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Sustainability of insect resistance management strategies for transgenic Bt corn

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Abstract

Increasing interest in the responsible management of technology in the industrial and agricultural sectors of the economy has been met thorough the development of broadly applicable tools to assess the "sustainability" of new technologies. An arena ripe for application of such analysis is the deployment of transgenic crops. The new transgenic pesticidal or plant-incorporated protectant (PIP) crops have seen widespread application in the United States based on the features of higher yield, lower applications of insecticides, and control of mycotoxin content. However, open rejection of these new crops in Europe and in other countries has been a surprising message and has limited their worldwide acceptance. The US Environmental Protection Agency's (USEPA) Office of Pesticide Programs (OPP) has worked on the development and analysis of insect resistance management (IRM) strategies and has mandated specific IRM requirements for *Bacillus thuringiensis* (Bt) crops since 1995 under the Food, Fungicide, Insecticide, and Rodenticide Act. Improvement of data quality and sustainability of IRM strategies have been targeted in an ongoing partnership between the USEPA Office of Research and Development and the Office of Pesticide Programs that will further enhance the agency's ability to develop sustainable insect resistance management strategies for transgenic field corn (Bt corn) producing *B. thuringiensis* (Bt) insecticidal proteins.

Keywords: Plant-incorporated protectants; Transgenic crops; *Bacillus thuringiensis*; Bt corn

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1. Introduction

Agricultural biotechnology is beginning to show remarkable success in the deployment of new crops (Krimsky and Wrubel, 1996). In 2001, the US grew 35.7 million hectares (68% of total global transgenic crops), which is an increase of 18% over 2000 (James, 2001). More than half of the 72 million acres (29.9 million hectares) of soybeans and significant percentages of the 80 million acres (33 million hectares) of corn and 13 million acres (5.4 million hectares) of cotton in the US are planted in transgenic insect-resistant crops (James, 2001). The new transgenic cultivars are mainly genetic constructs formulated from selected non-transgenic stocks and genetic elements derived from the ubiquitous soil microorganism, *Bacillus thuringiensis* (Bt) (Boulter, 1993; Jouanin et al., 1998; Mazier et al., 1997; Meeusen and Warren, 1989). The genetic elements from Bt are selected for their expression of protein toxin activity as larvicides for crop pests.

Documentation of Bt insect resistance was first reported for *Plodia interpunctella* (Hübner) (Indian meal moth) in stored grain (McGaughy, 1985) and in the field, for *Plutella xylostella* (L.) (diamondback moth) (Tabashnik et al., 1990). Research with laboratory-selected colonies has demonstrated the possibility of substantial insect adaptation to Bt proteins (Alstad and Andow, 1995; Bauer, 1995; Bergelson et al., 1996; Forrester, 1994; Mallet and Porter, 1992; McGaughy and Whalon, 1992; Mellon and Rissler, 1998; Tabashnik, 1994a,b). Should insect resistance develop to Bt proteins expressed in transgenic corn or other transgenic crops and not mitigated, then the functional utility of Bt sprays and Bt crops will be lost. This scenario can be envisioned to require strategies for economic recovery of agricultural production that may involve the use of larger quantities of higher risk chemical-based conventional pesticides. Therefore, predicting the likelihood of resistance development and providing sustainable insect resistance management (IRM) strategies for crops such as Bt corn is critical to the sustainability and public acceptance of this new promising technology.

The search for answers in this debate lies in the concepts of a sustainable technology for agriculture and specific plans to implement controls over the use of Bt transgenic crops (Estruch et al., 1997; Gould, 1998a,b; Hall and Crowther, 1998; Hubbell and Welsh, 1998; Lewis et al., 1997; Nap et al., 2003; NRC [National Research Council], 2000; Riebe, 1999; Rissler and Mellon, 1996; Von Wiren-Lehr, 2001; Zechendorf, 1999). Clearly, insect resistance management falls within the general format of integrated pest management (Kogan, 1998; Levins and Wilson, 1980; Mumford, 1984; Stern et al., 1959; Way and van Emden, 2000).

Insect resistance management (IRM) is the term used to describe practices aimed at reducing the potential for insect pests to become resistant to a pesticide. IRM is important for transgenic crops expressing *B. thuringiensis* (Bt) insecticidal proteins (Bt crops) because insect resistance poses a threat to future use of microbial Bt pesticides and Bt technology as a whole. Academic and government scientists, public interest groups, organic and other farmers have expressed concern that the widespread planting of these genetically transformed plants will hasten the development of resistance to Bt endotoxins (Carlson et al., 1997; Lipson, 1999; Nelson, 2001; Williamson, 1996).

The aim of current US Environmental Protection Agency (USEPA) regulation and supporting research is to provide requirements for guidance and best management practices for IRM in Bt transgenic crops. These efforts will be organized into accessible forms such as protocols that provide a means to gain verifiable information useful to a more comprehensive understanding of insect resistance management options for transgenic crops, especially Bt corn. Part of this development effort will be devoted to the formulation of tools useful for the evaluation of the protocol requirements being sought. Gaps in the available predictive tools will be prioritized, and supplemental support will be sought for their development. As part of USEPA research efforts, the Office of Pesticide Programs (OPP) and the Office of Research Development (ORD) have forged a partnership to enhance the agency's ability to improve data quality and sustainability of insect resistance management strategies for Bt crops.

The USEPA is mandated to ensure that there will be no unreasonable adverse effects from the use of a pesticide when economic factors are taken into account under the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA). For Bt, in general, USEPA has stated that maintaining the susceptibility of Bt is in the "public good" (USEPA, 1998, 2001). By requiring specific IRM strategies for Bt crops, USEPA is working to the prevention of adverse effects such as the prohibition of Bt use because of insect resistance and replacement with more toxic compounds for the control of the insect pests. Sound IRM is expected to prolong the life of Bt pesticides, and adherence to the plans is to the advantage of growers, producers, researchers, and the public. The current strategy design to meet the challenge of insect resistance to Bt is twofold: (1) mitigate any significant potential for pest resistance development in the field by instituting IRM plans, and (2) develop a better understanding of the mechanisms behind pest resistance (USEPA, 2000b, 2001).

2. Perspective: global adoption of Bt crops

The total global area devoted to transgenic crops increased by 19% or 8.4 million ha between the 2000 and 2001 growing seasons (James, 2001). Herbicide tolerance was the dominant trait, with insect control traits following second (Table 1). This increase has been interpreted as reflective of increased political, policy, and institutional support for these crops with possible contribution due to global food security considerations. The higher adoption rates are assumed to reflect greater grower satisfaction with the crop benefits of greater returns commensurate with higher productivity and a safer operational environment.

Table 1
World distribution of transgenic crop planting (James, 2001)

Country	Area (10^6) [acres (ha)]	Crops
US	74.8 (31.0)	soybeans, corn, cotton, canola
Argentina	24.7 (10.2)	soybeans, corn, cotton
Canada	7.2 (2.3)	soybeans, corn, canola
China	1.2 (0.5)	cotton
South Africa	0.5 (0.2)	corn, cotton
Australia	0.44 (0.2)	cotton

Table 2
US plant incorporate protectant corn acreage (Fernandez-Cornejo and McBride, 2002)

Year	Area (10^6) [acres (ha)]	% total corn
1996	0.4 (0.2)	1
1997	4.4 (1.8)	8
1998	14.5 (6.0)	18
1999	19.8 (8.2)	26
2000	19.5 (8.1)	19
2001	19.5 (8.1)	19

Globally, the area planted with transgenic corn decreased by about 500,000 ha between the 2000 and 2001 growing seasons. The entire area associated with this decrease was in the United States (Table 2). Reasons for this change have been attributed to historically low European Corn Borer (ECB) infestation in the previous two growing seasons (USDA-ERS [US Department of Agriculture and National Agricultural Statistics Service], 2002a,b). Lack of global approval, especially in Europe, for Bt corn may have had an ancillary effect on the crop adoption level.

3. Sustainability

Sustainable development has been adopted as a major global environmental goal. The Brundtland Commission has enunciated the perspective that “Sustainable Development is development that meets the needs of the present without compromising the ability of future generations to meet their own needs” (WCED, 1987). The features of sustainability extend across multiple dimensions that have traditionally been evaluated separately but have enjoyed little cross comparison (Fig. 1). However, considerable debate ensues over the salient features to be considered for sustainable development (Costanza et al., 2000; Kates et al., 2001).

The 1990 US Farm Bill called for “an integrated system of plant and animal production practices having a site-specific application that will, over the long term: satisfy human food and fiber needs; enhance environmental quality and the natural resource base upon which the agricultural economy depends; make the most efficient use of nonrenewable resources and on-farm resources and integrate, where appropriate, natural biological cycles and controls; sustain the economic viability of farm operations; enhance the quality of life for farmers and society as a whole.” (USFB, 1990) The goal of agricultural sustainability must be the minimization of environmental impacts while sustaining production levels to meet society’s needs (Ruttan, 1999). The PIP crops have been designated as in the “public good” and as such can be considered to be environmental assets. The sustainability considerations for these crops can be narrowed to the consideration of the general extension of usefulness of lifetimes for these crops. Hence, IRM would presumably be part of agricultural sustainability of Bt corn and other Bt crops. The Conference on Environment and Development held in Rio de Janeiro espoused objectives for the environmentally sound management of biotechnology in terms of evaluating biotechnology for its place in the environment, engendering public trust and confidence, developing

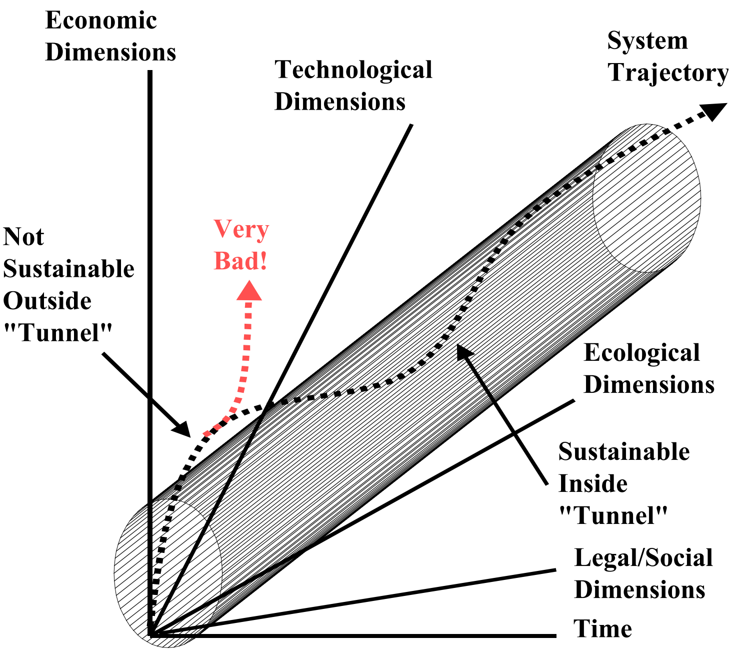


Fig. 1. Dimensions of sustainability.

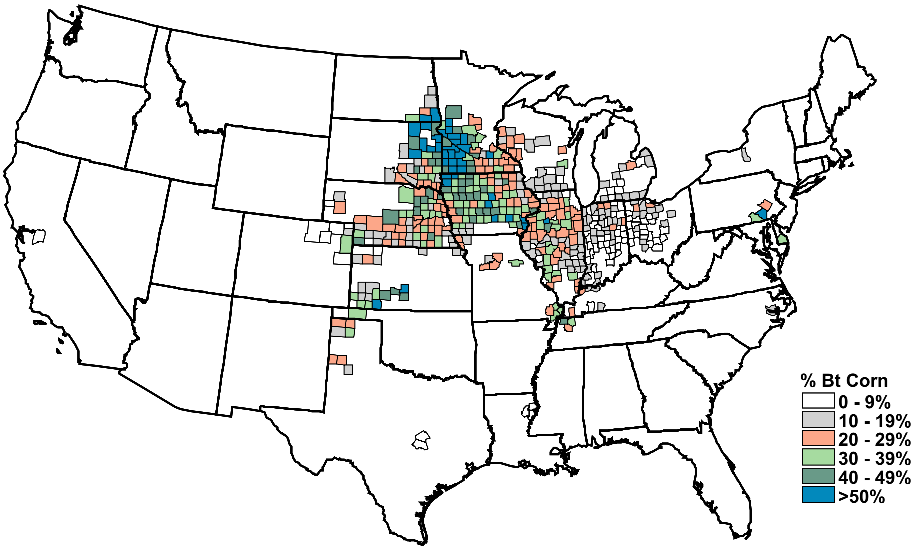
sustainable applications of biotechnology, and establishing mechanisms that enable biotechnology development (UNCED [United Nations Conference on Environment and Development], 1992).

3.1. *Differences of opinion*

The acceptability of this new form of biotechnology differs widely across the globe. The differences in philosophy as clearly portrayed when contrasting the positions of the European Union in contrast to the US. The organic production advocates continue their objection to PIP crops since it is their belief that the widespread use of a single Bt toxin will destroy the utility of Bt sprays that are part of the organic production pest control strategies. (Lipson, 1999; Wilkins, 2001; Young, 1994).

4. Bt corn hybrids

The introduction of transgenic hybrid corn varieties began in 1996 with the registration of Event 176 (Cry1Ab protein) in NatureGard and Knock*Out* products (Fig. 2). The protection offered by this event controlled first-generation insect larvae but only partial control for second-generation larvae. The late-stage larvae bore into the stalks and ear shanks to feed on the developing corn kernels. Event 176 hybrids containing the Cry1Ab PIP expressed in corn are no longer registered.



*** Percentage of total corn acreage in Bt corn hybrids based on counties having > 50,000 total acres of corn**

Fig. 2. 1999 distribution of PIP corn crop.

Two different Bt delta-endotoxins expressed in registered Bt PIPs, for lepidopteran control, produced in corn are Cry1Ab and Cry1F. Each of these corn hybrids produce slightly truncated versions of either the Cry1Ab delta-endotoxin or the Cry1F delta-endotoxin in contrast to the bacterial isolates from which they were derived. (USEPA, 2001). Current US registered Bt PIPs in corn that target a number of lepidopteran stalk-boring pests are listed in Table 3. The major stalk-boring corn pests controlled by current transgenic Bt corn hybrids are the European corn borer (CEB) *Ostrinia nubilalis* (Huebner), corn earworm (CEW) *Helicoverpa zea* (Boddie), and the secondary pests of southwestern corn borer (SWCB) *Diatraea crambidoides* (Grote), and fall armyworm (FAW) *Spodoptera frugiperda* (J.E. Smith) that is a sweet corn pest. ECB is one of the major corn insect pests in the United States. Its infestation can be characterized by stalk tunneling that leads to lodging, reduced nutrient and moisture flow, and reduced crop yield. In the US, the climate supports one (univoltine) to fur (multivoltine) generations of ECB across the North American latitudes extending from the Gulf Coast to the Canadian border (Mason et al., 1996).

Table 3
Plant incorporated protectant corn events registered in the US

Event	Trademark	Cry protein
Bt 11	Attribute (sweet corn)	Cry1Ab
	Yieldgard (field corn)	Cry1Ab
Mon 810	Yieldgard (field corn)	Cry1Ab
PHI8999 TC 1507	Herculex I (field corn)	Cry1F

Toxins are produced by the bacterium, *B. thuringiensis*, as crystal protoxins and expressed by the transgenic corn operate in an incompletely understood mode of action. The toxins ingested by the larval stage of the pest are solubilized and activated by enzymes of the insect's midgut. (Aronson and Shai, 2001). The activated toxins bind to the midgut of susceptible insects, leading to the formation of pores, loss of cellular fluids, and mortality of the larvae.

5. Benefits of Bt corn

Significant benefits to growers, the public, and the environment are expected to accrue from the availability and use of Bt crops including Bt corn (Carpenter and Gianessi, 2001; Gianessi and Carpenter, 1999; Johnson and Hope, 2000; USEPA, 2001; CAST [Council for Agricultural Science and Technology], 2002; Pilcher et al., 2002; Shelton et al., 2002; Wolfenbarger and Phiefer, 2000). Major environmental benefits can occur as a result of a reduction in insecticide use that addresses human health and ecological issues. One advantage of Bt crops is the narrow targeted range of crop pests that avoids affecting other beneficial insects associated with the crop. The broad-spectrum conventional pesticides can have a wide range of effects in the agroecosystem on birds, insects, and mammals. Bt crops affect only the target pest and closely related organisms; hence, they often result in lower exposures, while the pollution problems caused by conventional pesticides are controlled (Oerke, 1994; USEPA, 2000a, 2001).

5.1. Yield enhancement

A multistate survey of US growers over 3 years has shown that Bt corn was selected to eliminate losses due to the European corn borer (Pilcher et al., 2002). Insecticide use was not a determinant for adoption of PIP corn. Where insecticide use was employed to manage the pest infestation, adoption of the Bt corn led to nearly 200% reduction in the number of growers using insecticide controls. Stalk-tunneling larva can inflict physiological damage on the unprotected corn plant that leads to reduced production by 2.4–6.6% per plant (Bode and Calvin, 1990). Yield losses in Iowa conventional cornfields infested with multiple larvae could be as much as 32.6 bushels per acre (Rice, 1994, 1997). Even with a series of ancillary management practices to reduce plant injury caused by corn borers, the densities of corn borers often exceed established economic thresholds. The 3-year study pointed out that growers appear to recognize that losses with the non-Bt crop were greater than originally perceived.

5.2. Mycotoxin reduction

One of the benefits of Bt corn is that it has been demonstrated to dramatically reduce the occurrence of food contamination by the mycotoxin, fumonisin (Munkvold and Desjardins, 1997; Munkvold et al., 1997, 1998, 1999; Munkvold and Hellmich, 2000; USEPA, 2001; CAST [Council for Agricultural Science and Technology], 2002, 2003). It is widely recognized that corn crop quality is affected by the infestation by fungi (Dowd, 1995, 2000, 2001). Mycotoxigenic fungi can infest the corn ear and produce chemicals that are toxic and possibly carcinogenic to humans and animals (CAST [Council for Agricultural Science and Technology], 2003; Nelson et al., 1993; Masoero et al., 1999). Control of these fungi with malathion has been exploited (Dowd et al., 1998, 1999, 2000). The infestation by the various pests may present an opening for subsequent fungal infestation and mycotoxin contamination to the corn crop (Munkvold and Desjardins, 1997; Munkvold et al., 1997, 1999; Nelson et al., 1993).

5.3. Reduced pesticide application

Six biotech crops, including corn, planted in the United States were found to produce an additional 4 billion lbs (1.8 billion kg) of food and fiber on the same acreage, improved farm income by $1.5 billion, and reduced pesticide use by 46 million lbs (21 million kg) (Gianessi et al., 2002). This study projects that if the 21 additional biotech crops known to be in the development pipeline have been planted in 2001, production would have increased an additional 10 billion lbs (4.5 billion kg), farm income would have risen another $1 billion, and pesticide use would have decreased by an extra 117 million lbs (53 million kg).

Economic benefit analysis has been modeled using a partial budgeting approach as part of the risks and benefits reassessment of Bt crops (USEPA, 2001). Utilizing conditions of two ECB infestation levels, low and high, a 5.4-bushel-per-acre increase over non-Bt corn and a 10.8-bushel-per-acre increase over non-Bt corn were calculated. Using a technology fee of $8, the per-acre benefit was $2.11 and $12.21 for low and high ECB pressure, respectively. Aggregate benefits received by farmers were $38 million and $219 million for low and high ECB pressure, respectively. Assuming low ECB infestation levels, $38 million per year across 19.5 million acres of Bt corn implies benefits of less than $2 per acre may not make economic sense for many farmers. A number of other economic analyses have projected the net economic return of Bt corn (CAST [Council for Agricultural Science and Technology], 2002). All of these studies indicate that the economic projection models are sensitive to ECB infestation levels (Benbrook and Do, 2001).

6. Bt corn insect resistance management

Prior to the introduction of Bt crops in 1995, insect resistance management was rarely implemented before field resistance occurred, and generally, another insecticide was available to replace the old one. Only two examples of a proactive IRM strategy occurred before 1988 (Andow and Ives, 2002). With the advent of Bt crops, more focus has been on proactive IRM strategies.

The Bt toxins installed in these crops are proteins expressed by the microbe *B. thuringiensis* and have been used in pest control strategies as a spray. The Bt toxins are lethal to larval forms of certain insects and can be selected for their toxicity toward economically significant insects. The earlier instar stages of larval development are more sensitive to the toxin activity. The mode of action is still a matter of some speculation, but a general picture is available. The ingested toxin is cleaved in the insect gut to the active form, which attaches to the midgut forming a pore that releases cellular fluids and ends in larval mortality. Like other insecticides, Bt toxins appear to be vulnerable to pest adaptation (Gould, 1988, 1994, 1998a,b; Gyslayne et al., 1998; Hubbell and Welsh, 1998; Matten et al., 2003; McGaughey and Gould, 1998; Parker and Kareiva, 1996, Shelton and Roush, 2000; USEPA, 2001). An individual toxin has a very specific target site within the insect (Table 4). Resistance literature points out that toxic factors tethered to single, specific target site may provide a smaller evolutionary barrier to resistance than toxins having multiple site effects (Gould, 1994; Ives et al., 1996; Roush and Miller, 1986; Roush, 1998). Technological advancements in Bt toxicity, host range, stability, formulation, application, and expression in transgenic plants are greatly improving biopesticide potency and efficacy. Unfortunately, while providing high levels of pest suppression, improved efficacy will rapidly help select for the segment of the population that is capable of withstanding Bt intoxication. Putative shifts in susceptibility were noted (Mahler, 1999). The possibility of widespread resistance to Bt was generally not acknowledged until field resistance was reported to Bt in Hawaiian populations of *P. xylostella* (L.), the diamondback moth (Tabashnik et al., 1990). Insect resistance is a real concern for Bt crops especially those that have widespread adoption, and consequently, high selection intensity caused the production of high, season long dose of single Bt proteins.

6.1. High-dose/structured refuge strategy

A series of resistance management strategies, such as those found in Table 5, have been considered to minimize the selection pressure for insect resistance (Caprio, 1998, 2000; Gould, 1998a,b; Mahler, 1999; Meadows, 1993; Roush, 1997a,b; Tabashnik, 1994a,b). The insect resistance management issues and strategies have been extensively scrutinized for Bt crops including corn as part of the risk/benefit analysis under FIFRA (USEPA, 1998, 2001). Generally, the high-dose/structured refuge strategy has received the most attention as an optimal insect resistance management strategy. This strategy (Table 6) assumes that resistance is recessive and is conferred by a single locus with two alleles in three insect genotypes (RR, SS, RS), and that resistance alleles are initially rare and that there will be random mating between resistant and susceptible adults (Roush, 1994; Gould, 1998b; USEPA, 1998, 2001). Under ideal field conditions, only rare RR homozygous recessive individuals will survive a high dose produced by the Bt crop. Resistance can then

Table 4
Bt toxin specificity

Prototoxin	Molecular mass (kDa)	Toxicity
Cry 1Aa, Cry 1Ab, Cry 1Ac	131–133	Lepidoptera
Cry 1B, Cry 1C, Cry 1D	132–138	Lepidoptera, Diptera
Cry 2	70–71	Lepidoptera, Diptera
Cry 3	73–74	Coleoptera
Cry 4	72–134	Diptera
Cry 5	80	Lepidoptera, Coleoptera

Table 5
Potential options for PIP crop management

Seed mixtures
Crop rotation
Temporal plantings (early plantings)
Dose (high) structure
Spatial configuration and proximity
Proportion of refuge
Timing and implementation of structured refuges
Alternative hosts
Weeds
Noncrops
Non-Bt crops

be diluted when RR individuals mate with SS homozygous susceptible individuals from the structure refuge to produce fully susceptible RS heterozygous individuals. A refuge is composed of a non-Bt host plant that produces susceptible (SS) insects. In the case of corn, the refuge is composed of non-Bt corn.

A “25 × ” definition of high-dose criterion has been employed since 1998 by USEPA. A high dose was defined as 25 times the toxin concentration needed to kill susceptible insects at an LD_{99} (USEPA-SAP, 1998). The 1998 SAP determined that there are five possible but imperfect techniques to verify high-dose expression by the Bt crop for lepidopteran control: (1) serial dilution larval bioassay with artificial diet containing lyophilized tissue of Bt plants with tissue from non-Bt plants as control; (2) determination of toxin expression using plant lines having expression levels 25-fold lower than a non-PIP cultivar as quantified by ELISA or technique of comparable reliability; (3) sufficient plants must be sampled in any survey of field plants to ensure that the cultivar is expressing a dose at the $LD_{99.9}$ or higher to assure that 95% of heterozygotes would be killed (Andow and Hutchison, 1998); (4) the use of controlled infestation, using the conditions of specification (3), with a laboratory strain of the pest having an LD_{50} value similar to field strains; or (5) determination of a later larval instar survivability for the targeted pest found with an LD_{50} value that was 25-fold higher than that of the neonate larvae. The last technique can be expanded to include later stage of larval development and its survivability on the Bt crop plants at a 95% or greater level.

The technical feasibility of producing high-dose-expressing Bt crops has contributed to the acceptance of the high-dose/structured refuge strategy, but more importantly, grower acceptance of the use of structured refuges and the desire to protect this technology have

Table 6
Basic requirements for the high-dose toxin strategy

Assumptions
Resistance genes must be nearly recessive
Heterozygote survival is less than 5% of RR
Resistance genes are rare
Non-transgenic refuge will maintain susceptible population
Refuge proximity is sufficient to ensure nearly random mating
Crop will express 25 times the toxin required to kill 99% of the susceptible pests

also contributed to the success of IRM for Bt crops. Knowledge of factors controlling insect resistance to conventional pesticides can provide instructive insight to guide efforts to preserve the utility of these unique insecticidal proteins. The crop management goal then becomes how to design and manipulate operational strategies to best conserve susceptibility, thereby delaying resistance (Levins and Wilson, 1980; Mazier et al., 1997; McGaughy, 1985; Tabashnik, 1994a,b).

6.2. High-dose evaluation for Bt corn hybrids

Information supporting the claim to high-dose control of the major stalk-boring target pests for certain transgenic Bt corn events has been reviewed (USEPA, 2001). High-dose expression to European corn borer, *O. nubilalis* (ECB), the primary target pest, has been shown for the following currently registered Bt corn products: Events Bt11, MON 810, and TC 1507 (Walker et al., 2000). However, none of these registered Bt corn products express a high toxin dose for corn earworm, *H. zea* (CEW), which is known to be less susceptible to Bt proteins than other targeted lepidopteran pests. Evaluation of toxin dose levels for other secondary pests (e.g., southwestern corn borer, *Diatraea grandiosella* (SWCB) and fall armyworm, *S. frugiperda* (FAW)) has been sporadic.

The lack of a high dose could allow partially resistant, i.e. heterozygous (RS), insects to survive and increase the frequency of resistance genes in an insect population. For this reason, there is concurrence among IRM researchers and expert groups that non-high-dose Bt expression presents a substantial resistance risk relative to high-dose expression (Roush, 1994; Gould, 1998b; Onstad and Gould, 1998; USEPA-SAP, 1998; ILSI [International Life Sciences Institute], 1998; Mellon and Rissler, 1998; USEPA-SAP, 2001). Although the high-dose/refuge strategy is the preferred strategy for IRM in Bt crops, effective IRM may still be possible under non-high-dose conditions. For example, management option such as increasing refuge size, increasing the use of alternate hosts, and limiting total acres may provide adequate protection to Bt corn.

6.3. Refuge requirements

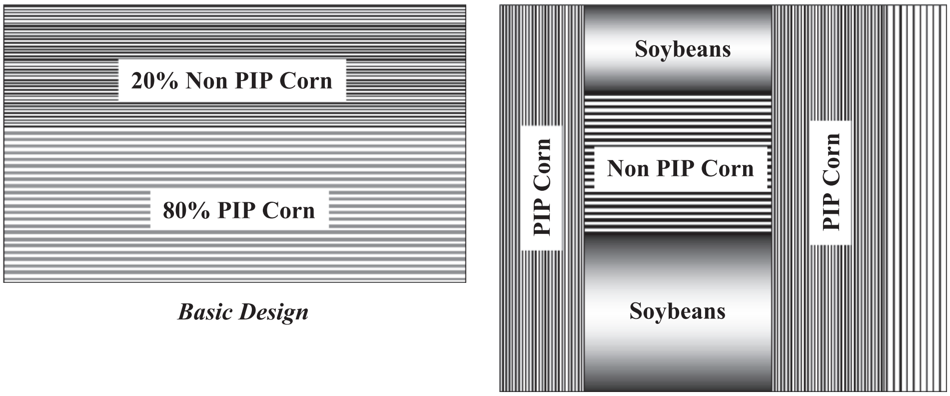
Specific refuge requirements are required as part of the 2001 reassessment of the risks and benefits of Bt PIPs expressed in corn (USEPA, 2001). The registrations for the Cry1Ab and Cry1F PIPs and genetically related materials necessary for their expression in corn expire October 15, 2008. These requirements reflect the current state of the art and science regarding IRM for Bt corn. Prior to these registration expirations, USEPA will consider new scientific data developed after 2001 to determine if modifications to the required IRM strategies will be necessary. This type of reassessment action increases the opportunities for enhancing the sustainability of IRM strategies.

The development of scientifically sound and sustainable IRM strategies for Bt corn has been the subject of open discussion at meetings, workshops, and information exchange with a range of stakeholders: growers, seed suppliers, scientists, regulators, technology providers, and public interest groups. Multiple public meetings have been held that focused on IRM for Bt crops including three FIFRA SAP meetings (USEPA-SAP [US Environmental Protection Agency Science Advisory Panel], 1995, 1998, 2001), six public workshops (USEPA/USDA, 1999a,b), two public hearings in 1997, two Office of Pesticide Program Dialogue Committee Meetings in 1996 and 1999, and one technical briefing in 2001. A strong contributor to the development of science-based practical IRM strategies for Bt corn was the United States Department of Agriculture's NC205 research committee that formally addresses research on the ecology and management of European corn borer and other stalk-boring Lepidoptera. Since 1995, the NC205 has sponsored annual IRM meetings with industry, academics, and USEPA. Crop registrants having different Bt corn technologies assembled to form the Agricultural Biotechnology Stewardship Technical Committee (ABSTC), in part to address technical and practical issues for IRM for Bt corn. The National Corn Growers Association has supported all of the efforts to provide scientifically sound and practical IRM strategies to its growers. Environmental and public interest groups have provided informative input and comments to the Agency during development of the IRM requirements for Bt corn.

The size, placement, and management of refuge plantings are critical to the success of IRM strategies relying on the high-dose/structured refuge option or non-high dose with suitable structured refuges option for management of insect resistance to Bt proteins expressed in corn (Gould, 1998b; Roush, 1997a,b). Sustainable refuge options may vary among regions. Current research suggests that non-Bt field corn provides the best refuge to increase the probability that susceptible insects will mate with potentially resistant European corn borer emerging from Bt corn. A structured refuge should include all suitable non-Bt host plants for targeted pests that are planted and managed by people (USEPA-SAP, 1998).

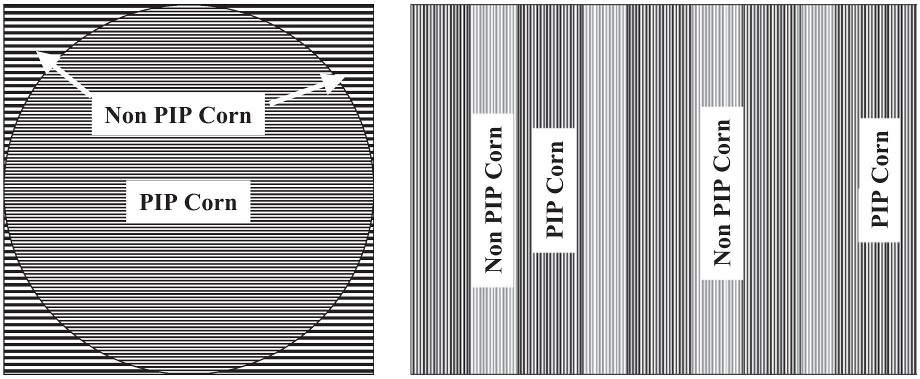
Refuge plantings must be located proximate to Bt fields to promote optimal random mating between susceptible moths from the refuge and any resistant survivors from the Bt cornfields. Information regarding insect flight and ovipositional behavior is critical to establish the separation distance between the Bt corn planting and refuge. External blocks, in-field strips, seed mixes, temporal, and non-corn hosts as suitable refuges have been considered in a series of studies (USEPA, 2001; Matten et al., 2003). One important factor is the trade-off between movement of larvae versus adults relative to refuge placement or proximity. Distant and temporal planting of Bt and refuge plants eliminate problems with larval movement, but potentially compromise random mating of susceptible and resistant adults. Simulation modeling of insect movement and mating identifies a design of strips of at least six rows in width as effective for European corn borer IRM in adjacent blocks when a 20% refuge employed (Onstad and Guse, 1999). Non-Bt corn refuges are required to be planted as either external blocks, 0.5 mile (0.25 mile or closer is preferred) or as in-field strips (across the entire field) that must be at least four rows in width, but greater than six rows is preferred (USEPA, 2001; Matten et al., 2003).

A structured refuge should provide sufficient numbers of susceptible adult insects to mate with potential Bt-resistant adult insects to dilute the frequency of resistance genes (Fig. 3). A 500:1 ratio of susceptible-to-resistant insects as a suitable goal was recommended, assuming a resistance allele frequency of 0.05 (USEPA-SAP, 1998). The size of the refuge is based on understanding the dose level expressed by the transgenic crop, the predicted genetics of resistance inheritance, population genetics and ecology for a given target pest, and estimation of the initial resistance allele frequency. Since there is a wide range for these factors, simulation models were used to predict possible outcomes if



Basic Design

Typical Mosaic



Pivot Fields

Split Plantings

Fig. 3. Current PIP corn refuge designs.

certain refuge sizes were implemented. One model, used in making the specifications of refuge size, was that of Onstad and Gould (1998). The current mandated refuge size requirements are at least a 20% non-Bt corn refuge in Corn Belt areas and at least a 50% non-Bt corn refuge in cotton-growing area (USEPA, 2001; Matten et al., 2003). Spraying the refuge should be based on whether economic thresholds are reached for one or more target pests.

The sustainability of IRM for Bt crops depends on a number of factors. These factors are grower adoption, grower education, grower compliance, selection intensity, the genetics of resistance should it occur in the field, initial resistance allele frequency, the mechanism(s) of resistance, the sensitivity and accuracy of resistance detection, the timing of corrective measures to ensure that the IRM plan is adaptive and dynamic, and research data to address some of the knowledge gaps related to population biology, genetics, and ecology of the target pests. For instance, there are little data regarding the mating and ovipositional behavior of European corn borers, and certain data are lacking regarding population dynamics, mortality factors, and larval/adult movement. The unconfirmed assumptions of the high-dose/structured refuge strategy that include that resistance will be nearly completely recessive and rarely need to be tested. The assumptions of complete grower compliance require confirmation. Continued research is necessary to alleviate data gaps and to validate assumptions related to the high-dose/structured refuge strategy. New IRM research directed to understanding the effect of north to south movement by corn earworm and high use of insecticide sprays has been required to permit the improvement of current IRM strategies for greater long-term sustainability (USEPA, 2001).

7. Resistance monitoring

Bt corn registrants in the United States are required to monitor for insect resistance to the Bt proteins, as indicated by shifts in the frequency of resistance-conferring alleles, to detect early warning signs indicating resistance development in the field and determine whether IRM strategies are working on an annual basis. Rarely has crop resistance monitoring been approached in a proactive fashion except for the case of Bt crops. Current resistance monitoring requirements were developed as part of the reassessment of risks and benefits of Bt plant-incorporated protectants (PIPs) (USEPA, 2001). Current US resistance monitoring efforts are coordinated by the ABSTC focusing on the European corn borer, southwestern corn borer, and corn earworm and are concentrated in areas where Bt corn adoption is highest as well as areas with the highest insecticide use. Four corn-growing regions have been identified: two regions primarily focusing on the European corn borer, one region co infested with European corn borer and southwestern corn borer, and one region primarily infested with southwestern corn borer. Sampling goals have been selected to target a range of four to six locations in areas in which the European corn borer is the primary pest (northeastern US) and two to three locations in areas co-infested with European corn borer and southwestern corn borer (mid-Atlantic US) or in areas predominantly infested with southwestern corn borer (south-central US). The sampling strategy is aimed to collect when possible, at least 200 first- or second-flight adults (100 females), 100 second-flight egg masses, or 100 diapausing larvae per site in each region. The ABSTC resistance monitoring plan uses primarily the diagnostic dose or discriminating dose bioassays to detect resistance alleles once they reach a frequency of 1 in 100 (Hawthorne et al., 2001).

Resistance monitoring may provide validation of parameters used in models as an additional dividend. Effective monitoring programs should have well-established baseline susceptibility data, sensitive detection methods, and a reliable collection network. Detection of resistant larvae in Bt corn will depend on level of pest pressure, frequency of resistant individuals, number of samples, and sensitivity of the detection technique. Therefore, as the frequency of resistant individuals or the number of collected samples increases, the likelihood of locating a resistant individual increases (Roush and Miller, 1986). The management goal to detect resistance in insect populations before the occurrence of widespread crop failures is critical to the use of mitigation practices that can delay the development of resistance.

Monitoring for insect resistance should be undertaken in areas where the pests are known to regularly overwinter. Secondary pests may need to be monitored on a case-to-case basis, since these pests may be of local or regional significance. Conventional insecticide use has shown that once resistant phenotypes are detected at a frequency >10%, control or crop failures are common (Roush and Miller, 1986). Resistance to Bt toxins could develop prior to detection in the field due to sampling and detection technique sensitivity limitations. Sampling locations should be selected to reflect all crop production practices and should be separated by a sufficient distance to reflect distinct populations, but should focus on intensively planted Bt crop areas in which selection pressure is expected to be higher.

The detection and monitoring of resistance in the field can be daunting. Analytical techniques having high sensitivity and accuracy are required to detect rare genes (Venette et al., 2002). Sampling statistics are difficult for the large planted areas with these crops (Binns et al., 2000). For example, if the phenotypic frequency of resistance is 1 in 1000, then more than 3000 individuals must be sampled to have a 95% probability of one resistant individual (Roush and Miller, 1986). Hence, as the planted area for these crops increases, it becomes more difficult to install the resistance monitoring necessary to meet specified management objectives.

A series of monitoring methods have been proposed: (1) grower reports of unexpected damage and seed registrants must investigate unexpected damage as part of the mandated resistance monitoring requirements; (2) systematic field surveying of Bt corn; (3) discriminating concentration assay (Marçon et al., 2000; USEPA, 2001); (4) F_2 screen (Andow and Alstad, 1998; Andow et al., 1998; 2000; Bentur et al., 2000; Zhao et al., 2002); (5) screening against resistant colonies (Gould et al., 1997); and (6) sentinel Bt-crop field plots (Venette et al., 2000).

Baseline susceptibility and a diagnostic concentration that have been developed for the three primary target pests, European corn borer, southwestern corn borer, and corn earworm, are required (USEPA, 2001). This information is essential to managing resistance in pest populations and especially in assessing whether a field control failure was due to actual resistance or other factors affecting expression of the Bt protein. The results of the baseline susceptibility and resistance monitoring studies for Bt corn have been summarized (USEPA, 2001; Matten et al., 2003). Ultimately, the sensitivity and accuracy of detection of resistance will directly affect the sustainability of long-term IRM strategies. The confidence that can be achieved in the sampling plan, detection methodology, and interpretation in the results will determine whether IRM plans will be dynamic and therefore sustainable. If an adaptive management strategy is possible, refuge options can be modified to manage the resistance of the European corn borer, for example, to Bt corn and hedge against the uncertainties of the high-dose/structure refuge strategy (Andow and Ives, 2002).

8. Remedial action plans

Remedial action plans are required to be developed for responding to possible Bt resistance (USEPA, 2001). These measures are intended to either mitigate the further development of Bt resistance in other areas (prevent its spread) or eradicate resistance (if detected early enough). The ability to remediate is directly related to the population biology and ecology of the insect, the genetics of resistance, and the mechanism of resistance. It is considered very difficult to eradicate resistance, but slowing the spread of resistance genes may be practical (USEPA-SAP, 2001). The following elements should be considered as part of a remedial action plan: (1) education of growers and crop consultants to look for unexpected pest damage; (2) monitoring for plant damage, pest susceptibility, and resistance allele frequency (with rapid verification and alternate control strategies for verified resistance); (3) sales suspensions of the affected product in the region until it can be shown that the product's benefits will outweigh its risks; (4) continual monitoring to determine the effectiveness of the remedial action plan; and (5) an assessment of how the resistance problem occurred (USEPA-SAP, 2001). The more sensitive and accurate the resistance monitoring program is, the more likely the remedial action plan can successfully eradicate or limit the spread of Bt resistance (Carriere et al., 2001; Carriere and Tabashnik, 2001). Therefore, the sustainability of IRM for Bt corn is primarily dependent on the validity of the assumptions of the high-dose/structure refuge strategy, the sensitivity and accuracy of detection of resistance, responses to changes in susceptibility which will alter the IRM strategy as a dynamic rather than static IRM program, and the sensitivity and accuracy of the remedial action plan to swiftly limit or eradicate Bt resistance.

The recommendations of the USEPA-SAP have been worked into requirements that the PIP Bt corn registrants conduct certain actions to investigate for both suspected and confirmed resistance. A resistance event becomes confirmed if the progeny of the sampled, European corn borer, southwestern corn borer, or corn earworm would exhibit all of the following characteristics in laboratory bioassays initiated with neonates: (1) there is >30% survival and >25% leaf area damaged in a 5-d bioassay using Cry1Ab-positive or Cry1F-positive leaf tissue under controlled laboratory conditions; (2) standardized laboratory bioassays using diagnostic doses for *O. nubilalis* (Marçon et al., 2000), *D. grandiosella* (MRID 450369-02), or *H. zea* demonstrate resistance has a genetic basis and survivorship in excess of 1% (gene frequency of population ≥ 0.1); and (3) an LC_{50} in a standard Cry1Ab or Cry1F diet bioassay exceeds the upper limit of the 95% confidence interval of the standard unselected laboratory population LC_{50} for susceptible *O. nubilalis*, *D. grandiosella*, or *H. zea* populations, as established by the ongoing baseline monitoring program. An annual resistance monitoring report is required to be submitted by the registrants to the USEPA following each growing season.

9. Compliance assurance and grower education

Corn growers are the most essential component of a successful IRM program because they are responsible for complying with terms of registration such as planting refuges and carrying out the operational details of an IRM plan. Education of growers is critical to conveying the importance of IRM to growers and, with compliance monitoring, form integral parts of any resistance management strategy. A consortium of stakeholders including the registrants, seed companies, the National Corn Growers Association and state grower associations, academia, consultants, seed dealers, and USDA conducts grower education programs. Seed registrants are required to design and implement a comprehensive, ongoing IRM education program to convey necessary information to Bt corn users relating to the importance of complying with the IRM plan. Bt corn registrants are required to establish a broad compliance program as part of the IRM requirements (USEPA, 2001). A compliance program is expected to (1) establish an enforcement structure that will maximize compliance, (2) monitor level of compliance, and (3) investigate effects of noncompliance on IRM. The current US compliance program consists of the following general elements that are implemented each crop season: grower education programs, grower affirmation of IRM requirements, a survey of growers conducted by an independent third party, and penalties for noncompliance such as the lack of access to the technology for deviations from the refuge requirements. An annual compliance report is required to be submitted by the registrants to USEPA following each growing season. A summary of the Bt corn compliance assurance program can be found at the National Corn Growers Association's website. Current grower education and compliance assurance programs must be revised and expanded on an annual basis and consider the results of the compliance assurance program. These annual revisions should provide additional support mechanisms for sustainability of the IRM programs.

10. Measurements of success

A partnership has been developed between the USEPA's Office of Research and Development (ORD) and the Office of Pesticide Programs (OPP) in an attempt to enhance the massive effort undertaken by OPP to provide the necessary controls for these very important crops. This partnership has been designed to focus on the continued development of quantitative measures for insect resistance management plans specifically devoted to Bt corn (Table 7). The ability to estimate the risk of resistance development without quantitative data describing the important factors governing resistance development is essentially unattainable. A series of information-gathering expert workshops focused on four topical areas: pest simulation model design and validation, resistance monitoring and detection design and validation, resistance estimation and refuge considerations, and remedial action strategies to assist the direction of the research program (Fig. 4).

The size of refuge as related to the effective dose and likely genetics of resistance, monitoring of resistance evolution, mating and ovipositional biology, heterozygote fitness, developmental time, number of yearly generations of pest, pest movement, any abiotic/

Table 7
Current options for refuge management of PIP corn

Refuge design and location
Blocks
Internal (i.e., within the Bt field)
External (i.e., separate fields)
Proximity: within 1/2 mile (1/4 mile preferred) of the Bt field to maximize random mating
In-field strips
Wider strips ≥4 rows are required, but ≥6 rows are preferable to reduce the effects of larval movement

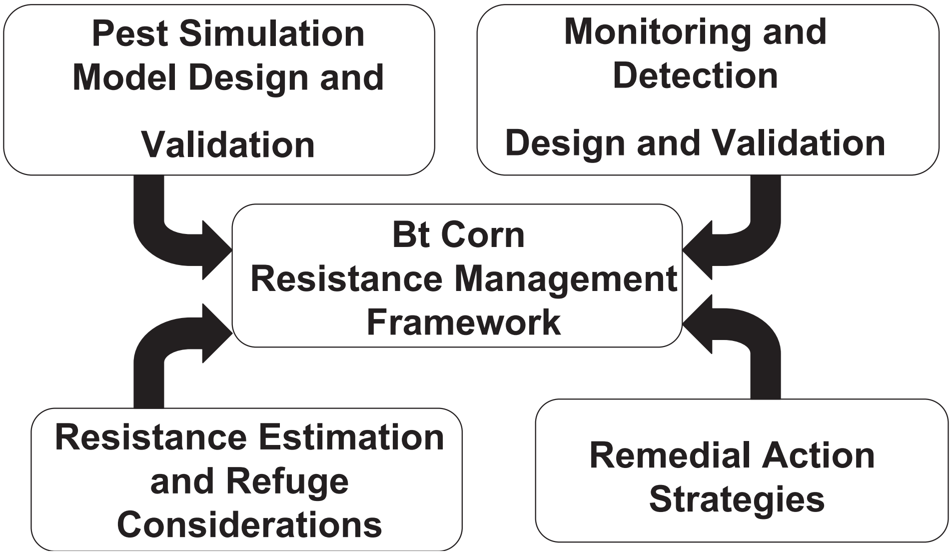


Fig. 4. Resistance management framework plan.

biotic mortality factors, and mortality of nontargeted insects are among the features of resistance management that are targeted for evaluation as part of this research.

Due to a general lack of field data, mathematical simulation models have been designed to conceptually and theoretically provide predictions of the longevity of insect resistance strategies for Bt transgenic crops. A thorough inspection of existing simulation models, including the assumptions of each model, devoted to this endeavor is critical to this research. The underlying assumptions and limitations of the analysis must be delineated to ensure that the nonexpert user of these potentially useful models is not misled by the computational results. The USEPA Data Quality Objectives Process is used in this research effort to help define and evaluate the level and quality of information as input into the simulation models and output from this simulation models. These efforts support the agency's efforts toward sustainability of reduced risk pesticide technologies (Table 8).

Table 8
Areas of risk management application (Hofstetter and Glaser, unpublished)

Risk management concerns
Manageability of risks
Ease of damage reduction
Excess of target damage
Index of manageability
Damage analysis
Proxy for unknown damage
Indices for known damage
Success of regulation

11. Conclusions

The development of plant-incorporated protectants as a significant contributor to agribusiness is in the early stages of realization. Scrutiny of this new technology is warranted in terms of sustainability and potential environmental contributions. For this technology, the major threat to its sustainability is the development of Bt resistance in pest populations targeted for control by the expression of Bt proteins in crops such as corn. The required IRM program for Bt crops is unprecedented in the management of pesticide application (Table 9). The specific IRM strategies and requirements for Bt corn in the US have been developed by a coalition of stakeholders including USEPA, USDA, academic researchers, seed producers, public interest groups, and growers. Many of the stakeholders recognize that the required IRM strategies to be scientifically sound, practical, flexible, implementable, and sustainable. IRM requirements for Bt corn include a 20% mandatory non-Bt corn refuge in the US Corn Belt and a 50% mandatory non-Bt corn refuge in cotton-growing areas to be planted within 2 miles, 0.25 mile or closer preferred, to mitigate insect resistance. In-field refuges consisting of strips of at least four rows are also allowed. There are also requirements for annual resistance monitoring, remedial action plan, grower education, grower compliance, research, and annual reporting. Improvements to the sensitivity and accuracy of detection of Bt resistance will enhance the possibility that adaptive measures can be made to current IRM strategies to keep them dynamic and sustainable. Continuing IRM research will also allow current IRM strategies to be further improved for greater long-term sustainability. In 2008, the registrations for the Cry1Ab and Cry1F PIPs with the genetically enhanced materials necessary for their production will expire, and the regulatory authorities will have the opportunity to review scientific data that have been developed since the 2001 reassessment to modify IRM strategies and, where necessary, make them more supportive of Bt corn crop sustainablity.

12. Disclaimer

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Table 9
Best management criteria for PIP corn

Management requirements
IRM plan
Structured refuge
Annual resistance monitoring
Remedial action plan
Grower education
Compliance assurance plan
New supporting research data
Monitoring and compliance annual reports
Seed sales data

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