Insect Sci. Applic. Vol. 17, No. 1, pp. 157–167, 1997
Printed in Kenya.

0191-9040/97 $3.00 + 0.00

EFFICACY AND DEPLOYMENT OF TRANSGENIC PLANTS FOR STEMBORER MANAGEMENT

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(*Accepted* 8 *October* 1997)

Abstract—Transgenic plants expressing *Bacillus thuringiensis* δ-endotoxins are now being used commercially in several crop species. These toxins have demonstrated good control of temperate (*Ostrinia nubilalis*) and tropical (*Diatraea grandiosella* and *D. saccharalis*) stemborers in maize. Resistance to *B. thuringiensis* toxins has been reported in over 11 species in both field and laboratory studies, demonstrating the need for resistance management strategies to prolong the efficacy of this valuable pest management tool within an integrated control programme. Resistance involves reduced binding of toxins to midgut epithelial cells and is generally considered to be a recessive trait. Resistance management will require the use of spatial and temporal refugia which may require unique schemes for each pest complex. Information is presented on the mode of action of *cry* toxins, resistance mechanisms, interaction of transgenic plants and biocontrol agents, and management/deployment strategies for transgenic maize in tropical ecologies.

Key Words: *Diatraea grandiosella, Diatraea saccharalis, Spodoptera fugiperda,* transgenic plants, *Bacillus thuringiensis,* δ-endotoxin, tritrophic interactions, management, refugia

Résumé—Les plantes transgéniques qui expriment les δ-endotoxines de *Bacillus thuringiensis* sont maintenant en train d'être utilisées commercialement dans plusieurs espèces de cultures. Ces toxines se sont révélées de bons agents de lutte contre les foreurs des tiges du maïs des zones tempérées (*Ostrinia nubilalis*) et des zones tropicales (*Diatrea grandiosella* et *D. saccharalis*). Une résistance aux toxines de *B. thuringiensis* a été rapportée chez plus de 11 espèces à partir des études de terrain et de laboratoire, démontrant le besoin des stratégies de contrôle de la résistance pour prolonger l'efficacité de cet outil de valeur dans le contrôle des ravageurs au sein d'un programme de lutte intégrée. La résistance implique la réduction de l'attachement des toxines sur les cellules épithéliales de l'estomac moyen et elle est généralement considérée comme un caractère récessif. Le contrôle de la résistance va nécessiter l'utilisation des refuges spatiaux et temporaires qui pourraient exiger des schémas uniques pour chaque complexe de ravageurs. Une information est fournie en ce qui concerne le mode d'action des *cry* toxines, les méchanismes de résistance, l'interaction entre plantes transgéniques et agents de lutte biologique ainsi que des stratégies du contrôle/déploiement pour le maïs transgénique.

Mots Clés: *Diatrea grandiosella, Diatrea saccharalis, Spodoptera fugiperda,* plantes transgéniques, *Bacillus thuringiensis,* δ-endotoxine, interactions tritrophiques, contrôle, refuges

INTRODUCTION

Integrated pest management has historically placed a great emphasis on the development of host plant resistance. The development of conventional host plant resistance often involved quantitative traits at several loci, making the resistance durable but difficult to achieve. With the advent of transformation techniques, genes which confer resistance to pest organisms have been inserted into crop plants including maize, rice, soybean, potato, tobacco and cotton (USDA, 1995; Bennett, 1994).

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Among the biological pesticides, bacteria have been the most successful group of organisms identified for insect control on commercial crops. The best examples of bacterial insecticides come from two soil bacteria, *Bacillus thuringiensis* and *B. sphaericus* (Gill et al., 1992; Charles et al., 1996). Insecticidal crystal proteins, called δ-endotoxins, produced by these bacteria are highly toxic to certain pests, yet cause little or no harm to humans, most beneficial insects, and other non-target organisms (Croft, 1990). After being activated by midgut proteases, *B. thuringiensis* toxins bind to epithelial brush border membrane vesicles (BBMV), creating pores that result in cell lyses (Gill et al., 1992). Incorporation of genes encoding δ-endotoxins into maize has provided high levels of resistance and tremendous excitement in crop protection. However, concerns over environmental hazards such as gene flow (Serratos et al., 1997) and widespread resistance in pest populations has restricted the deployment of toxin-producing plants.

Transgenic plants containing insecticidal proteins are set to feature prominently in agricultural systems in both developed and developing countries around the world. Entomologists, breeders, molecular biologists and population ecologists need to determine how to best deliver this technology to provide good pest control while at the same time reducing environmental hazards (including gene flow) and retarding the development of resistance in pest populations. To achieve these objects we need to have a greater understanding of the pest biology, behaviour and response to insecticidal proteins; the temporal and spatial expression of toxins in transgenic plants; the dynamics of different refugia strategies in resistance management; impact of toxin-producing plants on biological controls; and how to deliver this package to resource-poor farmers.

Nomenclature, Structure and Mode of Action of δ-Endotoxins

Bacillus thuringiensis is a gram-positive bacterium which produces a proteinaceous crystalline inclusion during sporulation. Several subspecies of *B. thuringiensis* produce these crystals which have insecticidal properties against lepidopteran, dipteran and coleopteran species. Because of the crystalline nature of these toxic proteins, the term 'cry' is used in gene and protein nomenclature. The current classification scheme was introduced by Höfte and Whiteley (1989). Toxin genes were then classified into four types based on insect specificity and sequence homology: Type-I genes encode proteins of 130 kDa and are usually specific for lepidopteran larvae. Type-II genes encode 70 kDa proteins that are specific to both lepidopteran and dipteran larvae. Type-III genes encode for 70 kDa proteins that are specific to coleopteran larvae. Type-IV genes are specific to dipteran larvae. The nomenclature system has since been extended to include Type-V genes which are specific to both lepidopteran and coleopteran larvae (Tailor et al., 1992).

These crystalline inclusions are solubilised in the midgut at high pH, releasing proteins called δ-endotoxins. Delta-endotoxins are protoxins of approximately 130 kDa, except for CryII and III protoxins which are 70–75 kDa (Gill et al., 1992). Protoxins are activated by midgut proteases to yield 60 to 75 kDa proteinase-resistant toxin fragments. The toxin portion is derived from the N-terminal half of the protoxin. The C-terminal sequence is involved in the formation of parasporal inclusions and is mostly hydrolysed into small peptides (Choma et al., 1990).

The region within the N-terminal toxic domain (amino acids 1–279) is composed of α-helices which are considered important in penetrating the peritrophic membrane. At least six major α-helices can be identified in most cry toxins. Based on the crystal structure of Cry IIIA, other cry proteins may adopt the same protein folding scheme with a central hydrophobic helix (helix 5) surrounded by six amphipathic helices (Li et al., 1991). Reducing the hydrophobicity of these regions can reduce toxicity (Wu and Aronson, 1990). The C-terminal region (amino acids 461–695) and the highly variable region (amino acids 280–460) are considered important in toxin specificity by coding for open β-sheets that bind to glycoprotein receptors in the midgut. Using ^{125}I-labelled toxins, brush border membrane vesicles (BBMV) were identified as the primary binding site for several insect species (Lee et al., 1992). BBMV studies show a positive correlation between toxin activity and ability to bind to BBMV (Gill et al., 1992). Furthermore, toxicity appears to be correlated with receptor number rather than receptor affinity (van Rie et al., 1989).

The toxicity of *B. thuringiensis* toxins lies in the organisation of the α-helices derived from domain

I. After binding to the midgut epithelial cells, the α-helices can penetrate the apical membrane to form an ion channel (Knowles and Dow, 1993). Studies using CryIC toxin have demonstrated these pores to possess both selective (only K^+ passes through) and non-selective (Na^+ and anions pass) properties depending on pH (Schwartz et al., 1993). Since the lepidopteran insect midgut is alkaline, the pores most probably permit K^+ leakage. Formation of this cation-selective channel destroys membrane potentials (English and Slatin, 1992), resulting in midgut necrosis, degeneration of the peritrophic membrane and epithelium, and ultimately bacterial septicemia which occurs after larval death due to toxins (Salama and Sharaby, 1985; Sneh and Schuster, 1981).

Development of Transgenic Plants containing Cry Genes

Plant transformation was first achieved less than 15 years ago with the *Agrobacterium*-mediated introduction of kanamycin resistance into tobacco (Bevan et al., 1983). Since then, transformation technology has developed rapidly with particular emphasis being placed on cereals. The basic requirements for plant transformation are: (1) a target genome; (2) a vector to carry the gene(s) of interest; (3) modification of foreign DNA (i.e. bacterial origin) to increase expression in plant tissue; (4) methodology to deliver plasmid DNA into plant cells; (5) selection methodology to identify transformed cells; and (6) tissue culture methodology to recover viable plants from transformed cells. For maize, the nuclear genome has been the target for *B. thuringiensis* genes to provide heritable resistance to insects.

Recently, a large selection of vectors have been developed from bacterial plasmids. These vectors contain antibiotic resistance as selectable markers, a replication gene and a multiple cloning site (MCS) with several restriction sites for DNA insertion. Foreign DNA can be inserted into the vector using restriction enzymes that recognise a specific DNA sequence. Insertion of foreign DNA interrupts gene expression of an identifiable protein product to indicate DNA incorporation.

Construction of a DNA sequence for incorporation into vectors consists of several components. The *B. thuringiensis* gene must first be converted from AT-rich (typical of bacteria) to CG-rich (typical of higher plants) in order increase toxin expression. Most changes are made to the third codon, thereby minimising changes in the amino acid sequence but increasing expression of the *B. thuringiensis* toxin by 10- to 100-fold over native gene expression (Perlak et al., 1991). For expression of *B. thuringiensis* genes in higher plants, a recognisable promoter and termination sequence must bracket the toxin gene. Popular constitutive promoters include the cauliflower mosaic virus (CaMV), 35S promoter and the ubiquitin promoter. Tissue-specific promoters used in maize include the PEP carboxylase promoter (green tissue) and a maize pollen-specific promoter (Koziel et al., 1993). Selectable markers, such as the *bar* gene which confers resistance to the herbicide phosphinothricin (PPT), are incorporated to facilitate the identification of transgenic plants. The size of successful vectors ranges from 5000 bp to 11000 bp depending on the promoters and *B. thuringiensis* genes incorporated into the vector (Koziel et al., 1993). Delivery of vectors into the nucleus of cereals has been achieved by using *Agrobacterium*-mediated transformation and the biolistic method (Raineri et al., 1990; Koziel et al., 1993).

Effectiveness of Transgenic Maize in Controlling Cereal Stemborers

The emergence of insect populations resistant to conventional insecticides and concern over pesticide loading in the environment has resulted in increased use of *B. thuringiensis* toxins for insect control. Commercial *B. thuringiensis* formulations contain a mixture of toxins. For example, Dipel 2X (Abbot Laboratories, North Chicago, IL) is derived from *B. thuringiensis* subsp. *kurstaki* and contains CryIA(a), CryIA(b), CryIA(c), CryIIA and CryIIB. Similarly, XenTari (Abbot Laboratories) is derived from subsp. *aizawai* and contains CryIA(a), CryIA(b), CryIC, CryID and Cry IIB (Shelton et al., 1993) In contrast to the commercial formulations of *B. thuringiensis*, transgenic plants usually contain only one or two toxins which are highly effective against a target species (Armstrong et al., 1995; Koziel et al., 1993).

Published field trials of transgenic maize containing *cry*I-type genes developed by the private sector have demonstrated unequivocally the effectiveness of this control method against the European corn borer, *Ostrinia nubilalis*. In 1993, Monstanto Co. (700 Chesterfield Parkway North, St. Louis, MO 63198) evaluated transgenic maize

by infesting plants three times with ca. 50 insects per application at the mid-whorl stage and again with 300 larvae applied at anthesis to simulate second-generation brood (Armstrong et al., 1995). Leaf-feeding damage was restricted to pin- and shot-holes while the second generation infestation generated 0.2 to 1 tunnel per plant compared to 9 tunnels per plant in untransformed plants (Armstrong et al., 1995). Field trials by CIBA-GEIGY (Research Triangle Park, NC 27709) were conducted under extremely high pest pressure, with 2400 European corn borer larvae per plant at the mid-whorl stage and 1200 larvae per plant at anthesis. Leaf-feeding damage in this trial rated as low as 1.6 while checks rated 7.2 (scale of 1–10) (Koziel et al., 1993). Tunnelling damage by the second-generation infestation resulted in 59 cm of tunnelling in susceptible checks while only 1.7 cm of tunnelling was recorded for a transgenic hybrid heterozygous for the *cry*IA(b) gene.

The effectiveness of transgenic plants against tropical stemborers is now being tested in both the public and private sectors. In collaboration with the private sector, transgenic maize is being tested at the International Center of Maize and Wheat Improvement (CIMMYT). Trials using three lepidopteran pests reared at CIMMYT were conducted in biosafety greenhouses designed to prevent pollen escape. The first trial investigated the level of leaf-feeding resistance of an Acquired Transgenic Maize (ATM) line containing *cry*IA(b) in comparison with a susceptible CIMMYT line (Table 1). Although the untransformed line used to carry the *cry*IA(b) gene was slightly more resistant to the two stemborers than the susceptible check, a dramatic reduction was observed for the line and hybrid containing the *cry*IA(b) gene. The hybrid showed a slightly lower damage rating which was probably due to hybrid vigour. The *cry*IA(b) gene did not have an impact on the fall armyworm, *Spodoptera frugiperda*.

Additional trials were conducted to compare the resistance levels conferred by *cry*IA(b) and conventional host plant resistance (HPR) developed at CIMMYT. Infestations with the southwestern corn borer, *Diatraea grandiosella*, demonstrated the superior level of resistance in transgenic maize (Table 2). Considerably lower leaf feeding damage ratings in the transgenic hybrid resulted in no larvae being collected from 30 infested plants. Larvae collected 25 days after infestation were smaller and fewer from the resistant hybrid CML139xCML67 than those on the susceptible hybrid. The effectiveness of the transgenic hybrid was less pronounced against the sugarcane borer, *Diatraea saccharalis*, with the level of resistance being comparable in both the *cry*IA(b) and hybrid with conventional resistance (Table 2). Low temperatures during the trial adversely affected *D. saccharalis* development. The final trial compared the levels of resistance against *S. frugiperda* (Table 2). Hybrid CML139xCML67 showed the best level of resistance, especially when comparing the mean larval weight which was an order of magnitude lower in larvae feeding on this hybrid. These studies show the effectiveness against two tropical stemborers of a *B. thuringiensis* toxin, CryIA(b), developed for a temperate stemborer, *Ostrinia nubilalis*.

Future work will focus on the identification of *B. thuringiensis* toxins that could be more affective against tropical cereal stemborers. Screening conducted at CIMMYT has identified CryIA(b) and CryIA(c) to be effective against *D. grandiosella*. Due to the binding specificity of *B. thuringiensis* toxins, each stemborer species of economic importance should be screened against a standard library of *B. thuringiensis* toxins to determine which constructs should be used within a region. Initial screening tests will have to be validated by testing the performance of transgenic lines against various stemborers of economic importance. Bioassays should also be conducted against predators and parasitoids to ensure the compatibility of *cry* genes and biological control strategies. The role of conventional resistance in transgenesis should

Table 1. Evaluation of maize lines and hybrids against *Diatraea grandiosella*, *D. saccharalis* and *Spodoptera frugiperda*

Material	*D. grandiosella*[1]	*D. saccharalis*[1]	*S. frugiperda*[1]
CML216 (Susceptible check)	10.0	10.0	9.5
Line without cryIA(b)	9.5	9.5	10.0
Line with cryIA(b) ATM[2]	2.6	2.5	8.7
Hybrid with cryIA(b) ATM	2.4	2.4	8.0

[1]Plants were infested with 50 larvae at the 6-leaf stage. Ratings were based on a 1–10 scale with 10 being dead (Mihm, 1989).
[2]ATM = Acquired Transgenic Maize.

Table 2. Leaf feeding damage and larval performance on maize hybrids against *Diatraea grandiosella*, *D. saccharalis*, *Spodoptera frugiperda*[+]

	Material	Leaf damage rating	Number of larvae	Total larval weight (mg)	Average larval weight (mg)
D. grandiosella	CML 216 x ATMcryIA(b)	2.5	0	0	0
	CML 139 x CML 67	6.2	13	310	27
	CML 78 x CML 216	7.7	27	3250	100
	Std Err. (SE)	0.19	6.7	700	15
D. saccharalis	CML 216 x ATMcryIA(b)	3.2	0.04	0	0
	CML 139 x CML 67	3.8	0	0	0
	CML 78 x CML 216	6.3	9.71	0.1	0.009
	Std Err. (SE)	0.33	1.2	0.144	0.031
S. frugiperda	CML 216 x ATMcryIA(b)	6.8	1.15	–	0.14
	CML 139 x CML 67	5.3	0.8	–	0.014
	CML 78 x CML 216	7.4	3.04	–	0.22
	Std Err. (SE)	0.24	1.45	–	0.056

[+]Plants were infested with 40 *S. frugiperda* larvae and 45 *Diatraea* larvae at the 5-leaf and 6-leaf stage in plant development, respectively. Ratings were based on a 1–10 scale with 10 being dead (Mihm, 1989). Number of larvae is based on a 10 plant sample, 3 replicates.

also be examined as synergism between HPR and *B. thuringiensis* has been reported for *Trichoplusia ni* control (Gibson et al., 1995) and such interactions could extend the effectiveness of transgenic plants containing *cry* genes for cereal stemborer control. Collaboration between International Agricultural Research Centres, National Agricultural Research Centres and the Private Sector is necessary to identify active *B. thuringiensis* toxins and other insecticidal polypeptides for stemborers of local importance, to assess their impact on insect ecosystems and to identify deployment strategies that delay resistance development in pest populations.

Compatibility of *B. thuringiensis* Toxins and Biological Control Strategies

Host plant resistance (HPR) and biological control are considered to be compatible components in an integrated pest management programme (Adkisson and Dyck, 1980). HPR reduces the need for insecticides which can have adverse effects on natural enemies. Conventional HPR can also slow the rate of increase of pest populations, thereby exposing pests to biological control agents for prolonged periods (Starks et al., 1972). However, some qualitative defense compounds such as nicotine can be antagonistic to biological controls (Barbosa et al., 1986). The introduction of transgenic plants brings a new system of HPR into play which could impact on tritrophic interactions.

Due to the recent release of transgenic plants for field testing, only a few studies have been conducted on the impact of transgenics on biological control agents. No studies have been published to date on the interaction between transformed cereals, stemborers and biological control agents. Field trials have been designed to assess the impact of transformed tobacco on parasitoids of *Heliothis virescens* (Johnson and Gould, 1992). This study consisted of four treatments: transgenic plants either exposed or excluded from natural enemies and toxin-free plants either exposed to or excluded from natural enemies. Toxin-expressing plants reduced the number of surviving larvae to approximately half that found on non-toxic plants. Percent parasitism was highly variable, but in one location percent parasitism by *Campoletis sonorensis* was significantly higher on toxin-producing plants. Two possible explanations for the observed biocontrol efficiency in toxin-producing plants are: (1) *H. virescens* development was 10–20% slower on toxin-producing plants, and (2) fewer larvae were available for parasitism on toxin-producing plants. Although the data supporting synergistic interactions was weak, there appeared to be no antagonistic interactions between toxin-producing plants and parasitoids. However, these transgenic plants caused only 80–90% mortality of *H. virescens* compared to 40–50% in non-toxic plants. Toxic plants which kill all larvae within 2 instars would probably impact parasite populations by not providing a sufficiently large host for parasite development. Likewise, laboratory studies of *Leptinotarsa decemlineata* and an active predator, *Coleomegilla maculata*, showed no adverse effect on predator performance when exposed to a *B. thuringiensis*-based insecticide (M-

One, Mycogen, San Diego, CA) at the recommended dosage (Giroux et al., 1994). However, in choice studies, *C. maculata* showed a significant preference for control eggs of *L. decemlineata* than those treated with M-One at a rate tenfold higher than the manufacturer's recommended rate.

Based on these and other preliminary studies, *B. thuringiensis*-based transgenic maize should not adversely affect biological control agents of cereal stemborers. Studies will be required to characterise the impact of biological controls first at a laboratory level using artificial diets and then at the field level once transgenic plants are made available and biosafety regulations are in place. The use of models to gain further understanding of the interaction between transgenic plants and biological controls will also assist in minimising adverse effects and capitalising on any synergism between these two control strategies (Gould, 1994). Modelling will also be of benefit when determining the optimal design for refugia in resistance management and the role biocontrol agents play in delaying the establishment of resistant individuals.

Management of *B. thuringiensis*-Based Transgenic Plants

Diamondback moth, *Plutella xyllostella*, populations in many parts of the world have already developed resistance to *B. thuringiensis* formulations (Tabashnik, 1994). Laboratory screening for *B. thuringiensis*-toxin resistance has resulted in the development of tolerant populations in the following species: (Lepidoptera) *Heliothis virescens, Spodoptera exigua, Spodoptera littoralis, Trichoplusia ni, Plutella xylostella, Anagasta kuehniella, Cadra cautella, Homoeosoma electellum, Plodia interpunctella, Choristoneura fumiferana;* (Coleoptera) *Chrysomela scripta, Leptinotarsa decemlineata;* (Diptera) *Aedes aegypti, Culex quinquefasciatus, Drosophila melanogaster,* and *Musca domestica* (Tabashnick, 1994). This rather impressive list of resistant populations highlights the fact that resistance development in field populations is a very real concern for transgenic plants containing *cry* genes. With transgenic cereals now being produced in both private and public sectors, the formidable task ahead is to determine the best deployment strategy for transgenic plants that will lead to sustained protection against tropical insect pests.

Cry-toxin-resistant populations of cereal stemborers have not been reported in the literature but the deployment of transgenic maize in the United States will prompt the publication of such reports for the European corn borer, *O. nubilalis*. Based on earlier publications, Tabashnik (1994) concluded that initial gene frequencies for Cry toxin resistance were higher than 0.5%. Estimates of resistant gene frequency may also be influenced by the nutritional status of eggs. Rossiter et al. (1990) has shown that the eggs of *Lymantria dispar* L. which are laid first have a 20–400 percent increase in Cry toxin tolerance than later egg masses from the same cohort. Therefore, genetic studies should include all individuals from a defined population and not just a fraction which emerge at a given time.

Studies conducted in biosafety greenhouses at CIMMYT have determined the initial frequency and rate of resistance development in tropical stemborer populations to transgenic maize containing *cry*IA(b). Based on these results, resistance could develop rather quickly for *D. saccharalis* as a moderately high proportion of neonate larvae in the population could survive 8 days on transgenic maize (Table 3). However, D. *grandiosella* had a significantly (*t*-test, $P < 0.05$) lower frequency of surviving individuals. Surviving larvae were transferred to artificial diet and their progeny will be applied to the same

Table 3. Larval survivorship of two Mexican stemborer species after 8 days of feeding on transgenic maize containing a single copy of cryIA(b)

	6-leaf stage infestation			8-leaf stage infestation		
Stemborer species	Larvae per plant	Number surviving per plant (N ± SE)*	Survival ratio*	Larvae per plant	Number surviving per plant *	Survival ratio+
Diatraea saccharalis	600	6.43 ± 1.57 a/a	0.011	250	27.12 ± 9.18 a/b	0.109
Diatraea grandiosella	550	0.56 ± 0.18 b/a	0.001	250	1.75 ± 0.25 b/b	0.007

*Means within columns and rows (column/row) with a different letter are significantly different ($P < 0.05$, *t*-test).

+Survivorship was determined on a population basis.

transgenic hybrid for 8 days in order to culture resistant populations and determine the rate and magnitude of resistance development in these two *Diatraea* spp.

Avoidance may be one strategy that larvae can employ against transgenic plants. Choice studies using *Heliothis virescens* have shown susceptible larvae to avoid diet containing *B. thuringiensis* toxin (Gould and Anderson, 1991). An interesting observation from Table 3 is the significant and consistent difference in reduced survival of larvae placed on 6-leaf compared to 8-leaf plants. One possible reason for this could involve an avoidance strategy as younger plants (6 leaf) tend to have more exposed whorls with greater light penetration. The current *cry*IA(b) construct employs a PEP-carboxylase promoter which enables Cry-toxin expression in green tissue only so toxin levels may be greater in younger plants which have all leaf tissue exposed to light. The two borer species reared at CIMMYT tend to migrate into the whorl with little feeding until reaching the leaf tissue that has incomplete chlorophyll formation in the leaf. If the toxin is expressed at a sufficiently low level inside the whorl, larvae could survive the duration of the screening. Live larvae from both borer species were collected only from the immature leaf tissue within the whorl. Given the feeding preference for inner whorl tissue, behavioral avoidance of toxic tissues will likely be one component in stemborer resistance to transgenic maize.

Reduced binding affinity of Cry toxins onto the BBMV of the midgut epithelium has been identified as the resistance mechanism operating in *Plutella interpunctella* (van Rie et al., 1990) and *P. xylostella* (Ferré et al., 1991). Van Rie et al. (1990) demonstrated that a 50-fold reduction in CryIA(b) binding was associated with >100-fold reduction in toxicity in the resistant versus susceptible populations of *P. interpunctella*. Midgut proteases were similar in both populations suggesting that altered proteolytic processing was not the mechanism of resistance (Johnson et al., 1990). Other resistance mechanisms are probably operating in some species as Gould et al. (1992) found no significant change in toxin binding to BBMV of a resistant population of *H. virescens*. Moreover, *H. virescens* selected on CryIA(c) showed >500-fold increase in resistance to CryIA(c) after 19 cycles but was also cross-resistant to CryIB, CryIC and CryIIA (Gould et al., 1995), implying a broader-based resistance mechanism. An additional resistance mechanism may also involve the complete degradation of Cry toxins by proteolytic enzymes, as has been reported for *S. frugiperda* (Keller et al., 1996). As work in resistance mechanisms continues, additional physiological, behavioral and biochemical mechanisms will likely be discovered. Resistance may include lower gut pH that would reduce crystal dissolution, altered protease enzymes that would reduce activation of protoxins or reduced sensitivity of epithelial cells to pore formation.

The inheritance of resistance traits appears to be mostly recessive and apparently due to one or a few major loci (Tabashnik, 1994). However, resistance in *H. virescens* (50-fold) was partially recessive and was thought to be inherited as an additive trait involving more than one locus (Gould et al., 1992), but a different strain (3000-fold resistance) demonstrated a single, recessive locus which conferred resistance and occurred at an initial frequency of 10^{-3} (Gould et al., 1995). Mapping of loci conferring resistance in pest populations will be the next step in genetic studies and the information obtained will provide further insight into resistance mechanisms as well as being used to monitor resistance development in pest populations.

Deployment of insecticidal plants should be couched within the philosophy of IPM. Such a strategy would not only consider gene construct and field design but would also consider alternate mortality mechanisms, means to reduce selection pressure on major mortality mechanisms, maintain susceptible individuals in the population and monitor pest populations for resistance to design more effective management strategies. This is especially true when considering food security in developing countries, as resistance break-through may leave resource-poor farmers in a vulnerable position unless other control strategies are developed as a backstop to insect-related yield losses. Those involved in the development and implementation of insecticidal plants have two major questions to address: (1) which *cry* genes will be used and how effective are they (expression level and toxicity) in controlling targeted pests and, (2) how will these plants be grown in the field to minimise resistance development? A list of the more popular tactics for deploying insecticidal plants is provided in Table 4. Gould (1986) has developed simulation models to compare the utility of different release strategies of two single gene factors: sequentially, cultivar mixture or a single pyramided resistant cultivar. The best option depends on the inheritance, epistasis and initial

Table 4. Tactics available for the deployment of insecticidal genes in crops

Category	Tactics
Gene strategies	Single gene Multiple genes (pyramid) Chimeric genes
Gene promoter	Constitutive Tissue-specific Inducible (wound, phenology, elicitor)
Gene expression	High dose Low dose Mixture (high/low)
Field design	Uniform single gene Mixture of genes Gene rotation Mosaic planting (susceptible, resistant cultivars) Refugia (spatial, temporal)

Adapted from McGaughey and Whalon (1992), p. 1453.

frequency of resistant alleles in the pest population. If resistance alleles are recessive and epistasis is strong, making the two resistance factors redundant, pyramided deployment was predicted to be the most durable. What is clear from this and other models is the importance of refugia in resistance durability.

Refugia can be defined as a food resource in time or space that does not impose a selection pressure so as to maintain a susceptible population base to mate with resistant individuals. The optimal spatial or temporal scale of refugia will likely be unique for each insect-plant interaction. For refugia to be effective, no selection pressure should be applied (i.e. insecticides) and individuals should be in suitable proximity to mate with survivors from insecticidal plants. Refugia can be placed within a given plant (tissue- or developmental-specific promoters), between plants (mixed seed) or between fields. In addition to maintaining resistance alleles at a low frequency, refugia also enhance the capacity of biological controls. Recent simulations (Gould, 1994) indicate that natural enemies can slow the rate of pest adaptation to plants with high levels of HPR if these plants are grown near other plants that are susceptible to the pest insect. This is especially true if predation is inversely density-dependent and insecticidal plants are mixed with non-transgenic plants at a plot-to-plot spatial scale. One practical example of refugia is found in transgenic cotton farming in which the farmers (contract with the seed producer to) either plant 20% of their land with conventional cotton and use non-*B. thuringiensis* insecticides to control *Helicoverpa zea* populations or plant 4% of their land with conventional cotton with no insecticide use. Using either method, sufficient insects survive to mate with individuals emerging from transgenic cotton to delay the appearance of homozygous resistant individuals.

Most models developed to date are simple, considering one crop, one pest and one biological control organism. In reality, cereal systems in tropical environments have a number of important pests which are often polyphagous with different biocontrol agents being active at different stages of pest and plant development. Given this complexity, constructs should be designed with these considerations in mind. Moreover, pests in tropical environments are capable of completing several life cycles per cropping season and tropical stemborers tend to be more aggressive than temperate species in their feeding behaviour. To address the complexity of transgenic management for cereal stemborers, there is a genuine need for more information on insect performance against different *B. thuringiensis* toxins and other insect-active polypeptides, species distribution within different regions as well as on a smaller scale between cultivated and wild hosts, and the impact of natural enemies on stemborer populations. As this basic information is obtained, prediction models can become more sophisticated in predicting the optimal deployment strategy for transgenic plants.

One possible product development strategy in maize is the production of targeted synthetics. By incorporating various constitutively expressed Cry toxins or other insect-active polypeptide genes into lines adapted for specific tropical environments, synthetics can quickly be formed which are effective against a pest complex and compatible with important biological control agents. For this to be effective, each tropical pest of maize and associated biological control agents must be properly characterised for their sensitivity to different constructs. Based on toxicity and line adaptation to the target environment, 6–10 lines expressing different toxins and specialty traits (tolerance to drought and/or low soil nitrogen, conventional HPR, disease resistance) would be recombined to form a homogeneous synthetic which would effectively 'pyramid' or 'stack' resistance genes into a maize synthetic. Once released to farmers, these synthetics could be maintained as narrow-based populations at the

farm level by eliminating tassels of plants showing insect damage. This system would also lend itself to the incorporation of lines with conventional resistance to reduce the selection pressure on Cry toxins, thereby increasing the durability of resistant germplasm. The number of *cry*-lines would depend on the expense of transformation and/or backcrossing to incorporate *cry* genes and the number of constructs available. Such a scheme could provide synthetics with good yield potential as well as stability from insect losses.

Conclusion

Incorporation of *B. thuringiensis* genes coding for insect toxins will have a tremendous global impact on cereal stemborer management in the next decade. Researchers in both the public and private sector as well as legislators need to actively pursue a management strategy that reflects the pest biology and interactions with the crop and associated biological controls to extend the lifetime of transgenic plants. Refugia will play a crucial role in resistance management and as such should be tailored to each pest complex and environment where transgenic plants are deployed. *Bacillus thuringiensis* toxins provide a unique source of insect control that delivers specificity, is environmentally sound, and can be effectively incorporated into important crop species. Loosing such a control strategy will also have a tremendous global impact, especially for the resource-poor farmer who may not have alternate control strategies in place once resistant insect populations develop.

Acknowledgments—The authors wish to thank Dr H. Kumar for assistance evaluating trials. Financial assistance provided by the UNDP project Nos. GLO/90/003 and GLO/91/014 for the development of stress-resistant and transgenic maize, respectively. The opinions expressed in this paper are the responsibility of the authors.

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