecticides on fitness among surviving individuals in the treated refuge. More research is needed to clarify the importance of spraying the refuge.

There are two types of refuges: structured and unstructured. Structured refuges are planned and arrayed on the landscape in such a way to they ensure a significant source of SS-individuals to mate with Bt-selected ones, and dilute the level of resistance. The unstructured refuge is constituted by the whole range of non Bt-plants in or surrounding the agricultural area; it includes weeds, other non Bt-crops, and wild host plants species.

The synchrony between refuge and Bt-crops represents one of the biggest concerns about the usefulness of this strategy. Most of the time, a refuge is conceived as an area with the following features: does not express Bt-toxins, is planted simultaneously with the Bt-crop, is close to the Bt-crop and is composed by the same cultivar. These features do not guarantee similarities in emergence between Bt-selected and unselected individuals.

The problem is critical when the target population has only one generation per crop season. This concern is supported by the fact that Bt-selected individuals are less fit and hence take longer time to become adults. It this is the case, farmers would be wasting time using refuge with the sole purpose of rearing insects.

More research is needed to estimate the amount of SS-insects produced in the refuge relative to the Bt-crop. According to the U. S. EPA (1998a) a 500:1 ratio of unselected to Bt-selected target pest is adequate to slow resistance.

As resistance to certain Bt-toxins develop, the availability of new Bt-toxins without cross resistance will be greatly appreciated. Companies are investing huge amount of money and scientific effort to find new genes with potential to be inserted in important crops. However this strategy is of limited importance when there is a high risk of field resistance. In consequence RM must not relay on the availability of new genes.

Cross resistance exists when protection against two or more selecting agents is conferred by a single mechanism of resistance. Unfortunately, selection with one Bt-toxin may produce cross resistance to others (Gould *et al.* 1992, McGaughey and Johnson 1992, Moar *et al.* 1995, Tabashnik *et al.* 1996). The level of cross resistance could also change depending on the target species; for example the cry1A(b) and the cry9c toxins act independently on the European corn borer but not on corn earworm or fall armyworm (Lambert *et al.*1996).

Knowledge about cross resistance among Bt-toxins may provide insights about their use in rotation or mixture scheme; for example, there is no sense in rotating Bt-toxins with high degree of cross resistance. However, the use of a refuge and rotation of unrelated toxins on a large geographic area have the potential to delay resistance. Sequential use of Bt-toxins could be similar or better than mosaic deployment (Roush 1989, 1997a, b). If the target pest is not highly sensitive, single Bt-toxin, crops are at risk (Roush 1997b, Gould and Tabashnik 1998). In absence of refuge, none of these strategies will be useful.

Stack Bt-genes deployment could be better than single Bt-genes because of the redundant killing (Curtis 1985, 1987; Mani 1985, Comins 1986, Taylor 1986); an insect able to survive to toxin “A” will be killed by toxin “B” and vice versa (Mani 1985).

The field efficacy of Bt-toxin influences the evolution of resistance. If the efficacy is high enough to kill heterozygous individuals, resistance will slow if the population is fully susceptible, otherwise it will speed up.

Social and economic factors also play an important role in the evolution of resistance. Some farmers reject the use transgenic crops, thus helping to preserve susceptible Bt-genes that are valuable for organic farming; these farmers understand that the use of Bt-crops is not the only way to produce healthy crops. This attitude must be respected and avoid the idea to convince them that by the fallacy that “to be a modern farmer, a transgenic crops is required”.

Growers’ acceptance is closely related to Bt-crop profitability; a Bt-crop unable to provide an acceptable money return is unlikely to share an important plant protection market. The risk of resistance exists when the transgenic crop reduces substantially the cost of pest management and hence its market penetration make possible to impose a significant selection pressure over a large geographic area.

The level of social organization influences the efficacy of refuges. Before planting, growers must sign an agreement with the respective company. In this agreement, farmers acquire the responsibility to deploy on site refuge. If the social organization is strong, it is easy to convince them about the importance of refuge as a RM strategy and as a consequence its deployment is not a big deal.

It is important to device simple and efficient RM strategies, otherwise it will be difficult to understand and deploy them. Companies must consider that farmers are not pesticide resistance experts and that they are not willing to waste time on something that is too complicated.

Dealing with few large scale farmers is easier than dealing with a lot small scale ones. Large scale farmers are, in general, more sensitive to new technology and prone to used it once profitability is demonstrated.

Implications of evolution of resistance to *Bacillus thuringiensis* toxins

In the Comarca Lagunera Region, Mexico, a dramatic reduction in insecticide intoxication cases have been observed after Bollgard® cotton were grown since 1996; indicating the benefits that are at risk if high levels of resistance develops to Bt-crops. To minimize the risk, evolution of resistance to Bt-toxins are closely monitored and effective RM strategies implemented wherever a Bt-crop in grown. In consequence remedial actions can be taken before Bt-resistant reaches intolerable levels.

According to Roush and Shelton (1997), Bt-crops may reduce the use of conventional insecticides in the range of 50-60%. This value is as high as 90% in Comarca Lagunera Region, Mexico where Bollgard® cotton is extensively grown. It resistance to Bt-toxins develops and spreads over the range of the growing area, farmers would have to go back to conventional insecticides.

In the cotton growing area of Reynosa-Tamaulipas, Mexico, during 1960, more than 710, 000 acres of cotton were grown (Table 2), and a significant amount of labor was employed. Cotton was called “black gold” because of its economic importance. In order to achieve the higher possible yield, farmers applied increasing amounts of insecticides against *Heliothis virescens*: the main cotton pest in Reynosa-Tamaulipas. Plant protections costs represented from 30 to 40% of the total production cost. Cotton price went down, making unprofitable to grow it. As the problem became worst, acreage decreased; in 1971, only 493 acres of cotton were grown. Other low profitable crops were grown, such as sorghum and maize. Huge amount of farm workers were fired out; in consequence, delinquency and poverty increased notoriously.

Table 2. Changes in cotton acreage in the area of Matamoros, Tamaulipas, Mexico, influenced by low international cotton price and high plant protection cost associated with insecticide resistance in *Heliothis virescens* (Lagunes 1985)

Year	Acreage
1960	710, 466
1961	539, 400
1962	499, 614
1963	508, 879
1964	191, 713
1965	102, 567
1966	43, 938
1967	24, 170
1971	493
1972	12, 395
1973	37, 190

About 10 years later, cotton was grown again, reaching more than 148,000 acres in 1994; insect pests developed insecticide resistance faster than they did before. Fortunately, Bt-cotton was introduced in 1996 and farmers were confident to grow cotton again. During the time Bollgard® cotton is being grown, resistance to pyrethroids is declining (Table 3); the same trend is being observed for many organophosphorous and carbamate insecticides. This phenomenon is beneficial not only to cotton crop, but also to the rest of the crops that share the same pests with cotton, regardless if they are or not Bt-susceptible.

The cost of using Bollgard® technology in Tamaulipas, Mexico, during 2000, was about $67 U.S. Dollars and provided protection against the main Lepidopteran pests: *Heliothis virescens* (Fabricius)*, Spodoptera exigua* (Hübner), *Trichoplusia ni* (Hübner), and *Pseudoplusia includens* (Walker). Controlling those pests with conventional insecticides cost $178 U.S. Dollars; thus, going back to conventional cotton would increase crop protection by more than 150%.

Problems do not end here; returning to conventional insecticides would represent a great risk to the environment and human health. On average, in the South part of Tamaulipas, Mexico, about 24,000 acres of cotton, are grown every year; considering an average of 4 rounds of insecticides per crop season and about 0.2 liters of formulated insecticides per acre; on average 4,800 liters of formulated insecticides would be released to the environment, every year, in absence of Bollgard® cotton.

Most of the people involved in field pesticide applications are not aware of the risk of handling insecticides; thus, they do not take precautions during and after using them. We have encounter cases in which a person uses his hands to mix the pesticide with the water. No shower is taken after application and they touch their kids with contaminated hands and cloths; so, the family is also highly exposed to dangerous chemicals.

Aflatoxins and fumonisins are mycotoxins that can be fatal to livestock and are probably human carcinogens. Bt-corn experience significant less mycotoxins than their non-Bt counterparts (Munkvold and Desjardins 1997, Dowd and Munkvold 1999, Windham *et al.* 1999). Despite that under intense disease pressure, Bt-corn is unable to keep these toxins at low levels, it constitute a valuable tool to reduce the amount of carcinogens in stored corn grain.

Bt-toxins are also valuable tools for pest control in organic farming. The use of formulated Bt-insecticides allow to control many economic insect pests and does not lead to toxic residue on treated crop, thus contributing to produce safe food. This type of agriculture has a profitable market and do not accept Bt-crops. However, if resistance to Bt-toxins develops, the potential use of this pesticide will also decline. Fortunately, Bt-toxins are not the only chemical option for organic farming. Despite of that, they have the right to get the benefits of susceptibility to Bt-toxins.

Table 3. Changes in DL_{50} and resistance ratio to pyrethroids in a population of *Heliothis virescens* from South Tamaulipas, Mexico.

Insecticide	Year	DI_{50}[a] (µg/larvae)	RR50[b]
Cypermethrin	1991	0.084	5.2
	1994	0.620	38.7
	1995	1.092	68.2
	1997	0.313	19.5
	1998	0.097	6.0
	1999	0.148	8.4
Deltamethrin	1991	0.038	9.5
	1994	0.086	21.5
	1995	0.079	19.7
	1997	0.049	12.2
	1998	0.030	7.5

Bollgard® Cotton was introduced in 1996
[a] Data taken 72 h after treatment
[b]RR50 = DL50 field strain/DL50 susceptible strain

Resistance management in transgenic crops in the USA and elsewhere

Resistance management to Bt-toxins is supported mainly by the use of a high dose strategy and the existence of a refuge able to provide enough susceptible individuals to dilute resistance (Roush and Daly 1990, Gould 1994, Alstad and Andow 1995, Roush 1996, 1997, 1998, Andow and Hutchison 1998, Gould 1998).

A panel of experts made the recommendations included on table 2. The refuge size depends on the perceived or scientifically estimated risk. For example, when only one Bt-crop is grown, the suggested size refuge is around 5%, and 10% if the targeted pest is controlled with insecticides, except with Bt-formulations. When two or more Bt-crops coexists in a given area, refuge size should be increased to the double or more; however if the risk ranges from medium to high, a treated refuge will not mitigate the evolution of resistance.

High dose strategy: in a fully susceptible population there is one heterozygous individual for 1000 homozygous susceptible ones (Gould *et al.* 1995, 1997; Gould and Tabashnik 1998); under this circumstance it is improbable to find a single homozygous resistant genotype. Considering that resistance to Bt-toxins is partially or completely recessive (Tabashnik *et al.* 1994, Ferré *et al.* 1995), and inherited as a single gene (Tabashnik 1994, Gould *et al.*1995, Gould 1998), field resistance will be evident until the frequency of RR individuals is enough to make Bt-crop inefficient to control target pests.

Genetics studies are carried out to determine the inheritability of a resistant trait and estimate if resistance is recessive, partially dominant or dominant. However, it is possible to make resistance functionally recessive by increasing the Bt-toxin concentration. For example, in the figure 1, if titer B is applied, resistance is functionally recessive because SS and RS individuals are killed; titer A makes resistance dominant because RS and RR-individuals are able to survive to it. In the case of conventional insecticides, the required dose to make resistance functionally recessive could be too high for practical, economical and safety purposes. Fortunately, with Bt-crops is possible to eliminate 99% or all RS-genotypes to maintain its frequency at very low levels (Gould *et al.* 1997, Roush 1994, Metz *et al.*1995).

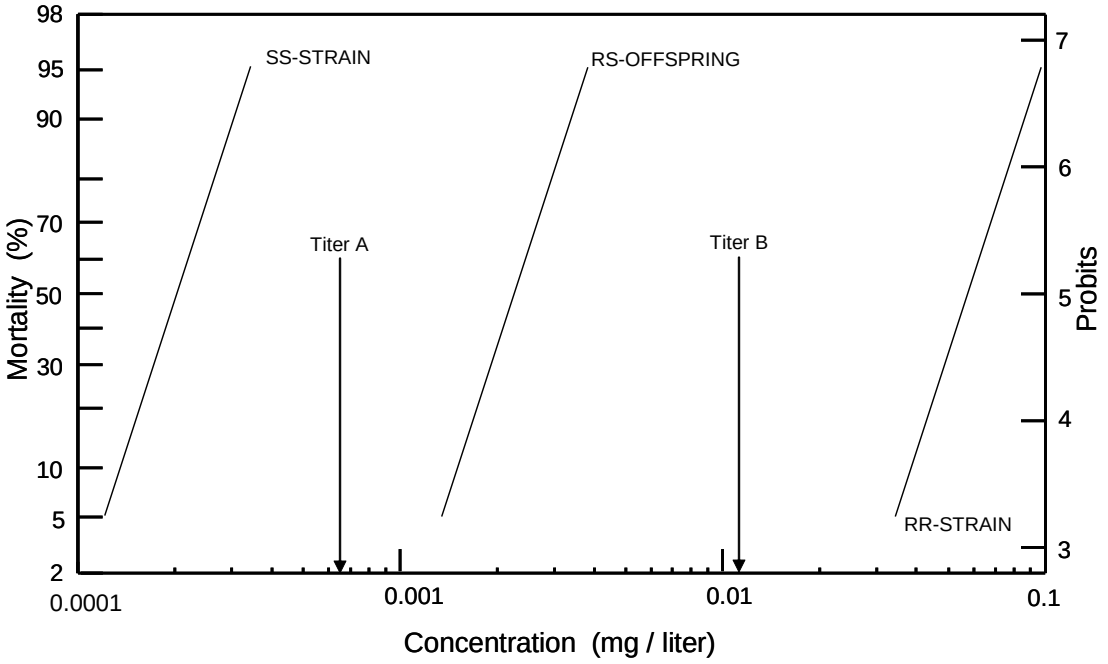

Figure 1. Dosage-response lines for SS, RS, and RR genotypes when resistance is recessive. Titer A kills most SS individuals, making resistance dominant. Titer B kills SS and RS individuals, making resistance functionally recessive

Resistance is well characterized until it develops, thus is difficult to ensure in advance that RS-genotypes would be killed by Bt-crops. According to U. S. EPA (1998), a high dose is defined as 25 times the concentration needed to kill all susceptible larvae. The problem still the same, however the consensus is that the higher the dose, the better.

Refuge: the main purpose of refuge is to provide enough SS-insects to mate with the RS-insects that probably emerge from the Bt-crop; SS-insects should outnumber SR-ones by a ratio of 500:1 (U. S. EPA 1998). To ensure this ratio, refuge must be adjacent to the Bt-crop (Peck *et al.* 1998), unless the target pests move long distances before mating as

happens with *Helicoverpa zea* (Boddie) (Anonymous 1999). Farmers must take direct responsibility for planting a refuge without relying on neighbors' non Bt-corn fields to mitigate Bt-resistance.

When other Bt-crops coexist, refuge size should increase from 5 to 10% if the perceived risk is low and remains unsprayed; if insect pests are controlled in the refuge, its size should increase from 10 to 20%. When risk is high and Bt-corn is the only insecticidal transgenic crop, the refuge size ranges from 40 to 80%, depending if they are unsprayed or not, respectively.

More research is needed to increase refuge efficiency and hence reduce its size; for example, perhaps we can use of other type of crop or increase plant density to sustain higher number of target insect pests.

Table 4. Refuge size recommendations (in % acreage) for Bt-crops

RISK	Bt–corn	Bt-corn + other Bt-crop
No concern	0	0
Low	5(10)	10(20)
Medium	20(40)	50(NR)
High	40(80)	60(NR)
Extremely high	100	100

The number in parenthesis indicates the recommendation for treated refuges (except with Bt-formulations)
Modified from Anonymous (1999).

It is a mistake to consider that refuge´s role will always be to provide susceptible individuals. Refuge is part of the same ecosystem and sooner or later it will be contaminated by resistant-genotypes and change its role in the evolution of resistance.

Conditions affecting resistance management plans in Mesoamerica

Deploying RM strategies is not an easy task, even in advanced agricultural systems. It requires excellent coordination among involved parts: government, farmers, consultants and companies. Government plays a central role to reinforce RM measures by imposing them as a condition to grow Bt-crops.

Mesoamerica is a mosaic of types of agriculture; some of them look like the modern U. S. agricultural systems and the conditions affecting RM plans are similar. Other group is constituted by small scale farmers that usually grow hybrids. They are unorganized and managing structured refuge is improbable; it is not feasible to inspect if refuge is deployed; in consequence, it is better to implement unstructured refuge. Unstructured refuge is

constituted by plots were non Bt-crops are grown, in addition to wild host plants in the agricultural area.

Small scale farming is represented by small plots, each of one to four acres, so it is possible to create a mosaic of Bt-crops and non Bt-crops; thus ensuring random mating between unselected and Bt-selected individuals. Since farmers growing non Bt-corn will control insect pests, the unstructured refuge is considered as sprayed. In consequence the adequate refuge size can be achieved by limiting the percentage of acreage available to Bt-corn, and increasing one category the perceived risk.

Suppose that a 10% refuge size is considered adequate because risk is low, there is no other Bt-crop, and the refuge is sprayed (Table 4). For small scale and unorganized farmers, the risk should be considered as medium and refuge size should increase to 40%. For practical purposes, at most only 60% of the total area should be allowed to grow with Bt-corn and avoid the burden of deploying a useless “structured refuge”. Lack of organization of many small scale farmers made extremely difficult to deploy a structured refuge close to Bt-crops. However, monitoring resistance must remain as a means of detecting resistant individuals as soon as possible.

Small scale farmers growing native maize constitute a major group of farmers in Mesoamerica. They have spent thousands of years improving maize to produce high yield under very stressing environmental conditions: drought, low or high temperatures, poor soil fertility, etc. These conditions are highly variable from place to place, even if they are relatively close to each other; thus in many cases they have selected the “best native maize” for each plot.

Bt-corn is improved in relation to one limiting factor: Bt-susceptible insect pests. In consequence, introducing Bt-corn to those areas would be a historic mistake because Bt-genes are unlikely to be inserted on each “best native maize” cultivar, and stressing environmental conditions will collapse corn yield.

Outline for resistance management for transgenic Mesoamerican maize

Aside from implementing high dose and refuge size discussed above, resistance must be surveyed. Monitoring the evolution of resistance is of paramount importance to detect early warning of pest adaptation (Ffrench-Constant and Roush 1990). The major challenge is having an effective, sensitive, simple, and quick essay method. Usually, surveillance of resistance is carried out by full bioassay, diagnostic dose and sentinel plots.

Since all Bt-selected populations should be monitored, it is important to define or get a consensus about population boundaries, and take samples for each one. Ideally, three samples should be taken: before, during and after Bt-crop is grown. Because of the difficulty to find enough individuals, sometimes is possible to take only one. More research is needed to determine the minimum amount of individuals to be collected and the sampling technique, to ensure that the sample adequately represents the population from where it was taken. We usually take samples from five distant places of the Bt-crop growing area and its individual number ranges from 100 to 1000 per sampling place, depending on the difficulty to find them. Biological samples are sent to the laboratory where they should be inspected to eliminate non target species and rear them to get the F1 generation to be used for evaluations. Sometimes mortality due to parasitoids and pathogens is very high and more samples are needed to do resistance studies.

Full bioassay: Sims *et al.* (1996) and Dulmage *et al.* (1976) provide the bioassay's methodology to obtain the ldp-line; any recognized method can be used. However, it is important to use the same one through time and for all the surveyed places; otherwise it will be impossible to make comparisons.

The first step consists on determining baseline susceptibility data for each Bt-susceptible pest before Bt-crop is grown. Bioassays are carried out, under lab conditions, with the purified Bt-toxin expressed by the respective transgenic insecticidal crop. At first, several well spaced dose are evaluated to find the window in which the cero and 100% response is found. Then several, usually from four to ten, concentrations are included to cover this range of response. Each concentration is replicated from three to five times at different days, and 10-32 neonate larvae are used for each replication per dose.

As a response, three variables are measured: percent mortality, number of larvae that reach third instar and percent of weight inhibition (stunting). To analyzing data, dose should be transformed to $Log_{10}(x+1)$ and response to probit units. Dose-response function of treatments is fit using probit analysis.

The base-line for several populations of *Heliothis virescens* from Tamaulipas, Mexico were obtained in 1996 (Table 5 to 7), the year Bt-cotton was introduced to that area. Mortality response to the cry1A(c) toxin, between field and susceptible population was similar (Table 5). DL50 values were from 13.53 to 15.79 μg cry1A(c)/mL water-agar, while DL95 oscillated between 280.3 to 527.3 μg cry1A(c)/mL water-agar. Resistance ratio, at 95% mortality (RR95), ranged from 0.65 to 1.13, indicating full susceptibility to this Bt-

toxin. However, mortality is not an important parameter because some larvae exhibits more than 99% reduction in weight and they are alive; larvae in this condition have not impact on crop production.

Table 5. Mortality response-line to the cry!A(c) toxin in Mexican populations of *Helicoverpa zea.* 1996.

Population	n	Slope± SE	CL_{50} [a] (95% CL)	CL_{95} [a] (95% CL)	χ^2	RR_{50} [b]	RR_{95} [b]
Costa	1440	1.32±0.06	15.79 (13.35-18.63)	280.30 (208.45-0398.71)	12.47	1.17	0.65
Gonzáles	1440	1.06±0.05	15.03 (12.36-18.24)	527.30 (359.25-0838.69)	04.29	1.11	1.21
Ponciano	1280	1.11±0.11	16.48 (10.34-26.25)	491.47 (182.04-1373.48)	18.57	1.22	1.13
Susceptible	1440	1.09±0.11	13.53 (08.47-21.57)	433.85 (164.61-1175.89)	23.80		

[a] μg of CryIA(c) toxin / mL water-agar
[b] $RR_{50(95)}$ = $DL_{50(95)}$ field strain / $DL_{50(95)}$ susceptible strain

The most important parameters to estimate the evolution of resistance are related to larval development under Bt-exposure: percent weight reduction (percent of stunting) and developmental inhibition (percent of Bt-exposed larvae that reach third instar).

As the Bt-concentration increases, less percentage of treated larvae reach the third instar. It is estimated that insect adaptation to Bt-toxins is highly correlated with this parameter. The importance to survey Bt-response before Bt-crop is deployed consists on knowing the range of susceptibility variation non-associated to the expression of R-genes. Through time we can determine how the response of field populations gets away from its susceptible counterpart.

In our studies on *H.* zea, the WI50 (median weight inhibition) for the susceptible strain was 0.002 μg cry1A(c)/mL water-agar; indicating the Bt-concentration required to inhibit 50% of larval weigh in relation to the untreated control. The WI50 values were pretty similar in all tested strains (Table 6). The RR95 values were between 0.2 and 0.8, showing no difference when compared with the susceptible population.

Table 6. Percent of stunting-response line to the cry!A(c) toxin in Mexican populations of *Helicoverpa zea.* 1996.

Population	n	Slope±SE	WI_{50} (95% CL)[a]	WI_{95} (95% CL)[a]	χ^2	RR_{50}[b]	RR_{95}[b]
Costa	1120	0.62±0.04	0.002 (0.001-0.004)	0.924 (0.29-3.01)	20.00	1.0	0.80
Gonzáles	1120	0.80±0.05	0.002 (0.001-0.004)	0.238 (0.10-0.59)	18.90	1.0	0.20
Ponciano	1120	0.66±0.02	0.003 (0.002-0.003)	0.816 (0.59-1.18)	07.70	1.5	0.70
Susceptible	1120	0.58±0.02	0.002 (0.001-0.002)	1.215 (0.83-1.86)	07.10		

[a] μg of CryIA(c) toxin / mL water-agar
[b] $RR_{50(95)} = DL_{50(95)}$ field strain / $DL_{50(95)}$ susceptible strain

The median developmental inhibition (DI50) for the susceptible strain was 0.004 μg cry1A(c)/mL water-agar; in other words 0.004 μg cry1A(c)/mL water-agar is needed to prevent 50% of treated larvae from reaching third instar, in relation to the untreated control.

We expect that, sooner or later, WI50 and WI95 values between field and susceptible populations will be different, indicating an increased ability of the field population to develop under Bt-toxin pressure. In the studied populations, RR95 values were from 2.8 to 5.30 (Table 7); this variation is not important and perhaps indicates very small differences in the genetic make up of each evaluated strain and differential level of adaptation to laboratory conditions. It is a relatively common mistake to associate with resistance any difference between susceptible and field strain; for this reason it is better to use the term “relative response”, rather than “resistance ratio”, when comparing those strains.

Table 7. Developmental inhibition response-line to the cry!A(c) toxin in Mexican populations of *Helicoverpa zea.* 1996.

Population	n	Slope±SE	DI_{50} (95% CL)[a]	DI_{95} (95% CL)[a]	χ^2	RR_{50}[b]	RR_{95}[b]
Costa	1120	0.66±0.04	0.01 (0.010-0.021)	4.68 (2.47-10.33)	05.20	02.5	5.30
Gonzáles	0968	0.93±0.06	0.04 (0.031-0.056)	2.53 (1.57-04.60)	04.60	10.0	2.80
Ponciano	1120	0.90±0.06	0.04 (0.023-0.052)	2.56 (1.55-04.86)	08.90	10.0	2.90
Susceptible	1120	0.71±0.04	0.004 (0.003-0.006)	0.89 (0.51-01.74)	03.60		

[a] μg of CryIA(c) toxin / mL water-agar
[b] $RR_{50(95)} = DL_{50(95)}$ field strain / $DL_{50(95)}$ susceptible strain

Response of a Mexican population of *Spodoptera frugiperda* (J. E. Smith) to the cry2Ab toxin was determined before Bollgard® II was deployed for the first time in Tamaulipas, Mexico. It was not possible to increase mortality beyond 57%, despite of using a concentration as high as 100 μg of cry2Ab toxin /mL water-agar; at this Bt-toxin concentration, weight inhibition was above 99% in both strains (Table 8).

In both susceptible and field strain no larvae reached third instar beyond the concentration of 5 μg of cry2Ab toxin /mL water-agar. In consequence, this concentration could be used as a diagnostic dose.

Table 8. Mortality, percent of stunting and developmental response to the cry2Ab toxin in a susceptible and a field Mexican population of *Spodoptera frugiperda* (J. E. Smith).

	POPULATIONS					
	TAMAULIPAS			**SUSCEPTIBLE**		
μg/mL	A	B	C	A	B	C
Control	0.00	97.50		0.00	95.63	
0.001	1.25	93.75	13.33	1.88	91.88	11.20
0.005	0.63	88.13	25.28	1.25	90.00	17.31
0.01	1.25	71.25	47.60	2.50	70.63	45.29
0.05	1.25	50.63	62.13	1.88	45.00	68.56
0.1	2.50	25.00	86.29	2.50	21.25	86.39
0.5	6.88	4.38	93.64	5.00	05.00	95.29
1	11.25	0.63	96.64	12.50	0.63	98.19
5	21.88	0.00	97.46	18.75	0.00	98.58
10	30.63	0.00	98.32	30.00	0.00	99.05
50	40.00	0.00	98.97	44.38	0.00	99.54
100	48.75	0.00	99.19	56.88	0.00	99.69

A = % mortality after 7 days of toxin treatment
B = % larvae that reached third instar
C = % stunting

Diagnostic dose: one or more diagnostic doses can be used to screen for resistance. The use of diagnostic dose has two main advantages over full bioassays: avoid using non-informative concentrations and it is more sensitive because sample size is larger. The methodology to expose insects to the toxins is the same as in full bioassay. About 100 larvae are used per replication and five replications are carried out on different days. At least 500 larvae are used per diagnostic dose. Differences among evaluated populations can be estimated by ANOVA and a multiple comparisons tests. Sometimes a variable transformation is required to get normal data distribution and hence apply the procedures of parametric statistics.

Sentinel plots involves sampling Bt-crops as wells as non Bt-crops for target larvae infestation, in order to detect early field pest adaptation to Bt-toxins. It is quite possible to find patches of high levels of infestation; if this is the case, the first taken action must be to determine the type of species involved. Quite frequently a non Bt-susceptible species increases its density and could give a false idea of ineffective Bt-crop. However, if larvae infestation is constituted by a Bt-target species, it is important to verify that the crop is a Bt-crop; samples of those individuals should be taken to the lab to carry out resistance studies.

The worst scenario would occur when the infested patch is composed of Bt-target individuals feeding successfully on a Bt-crop. Undoubtedly this an emergency and the best conventional insecticide should be applied not only to kill larvae from the patch but also to the surrounding area.

The best strategy to manage resistance in Mesoamerica consists on implementing government regulation on the use of Bt-crops. This agency must analyze all applications to deploy commercial Bt-crops and based on the estimated risk, require all the necessary biosafety and resistance measures. Since all decisions must be scientifically supported, scientist related to Bt-crops must be involved. Scientists and government should design the adequate RM to deploy, and delegate monitoring for resistance activities to trained people. It is highly desirable to have a good coordination among involved groups: farmers, companies, scientists and government.

Field efficacy trials should be carried out with Bt-crops in order to ensure that farmers would get benefits by deploying a useful control measure. The European corn borer is one of the main insect pest in the USA and Europe which does not occur in Central America,

where the fall armyworm is extremely important (Hruska 1996). It makes not sense to grow a useless Bt-crop.

Biotechnology represents an enormous risk for developing countries and this risk consists on remaining off this scientific area.

LITERATURE CITED

Alstad, D. N. and D. A. Andow. 1995. Implementing management of insect resistance to transgenic crops. AgBiotech news 8:177-181.

Andow, D. A. and W. D. Hutchinson. 1998. *Bt* corn resistance management. Now or never: serious new plans to save a natural pest control. Union of Concerned Scientist. M. Mellon and J. Rissler (Eds.).Cambridge, MA.

Anonymous. 1999. An evaluation of insect resistance management in Bt field corn: a science-based framework for risk assessment and risk management. Report of an Expert Panel. November 23, 1998. International Life Science Institute – Health and Environmental Sciences Institute. U.S.A.

Cannon, R. J. C. 1993. Prospects and progress for *Bacillus thuringiensis*-based pesticides. Pest. Sci. 37:331-335.

Comins, H. N. 1986. Tactics for resistance management using multiple pesticides. Agric. Ecosyst. Environ. 16:129-148.

Comins, H. N. 1977a. The development of insecticide resistance in the presence of migration. J. Theoret. Biol. 64:177-197.

Comins, H. N. 1977b. The management of pesticide resistance. J. Theoret. Biol. 65:399-420.

Curtis, C. F. 1985. Theoretical models of the use of insecticide mixtures for the management of resistance. Bull. Entomol. Res. 75:259-265.

Curtis, C. F. 1987. Genetic aspects for selection for resistance pp. 150.161. *In*: Combating resistance to xenobiotics: biological and chemical approaches. M. G. Ford, D. W. Hollman, B. P. S. Khambay, and R. M. Sawacki. Horwood, Chinchester, England.

Dowd, P. F. and G. P. Munkvold. 1999. Associations between insect damage and fumosin derived from field-based insect control strategies. Proceedings of the 40th Annual Corn Dry Milling Conference. June 3-4, 1999. Peoria, IL.

Dulmage, H. T., A. J. Martínez, and T Peña. 1976. Bioassay of *Bacillus thuringiensis* (Berliner) delta-endotoxin using the tobacco budworm. Tech. Bull. No. 1528. U. S. Dept. Agr. Res. Serv.

Feldman, J. and T. Stone 1997. The development of a comprehensive resistance management plan for potatoes expressing the cry3A endotoxin, pp 49-61. *In:* advances in insect control: the role of transgenic plants. N. Carozzi and M. Koziel (ed). London: Taylor and Francis.

Ferré, J., B. Escriche, Y. Bel., and J. Van Rie. 1995. Biochemistry and genetics of insect resistance to *Bacillus thuringiensis* insecticidal crystal proteins. FEMS Microbiol. Lett. 132:1-7.

Ffrench-Constant, R. H. and R. T. Roush. 1990. Resistance detection and documentation: the relative roles of pesticidal and biochemical assays, pp 4-38. *In*: Pesticide resistance in arthropods. R. T. Roush and B. E. Tabashnik (Eds.). Chapman and Hall, New York.

Fischhoff, D. A. 1996. Insect-resistant crop plants, pp. 214-227. *In*: Biotechnology and integrated pest management. CAB International, Wallingford, UK.

Fitt, G. P., C. L. Mares, and D. J. Llewellyn. 1994. Field evaluation and potential ecological impact of transgenic cottons (*Gossypium hirsutum*) in Australia. Biocont. Sci. and Tech. 4:535-548.

Gasser, C. S. and R. T. Fraley. 1989. Isolation of tissue specific cDNAs from tomato pistils. Plant Cell 1:15-24.

Georghiou, G. P. 1981. The occurrence of resistance to pesticides in arthropods: an index of cases reported through 1980. Rome: Food and Agriculture Organization of the United Nations.

Georghiou, G. P., and C. E. Taylor. 1977. Genetic and biological influences in the evolution of insecticide resistance. J. Econ. Entomol. 70:319-323.

Georghiou, G. P., and C. E. Taylor. 1986. Factors influencing the evolution of resistance, Pp. 157-169. *In*: Pesticide resistance: strategies and tactics for management. National Academy Press. Washington, D. C.

Gill, S. S., E. A. Cowles, and P. V. Pietrantonio. 1992. The mode of action of *Bacillus thuringiensis* endotoxins. Ann. Rev. Entomol. 37:615-636.

Gould, F. 1986. Simulation models for predicting durability of insect-resistant germ plasm: Hessian fly (Diptera: Cecidomyiidae)-resistant winter wheat. Environ. Entomol. 15:11-23.

Gould, F. 1994. Potential and problems with high-dose strategies for pesticidal engineered crops. Biocont. Sci. Technol. 4:451-461.

Gould, F. A. 1998. Sustainability of transgenic insecticidal cultivars: integrating pest genetics and ecology. Ann. Rev. Entomol. 43:701-726.

Gould, F. A., A. Anderson, A. Jones, D. Sumerford, D. Heckel, J. Lopez, S. Micinski, R. Leonard, and M. Laster. 1997. Initial frequency of alleles for resistance to *Bacillus thuringiensis* toxins in field populations of *Heliothis virescens*. Proc. Natl. Acad. Sci. U.S.A. 94: 3519-3523.

Gould, F. A., A. Anderson., A. Reynolds, L. Bumgarder, and W. Moar. 1995. Selection and genetic analysis of a *Heliothis virescens* (Lepidoptera: Noctuidae) strains with high levels of resistance to *Bacillus thuringiensis* toxins. J. Econ. Entomol. 88:1545-1559.

Gould, F. A., A. Martínez-Ramírez, A. Anderson, J. Ferré, F. J. Silva, and W. J. Moar. 1992. Broad-spectrum resistance to *Bacillus thuringiensis* toxins in *Heliothis virescens*. Proc. Natl. Acad. Sci. U.S.A. 89:7986-7990.

Gould, F. and B. E. Tabashnik. 1998. Bt-cotton resistance management, pp.67-105. *In*: Now or never: serious new plans to save a natural pest control. M. Mellon and J. Rissler (Eds.). Cambridge, MA: Union of Concerned Scientist.

Gould, F., G. G. Kennedy, and M. T. Johnson. 1991. Effects of natural enemies on the rate of herbivore adaptation to resistant host plant. Entomol. Experimentalis et applicata 58:1-14.

Höfte, H. and H. R. Whitely. 1989. Insecticidal crystal proteins of *Bacillus thuringiensis*. Microbiol. Rev. 53:242-255.

Hokkanen, H. M. and C. H. Wearing. 1994. The safe deployment of *Bacillus thuringiensis* genes in crop plants: conclusions and recommendations of OECD workshop on ecological implications of transgenic crops containing Bt-toxin genes. Biocontrol Sci. Technol. 4:399-404.

Hruska, A. J. 1996. Transgenic crops in Central American agriculture. Biotechnol. And develop. Monitor 29, p. 79

Jansens, S., A. Van Vliet, C. Dickburt, C. Buysse, C. Piens, B. Saey, A. De Wulf, A. Paez, E. Gobel, and M. Peferoen. 1997. Field evaluation of transgenic corn expressing a Cry9C insecticidal protein from *Bacillus thuringiensis*, protected from European corn borer. Crop. Sci. 37:1616-1624.

Knowles, B. 1994. Mechanisms of action of *Bacillus thuringiensis* insecticidal endotoxins. Adv. Insect. Physiol. 24:275-308.

Koziel, M. K., F. L. Belang, C. Bowman, N. B. Carozzi, R. Crenshaw, L. Crossland, J. Dawson, N. Desai, M. Hill, S. Kadwell, K. Launis, K. Lewis, D. ;addox, K. McPherson, M. R. Mefhji, E. Merlin, R. Rhodes, G. W. Warren, M. Wright, and S. Evola. 1993. Field performance of elite transgenic maize plants expressing an insecticidal protein derived from *Bacillus thuringiensis*. Biotechnology 11:194-200.

Lagunes, T. A. 1985. Perspectivas en el uso de insecticidas agrícolas en México pp. 1-22. *In:* Temas selectos de manejo de insecticidas agrícolas en México. Colegio de Postgraduados. A. Lagunes and J. C. Rodríguez (Eds.). México.

Lambert, B., L. Buysse, C. Decock, S. Jansens, C. Piensn B. Saey, J. Seurinck, K. Van Audenhove, J. Van Rie, A van Vliet, and M. Peferoen. 1996. A *Bacillus thuringiensis* insecticidal crystal protein with a high activity against members of the family Noctuidae. Appl. Environ. Microbiol. 62:80-86.

Mallet, J., and R. Porter. 1992. Preventing insect adaptation to insect-resistant crops: are seed mixtures or refugia the best strategy? Proc. Soc. London Ser B 250:165-169.

Mani, G. S. 1985. Evolution of resistance in the presence of two insecticides. Genetics 109:761-783.

McGaughey, W. H. and M. E. Whalon. 1992. Managing insect resistance to *Bacillus thuringiensis* toxins. Science 258:1451-1455.

Mckensie, J. A. 1996. Ecological and evolutionary aspects of insecticide resistance. Academic Press, Austin, TX. 185p.

Meeusen, R. L. and G. Warren. 1989. Insect control with genetically engineered crops. Ann. Rev. Entomol. 34:373-381.

Metz, T. D., R. T. Roush, J. Tang, A. M. Shelton, and E. D. Earle. 1995. Transgenic broccoli expressing a *Bacillus thuringiensis* insecticidal crystal protein: implications for pest resistance management strategies. Mol. Breeding 1:309-317.

Moar, W. J., M. Pusztai-Carey, H. van Faassen, D. Bosch, R. Frutos, C. Rang, K. Luo, and M. J. Adang. 1995. Development of *Bacillus thuringiensis* Cry1C resistance by *Spodoptera exigua* (Hübner) (Lepidoptera: Noctuidae). Appl. Environ. Microbiol. 61:2086-2092.

Munkvold, G. P. and A. E. Desjardins. 1997. Fumonisins in maize: can we reduce their occurrence? Plant Diseases 81:556-565.

Ostlie, K. R., W. D. Hutchinson, and R. L. Hellmich. 1997. Bt-corn abd European corn borer: long-term success through resistance management. North Central Reg. Ext. Publ. Univ. Minn. Ext. Serv., St. Paul, MN.

Perlak, F., T. B. Stone, Y. M. Muskopf, L. J. Petersen, G. B. Parker, S. A. McPherson, J. Wyman, S. Love, D. Biever, G. Reed, and D. Fischhoff. 1993. Genetically improved potatoes; protection from damage by Colorado potato beetles. Plant Molecular. Biol. 22:313-321.

Pilcher, C. D. and. M. E. Rice. 1998. Management of European corn borer (Lepidoptera: Noctuidae) and corn rootworms (Coleoptera: Chrysomelidae) with transgenic corn: a survey of farmer perceptions. Am. Entomol. 44:36-44.

Roush, R. T. 1989. Designing resistance management programs: How can you choose? Pestic. Sci. 26:423-441.

Roush, R. T. 1994. Managing pests and their resistance to *Bacillus thuringiensis*: can transgenic crops be better than sprays? Biocontrol Sci. Technol. 4:501-516.

Roush, R. T. 1996. Can we slow adaptation by pests to insect resistant transgenic crops pp. 242-263. *In*: Biotechnology and integrated pest management. G. J. Persley (Ed.). University Press, Cambridge, England.

Roush, R. T. 1997a. Managing resistance to transgenic crops, pp. 271-294.*In*: Advances in insect control: the role of transgenic plants. N. Carozzi and M. Koziel (Ed.). London: Taylor and Francis.

Roush, R. T. 1997b. Bt-transgenic crops: just another pretty insecticide or a chance for a new start in resistance management? Pestic. Sci. 51:328-334.

Roush, R. T. and A. M. Shelton. 1997. Assesing the odds; the emergence of resistance to Bt transgenic plants. Nature Biotech. 15:816-817.

Roush, R. T., and G. L. Miller. 1986. Considerations for design of insecticide resistance monitoring programs. J. Econ. Entomol. 79:293-298.

Roush, R. T., and J. C. Daly. 1990. The role of population genetics in resistance research and management, pp. 97-152.*In*: Pesticide resistance in arthropods. R. T. Roush and B. E. tabashnik (Eds.) Chapman and Hall, New York.

Starnes, R. L., C. L. Liu, and P. G. Marrone. 1993. History, use, and future of microbial insecticides. Am. Entomologist 39:83-91.

Sims B. S., J. T. Greenplate, T. B. Stone, M. A. Caprio, and F. L. Gould. 1996. Monitoring strategies for early detection of Lepidoptera resistance to *Bacillus thuringiensis* insecticidal proteins, pp.229-242. Molecular genetics and evolution of pesticide resistance. T. M. Brown (Ed.) American Chemical Society.

Tabashnik, B. E. 1990. Modeling and evaluation of resistance management tactics, pp. 153-182. *In*: Pesticide resistance in arthropods. R. T. Roush and B. E. Tabashnik (Eds.) Chapman and Hall, New York.

Tabashnik, B. E. 1994. Evolution of resistance to *Bacillus thuringiensis*. Ann. Rev. Entomol. 39:47-79.

Tabashnik, B. E., L. Yong-Biao, T. Malvar, D. G. Heckel, L. Masson, and J. ferré. 1999. Insect resistance to *Bacillus thuringiensis*: uniform or diverse? pp 75-80. *In:*Insecticide resistance: from mechanisms to management. I. Denholm, J. A. Pickett, and A. L. Devonshire (edit.). CABI- Publishing – The Royal Society. UK.

Tabashnik, B. E., T. Malvar, Y. B. Liu, N. Finson, D. Borthakur, B. S. Shin, S. H. Parks, L. Masson, R. A. de maagd, and D. Bosh. 1996. Cross resistance of the diamondback moth indicates altered interactions with domains II of *Bacillus thuringiensis* toxins. Appl. Environ. Microbiol. 62:2839-2844.

Tabashnik, B. E., Y. B. Liu, N. Finson, L. Masson, and D. G. Heckel. 1977a. One gene in diamondback moth confers resistance to four *Bacillus thuringiensis* toxins. Proc. Natl. Acad. Sci. U.S.A. 94:1640-1644.

Tabashnik, B. E., Y. B. Liu., T. Malvar, D. G. Heckel, L. Masson,, V. Ballester, F. Granero, J. L. Mensua, and J. Ferré. 1997b. Global variation in the genetic and biochemical basis of diamondback moth resistance to *Bacillus thuringiensis.* Proc. Natl. Acad. Sci. U.S.A. 94:12780-12785.

Tang, J. D., S. Gilboa, R. T. Roush, and A. M. Shelton. 1997. Inheritance, stability, and lack-of-fitness costs of field-selected resistance to *Bacillus thuringiensis* in diamondback moth (Lepidoptera: Plutellidae) from Florida. J. Econ. Entomol. 90:732-741.

Taylor, C. E. 1986. Genetics and evolution of resistance to insecticides. Biol. J. Linn. Soc. 27:103-112.

U. S. Environmental Protection Agency. 1998. FIFRA Scientific Advisory Panel, Subpanel on *Bacillus thuringiensis* (Bt) Plant Pesticides and Resistance Management. February 9-10, 1998. (Docket Number: OPP 00231).

Weinzierl, R., C. Pierce, and K. Steffy. 1997. Preliminary results of the 1997 summer survey for Bt resistant European corn borers. Pest. Manag. Crop. Dev. Bull. 22:183-184.

Whitten, M. J., and J. A. McKensie. 1982. The genetics basis for pesticide resistance, Pp, 1-16. *In*: Proc. 3rd Australas. Conf. Grassl. Invert. Ecol. K. E. lee (Ed.) Adelaide, Australia: S. A. Government Printer.

Windham, G. L., W. P. Williams, and F. M. Davis. 1999. Effects of the southwestern corn borer on *Aspergillus flavus* kernel infection and aflatoxin accumulation in maize hybrids. Plant Diseases 83: 535-540.