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Genetic Transformation of Crops for Insect Resistance: Potential and Limitations

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Transgenic resistance to insects has been demonstrated in plants expressing insecticidal genes such as δ-endotoxins from *Bacillus thuringiensis* (*Bt*), protease inhibitors, enzymes, secondary plant metabolites, and plant lectins. While transgenic plants with introduced *Bt* genes have been deployed in several crops on a global scale, the alternative genes have received considerably less attention. The protease inhibitor and lectin genes largely affect insect growth and development and, in most instances, do not result in insect mortality. The effective concentrations of these proteins are much greater than the *Bt* toxin proteins. Therefore, the potential of some of the alternative genes can only be realized by deploying them in combination with conventional host plant resistance and *Bt* genes. Genes conferring resistance to insects can also be deployed as multilines or synthetic varieties. Initial indications from deployment of transgenics with insect resistance in diverse cropping systems in USA, Canada, Argentina, China, India, Australia, and South Africa suggest that single transgene products in standard cultivar backgrounds are not a recipe for sustainable pest management. Instead, a much more complex approach may be needed, one which may involve deployment of a combination of different transgenes in different backgrounds. Under diverse climatic conditions and cropping systems of tropics, the success in the utilization of transgenics for pest management may involve decentralized national breeding programs and several small-scale seed companies. While several transgenic crops with insecticidal genes have been introduced in the temperate regions, very little has been done to use this technology for improving crop productivity in the harsh environments of the tropics, where the need for increasing food production is most urgent. There is a need to develop appropriate strategies for deployment of transgenics for pest management, keeping in view the pest spectrum involved, and the effects on nontarget organisms in the ecosystem.

Keywords biotechnology, genetic transformation, *Bacillus thuringiensis*, host plant resistance, insecticidal genes, pest management, nontarget effects, limitations of transgenics.

INTRODUCTION

There is a continuing need to increase food production, particularly in the developing countries of Asia, Africa, and Latin America. Losses due to insect pests represent one of the single largest constraints to crop productivity, estimated at 14% of the total agricultural production (Oerke *et al*., 1994). In addition, insects also act as vectors of various plant pathogens. The annual global cost of attempting to reduce pest damage through insecticide application is currently valued at US$10 billion. Large application of insecticides for insect control results in toxic residues in food and food products, in addition to adverse effects on nontarget organisms and the environment in general. Furthermore, the cost–benefit ratio of such practices can easily become negative in marginal cropping systems, particularly when other factors such as diseases or drought also become rate limiting in crop production.

The losses due to insect pests can be minimized effectively through host plant resistance to insects (environmentally safe seed-based technology through conventional plant breeding and/or biotechnological approaches) compared to other primary constraints to crop production such as low soil fertility and drought. The ability to isolate and manipulate single genes through recombinant DNA technology (Watson *et al*., 1987), together with the ability to insert specific genes into a chosen variety (Chilton, 1983) has opened a new era of targeted plant breeding. Significant progress has been made over the past two decades in introducing foreign genes into plants, and this has provided opportunities to modify crops to increase yields, impart resistance to biotic and abiotic stresses, and improve nutritional quality (Sharma *et al*., 2002a). Genes encoding δ-endotoxins from *Bacillus thuringiensis* (*Bt*) were cloned in the early 1980s (Schnepf and Whiteley, 1981), and genetically modified plants with resultant resistance to insects were developed by the mid-1990s (Hilder and Boulter, 1999; Sharma *et al*., 2000). In this article, we focus on candidate genes conferring resistance to insect pests and review the current progress in developing transgenics with insect resistance and their limitations in order to assess the future potential of this technology, with particular reference to the genetic improvement of crops for improving the livelihoods of poor people in developing countries.

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GENETIC TRANSFORMATION OF CROP PLANTS

The efficiency of tissue culture and transformation protocols is one of the most important components for successful generation of transgenic crops (Sharma and Ortiz, 2000b). The major components for the development of transgenic plants are: (1) development of reliable tissue culture and regeneration systems, (2) preparation of gene constructs and transformation with suitable vectors, (3) efficient techniques of transformation for introduction of genes into the crop plants, (4) recovery and multiplication of transgenic plants, (5) molecular and genetic characterization of transgenic plants for stable and efficient gene expression, (6) transfer of genes into elite cultivars by conventional breeding methods, and (7) evaluation of transgenic plants for their effectiveness in alleviating biotic and abiotic stresses without being an environmental hazard (Birch, 1997). Although several approaches have been tried successfully for integrative transformation (Potrykus, 1991), only four approaches are used widely and have enabled scientists to introduce genes into a wide range of crop plants (Dale *et al*., 1993). These include (1) *Agrobacterium*-mediated gene transfer, (2) microprojectile bombardment with DNA or biolistics, (3) microinjection of DNA, and (4) direct DNA transfer into isolated protoplasts. Of these techniques, the first two approaches have been used quite successfully.

- *Agrobacterium tumefaciens* has been used widely for transforming the desired genes into crop plants. It is a soil-inhabiting bacterium that has been implicated in gall formation at the wound sites in many dicotyledonous plants. This tumor-inducing capability is due to the presence of a large *Ti* (tumor-inducing) plasmid in virulent strains of *Agrobacterium*. Likewise, *Ri* (root-inducing) megaplasmids are found in virulent strains of *Agrobacterium rhizogenes*, the causative agent of "hairy root" disease. The *Ti* and *Ri* plasmids, and the molecular biology of crown gall and hairy root induction, have been studied in great detail (Zambryski *et al*., 1983; Zambryski, 1992). *Agrobacterium*-mediated transformation is brought about by incorporation of genes of interest from an independently replicating *Ti* plasmid within the *A. tumefaciens* cell, which then infects the plant cell and transfers the T-DNA containing the gene of interest into the chromosomes of the actively dividing cells of the host plant.
- Genetically engineered DNA can also be directly injected into nuclei of embryogenic single cells, which can be induced to regenerate plants in cell culture (Neuhaus *et al*., 1987). This requires micromanipulation of single cells or small colonies of cells under the microscope, and precise injection of small amounts of DNA solution with a thin glass micro pippette. Injected cells or clumps of cells are subsequently raised in *in vitro* culture systems and regenerated into plants.
- In the particle bombardment (biolistics) method, tungsten or gold particle microprojectiles are coated with the DNA to be inserted, and bombarded into cells/tissues capable of subsequent plant regeneration. Acceleration of heavy microprojectiles (0.5 to 5.0 μm diameter tungsten or gold particles) coated with DNA carries genes into virtually every type of cell and tissue (Klein *et al*., 1987; Sanford, 1990). The DNA-coated particles enter the plant cells, the DNA is incorporated in a small proportion of the treated cells, and the transformed cells are selected for plant regeneration.
- In the protoplast transformation, the cell wall of the target cells is removed by enzymatic treatment, and the cells are bounded by a plasma membrane (Zhang and Wu, 1988). The DNA can be added into cell suspension, which can be introduced by affecting the plasma membrane by polyethylene glycol or by passing an electric current through the protoplast suspension. The DNA gets incorporated into the genome of a few cells. A suitable marker may be inserted to select the transformed protoplasts and the cell colonies that develop from them (Shimamoto *et al*., 1989). The *Cry2Aa2* operon expressed in tobacco chloroplasts resulted in *Bt* protein content of up to 45.3% of the total protein in mature leaves, which resulted in 100% mortality of cotton bollworm and beet armyworm (Cosa *et al*., 2002).

GENE EXPRESSION

Efficient genetic engineering relies on being able to generate a specific gene product at the desired level of expression, in the appropriate tissues, at the right time. This can be accomplished by creating gene constructs that include promoters and/or transcription regulation elements that control the level, location, and timing of gene expression. A major constraint in the development of effective transgenic products has been the lack of promoters that can offer a high level of gene expression at this degree of specificity in the crop species of interest. Traditionally transgene expression has been driven by strong constitutive promoters such as cauliflower mosaic virus 35S promoter (*CaMV35S*) (Benfey and Chua, 1989, 1990) and *Actin 1* (McElory *et al*., 1990). Although *CaMV35S* has been widely used in a number of dicotyledonous plant transformation systems, it has low activity in monocotyledonous systems (Wilmink *et al*., 1995). Moreover, the pattern of *CaMV35S* promoter activity in different tissues of transgenic plants is difficult to predict (Benfey and Chua, 1990). In general, it has been found that monocot promoters are more active in monocot tissues than in dicot tissues (Wilmink *et al*., 1995).

More recently, tissue-specific promoters have been successfully employed for driving transgene expression solely in pith tissue. Phosphoenolpyruvate carboxylase (*PEPC*) from maize can be used for gene expression in green tissue (Hudspeth and Grula, 1989). From a crop-yield–potential perspective, insect-resistant

transgenes should be expressed only in those organs likely to be attacked by the insects. Otherwise, plants may be highly resistant, yet the metabolic cost may substantially reduce the crop yield. This approach also reduces the probability of unexpected negative effects on nontarget organisms. Often, it may not be possible to extrapolate results on gene expression levels from one species to another, and each crop should be tested with a set of promoters. Although the constitutive promoters such as *CaMV35S* are effective in providing high levels of gene expression, such expressions in some cases are not only unnecessary, but could also have unanticipated negative consequences towards nontarget organisms. On the contrary, a more targeted expression of insecticidal genes by using tissue- and organ-specific promoters can form an important component for developing transgenic plants with resistance to insects (Wong *et al*., 1992; Svab and Maliga, 1993; McBride *et al*., 1995).

Transposon-mediated repositioning of transgenes is an attractive strategy to generate plants that are free of selectable markers and T-DNA inserts (Cotsaftis *et al*., 2002). By using a minimal number of transformation events, a large number of transgene insertions in the genome can be obtained so as to benefit from position effects in the genome that can contribute to higher levels of expression. *Cry1B* gene expressed under the control of maize *ubiquitin* promoter between minimal terminal inverted repeats of the maize *Ac-Ds* transposon system was cloned in the 5′ untranslated sequence of a *gfp* gene used as an excision marker. The results indicated that transposon-mediated relocation of the gene of interest is a powerful method for generating T-DNA integration site-free transgenic plants and exploiting favorable position effects in the plant genome.

BIO-EFFICACY OF TOXIN GENES EXPRESSED IN TRANSGENIC PLANTS AGAINST INSECT PESTS

Genes from bacteria such as *Bacillus thuringiensis* (*Bt*) and *Bacillus sphaericus*, protease inhibitors, plant lectins, ribosome-inactivating proteins, secondary plant metabolites, and small RNA viruses have been used alone or in combination with conventional host plant resistance to develop crop cultivars that suffer less damage from insect pests (Hilder and Boulter, 1999). Genes conferring resistance to insects have been inserted into crop plants such as maize (*Zea mays*), rice (*Oryza sativa*), wheat (*Triticum aestivum*), sorghum (*Sorghum bicolor*), sugarcane (*Saccharam officinarum*), cotton (*Gossypium hirsutum*), potato (*Solanum tuberosum*), tobacco (*Nicotiana tabacum*), broccoli (*Brassica oleracea* var *italica*), cabbage (*Brassica oleracea* var *capitata*), chickpea (*Cicer arietinum*), pigeonpea (*Cajanus cajan*), cowpea (*Vigna unguiculata*), groundnut (*Arachis hypogea*), tomato (*Lycopersicon esculentum*), brinjal (*Solanum melongena*), and soybean (*Glycine max*) (Sharma *et al*., 2000). Genetically transformed crops with *Bt* genes have been deployed for cultivation in USA, Argentina, Canada, China, South Africa, Australia, Romania, Mexico, Bulgaria, Spain, Germany, France, Uruguay, Indonesia, Ukraine, Portugal, and India.

While several transgenic crops with insecticidal genes have been introduced in the temperate regions, very little has been done to use this technology for improving crop productivity in the harsh environments of the tropics, where the need for increasing food production is most urgent. Transgenic *Bt* cotton and maize have largely been grown on a commercial scale under high input temperate or subtropical cropping systems. The most urgent need to use this technology is in the tropical regions, where soil fertility, water availability, insect pests, and diseases severely constrain crop production. For transgenic plants to be successful in integrated insect pest management they have to substitute, completely or partially, for the use of insecticides in crop production, and then result in increased crop production and environment conservation. The bioefficacy of different toxin genes expressed in transgenic plants has been discussed below.

δ-Endotoxins from *Bacillus thuringiensis*

Bacillus thuringiensis was isolated from diseased larvae of Mediterranean meal moth (*Ephetia kuhniella*) (Berliner, 1915). It is a gram-positive bacterium that produces proteinaceuos crystalline (*Cry*) inclusion bodies during sporulation. It also produces cytotoxins that synergize the activity of *Cry* toxins. There are several subspecies of this bacterium that are effective against lepidopteran, dipteran, and coleopteran insects. The identification of the *kurstaki* strain, which is highly effective against the lepidopteran insects, provided a boost for commercialization of *Bt*. Since then, a vast array of *Bt* strains have been isolated, of which HD 1 strain is the most important product in the market (Dulmage, 1981). The *Bt* toxins were earlier classified into four types, based on insect specificity and sequence homology (Hofte and Whiteley, 1989). *Cry*-I–type genes encode proteins of 130 kDa and are usually specific to lepidopteran larvae, type II genes encode for 70 kDa proteins that are specific to lepidopteran and dipteran larvae, and type III genes encode for 70 kDa proteins specific to coleopteran larvae. *Cry*-IV–type genes are specific to the dipteran larvae. The system was further extended to include *Cry*-V–type genes that encode for proteins effective against lepidopteran and coleopteran larvae (Tailor *et al*., 1992). The *Bt* δ-endotoxins are now known to constitute a family of related proteins for which 140 genes have been described (Crickmore *et al*., 1998), with specificities for Lepidoptera, Coleoptera, and Diptera. Expression of *Bt* genes in tobacco and tomato provided the first example of genetically modified plants with resistance to insects (Barton *et al*., 1987; Fischhoff *et al*., 1987; Vaeck *et al*., 1987). Progress made in developing transgenic plants with *Bt* genes has been discussed below.

Cotton

Considerable progress has been made in developing cotton cultivars with *Bt* genes for resistance to bollworms, and there is a clear advantage of growing transgenic cotton in reducing bollworm damage and increasing cottonseed yield (Hilder and Boulter, 1999). Cotton plants with *Bt* genes are effective

against pink bollworm (*Pectinophora gossypiella*) (Wilson *et al*., 1992). Cotton cultivar Coker 312, transformed with the *Cry1A(c)* gene (having 0.1% toxin protein), has shown high levels of resistance to cabbage looper (*Trichoplusia ni*), tobacco caterpillar (*Spodoptera exigua*), and cotton bollworms (*Helicoverpa zea*/*Heliothis virescens*). In transgenic cotton, cotton bollworm damage was reduced to 2.3% in flowers and 1.1% in bolls compared to 23% damage in flowers and 12% damage in bolls in the commercial cultivar, Coker 312 (Benedict *et al*., 1996). The cottonseed yield was 1050 kg ha^{-1} in Coker 312 compared to 1460 kg in *Bt* cotton. In China, cotton cultivars Shiyuan 321 and Zhongmiansuo 19, 3517, and 541 (transformed with *Bt* genes) have resulted in up to 96% mortality of cotton bollworm (*Helicoverpa armigera*) (Guo *et al*., 1999). Transgenic cotton lines S 545, S 591, S 636, and S 1001 from Simian 3 and 1109 from Zhongmiansuo 12 with *Bt* genes have shown adverse affects on survival and development of *H. armigera* (Ni *et al*., 1996). Cotton bollworm (*H. zea* and *Heliothis virescens*) survival has been found to be greater on squares and flower anthers than on other floral structures in Deltapine 5415 conventional cotton and transgenic NuCOTN 33B (Cotsaftis *et al*., 2002). ELIZA tests indicated that *Cry1A(c)* expression varied in different plant parts, but bollworm survival did not correlate with the protein expression (Gore *et al*., 2001). Trends in Bollgard II were similar to Bollgard I and nontransgenic cotton. These data support the observations under field conditions that white flowers and small bolls of cotton suffer greater damage than the older bolls.

First- to fourth-instar larvae of *H. armigera* died on transgenic *Bt* cotton, while in fifth- and sixth-instar larvae the pupation decreased by 48.2 and 87.5%, and adult emergence by 66.7 and 100%, respectively. Egg laying decreased by 50.1 to 69.7%, and egg hatching by 80.6 to 87.8% (Cui and Xia, 1999). Feeding dust of transgenic cotton to the adults decreased the number of eggs and egg hatching by 59.8 and 72.1%, respectively. *Cry1A(c)* levels can be quantified by cotton budworm (*H. virescens*) growth inhibition bioassay through concurrently run concentration-response curves using purified *Cry1A(c)* protein (Greenplate, 1999). The assay is amenable to large number of samples, uses small amounts of plant tissue, and avoids some of the concerns associated with immuno-based quantitative assays. The bioassay sensitivity ranged from 0.1 to 10.0 ng per ml. Accurate measurements of *Bt* toxins through immunoassay requires the production of quality antibodies, as well as optimization and validation of protein extraction from the specific tissue. Bioassays over the crop-growing season give a better indication of active toxins in the plant than the immunological recognition of the toxins, which may be influenced by other chemical constituents of the plant.

Maize

Bacillus thuringiensis toxins expressed in maize plants are highly effective against the European corn borer (ECB) (*Ostrinia nubilalis*) (Koziel *et al*., 1993; Armstrong *et al*., 1995; Archer *et al*., 2000). Transgenic maize expressing *Cry9C* (from *Bacillus thuringiensis* subsp. *tolworthi*) is highly effective against ECB (Jansens *et al*., 1997). Maize plants transformed with *Bt* genes have also been found to be effective against the spotted stem borer (*Chilo partellus*) and the maize stalk borer (*Busseola fusca*) in Southern Africa (Rensburg van, 1999). Spotted stem borer is more susceptible than the maize stalk borer to transgenic maize with *Bt* genes. Maize plants with *Cry1A(b)* gene are also resistant to the sugarcane borers (*Diatraea grandiosella* and *Diatraea saccharalis*) (Bergvinson *et al.,* 1997). The *Bt*-transformed plants exhibit greater resistance to *D. grandiosella* than those derived from conventional host plant resistance breeding. Williams *et al*. (1997) developed transgenic corn hybrids, which sustained significantly less leaf feeding damage by fall armyworm (*Spodoptera frugiperda*) and Southwestern corn borer (*Diabrotica undecimpuncta howardi*) than the resistant cultivars derived through conventional breeding. Resistance to fall armyworm and near immunity to Southwestern maize borer observed in these transgenic maize hybrids is the highest level of resistance documented for these insect pests. Transgenic tropical maize inbred lines with *Cry1A(b)* or *Cry1A(c)* genes with resistance to corn earworm, fall armyworm, Southwestern corn borer, and sugarcane borer have also been developed (Bohorova *et al*., 1999). A binary insecticidal crystal protein (*bICP*) from *B. thuringiensis* strain PS149B1, composed of a 14-kDa protein (*Cry34Ab1*) and a 44-kDa protein (*Cry35Ab1*), have been coexpressed in transgenic maize plants and provide effective control of Western maize rootworm, (*Diabrotica virgifera virgifera*) under field conditions (Herman *et al*., 2002). The 14-kDa protein is also active alone against the southern maize rootworm (*Diabrotica undecimpunctata howardi*), and was synergized by a 44-kDa protein.

Bt-maize is quite effective in preventing ECB damage, and generally produces higher grain yields than the nontransgenic crop (Clark *et al*., 2000). First generation ECB damage is reduced or eliminated with the use of the *Bt* hybrids. In the absence of ECB pressure, the performance of transgenic hybrids is similar to their nontransgenic counterparts. Yield of isoline hybrids is 10% lower than the standard and *Bt* hybrids regardless of ECB infestation (Lauer and Wedberg, 1999), but *Bt* hybrids generally yield 4 to 8% greater than the standard hybrids under severe ECB pressure. Transgenic crops have also been observed to have beneficial effects on nontarget pests; for example, maize hybrids with *CryIA(b)* also suffer less *Fusarium* ear rot than their nontransgenic counterparts (Munkvold *et al*., 1999). Novartis Sweetcorn and GH 0937 hybrids containing the *Bt* gene are highly resistant to *H. zea* and *S. frugiperda* (Wiseman *et al*., 1999; Lynch *et al*., 1999b).

Rice

Rice plants having 0.05% toxin of the total soluble leaf protein have shown high levels of resistance to the striped stem borer (*Chilo suppressalis*) and rice leaf folder (*Cnaphalocrosis medinalis*) (Fujimoto *et al*., 1993). Scented varieties of rice (Basmati 370 and M 7) have been transformed with *Cry II(a)* and are resistant to yellow rice stem borer (*Scirpophaga incertulas*) and the

rice leaf folder (Mqbool *et al*., 1998). Truncated *Cry1A(b)* gene has been introduced into several *indica* and *japonica* rice cultivars by microprojectile bombardment and protoplast systems (Datta *et al*., 1998). Rice lines transformed with the synthetic *Cry1A(c)* gene are highly resistant to yellow stem borer (Nayak *et al*., 1997), and those with the *Cry1A(b)* gene are resistant to the striped stem borer and the yellow stem borer (Ghareyazie *et al*., 1997). *Cry1A(b)* gene has also been inserted into the maintainer line, R 68899B, with enhanced resistance to yellow stem borer (Alam *et al*., 1999). Khanna and Raina (2002) developed *Bt*-transgenics of elite indica rice breeding lines (IR 64, Pusa Basmati 1 and Karnal Local) with synthetic *Cry1A(c)* gene. Selected *Bt*-lines of IR 64 and Pusa Basmati 1, having *Bt*-titres of 0.1% (of total soluble protein), showed 100% mortality of yellow stem borer larvae within 4 days of infestation in cut-stems as well as at the vegetative stage in whole plant assays. Husnain *et al*. (2002) expressed *Cry1A(b*) in Basmati rice under the control of three promoters (*PEPC*, *ubiquitin*, and pollen-specific promotor derived from *Bp10* gene of *Brassica napus* in *pGEM 4Z*). Toxin protein expression was 0.05% of the total protein in stems under the control of *PEPC* promotor alone or in combination with the pollen-specific promotor, but was nearly 0.15% of the total protein under the control of *ubiquitin* promotor, suggesting that a specific promotor can be used to limit the expression on *Cry1A(b)* gene in desired plant parts. The *GUS* histochemical assay, larval mortality, leaf area consumed, and leaf disc and whole-plant bioassays have been found to give similar results (Ye *et al*., 2000). Detached leaf assay for evaluating the resistance of transgenic rice to striped stem borer overcomes the difficulty of maintaining fresh stems for a long time and frequent escape of striped stem borer larvae (Yao *et al*., 2002).

Sorghum

Toxins from *B. thuringiensis* var *morrisoni* have shown biological activity against the sorghum shoot fly (*Atherigona soccata*). *Cry1A(c)*, *Cry1C, Cry1E*, and *Cry2A* are moderately effective against spotted stem borer (*C. partellus*), while *Cry1A(c)* is effective against *H. armigera* (Sharma *et al*., 1999). Sorghum plants having the *Cry1A(c)* gene have been developed at ICRISAT and are presently being tested for their resistance to the spotted stem borer (Harshavardhan *et al*., 2002).

Sugarcane

The truncated *Cry1A(b)* gene in sugarcane has shown significant activity against the sugarcane borer (*D. saccharalis*) despite low expression of the *Bt* protein (Arencibia *et al*., 1997).

Oilseed Crops

A codon-modified *Cry1A(c)* gene has been introduced into groundnut by using microprojectile bombardment (Singsit *et al*., 1997). The immunoassay of plants selected with hygromycin has shown the expression of *Cry1A(c)* protein up to 0.16% of the total soluble protein. Complete mortality or up to 66% reduction in larval weight has been recorded in the lesser corn stalk borer (*Elasmopalpus lignosellus*). There is a negative correlation between larval survival and larval weight of the lesser corn stalk borer with the amount of *Bt* protein.

Grain Legumes

A tissue culture and regeneration protocol has been developed for chickpea, which has been found to be useful for genetic transformation of this crop (Jayanand *et al*., 2003). Chickpea cultivars ICCV 1 and ICCV 6, transformed with *Cry1A(c)* gene, have been found to inhibit the development of and feeding by *H. armigera* (Kar *et al*., 1997). Pigeonpea plants with *Cry1A(b)* and soybean trypsin inhibitor (*SBTI*) genes developed at ICRISAT are being tested against *H. armigera*.

Tobacco

Expression of *Bt* genes in tobacco provided the first example of genetically modified plants with resistance to insects (Barton *et al*., 1987). Synthetic *CryIII* genes in tobacco are effective for the control of Colorado potato beetle (*Leptinotarsa decemlineata*) (Perlak *et al*., 1993). Tobacco plants containing the *CryIIa5* gene are highly resistant to *H. armigera* (Selvapandian *et al*., 1998), and the effectiveness of this toxin is comparable to *Cry1A(b)* or *Cry1A(c)*.

Potato

Synthetic *CryIII* gene has been expressed in potato plants with resistance to Colorado potato beetle (*L. decemlineata*) (Jansens *et al*., 1995). Transgenic potato plants containing the *Cry1A(b)* gene (*Bt* 884), and a truncated gene *Cry1A(b)6* resulted in less damage to the leaves by the potato tuber moth (*Pthorimaea operculella*). However, the size of the leaf tunnels increased over time in plants containing only the *Bt* 884 gene, while there was no increase in tunnel length in those containing *Cry1A(b)*6 (Arpaia *et al*., 2000). The latter also resulted in 100% mortality of the insects in tubers stored up to six months. Transgenic LT 8 and Sangema tubers remained uninfested by *P. operculella* for 6 months. However, no significant effects were observed on the nontarget species such as *Liriomyza huidobrensis, Russelliana solanicola* and *Myzus persicae*. Damage to the 4th terminal leaf by *Epitrix cucumeris* was 20 to 31% lower than in nontransgenic plants (Stoger *et al*., 1999). Davidson *et al*. (2002) developed transgenic lines of Ilam Hardy and Iwa with *Cry1Ac9* gene. A transgenic line from each cultivar inhibited larval growth of *P. operculella* by over 40%, and the line derived from Ilam Hardy prevented pupation of all larvae. A modified gene of *B. thuringiensis* var *tolworthi* (*CryIIIB*) has shown insecticidal activity toward neonate larvae of Colorado potato beetle (Arpaia *et al*., 1997). Picentia and the wild species, *Solanum integrifolium,* have also been transformed with a wild-type (wt) and four mutagenized versions of *Bt* 43 belonging to the *CryIII* class (Innacone *et al*., 1997). Adult males feeding on high-level *Bt*-expressing transgenic potatoes were able to mate and produce mobile sperm, but the females were impaired in their reproductive ability since their ovaries were not fully developed (Stewart *et al*., 1999). New Leaf *Bt*-transgenic potatoes provide substantial ecological and economic benefits to potato growers (Hoy,

1999). *Cry5*-Lemhi Russet and *Cry5*-Atlantic potato lines have shown up to 100% mortality of first instar larvae of the potato tuber moth (Mohammed *et al*., 2000). The insertion and expression of the *Cry1A(b)* into potato cultivars Sangema, Cruza 148, and LT 8 has resulted in up to 100% larval mortality of *P. operculella* (Canedo *et al*., 1999).

Vegetable Crops

Expression of *Bt* genes in tomato was one of the first examples of genetically modified plants with resistance to insects (Fischhoff *et al*., 1987). Tomato plants expressing *Cry1A(b)* and *Cry1A(c)* genes are effective against the lepidopteran insects (Delannay *et al*., 1989; Van der Salm *et al*., 1994). Expression of *Cry1A(c)* gene in tomato is highly effective against *H. armigera* (Mandaokar *et al*., 2000). Transformed brinjal plants have shown significant insecticidal activity against the fruit borer (*Leucinodes orbonalis*) (Kumar *et al*., 1998). Synthetic *Cry1C* gene introduced into broccoli (*Brassica oleracea* subsp. *italica*) provides protection not only from the susceptible diamond back moth (*Plutella xylostella*) larvae, but also from diamond back moth selected for moderate levels of resistance to *Cry1C* (Cao *et al*., 1999). Transgenic broccoli containing *Cry1C* is also resistant to the cabbage looper (*Trichoplusia ni*) and cabbage butterfly (*Pieris rapae*). *Brassica campestris* subsp. *parachinensis* transformed with *Cry1A(b)* or *Cry1A(c)* is resistant to *P. xylostella* (Xiang *et al*., 2000).

Vegetative Insecticidal Proteins

Supernatant of vegetative *Bacillus cereus* culture have two compounds, *VIP 1* and *VIP 2*, which have been shown to possess toxic effects toward insects (Estruch *et al*., 1997). *VIP 3* is highly toxic to *Agrotis* and *Spodoptera* (Estruch *et al*., 1996). The activity of these proteins is similar to δ-endotoxins. The acute toxicity of vegetative insecticidal proteins is in the same range as that of the δ-endotoxins from *Bt*. They induce gut paralysis, followed by complete lysis of the gut epithelium cells, resulting in larval mortality. Efforts are underway to use these proteins for inducing resistance to insect pests.

Secondary Plant Metabolites

Many secondary plant metabolites such as alkaloids, steroids, foliar phenolic esters, terpenoids, saponins, flavonoids, and nonprotein amino acids act as potent protective chemicals. Some of the secondary plant metabolites are produced in response to insect feeding (Sharma and Agarwal, 1983; Ebel, 1986; Sharma and Norris, 1991). Systemically induced responses are modified through synthesis and action of jasmonic acid via its lipid precursor, *e.g*., linoleic acid in tomato. Application of exogenous jasmonate induces the production of proteinase inhibitors. Xu *et al*. (1993) observed enhanced resistance in rice by wounding methyl jasmonate and abscisic acid in transgenic plants. Effective manipulation of secondary metabolites by introduction (or elimination by antisense RNA technology) of enzyme-encoding sequences is quite difficult (Hallahan *et al*., 1992; McCaskill and Croteau, 1998), and increased production of many of these chemicals may impose a measurable cost in productivity potential of crop plants (Vrieling *et al*., 1991). Such cost is not involved in natural protection mechanisms based on protective proteins (Brown, 1988). Expression of relatively large amounts of a foreign protein such as cowpea proteinase inhibitor (*CpTi*) does not impose a cost in yield in transgenic plants (Hilder and Gatehouse, 1991).

Enzyme Inhibitors

The enzyme inhibitors act on key insect gut digestive enzymes such as α-amylase and proteinases. Several kinds of α-amylase and proteinase inhibitors present in seeds and vegetative organs in plants influence food utilization by the phytophagous insects (Ryan, 1990; Konarev, 1996; Chrispeels *et al*., 1998; Gatehouse and Gatehouse, 1998). The usefulness of some of these chemical compounds for developing insect-resistant transgenic plants is discussed in the following sections.

Protease Inhibitors

Protease inhibitors of plants are involved in a number of functions, including the control of endogenous proteolytic enzymes (Richardson, 1977), the reserve of ammonia and sulphur amino acids within the storage organs (Pusztai, 1972; Tan-Wilson *et al*., 1985), and the plant defense against insect and nematode attack (Sijmons, 1993; Urwin *et al*., 1995; Lawrence and Koundal, 2002)). There is considerable diversity in protease inhibitors of cultivated chickpea and its wild relatives (Patankar *et al*., 1999). In tomato and tobacco plants, protease inhibitors have been found to accumulate in response to infection by pathogenic microorganisms (Peng and Black, 1976; Rickauer *et al*., 1989). Since protease inhibitors are primary gene products, they are excellent candidates for engineering insect resistance into plants. Disruption of amino acid metabolism by inhibition of protein digestion has been one of the targets for use in insect control (Johnson *et al*., 1989). Genes encoding inhibitors specific for serine-proteases are the main digestive proteases in most lepidopteran insects (Boulter, 1993). Deployment of protease inhibitors for insect control requires a detailed analysis of particular insect-plant interactions. The ability of some insect species to compensate for protease inhibition by switching onto an alternative proteolytic activity or overproducing the existing proteases may limit the application of protease inhibitors in such species (Jongsma *et al*., 1995). Adaptive mechanisms elevate the levels of other classes of proteinases to compensate for the trypsin activity inhibited by dietary proteinase inhibitors.

Soybean Kunitz-type trypsin inhibitor (*SBTI*) and soybean Bowman-Birk–type trypsin-chymotrypsin inhibitor (*SBBI*) reduced the larval weight of *H. armigera*, and such effects were greater for *SBTI* than *SBBI* (Johnston *et al*., 1993). Larvae feeding on diet containing 0.234 mM *SBTI* also reduced the trypsin-like enzyme activity in the gut of *H. armigera*. Transgenic tobacco plants expressing cowpea trypsin inhibitor (*CpTi*) have shown resistance to *H. armigera* (Zhang *et al*., 1998). Transgenic tobacco plants expressing high levels of *SBTI* have shown greater resistance than the tobacco plants expressing *CpTi*

against *H. virescens*. The *SBTI* is also more effective than *CpTi* in reducing the proteolytic activity of gut extracts obtained from full-grown larvae of *H. armigera*. Proteolysis by gut extracts showed 40-fold more inhibition by *SBTI* than *CpTi* (Gatehouse *et al*., 1993). However, *CpTi* is considered to be more useful for genetic transformation, because unlike many serine protease inhibitors (*SPIs*), it is not deleterious to mammals (Pusztai *et al*., 1992). Transgenic tobacco plants expressing *SBTI* have shown high levels of resistance to *H. armigera* (Sharma, H.C. and coworkers, unpublished). In another study, *H. armigera* larvae fed on transgenic tobacco expressing *SBTI* gene showed normal growth and development (Nandi *et al*., 1999).

Transgenic tobacco plants expressing *SBTI* result in increased insect mortality, reduced larval growth, and reduced plant damage by *H. virescens* (Hilder *et al*., 1987), *H. zea* (Hoffman *et al*., 1992), *Spodoptera littoralis* and *Manduca sexta* (Yeh *et al*., 1997; McManus *et al*., 1999), and *H. armigera* (Zhao *et al*., 1998a; Charity *et al*., 1999). *Helicoverpa armigera* larvae fed on plants expressing the giant taro (*Alocasia macrorrhiza*) proteinase inhibitor (*GTPI*) have shown growth inhibition of 22 to 40%, but no larval mortality has been observed (Wu *et al*., 1997b). Three soybean protease inhibitor genes (KTi_3, *C-II*, and *PI-IV*), when transformed into tobacco and potato, showed variable expression among different plants (Marchetti *et al*., 2000). The level of resistance to *S. littoralis* was particularly high in tobacco, where many plants caused complete mortality of the larvae, while in potatoes the larval mortality was much less frequently achieved, but resulted in a reduction of larval weight gain by 50%. A highly significant correlation was observed between inhibitor content and larval weight. Larval weight gain was found to be dependent on mid-gut proteolytic activity. Accumulation of Kunitz-type *SBTI* in rice also confers resistance to the brown plant hopper (*Nilaparvata lugens*) (Lee *et al*., 1999).

Cowpea trypsin inhibitor (*CpTi*) transgenic cotton lines are resistant to *H. armigera* (Li *et al*., 1998). The cysteine protease inhibitor oryzacystatin is effective for controlling the Southern maize rootworm (*Diabrotica undecimpunctata*) (Edmonds *et al*., 1996). Expression of the potato trypsin inhibitor gene confers resistance to insects in rice (Duan *et al*., 1996). Constitutive expression of *CpTi* increases resistance to *C. suppressalis* and *Sesamia inferens* in rice (Xu *et al*., 1996). A synthetic gene (*mwti1b*) coding for a winged bean trypsin inhibitor (*WTI-1B*) significantly retarded the larval growth of *C. suppressalis* in rice (Mochizuki *et al*., 1999).

Transgenic sugarcane plants expressing potato proteinase inhibitor II have shown increased antibiosis to larvae of sugarcane grubs (*Antitrogus consanguineus*) (Nutt *et al*., 1999). The *CpTi* gene in *Brassica oleracea* var *capitata* (cultivars Yingchun and Jingfeng) has shown resistance to *Pieris rapae* (Fang *et al*., 1997). Adults of *Psylliodes chrysocephala* feed identically on leaf discs from control or transformed plants of oilseed rape expressing constitutively the cysteine proteinase inhibitor, oryzacystatin I (*OCI*) (Girard *et al*., 1998a). Transgenic potato expressing the *OCI* gene resulted in up to 53% mortality of *L. decemlineata* (Lecardonnel *et al*., 1999). Feeding young female *L. decemlineata* beetles with foliage from "Kennebec" potato (K 52) transformed with *OCI* did not affect female survival, incidence of diapause, relative growth rate, and female reproductive fitness (Cloutier *et al*., 2000). However, efficiency of conversion of ingested foliage during postemergence growth, and adaptation of the digestive proteolytic system to the inhibitory effect of *OCI* were reduced. A significant reduction in survival rate of the Angoumois grain moth (*Sitotroga cerealella*) has been observed on transgenic wheat seeds expressing the trypsin inhibitor from barley (Altpeter *et al*., 1999). However, it did not have a significant protective effect against leaf-feeding insects. Insects also produce serine protease inhibitors, which can also be used against other insects by expressing them in transgenic plants (Thomas *et al*., 1994, 1995a, 1995b).

Cysteine proteinase inhibitor *(CPI)* from potato (multicystatin, *PMC*) has been expressed in chrysanthemum (*Dendranthema grandiflorum*) at a level of 0.13% of total protein (Seetharam *et al*., 2002). No correlation between reduction in oviposition rate by the western flower thrips (*Frankliniella occidentalis*) and *PMC* expression could be established, which may be due to the relatively low expression level of *PMC* in chrysanthemum. Modified *CpTi* gene (*sck*) has been successfully transferred into Chinese cabbage (*Brassica campestris* subsp. *pekinensis* (*Brassica pekinensis*)) cultivars (GP 11 and Zhongbai 4) (Yang *et al*., 2002). The resistance of the transgenic plants to *P. rapae* was observed in the laboratory and field conditions. A trypsin inhibitor from Indian mustard, *Brassica juncea* (*BjTi*), a precursor of a 2S seed storage protein, showed a soybean trypsin inhibitor active site like motif *(GPFRI)* at the expected processing site (Mandal *et al*., 2002). The *BjTi* is a thermo-stable Kunitz-type trypsin inhibitor that inhibits trypsin at a molar ratio of 1:1. The 20-kDa *BjTi* was purified from mid-mature seeds. Third generation transgenics expressing *BjTi* at 0.28 to 0.83% of soluble leaf protein showed remarkable resistance against the tobacco cutworm, (*Spodoptera litura*). This novel trypsin inhibitor can be used in transforming seed crops for protection to their vegetative parts and early seed stages, when insect damage is maximal; as the seeds mature, the trypsin inhibitor will be naturally processed to the inactive storage protein that is safe for consumption.

The high level of mustard trypsin *PI 2*(*MTI-2*) expressed in tobacco and *Arabidopsis* has deleterious effects on larvae of *S. littoralis*, causing mortality and decreasing mean larval weight, and was correlated with a decrease in the leaf area consumed (De Leo *et al*., 1998). However, larvae fed on leaves from plants expressing *MTI-2* at the low expression level did not show increased mortality but instead a net gain in weight and faster development compared with the control larvae. These observations were correlated with the differential expression of digestive proteases in the larval gut, overexpression of existing proteases on low *MTI-2*–expression level plants, and induction of new proteases on high *MTI-2*–expression level plants. Such observations emphasize the critical need for the development of a proteinase inhibitor-based defense strategy for plants obtaining

the appropriate proteinase inhibitor-expression level relative to the pest's sensitivity threshold to that proteinase inhibitor.

Prosystemin, a compound biologically active as systemin (Ryan and Pearce, 1998), when assayed for proteinase inhibitor induction in young tomato plants, has been found to be active in the alkalinization response in cultured cells (Dombrowski *et al*., 1999). Prosystemin and/or large fragments of prosystemin can be active inducers of defense responses in tomato leaves. However, *M. sexta* larvae feeding on tomato plants, constitutively expressing a prosystemin antisense gene, had approximately 3 times higher growth rates than the larvae feeding on nontransformed control plants (Orozco-Cardenas *et al*., 1993). Prosystemin mRNA levels in antisense and control plants were correlated with levels of proteinase inhibitor I and II protein levels after 6 and 12 days of larval feeding, indicating that plant resistance to insects can be modulated by genetically engineering a gene encoding a component of the inducible systemic signaling system regulating a plant defensive response. Despite several reports on successful protection of plants and trees against phytophagous insects, defense strategies based on protease inhibitor expression in plants have not resulted in any commercial applications so far. This could be due to the insects' capacity to react to protease inhibitors, and the protease inhibitor expression levels in transgenic plants.

Alpha Amylase Inhibitors

Alpha-amylase inhibitors are attractive candidates for the control of seed weevils because they are highly dependent on starch as an energy source. Insect α-amylases (α-1, 4-glucan-4-glucanohydrolases, *EC 3.2.1.1*) constitute a family of endoamylases that catalyze the hydrolysis of α-1, 4-glycosidic linkages in starch components, glycogen, and other carbohydrates. The enzyme plays a key role in carbohydrate metabolism of microorganisms, plants, and animals. Moreover, several insects, especially those similar to weevils that feed on starchy seeds during larval and/or adult stages, depend on their α-amylases for survival. The finding that α-amylase inhibitors from *Phaseolus vulgaris* seeds are detrimental to the development of Mexican bean weevil (*Callosobruchus maculatus*) (Ishimoto and Chrispeels, 1996)), stimulated their use in developing transgenic pea against bruchid beetles (Shade *et al*., 1994). Amylase inhibitors from pigeonpea inhibit 22% amylase activity in *H. armigera* (Giri and Kachole, 1998). Amylase and protease inhibitors in combination in the artificial diet increased insect mortality and showed adverse effects on growth and development of larvae. Transgenic tobacco plants expressing amylase inhibitors from wheat (*WAAI*) increase the mortality of the lepidopteran larvae by 30 to 40% (Carbonero *et al*., 1993), and those from bean (*BAAI*) to *Collasobruchus* spp. (Shade *et al*., 1994; Schroeder *et al*., 1995). Enhanced levels of resistance to the bruchids have also been obtained in seeds of transgenic adzuki beans with alpha amylase gene (Ishimoto *et al*., 1996). Alpha-amylase inhibitors (*alpha Al-1* and *alpha Al-2*) are effective in protecting peas from the weevil damage under field conditions (Morton *et al*., 2000). *Alpha Al-1* inhibits pea bruchid alpha-amylase by 80%, while *alpha Al-2* inhibits the enzyme by 40%. *Alpha Al-2* delays the maturation of the larvae, while *alpha Al-1* results in larval mortality.

Enzymes

Several enzymes expressed in transgenic plants have shown resistance to lepidopteran insects (Purcell *et al*., 1993; Corbin *et al*., 1994; Smigocki *et al*., 1993; Ding *et al*., 1998). Cholesterol oxidase from *Streptomyces* is highly toxic to cotton boll weevil (*Anthonomus grandis*) (Cho *et al*., 1995), while polyphenol oxidases and peroxidases increase the inhibitory effect of *5CQA* (5-caffeoyl quinic acid) and cholorogenic acid by oxidizing the dihydroxy groups to ubiquinones that covalently bind to nucleophilic (-SH2 and -NH2) groups of proteins, peptides, and amino acids. Mechanical wounding and insect damage resulted in transient increase in activity of polyphenol oxidase (Dhankher and Gatehouse, 2003). However, there is no systemic induction of this enzyme following wounding, insect damage, or application of methyl jasmonate. Lipoxigenase from soybean has also been shown to exhibit toxic effects towards insects (Shukle and Murdock, 1983) and has been expressed in transgenic plants, but resistance to insects has not been demonstrated. Use of the bacterial isopentenyl transferase (*ipt*) gene, involved in cytokinin biosynthesis (fused proteinase inhibitor II (*PI-IIK gene*)) in *Nicotiana plumbaginifolia*, reduces *M. sexta* larval feeding by 70% (Smigocki *et al*., 1993) and retards the development of peach potato aphid, *M. persicae*. Zeatin and zeatin-riboside levels in leaves remaining on *PI-II-ipt* plants after hornworm feeding are elevated by about 70-fold. Exogenous application of zeatin to the *PI-II-ipt* leaves enhances the level of resistance to the tobacco hornworm and completely inhibits the normal development of the green peach aphid. Transgenic tobacco plants expressing chitinase gene have also shown resistance to several lepidopteran insects (Ding *et al*., 1998).

Plant Lectins

Plant lectins are a heterogeneous group of sugar-binding proteins that have a protective function against a range of organisms. Plant lectins are particularly effective against the sap sucking Hemiptera (Hilder *et al*., 1995; Shukle and Murdock, 1983; Czapla and Lang, 1990; Powell *et al*., 1993, 1995). A gene encoding the mannose-specific lectin from snowdrop (*Galanthus nivalis; GNA*) expressed in tobacco has shown enhanced resistance to peach potato aphid (*M. persicae*), and pea lectin in tobacco has shown enhanced resistance to *H. virescens* (Boulter *et al*., 1990). Greater insecticidal activity has been observed in chitin-binding lectins and the lectin gene in wheat germ and common bean. Larvae of cotton budworm fed on transformed cotton with the lectin gene have a reduced weight, but there was no effect on larval survival (Satyendra *et al*., 1998). Transgenic haploid rice shoots with *GNA* have shown resistance to brown plant hopper (BPH) (*Nilaparvata lugens*) and the green plant hopper (GLH) (*Nephotettix virescens*) (Yang *et al*., 1998) and potato leafhoppers (*Empoasca fabae*) (Habibi *et al*., 1992). In plants where *GNA* expression is tissue-specific (phloem and

epidermal layer) or constitutive, the green plant hopper survival has been reduced by 23 and 53%, respectively (Foissac *et al*., 2000). BPH nymphs tended to avoid plants expressing *GNA*, and avoidance was less pronounced and took longer time to develop on plants where *GNA* expression was tissue-specific. In contrast to BPH, the GLH nymphs were attracted to plants expressing *GNA*. Addition of transgenic sugarcane tissue with *GNA* into artificial diet enhanced larval growth in *D. saccharalis*, resulting in higher larval and pupal weight compared with diet with nontransgenic sugarcane, but this effect was not observed in the second generation (Setamou *et al*., 2002). In contrast, larval survival, percentage of adult emergence, and female fecundity of *Eoreuma loftini* were significantly reduced when fed a transgenic sugarcane diet as compared with a nontransgenic sugarcane diet.

Transgenic potatoes expressing *GNA* and concanavalin A (*ConA*) were less susceptible to peach potato aphid, *M. persicae* (Gatehouse *et al*., 1995, 1996, 1999). Larval biomass of the tomato moth (*Lacanobia oleracea*) is reduced in artificial diet containing *GNA*, and on excised leaves of transgenic tomato (Fitches *et al*., 1997), which may result in lower fecundity of the female moths. Transgenic maize expressing wheat agglutinin has shown moderate activity against *O. nubilalis* and *Diabrotica* sp. (Maddock *et al*., 1991). Wheat germ agglutinin (*WGA*) is antimetabolic, antifeedant, and insecticidal to the mustard aphid (*Lipaphis erysimi*) (Kanrar *et al*., 2002). Bioassays using leaf discs showed that feeding on transgenics induced high mortality and significantly reduced fecundity of aphids. However, mammalian toxicity of this lectin is high, and it may not be a good candidate for use in genetic transformation. Transgenic sugarcane plants engineered to express *GNA* have shown increased antibiosis to larvae of sugarcane grubs (*Antitrogus consanguineus*) (Nutt *et al*., 1999). Snowdrop lectin at levels greater than 0.04% decreases the fecundity but not the survival of the grain aphid, *Sitobion avenae* (Stoger *et al*., 1999). Plant lectins have shown biological activity against a range of insects. However, consideration should be given with regard to their deployment in transgenic plants because of their known toxicity to mammals and humans.

Toxins from Predators

Spiders and scorpions produce powerful neurotoxins that have been expressed in transgenic organisms (Barton and Miller, 1991). Genes encoding neurotoxins from predatory mites (Tomalski and Miller, 1991), and scorpion (Stewart *et al*., 1991) have been deployed in recombinant baculoviruses to increase their biological activity. Insect-specific neurotoxin *AaIT* from the venom of the scorpion *Androctonus australis* in tobacco has shown insecticidal activity against *H. armigera* larvae (up to 100% mortality after 6 days) (Yao *et al*., 1996). Transgenic plants of tobacco have been obtained containing an insecticidal spider peptide gene, and some of these plants have shown resistance to *H. armigera* (Jiang *et al*., 1996). The role of neurotoxins from insects and spiders need to be studied in greater detail before they are deployed in other organisms and plants because of their possible toxicity to mammals.

Neuropeptides and Peptidic Hormones

Neuropeptides and small peptidic hormones are another interesting class of molecules to be used as possible insecticides through transgenic plants (Tortiglione *et al*., 1999; Altstein *et al*., 2000). These molecules regulate several insect physiological processes and are active at very low concentrations. An alteration of their titer in the insect could cause severe functional modifications. There are several examples of neuropeptides encoded by a single gene coding for multiple copies of one or more peptides. Backbone cyclic (*BBC*) neuropeptide-based antagonists (*NBA*) has been applied to insect pyrokinin/pheromone biosynthesis-activating neuropeptide (*PBAN*) family. It has led to the discovery of potent antagonists and metabolically stable peptidomimetitic antagonists devoid of agonistic activity, which *in vivo* inhibited *PBAN*-mediated activities in moths (Altstein *et al*., 2000). There are possibilities for deploying these molecules through transgenic plants to disrupt physiological processes of insects.

Antibodies

Genes that are based on antibody technology can also be exploited for genetic transformation of crop plants (Hilder and Boulter, 1999). Single chain antibodies (*ScFvs*) can be used to block the function of essential insect proteins, which serve as control agents against nematodes, pathogens, and viruses (Rosso *et al*., 1996; Van Engelen *et al*., 1994). This approach to controlling insects would offer the advantage of allowing some degree of selection for specificity effects, so that insect pests, not the beneficial organisms, are targeted. The development of a delivery system from transgenic plants to the insect haemolymph will remove a key constraint in the transgenic approach to crop protection.

GENE PYRAMIDING

Many of the candidate genes that have been used in genetic transformation of crops are highly specific or are only mildly effective against the target insect pests. In addition, crops frequently suffer from a number of primary herbivores. This suggests that single and multiple transgenes will need to be combined in the same variety with other sources, mechanisms, and targets of insect pest resistance in order to generate highly effective and sustainable seed-based technologies. In this context, it is important to examine whether coexpression of multiple toxins in the same plant will have a synergistic or antagonistic effect, *e.g*., combination of *Cry1A(a)* and *Cry1A(c)* has a synergistic effect, while a combination of *Cry1A(a)* and *Cry1A(b)* produces an antagonistic effect against the gypsy moth (*Lymantria dispar*) (Lee *et al*., 1996). However, in tobacco plants, a combination of *Cry1A(b)* and *Cry1A(c)* genes has been shown to be effective for controlling the lepidopteran insects (Van der Salm *et al*., 1994).

The *Cry1A(c)*-resistant pink bollworm had little or no survival on second-generation transgenic cotton with *Cry2A(b)* alone or in combination with *Cry1A(c)* plus *Cry2A(b)* (Tabashnik *et al*., 2002). Similarly, a mixture of *Cry1A(c)* and *Cry1F* decreased the EC_{50} to *H. armigera* by 26 times (Chakrabarti *et al*., 1998). The expression of *Cry1A(b)–Cry1A(c)* genes results in increased protection against *S. exigua, M. sexta*, and *H. virescens*. In addition, a chitinase gene from *Serratia marcesens* has been shown to act synergistically with *Bt* toxins against *S. littoralis* (Rigev *et al*., 1996).

The activity of *Bt* genes in transgenic plants is enhanced by the serine protease inhibitors (MacIntosh *et al*., 1990; Zhao *et al*., 1997, 1998a) and tannic acid (Gibson *et al*., 1995). Protease inhibitors engineered into cotton with high gossypol and/or tannin content may achieve greater protection against *H. armigera* (Wang and Qin, 1996). Loc *et al*. (2002) inserted *Cry1A(c)* and *GNA* into rice plants with the selectable marker (*hpt*). Higher levels of accumulation of the insecticidal gene products *GNA* and *Cry1A(c)* were observed in plants resulting from minimal gene cassette transformation. Transgenic plants expressing *GNA* showed enhanced resistance to brown planthopper (*N. lugens*), while the plants expressing *Cry1A(c)* were protected against striped stem borer (*C. suppressalis*). Expression of both transgenes gave protection against both pests but did not increase protection against either pest significantly over the levels observed in plants containing a single insecticidal transgene. Foliage of five experimental transgenic lines expressing three different insecticidal proteins (snowdrop lectin, jackbean lectin, and cowpea trypsin inhibitor), tissue-cultured control plants, and standard control indicated that genetic transformation resulted in a lower level of leaf glycoalkaloids than that found in either the tissue-cultured controls or standard controls (Birch *et al*., 2002). However, the distribution of glycoalkaloids was unaffected by genetic transformation and tissue culture, with the highest glycoalkaloid levels being observed in the top third of the plant. From an evolutionary point of view, the development of multiple toxin systems in transgenic plants will be expected to decrease the probability of insect pests to overcome newly deployed seed-based resistance technologies and thereby prolong the life of such new varieties (Hadi *et al*., 1996; Karim *et al*., 1999).

TRANSGENIC CROPS *VIS-À-VIS* INTEGRATED PEST MANAGEMENT

The first transgenic crop was field tested in 1994, with large-scale commercial cultivation starting in 1996 in the USA (McLaren, 1998). Current global R&D expenditure in the private and public sectors is nearly $4.4 billion, with over 95% of the total investment being incurred in the industrial countries. China is a leading investor in R&D in crop biotechnology in developing countries, followed by India. The area planted to transgenic crops has increased from 1.7 million ha in 1996 to 39.5 million ha in 1999, to over 50 million ha in 2002 (James, 2002a). The commercial production of transgenic crops quickly advanced to 12 countries by 1997. Despite a moratorium in the European Union, over 20 countries were growing biotechnology-based products by 2002. The crops produced included insect-resistant cotton and maize, herbicide-resistant soybean and canola, and tomatoes with a longer shelf-life (Federici, 1998; Griffiths, 1998; James 2002a). Adoption rates, based on percentage of area planted to *Bt* maize, increased dramatically from 10.5% in 1996 to 40.7% in 1998 (Pilcher *et al*., 2002). In 2001, global area planted to transgenic crops was 52.6 million hectares grown in 13 countries by about 5 million farmers. Nearly one quarter of the crop area planted to transgenics was grown in developing countries, mainly in Argentina and China, and over 75% of the farmers were the small resource-poor farmers in developing countries. On a global basis, 46% of the 72 million hectares of soybean, 20% of the 34 million hectares of cotton, 11% of the 140 million hectares of maize, and 11% of the 25 million hectares of canola were based on transgenic crops (James, 2002a). The value of the global transgenic seed market in 2001 has been estimated to be US$3.8 billion, which accounted for approximately 13% of the $30 billion global seed market.

The benefits of growing transgenic crops to growers have been higher yield, lower input costs, and easier agronomic management. These factors are likely to have substantial impact on the livelihoods of farmers in both industrial and developing countries. On-farm trials carried out with *Bt* cotton in different states of India have shown that the technology substantially reduces pest damage and increases cottonseed yield (Qaim and Zilberman, 2003). The yield gains are much greater than those reported for other countries where genetically modified crops were used mostly to replace and/or enhance chemical pest control. However, there is considerable public debate on the issue, and several claims to the contrary have also been published in the public media. In many developing countries, small-scale farmers suffer pest-related yield losses because of technical and economic constraints. Pest-resistant genetically modified crops can contribute to increased yields and agricultural growth in such situations. Adopting transgenic crops offers the additional advantage of controlling insect pests that have become resistant to commonly used insecticides (Sharma *et al*., 2002a). In addition to the reduction in losses due to insect pests, the deployment of transgenic plants with insecticidal genes will also lead to: (1) reduction in insecticide usage, (2) reduced exposure of farm labor to insecticides, (3) reduction in harmful effects of insecticides on nontarget organisms, and thereby increased abundance of natural enemies and (4) reduced amounts of insecticide residues in food and food products. The advantages of adopting transgenic crops are discussed in detail below.

Effect on Pest Population Dynamics and Economic Thresholds

The effects of transgenic crops with insect resistance on insect population dynamics are expected to be similar to the varieties derived through conventional breeding (Sharma and Ortiz, 2002). Resistance to insect pests in the cultivated germplasm is

low to moderate, while that from the wild relatives of crops is frequently based on toxins and provides a high level of protection against the target pests. However, this level of resistance is often diluted during the introgression and varietal development process. Thus, the effects of transgenic resistance are expected to be higher than those of conventional varieties, and such effects would be cumulative over time. Insect populations in farmers' fields would be reduced to below the economic threshold levels with minimal use of insecticides. Continuous planting of conventionally bred Hessian fly-resistant (*Mayetiola destructor*) wheat cultivars in the USA has suppressed its population to below economic threshold levels in six years (Maxwell *et al*., 1973). Models predicting the effect of insect-resistant cultivars on insect abundance have been developed for several insect pests (Luginbill and Knipling, 1969; Knipling, 1979; Sharma, 1993; Sharma and Ortiz, 2002). Expression of resistance and its effect on insect populations varies between crops and environmental conditions, *e.g*., no direct relationship has been observed between the planting of Hessian fly-resistant cultivars and its population density in wheat (Foster *et al*., 1991). However, in case of green bug (*Schizaphis graminum*), the breeding programs continue to struggle consistently to keep pace with the evolution of new biotypes (Wood, 1971). Therefore, it is pertinent to know whether the transgenic crops have characteristics that might predispose them to unusually short or long durability. In transgenic plants, the insects are continuously exposed to the exotic genes, and there are distinct possibilities of a different type of effect on the population dynamics of the target and nontarget pests. Deployment of transgenic crops will lead either to an increase in the economic threshold level (ETL) or delay the time required by the insects to reach the ETL, depending on the nature of resistance, and the stage of the insect on which the ETL is based (Sharma, 1993). There is a need to develop a better understanding of the field performance of insect-resistant cultivars (from conventional and transgenic breeding programs) under diverse environmental conditions, and the long-term effects of resistant cultivars on insect populations.

Reduction in Dosage and Frequency of Insecticide Sprays

Deployment of transgenic insect-resistant crops has been shown to significantly reduce yield losses under severe insect pest pressure. For rational pest management, transgenic cultivars have to be deployed in combination with low dosages of insecticides (Schell, 1997). On an average, farmers spray more than 20 times to protect the cotton and many other crops from the ravages of insect pests. Transgenic crops can make a critical contribution in reducing the dosage and frequency of insecticide application. Kernel damage in transgenic maize is reduced to 17 cm^2 with 5 applications of methomyl compared to 172 cm^2 in the conventional hybrid, Silver Queen (Lynch *et al*., 1999a). Similarly, in *Bt* cotton, and insecticides are highly effective for bollworm control even at lower rates of application (Brickle *et al*., 1999). Susceptibility of *S. exigua* to alpha-cypermethrin, methomyl, profenofos, and chlorfluazuron was 1- to 1.6-fold lower in the larvae fed with *Bt* cotton than in the larvae fed with common cotton (Xue *et al*., 2002). However, the activities of acetylcholinesterase and carboxylesterase were greater in the larvae fed with *Bt* cotton than those fed with common cotton.

The yield losses and the cost of controlling insect pests in cotton are estimated at $5 billion annually, and the cotton-growing farmers used nearly $1.7 billion worth of insecticides in 2001 (James, 2002b). The economic benefit of growing *Bt* cotton has been estimated to be at $103 million or $50 per hectare in USA. In China, *Bt* cotton increased yield on 1.5 million hectares and reduced insecticide use by 78,000 tons (formulated product), resulting in significantly fewer poisonings due to insecticides. *Bt* cotton increased the annual income by $500 per hectare, which is equivalent to a national benefit of $750 million. Diao and Xie (1997) estimated that cropping with transgenic cotton R 93-4 resulted in a net income of over $1 billion and also led to a reduction in environmental pollution. On a global basis, the benefits from the deployment of *Bt* cotton between 1998 and 2001 were estimated to be $1.7 billion (James, 2002b). Cultivation of transgenic cotton with insect resistance reduced insecticide use by 10.0 to 15.8 liters ha^{-1} or 600 tons of insecticides per year (Costa *et al*., 2002). Similar estimates have also been made for genetically modified maize with resistance to corn borers. In addition, savings were also achieved in the use of soil, water, fertilizers, and energy per kg of cotton fiber and maize grain.

LIMITATIONS AND RISKS OF INSECT-RESISTANT TRANSGENIC CROPS

Recent developments in plant biotechnology offer both promises and challenges. Close proximity of transgenic crops to the sprayed fields of nontransgenic crops may suffer from insect migration from sprayed fields to the transgenic crops, and the resultant increase in pest pressure may reduce the benefits of transgenics. *Bt* toxins have been widely used as "natural" insecticides for many years with no reports of spontaneous development of resistance in the insect populations. However, with the dramatic increase in the presence of *Bt* toxins in the ecosystem (via transgenic crops) may significantly increase the pressure on insect populations to evolve resistant biotypes. The evidence on these issues is still inconclusive, and there is a need for careful monitoring before the transgenic crops are deployed on a large scale under subsistence farming conditions. One of the approaches to overcome these problems is to develop a new generation of transgenics with better genes, and use combinations of genes to delay the development of resistance in insect populations. The problems that limit the usefulness of transgenic crops for insect control include: (1) performance limitations, (2) secondary pest problems, (3) insect sensitivity, (4) development of resistance and evolution of new biotypes, (5) environmental influences on gene expression, (6) gene escape into the environment, (7) effects on nontarget organisms, (8) biosafety of food from transgenic crops, and (9) socioeconomic and ethical issues.

Performance Limitations

Bt toxins cannot produce the same dramatic effects on insect mortality as the synthetic insecticides, and therefore farmers need to be educated about the efficacy and mode of action of transgenic crops. Transgenic crops may not be able to withstand heavy insect density in some seasons, and therefore careful monitoring of insect populations should be an essential component of insect pest management involving transgenic crops. It may be necessary for national governments to develop legislation forcing commercial companies (or to fund the national programs) to ensure that this type of monitoring is carried out systematically across all cropping regions. The value of transgenic crops can be best realized when deployed as a component of insect pest management (Sharma and Ortiz, 2000a). In some cases, the yield of the transgenic cottons with resistance to *H. armigera* is lower than that of the traditional varieties (Wu *et al*., 1999). Therefore, the real benefits have to be seen in relation to the reduction in frequency and dosage of insecticide application. The value of protection offered by *Bt* maize has generally been found to be lower than the current seed premiums in Indiana, USA (Hyde *et al*., 1999). Therefore, there is an urgent need for more stringent scientific information on the performance of transgenic crops with insect resistance within a genuine integrated insect pest management system that quantifies their long-term performance and precisely models their interaction with various environmental conditions. The gene promoters currently in use direct the gene expression in green tissue, and the expression is greater in young plants. Some insects such as bollworms, pod borers, and stem borers enter into plant tissue with incomplete chlorophyll formation, which may have insufficient expression of the toxin proteins. Insufficient toxin expression may lead to development of resistance, and therefore care should be taken that the toxins are expressed in sufficient amounts at the site of damage/feeding by the insects.

Secondary Insect Pest Problems

The large-scale cultivation of transgenic plants with resistance to insect pests may result in secondary nontarget insects becoming a serious constraint in crop production in the absence of insecticide applications for the control of major pests. Therefore, it may become necessary to resume spraying in order to control the secondary insect pests. Chemical sprays applied for the control of secondary pests may kill the natural enemies and thus offset one of the advantages of transgenics. Most field crops are attacked by several insect species, and in the absence of competition from the major insect pests, the secondary insects may assume a major pest status (Hilder and Boulter, 1999). The *Bt* toxins may also be ineffective against certain insect pests, *e.g*., leaf hoppers, mirid bugs, root feeders, mites, etc. This may offset some of the expected advantages of the insect-resistant transgenic crops. Management of stinkbugs is necessary in transgenic cotton with resistance to bollworms (Greene *et al*., 1997). However, there are no differences in the susceptibility of transgenic and nontransgenic cotton varieties to boll weevil and aphids (Parker and Huffman, 1997). There is a need to identify genes that could be deployed to control the insects that are not susceptible to the *Bt* toxins. It will be desirable to use genes having a broad spectrum of activity, provided such genes do not influence the activity and abundance of beneficial and nontarget organisms.

Insect Insensitivity

The first generation transgenics contain just one *Bt* toxin gene, and lack of control of less sensitive species may present additional problems in insect pest management. There is considerable variation in the effectiveness of various *Bt* toxins towards different insect species. *Helicoverpa virescens* is less sensitive to *Cry1A(a), Cry1C*, and *Cry1E*, while *Spodoptera littoralis* is insensitive to most of the *Bt* toxins (Gill *et al*., 1992). *Spodoptera litura* is less sensitive to *Bt* toxins than *H. armigera, Achoea janata, Plutella xylostella*, and *Spilosoma obliqua* (Meenakshisundaram and Gujar, 1998). *Cry1B* is slightly active against *H. armigera*, but is inactive against *H. virescens* (Hofte and Whiteley, 1989). *CryIC* and *Cry1E* are active against *H. virescens* (MacIntosh *et al*., 1991), but are ineffective against *H. armigera* (Chakrabarti *et al*., 1998). Transgenic cotton with *Bt* genes is ineffective against some insect species, *e.g*., no differences have been observed in the survival and development of fall armyworm (*S. frugiperda*) larvae between the normal and *Cry1A(c)* transformed cottons (Adamczyk *et al*., 1998). Transgenic cotton and maize plants with *Bt* genes developed earlier were not effective against the fall armyworm (Bergvinson *et al*., 1997). Therefore, due care should be taken when considering the deployment of various genes for resistance to insect pests in different crops.

Development of Resistance and Evolution of New Biotypes

Insect-resistant cultivars derived through conventional breeding have not shown any direct relationship between insect resistance and the evolution of new biotypes, *e.g*., deployment of Hessian-fly (*Myetiola destructor*)–resistant cultivars has not led to the evolution of new insect biotypes in wheat. However, in the case of greenbug (*Schizaphis graminum*), the breeding programs struggle continuously to keep pace with the evolution of new biotypes (Daniels, 1981; Wood, 1971). In sorghum, only 3 of the 11 biotypes of greenbug have shown a correlation between the use of resistant hybrids and the development of new biotypes. Insect–plant interactions are quite specific, and future efforts should focus on the use of the most effective resistant genes or deploy multiple genes to delay the evolution of new insect biotypes. The ability of insects to overcome host plant resistance (as with their ability to develop resistance to insecticides) is always an important risk. In transgenic crops, the insects are exposed to the toxin proteins throughout the feeding cycle/season and, as a result, the insect populations are under continuous selection pressure. Most of the transgenic plants produced so far have *Bt* genes under the control of a constitutive promoter such as

CaMV35S, and this system may lead to development of resistance in the target and the nontarget insects, as the toxins are expressed in all parts of the plant (Harris, 1991). Toxin production may also decrease over the crop-growing season. Low doses of the toxins eliminate the most sensitive individuals of a population, leaving a population in which resistance can develop much faster. Since most *Bt* toxins have a similar mode of action, resistance developed against one toxin can also lead to development of cross-resistance to other toxins. However, there are reports that insects selected for resistance to one *Bt* toxin may not be resistant to other *Bt* toxins (Hilder and Boulter, 1999; Sharma and Ortiz, 2000a).

Different insect species react to *Bt* toxins differently. Resistance to *Bt* is inherited as a recessive trait in Indian meal moth (*Plodia interpunctella*) (McGaughey, 1985). Resistance increased nearly 30-fold in two generations and 100-fold after 15 generations of selection on diet treated with *Bt*. However, in *O. nubilalis* resistance appears to be inherited as a incompletely dominant autosomal gene (Huang *et al*., 1999). Laboratory selection with *Cry1A(c)* in transgenic cotton produced 300-fold resistance in a field-derived strain of pink bollworm, *P. gossypiella* (Tabashnik *et al*., 2002). Additional selection increased resistance to 3100-fold. The progeny of reciprocal F_1 crosses (resistant male × susceptible female and vice versa) responded alike in bioassays, indicating autosomal inheritance. Resistance was recessive at a high concentration of *Cry1A(c)*. However, the dominance of resistance increased as the concentration of *Cry1A(c)* decreased. Analysis of survival and growth of progeny from backcrosses (F_1 × resistant strain) suggested that resistance was controlled primarily by one or a few major loci. The progression of resistance from 300- to 3100-fold rules out the simplest model with one locus and two alleles. Overall, the patterns observed can be explained by either a single resistance gene with three or more alleles or by more than one resistance gene.

Resistance can develop quickly in *Diatraea saccharalis*, as a considerable proportion of the larvae survive up to 8 days on transgenic maize (Bergvinson *et al*., 1997). However, *D. grandiosella* has shown a considerably lower frequency of surviving individuals. There is considerable genetic variation in populations of diamondback moth in their susceptibility to *Bt* formulations (Tabashnik *et al*., 1991). Diamond back moth populations have developed resistance to *Bt* (Tang *et al*., 1999), and laboratory selection for resistance to *Bt* has shown a rapid response. Soybean looper collected from soybean and *Bt*-cotton is less susceptible to *Bt* (Condor XL^R) than the reference strain (Mascarenhas *et al*., 1998). The probability of development of resistance may be very low in some insect species, *e.g*., *O. nubilalis* (Lang *et al*., 1996). After 13 generations of selection pressure, no colony of *O. nubilalis* survived on transgenic *Bt* maize hybrids in the greenhouse. Development of resistance to *Bt* may not be a serious issue in some cases since the *Bt* and the insect pests have coevolved for millions of years (Bauer, 1995; Tabashnik, 1994). However, because of limited exposure and several toxins produced by *Bt*, the rate of development of resistance under natural conditions may not be high. In transgenic plants, the insects are continuously exposed to the transgene, and there are possibilities of development of resistance in the target insect pests over a period of time. One of the approaches to avoid the development of resistance will be to use two or more insecticidal genes with different modes of action or differentially expressed genes with tissue-specific or induced promoters. Overexpression of insecticidal toxin-coding genes in chloroplasts would be an effective strategy to delay the emergence of resistance among phytophagous insect pests. Reddy *et al*. (2002) transformed tobacco chloroplasts with *Cry1Ia5*, and accumulated up to 3% of total soluble protein in leaf tissue, which is 300-fold more when compared to the expression of the same protein in the nuclear-transformed plants. Transgenic plants offered complete protection against larvae of *H. armigera*. Analysis of T_0, T_1, and T_2 generation plants revealed site-specific integration, maternal inheritance, and uniform expression of transgenes without imposing any yield penalty.

The potential development of resistance poses the biggest challenge to insect-resistant transgenic plants and the development and implementation of Insect Resistance Management (IRM) strategies is essential. Countries that have adopted *Bt* cotton have successfully implemented different IRM strategies, and no resistance to *Bt* cotton has been detected to date, despite the fact that 13 million hectares of *Bt* cotton have been grown worldwide since 1996; several claims from critics have been proved to be unfounded. From a global viewpoint, any international initiative to substantially extend the adoption of *Bt* cotton must also anticipate and consider the implications of a significant expansion in the global area of *Bt* cotton. An effective mechanism to formulate, coordinate, and oversee a global strategy for deploying *Bt* cotton responsibly and effectively could play a seminal role if it could be operated without onerous bureaucracy.

Environmental Influences on Gene Expression

There is considerable variation in the production of *Bt* toxins over seasons and locations, which may be influenced by the site of gene insertion, epistasis, somaclonal mutations, and the environmental conditions during crop growth (Kaiser, 1996; Wu *et al*., 1997a). *Cry1A* gene expression is influenced by genetic and environmental factors. Resistance to insects in cotton crop flooded with 3 to 4 cm deep water for over 12 days is lost as compared to the crop irrigated normally (Wu *et al*., 1997a). However, the cotton plants recovered gradually after the water logging was over, and the resistance levels increased over time. Similar variation in gene expression has also been observed in *Bt* cotton under overcast and rainy conditions. The inheritance and expression patterns of the *Cry1A(b)* gene have been studied in the progenies derived from different *Bt* genes, where both Mendelian and distorted segregation ratios were observed in some selfed and crossed F_2 populations. The *Cry1A(b)* gene, driven by the maize *ubiquitin* promoter in transgenic *japonica* rice lines, displayed certain kinds of spatial and temporal

expression patterns under field conditions (Wu *et al*., 2002). The *Cry1A(b)* expression in different tissues of transgenic rice varied with temperature. The highest content of *Cry1A(b)* protein has been found in roots of plants grown at 35°C and the lowest at 40°C, while in the leaf, the highest content was recorded at 25°C and the lowest at 30°C. The content of *Cry1A(b)* protein in the leaf sheath tended to decrease with an increase in temperature.

Epistatic and environmental effects may also influence the stability, efficacy, and durability of the transgenes (Sachs *et al*., 1998). There have been some failures in insect control through the use of transgenic crops. Cotton bollworm (*H. zea*) destroyed *Bt* cottons due to its high tolerance to *CryIA(c)* in Texas, USA (Kaiser, 1996). Similarly, *H. armigera*, and *H. punctigera* destroyed the cotton crop in the second half of the growing season in Australia because of reduced production of *Bt* toxins (Hilder and Boulter, 1999). Possible causes for the failure of insect control may be inadequate production of the toxin proteins, effect of environment on expression of the transgene, locally resistant insect populations, and development of resistance due to inadequate pest management. Adamczyk and Sumerford (2002) suggested that factors such as parental background had stronger impact on expression of *Cry1A(c)* than the environment. In a set of 13 transgenic cultivars tested across locations, NuCOTN 33B and DP 458B/RR expressed more *Cry1A(c)* than the other 11 varieties tested. These two varieties have the same parental background of DP 5415. Similar varietal differences were also observed in the greenhouse tests in the following season.

Production of *Bt* toxins in transgenic plants also varies over the crop-growing season, and in different plant parts. Laboratory bioassays have indicated that there was a decline in efficacy with plant age (Sun *et al*., 2002). Amounts of *Cry1A(c)* in the fruiting structures decline from 57.1 μg per g of dry weight at 53 days after planting to 6.7 μg per g at 116 days after planting. Mean terminal *Cry1A(c)* levels declined from 163.4 μg per g at 53 days after planting to 34.5 μg per g at 116 days after planting. In general, *Cry1A(c)* levels in the fruits and terminals of Bollgard cotton declined steadily as the growing season progresses. Greatest activity has always been recorded in the terminal foliage (Greenplate, 1999; Zhao *et al*., 1998b), and there are no significant differences between *Cry1A(b)* and *Cry1A(c)* cottons (Parker *et al*., 2000). There is some variation in expression of resistance to insects in rice at the vegetative and flowering stages of the crop. Transgenic rice line 827 is more resistant to young larvae of *S. incertulas*, *C. suppressalis*, and *C. medinalis* than the control plants at the vegetative stage, but not at the flowering stage (Cohen *et al*., 2000). No borer infestation has been observed in rice cultivars TR 30-1 and KMD 1, homozygous for the *Bt* gene, while the commercial and *GUS*-negative controls have shown 83.3 and 56.7% damage at the tillering stage, respectively (Wu *et al*., 2000).

Gene Escape into the Environment

A major risk for large-scale deployment of transgenic plants is the potential spread of the transgene into the related weed community. Genes from transgenic maize, have already moved into local landraces in Mexico (Quist and Chapela, 2001). The introgression events are relatively common in maize, and the transgenic DNA constructs are maintained in the population from one generation to the next. Therefore, there is a need to study the impact of gene flow from commercial hybrids to the traditional landraces in the centers of origin in order to know the period for which the integrity of transgene construct is retained and the increase and or decrease in the abundance of the transgene construct overtime. However, it appears that there are no explicit reports of a plant becoming a weed as a result of plant breeding (Cook, 2000). This may be because crop plants generally have very low competitive ability in natural environments. Plant breeding efforts have tended to decrease rather than increase the toxic substances. As a result, improved varieties have become more susceptible to insect pests and are less competitive than the weeds. There is a feeling that genes introduced from outside the range of sexual compatibility might present new risks to the environment. A study conducted by the National Academy of Sciences, USA (NAS, 1987) concluded that there is no evidence of hazards associated with DNA techniques. The risks involved are related to the nature of the organism rather than the process, and there is an urgent need for systematic large-scale stringent research to better understand and predict these risks and thereby allow legislators and plant breeders to better plan the introduction of modified organisms into the environment.

One of the hazards in gene transfer from the transgenic plants to wild relatives is the consequence on natural plant population dynamics if the wild relatives are under selection pressure (biological control) from the target insect pest. Clearly, if the target insect does not play any role in regulating the wild plant population, then gene transfer is unlikely to constitute a significant hazard. Buildup of resistance in the wild relatives can also act as a component of insect pest management to the target insect species. Genes from unrelated sources may change the fitness and population dynamics of the hybrids between native plants and the wild species (Gregorius and Steiner, 1993; Serratos *et al*., 1997). However, interspecific hybridization is a common process, but sustainable hybrids are rare, and most are sterile. For some crops grown in some regions, there is very low chance of gene introgression into the wild relatives (Fitter *et al*., 1990). However, for others the probability is higher or as yet unquantified. Pollen dispersal from transgenic cotton is low, but increases with an increase in the size of the source plot (Llewellyn and Fitt, 1996).

There is a possibility of transfer of herbicide resistance genes to closely related wild species, which could create super weeds (Chevre *et al*., 1997). Studies in Norway and the United States have shown that the gene for herbicide resistance can move from cultivated canola to wild relatives. Genes from the conventionally bred *Brassica napus* have been moving to the wild turnip, *B. rapa* (Raybould and Gray, 1993). Therefore, for deploying crops with genes for resistance to herbicides, there is a need

to know whether the herbicide-resistant plants can establish as weeds, and the possibility of gene transfer into the wild relatives of the crop plants.

Some of the genetically engineered plants also contain a gene for antibiotic resistance as marker. Several studies have established that there is little chance that such a transfer would occur, but there is a continuing debate whether such a gene should be present in the commercial varieties. Gene transfer from plants to microorganisms is possible in laboratory studies (Gebhard and Smalla, 1998), and possibly has happened during evolution (Doolittle, 1999). Plasmid transfer between *B. thuringiensis* var *tenebrionis* and *B. thuringiensis* var *kurstaki* HD 1 (resistant to streptomycin) occurs at 10^{-2} (Thomas *et al*., 1997). However, no plasmid transfer has been observed in soil release experiments and in insects on leaf discs. Methods have now been developed for removing the selectable marker genes (Yoder and Goldsbrough, 1994; Ebinuma *et al*., 1997; Dale and Ow, 1991). There are a few alternatives to the antibiotic and herbicide markers, and systems are also available to carry out the transformation without involving any marker genes.

Effects on Nontarget Organisms

One of the major public concerns about transgenic crops is their effect on nontarget organisms. Most *Bt* toxins are specific to insects because they are activated in the alkaline medium of the insect gut and therefore are unlikely to have a detrimental effect on humans, other mammals, or birds. However, *Bt* proteins can have harmful effects on beneficial insects, although such effects are much less severe than those of the broad-spectrum insecticides. Root exudates from the transgenic maize release *Bt* toxins into the soil and retain insecticidal activity for 180 days (Saxena *et al*., 2002a, 2002b). *Bt* toxins bound to clay minerals are no longer available for uptake by microorganisms. Binding of the *Bt* toxins to humic acids reduces their potential for microbial biodegradation (Crecchio and Stotzky, 1998). Therefore, *Bt* toxins from transgenic plants and microbes could persist, accumulate, and later be released *en masse* to result in significant insecticidal activity in the soil.

The issue of genetically modified corn's toxic effects on larvae of the monarch butterfly, *Danaus plexippus* (Losey *et al*., 1999), deserves particular discussion. The initial report generated a huge amount of publicity and in turn a dramatic amount of misinformation. There is a temporal overlap between monarch butterfly and flowering of maize in the northern as compared to the southern parts of USA (Oberhauser *et al*., 2001). Agricultural practices such as weed control and foliar insecticide application have a much larger impact on the monarch population than the genetically modified crops. Wraight *et al*. (2000) concluded that there was no relationship between mortality of *Papilio polyxenes* and pollen deposition from transgenic maize. Although the findings of Losey *et al*. (1999) have been systematically discredited, the counterfindings have not been reported in the media at a level similar to the initial report. This is a recurrent problem in this dialogue, which is leaving the public psyche increasingly biased by high profile misinformation and half-truths.

Some reports suggest that transgenic oilseed rape does not appear to have harmful effects on the lifespan and behavior of honeybees (Pham-Delegue and Jouanin, 1997), but analysis of a wider range of transgenes suggests otherwise. The *chitinase* transgene in genetically modified oilseed rape did not affect learning performance of honeybees; *beta-1,3 glucanase* affected the level of conditioned responses (the extinction process occurring more rapidly as the concentration increased), and *CpTi* induced marked effects in both conditioning and testing phases, especially at high concentrations (Picard-Nizou *et al*., 1997). Trypsin inhibitor, wheat germ agglutinin (*WGA*), serine proteinase inhibitor from soybean, cysteine protease inhibitor from rice, chicken egg white cystatin, and Bowman-Birk type *SBTI* do not produce harmful effects on honeybees at the concentrations expressed in transgenic plants (Pham-Delegue and Jouanin, 1997; Bottino *et al*., 1988; Girard *et al*., 1998b). However, soybean trypsin inhibitor at 1% concentration is toxic to adult honeybees (Malone *et al*., 1995). Consumption of high doses of protease inhibitors induces overproduction of proteinase (Jouanin *et al*., 1998). However, what most of these studies miss is a direct comparison with the effects of commercial insecticide alternatives to transgenic crops.

Initial reports suggested that there were no major differences in predator abundance between transgenic and nontransgenic crops of tobacco (Hoffmann *et al*., 1992) and of cotton (Wang and Xia, 1997), and between transgenic and mixed-seed potato fields (Riddick *et al*., 1998). However, abundance of *Labia grandis* was observed to be lower in pure and mixed crops of transgenic potatoes than in a pure nontransgenic potato crop (Riddick *et al*., 1998). Similarly, a reduction in the fitness of the predatory chrysopid larvae has been observed when fed on prey reared on *Bt*-maize (Hilbeck *et al*., 1998; Hoffmann *et al*., 1992). There is no effect on preimaginal development or mortality of *Chrysoperla carnea* when reared on *Rhopalosiphum padi* fed on *Bt*-maize (Lozzia *et al*., 1998). Similarly, survival, aphid consumption, development, and reproduction are not influenced in *Hippodamia convergens* fed on *M. persicae* reared on potatoes expressing δ-endotoxin (Dogan *et al*., 1996). However, two spotted ladybird beetles (*Adalia bipunctata*) fed on peach-potato aphids (*M. persicae*) colonizing transgenic potatoes expressing the lectin gene from *Galanthus nivalis* have shown a decrease in fecundity, egg viability, and longevity (Birch *et al*., 1999). The adverse effects on ladybird reproduction were reversed after switching the ladybirds to pea aphids from nontransgenic bean plants. Feeding *C. carnea* on *Tetranychus urticae* (which ingested *Bt* toxin from the transgenic plants) or *R. padi* (which did not ingest the *Bt* toxin) did not affect survival or development of the predator (Dutton *et al*., 2002). However, a significant increase in the mortality and delay in development of *C. carnea* was observed when fed on *S. littoralis* (which also ingested the *Bt* toxins). Clearly, the effects of transgenic plants on the activity and abundance of

predators vary across crops, insect species, and the transgenes in question.

Bacillus thuringiensis toxin-mediated partial resistance is compatible with natural enemies for the control of *H. virescens*. Increased levels of parasitism by *Campoletis sonorensis* on *Helicoverpa* have been observed on the transgenic plants compared to the nontransgenic plants, which may be due to fewer larvae on the transgenic plants (Johnson and Gould, 1992). Activity of *Cardiochiles nigriceps* is not influenced by transgenic plants (Johnson, 1997; Johnson *et al*., 1997). Percentage of parasitism by *Diadegma insulare* is not significantly different between the mixed and pure transgenic crops (Riggin Bucci and Gould, 1997). Transgenic corn has no adverse effects on the parasitization of *O. nubilalis* by *Eriborus tenebrans* and *Macrocentrus grandii* (Orr and Landis, 1997). The survival of *Aphidius colemani*, *Trichogramma brassicae*, and *Cotesia glomerata* was reduced when fed on sucrose solution containing *GNA* (Romeis *et al*., 2003). Negative effects on fecundity were observed only in case of *T. brassicae*. Thus, the effects of *GNA* consumption through the honeydew may depend on the species biology and mode of egg maturation. The effects of transgenic crops on the parasitoid activity and abundance vary across crops and the cropping systems. Some of this variation may be due to differences in insect abundance between transgenic and nontransgenic crops. Wherever transgenic crops have shown adverse effects on natural enemies, these effects may still be far lower than the large-scale application of broad-spectrum insecticides (Sharma and Ortiz, 2000a).

Biosafety of Food from Transgenic Crops

Genetic engineering of a plant can also have unexpected effects on the ecosystem that cradles it. It is consumers' grasp of this fundamental yet unpredictable phenomenon that underlines much of the concern over transgenic crops that is now spilling over into more general concern about any biotechnology-based food product. There is clearly a need for new food technologies to be tested rigorously for their potential allergenic, toxic, and antimetabolic effects in a transparent manner, in a way similar to modern pharmaceuticals (Gillard *et al*., 1999; Sharma *et al*., 2002b). Most *Bt* toxins are specific to insects, as they are activated in the alkaline medium of the insect gut. There are no specific receptors for *Bt* protein in the gastrointestinal tract of mammals, including humans (Kuiper and Noteborn, 1994). The *Bt* proteins are rapidly degraded by stomach juices in vertebrates. There are no major changes in the composition of *Bt* tomatoes and potatoes. Thus, transgenic *Bt* tomatoes are considered to pose no additional risk to human and animal health as compared to conventional tomatoes (Noteborn *et al*., 1996). However, a number of aspects concerning the safety assessment of transgenic food would require further study. The seed from the *Bt*-transformed cotton lines is compositionally equivalent to and as nutritious as the seed from the parental lines and other commercial cotton varieties (Berberich *et al*., 1996). Processing removes over 97% of the *Bt* proteins in transgenic cottonseed (Sims and Berberich, 1996). *CryIA(b)* protein dissipates readily on the surface of soil, or is cultivated into the soil (Sims and Holden, 1996), and has not been detected in silage prepared from transgenic plants (Fearing *et al*., 1997). Intact transgenes from maize silage are unlikely to survive significantly in the sheep rumen (Duggan *et al*., 2003). However, DNA released from the diet within the mouth may retain sufficient biological activity for the transformation of competent oral bacteria. Histopathological effects have been observed in the gut mucosa in mice and rabbits, but no systemic adverse effects have been observed following oral administration. There are no differences in the survival and body weight of broilers reared on meshed or pelletted diets prepared with *Bt* transgenic and non-transgenic maize (Brake and Vlachos, 1998).

Several protein families that contribute to the natural defense mechanisms in crop plants are allergens or putative allergens and may be toxic to mammals and humans. These include α-amylase and trypsin inhibitors, lectins, and pathogenesis-related proteins (Franck-Oberaspach and Keller, 1997). Thus, there is a trade off between nature's insecticides produced by transgenic plants, varieties from traditional breeding programs, synthetic insecticides, mycotoxins, and other poisonous products of insect pests. Rats fed on purified cowpea trypsin inhibitor in a semisynthetic diet have shown a moderate reduction in weight gain, despite identical food intake (Pusztai *et al*., 1992). Most of the *CpTi* was rapidly broken down in the digestive tract, and its inclusion in the diet led to a slight increase in the nitrogen content of feces, but not of urine. The nutritional penalty for increased insect-resistance after the transfer of the *CpTi* gene into food plants is quite low in the short-term. The level of *GNA* lectin expression that provides insecticidal protection to plants does not reduce the growth of young rats, but shows a negligible effect on weight and length of the small intestine, and a slight hypertrophy of this tissue (Pusztai *et al*., 1996). Activity of brush border enzymes was affected; sucrase-isomaltase was nearly halved, and alkaline phosphatase and aminopeptidase activity showed a significant increase. Agglutinins from wheat germ (*WGA*), thorn apple (*Datura stramonium*), and nettle (*Urtica dioica*) interfere with metabolism to varying degrees (Pusztai *et al*., 1993). Expression of a new gene in a crop could also introduce new allergens normally not present in the nontransformed plants. Biotechnology can introduce new proteins into food crops from plants, bacteria, and viruses whose allergenicity is unknown. If the introduced proteins are from known sources, then predicting and assessing the allergenicity of genetically modified plants is easier. Eight commonly allergenic and 160 less allergenic foods have been identified, and scientists can certainly avoid the transfer of genes associated with these allergenic effects (Lehrer, 2000). While there are to date no documented serious effects of transgenics on mammals, extensive studies should still be undertaken on a case-by-case basis before a transgenic crop is released for large-scale cultivation by farmers (Sharma *et al*., 2002b). Conversely, genetic

transformation offers the potential of eliminating the allergenicity of conventional foods. For example, antisense technology may hold promise for reducing the dramatic allergenicity of peanuts and other nuts.

Socioeconomic and Ethical Issues

If transgenic crops are suddenly and seriously affected by the breakdown of resistance to the target insect pests, there may be dramatic effects on the lives of poor farmers. Under such circumstances, government agencies and the private companies must compensate the farmers for crop loss. However, widespread sudden and severe crop losses due to insect and disease epidemics are not uncommon in conventional crops. There is clearly an urgent need for better prediction of these catastrophes whether they are affecting transgenic or conventional crops. In addition, there is a need for equity in benefit sharing between biotechnologists and the primary conservers of genetic resources and the holders of traditional knowledge. The primary conservers have so far remained poor, while those who use their knowledge to manipulate the valuable genes encapsulated therein have become rich. Unless research and development efforts on transgenics are based on principles of bioethics, there will be serious public concerns about the social, ecological, and economic consequences of replacing local varieties with a few genetically improved crop varieties.

Public perceptions about biotechnology are often not based on fact, and there is a tendency of the media to overemphasize the risks in order to gain public attention (Sharma *et al*., 2001, 2002b). Research in biotechnology should be integrated with appropriate policies and conventional breeding. The benefits and the risks associated with the use of biotechnology to increase agricultural production need to be presented to the general public in a balanced manner, and concern for moral and ethical issues of relevance to different societies needs to be addressed. Public trust in the application of biotechnology for increasing and stabilizing agricultural production needs to be improved to use modern science for the maximum benefit of subsistence farmers in developing countries.

Patenting and application of intellectual property rights will limit the access to germplasm, control the research process, and lead to a focus of research effort on global commodities. There is no difference for farmers in industrial nations between buying the seed of a transgenic cultivar or that of a hybrid variety based on genetic or cytoplasmic male-sterility produced through conventional technology. However, it is widely articulated that transgenic crops may have serious implications in developing countries where the farmers depend on seeds saved from the previous season. The major risk of modern biotechnology is that technological developments may bypass the poor farmers in the developing countries because of a lack of enlightened adaptation and an absence of focus on the problems of small farmers in the developing countries. The private sector is unlikely to undertake research on problems with low profits, and without a stronger public sector role, a form of scientific apartheid may develop, in which cutting edge science might become oriented exclusively towards industrial countries and large-scale farming. Therefore, there is a need for establishing a strong cooperation between private sector and public institutions to solve the insect pest problems confronting crop production in the developing countries. The critical issue is that every tool in crop improvement and resource management needs to be mobilized to feed the hungry, help the poor, and protect the environment. Therefore, we must find ways of realizing the promise of biotechnology while avoiding the pitfalls.

MANAGING THE RISK OF GENE FLOW

Many agricultural practices are in place for risk management. The risk of gene transfer by out-crossing from a transgenic crop to a weed, *e.g*., from canola to weedy mustard, can be managed by spraying a herbicide with a different mode of action. Crop rotations can also be used to control such weeds. The risk of introducing a fertile hybrid between transgenic plants and weedy relatives can be managed by growing the seeds under strict certification procedures to identify crop weed hybrids in seed production plots. Gene transfer within the same species can be avoided by keeping a safe distance between the adjacent plots. Such information to avoid out-crossing is available for most of the cultivated crops. In areas where there is a greater chance of gene transfer, *e.g*., in the center of origin of a crop plant, serious scientific studies should be conducted before introducing a transgenic crop with certain genes. Varieties or crops that are likely to be carried to next crop season or contaminate the same crop next season can be replaced by crop varieties with less or no carryover of seed to the next season. However, the success of many of these actions relies on collective community action and/or strong national legislation.

FUTURE OUTLOOK

The ideal transgenic technology should be commercially viable, environmentally benign (biodegradable), easy to use in diverse agro-ecosystems, and have a wide spectrum of activity against the target insect pests. It should also be harmless to the natural enemies and nontarget organisms, target the sites in insects that have developed resistance to the conventional insecticides, be flexible enough to allow ready deployment of alternatives (if and when the resistance is developed in insect populations), and preferably produce acute rather than chronic effects on the target insects. Some of the criteria can be achieved by exploiting genes that are based on antibody technology. Single-chain antibodies can be used to block the function of essential insect proteins. The potential of plant-expressed antibodies or antibody fragments to serve as insect control agents against nematodes, pathogens, and viruses has been demonstrated. This approach of controlling insects would offer the advantage of allowing some degree of selection for specificity effects so that insect pests, but not the beneficial organisms, are targeted. The development of a delivery system for toxins from the transgenic

plants to the insect haemolymph will remove a key constraint in the transgenic approach to crop protection.

Incorporation of insecticidal genes in crop plants will have a tremendous effect on pest management. We need to pursue the management strategy that reflects the insect biology, insect–plant interactions, and their influence on natural enemies to prolong the life span of transgenic crops. Refugia (insects emerging from nontransgenic crops) can play an important role in resistance management and should take into account the insect pest complex, the insect hosts, cropping system, and the environment. Emphasis should also be placed on combining exotic genes with conventional host plant resistance and also with traits conferring resistance to other insect pests and diseases of importance in a crop in the target region. Several genes conferring resistance to insects can also be deployed as multilines or synthetics. Walker *et al*. (2002) combined a QTL conditioning corn earworm resistance in soybean PI 229358 and *Cry1A(c)* transgene from the recurrent parent Jack-*Bt* into BC_2F_3 plants by marker-assisted selection. The segregating individuals were genotyped and SSR markers linked to an antibiosis/antixenosis QTL on linkage group M, and they were tested for the presence of *Cry1A(c)*. Few larvae of corn earworm and soybean looper survived on leaves expressing the *Cry1A(c)* protein. Though not as great as the effect of *Cry1A(c)*, the PI 229358-derived LG M QTL also had a detrimental effect on larval weights of both the species, and on defoliation by corn earworm, but did not reduce defoliation by soybean looper. This work demonstrates that combining transgene- and QTL-mediated resistance to lepidopteran pests may be a viable strategy for insect control.

The vast majority of the area of transgenic crops remains concentrated in massive agricultural systems of Australia, Canada, Argentina, China, and the U.S. While several crops with commercial viability have been transformed in the developed world, very little has been done to use this technology to increase food production in the harsh environments of the tropics. There is a need to use these tools to provide resistance to insects in cereals, legumes, and oil seed crops that are a source of sustenance for poorer sections of the society. Equally important is the need to follow the biosafety regulations and make this technology available to farmers, who cannot afford the high cost of seeds and chemical pesticides. International research centers, advanced research institutions, and the national agricultural research systems need to play a major role in promoting biotechnology for food security of poor people in the developing countries.

REFERENCES

Adamczyk, J. J., Jr., Holloway, J. W., Church, G. E., Leonard, B. R., and Graves, B. 1998. Larval survival and development of the fall armyworm (Lepidoptera: Noctuidae) on normal and transgenic cotton possessing the *Bacillus thuringiensis* CryIA(c) delta-endotoxin. *J. Econ. Entomol.* **91**: 539–545.

Adamczyk, J. J., Jr. and Sumerford, D. V. 2002. Potential factors impacting season expression of Cry1Ac in 13 commercial varieties of Bollgard cotton. *Insect Sci.* **1.13**. *http://insectscience.org/1.13*

Alam, M. F., Datta, K., Abrigo, E., Oliva, N., Tu, J., Virmani, S. S., and Datta, S. K. 1999. Transgenic insect-resistant maintainer line (IR68899B) for improvement of hybrid rice. *Plant Cell Rep.* **18**: 572–575.

Altpeter, F., Diaz, I., McAuslane, H., Gaddour, K., Carbonero, P., and Vasil, I. K. 1999. Increased insect resistance in transgenic wheat stably expressing trypsin inhibitor *CMe*. *Mol. Breed.* **5**: 53–63.

Altstein, M., Ben-Aziz, O., Schefler, I., Zeltser, I., and Gilon, C. 2000. Advances in the application of neuropeptides in insect control. *Crop Prot.* **19**: 547–555.

Archer, T. L., Schuster, G., Patrick, C., Cronholm, G., Bynum, E. D. Jr., and Morrison, W. P. 2000. Whorl and stalk damage by European and Southwestern corn borers to four events of *Bacillus thuringiensis* transgenic maize. *Crop Prot.* **19**: 181–190.

Arencibia, A., Vazquez, R. I., Prieto, D., Tellez, P., Carmona, E. R., Coego, A., Hernandez, L., Riva, G. A. de-la, and Selman-Housein, G. 1997. Transgenic sugarcane plants resistant to stem borer attack. *Mol. Breed.* **3**: 247–255.

Armstrong, C. L., Parker, G. B., Pershing, J. C., Brown, S. M., Sanders, P. R., Duncan, D. R., Stone, T., Dean, D. A., DeBoer, D. L., Hart, J., Howe, A. R., Morrish, F. M., Pajeau, M. E., Peterse, W. L., Reich, B. J., Rodriguez, R., Santino, C. G., Sato, S. J., Schuler, W., Sims, S. R., Stehling, S., Tarochione, L. J., and Fromm, M. E. 1995. Field evaluation of European corn borer control in progeny of 173 transgenic corn events expressing an insecticidal protein from *Bacillus thuringiensis*. *Crop Sci.* **35**: 550–557.

Arpaia, S., De Marzo, L., Di Leo, G. M., Santoro, M. E., Mennella, G., and Vanloon, J. J. A. 2000. Feeding behaviour and reproductive biology of Colorado potato beetle adults fed transgenic potatoes expressing the *Bacillus thuringiensis* Cry3B endotoxin. *Entomol. Exp. Applic.* **95**: 31–37.

Arpaia, S., Mennella, G., Onofaro, V., Perri, E., Sunseri, F., and Rotino, G. L. 1997. Production of transgenic eggplant (*Solanum melongena* L.) resistant to Colorado potato beetle (*Leptinotarsa decemlineata* Say). *Theor. Appl. Genet.* **95**: 329–334.

Barton, K., Whiteley, H., and Yang, N. S. 1987. *Bacillus thuringiensis* δ-endotoxin in transgenic *Nicotiana tabacum* provides resistance to lepidopteran insects. *Plant Physiol.* **85**: 1103–1109.

Barton, K. A. and Miller, M. J. 1991. Insecticidal toxins in plants. *EPA 0431829 A1 910612*.

Bauer, L. S. 1995. Resistance: a threat to the insecticidal crystal proteins of *Bacillus thuringiensis*. *Fla. Entomol.* **78**: 415–443.

Benedict, J. H., Sachs, E. S., Altman, D. W., Deaton, D. R., Kohel, R. J., Ring, D. R., and Berberich, B. A. 1996. Field performance of cotton expressing Cry IA insecticidal crystal protein for resistance to *Heliothis virescens* and *Helicoverpa zea* (Lepidoptera: Noctiudae). *J. Econ. Entomol.* **89**: 230–238.

Benfey, P. N. and Chua, N. H. 1989. Regulated genes in transformed plants. *Science* **244**: 174–181.

Benfey, P. N. and Chua, N. H. 1990. The cauliflower mosaic virus 35S promotor combinatorial regulation of transcription in plants. *Science* **250**: 959–966.

Berberich, S. A., Ream, J. E., Jackson, T. L., Wood, R., Stipanovic, R., Harvey, P., Patzer, S., and Fuchs, R. L. 1996. The composition of insect-protected cottonseed is equivalent to that of conventional cottonseed. *J. Agric. Food Chem.* **44**: 365–371.

Bergvinson, D., Willcox, M. N., and Hoisington, D. 1997. Efficacy and deployment of transgenic plants for stem borer management. *Insect Sci. Appl.* **17**: 157–167.

Berliner, E. 1915. Uber die Schalffsuchi der Mehlmottenraupo (*Ephestia kuhniella* Zell.) und thren Erreger, *Bacillus thuringiensis* n. sp. *Z. Angew. Entomol.* **2**: 29–56.

Birch, R. G. 1997. Plant transformation; problems and strategies for practical application. *Annu. Rev. Plant. Physiol. Mol. Bio.* **48**: 297–326.

Birch, A. N. E., Geoghegan, I. E., Griffiths, D. W., and McNicol, J. W. 2002. The effect of genetic transformations for pest resistance on foliar solanidine-based glycoalkaloids of potato (*Solanum tuberosum*). *Ann. Appl. Biol.***140**: 143–149.

Birch, A. N. E., Geoghegan, I. E., Majerus, M. E. N., McNicol, J. W., Hackett, C. A., Gatehouse, A. M. R., and Gatehouse J. A. 1999. Tri-trophic interactions involving pest aphids, predatory 2-spot ladybirds and transgenic potatoes expressing snowdrop lectin for aphid resistance. *Mol. Breed.* **5**: 75–83.

Bohorova, N., Zhang, W., Julstrum, P., McLean, S., Luna, B., Brito, R. M., Diaz, L., Ramos, M. E., Estanol, P., and Pacheco, M. 1999. Production of transgenic tropical maize with cryIAb and cryIAc genes via microprojectile bombardment of immature embryos. *Theor. Appl. Genet.* **99**: 437–444.

Bottino, M. B., Girard, C., Jouanin, L., Metayer, M. le, Picard-Nizou, A. L., Sandoz, G., Pham-Delegue, M. H., Lerin, J., and Thomas, G. 1998. Effects of transgenic oilseed rape expressing proteinase inhibitors on pest and beneficial insects. *Acta Hort.* **459**: 235–239.

Boulter, D. 1993. Insect pest control by copying nature using genetically engineered crops. *Phytochem.* **34**: 1453–1466.

Boulter, D., Edwards, G. A., Gatehouse, A. M. R., Gatehouse, J. A., and Hilder, V. A. 1990. Additive protective effects of incorporating two different higher plant derived insect resistance genes in transgenic tobacco plants. *Crop Prot.* **9**: 351–354.

Brake, J. and Vlachos, D. 1998. Evaluation of transgenic event 176 "*Bt*" corn in broiler chickens. *Poult. Sci.* **77**: 648–653.

Brickle, D. S., Turnipseed, S. G., Sullivan, M. J., and Dugger, P. 1999. The efficacy of different insecticides and rates against bollworms (Lepidoptera: Noctuidae) in B.T. and conventional cotton. **In**: *Proceedings, Beltwide Cotton Production and Research Conference*, 3–7 January 1999, Orlando, Florida, USA, pp. 934–936. Richter, D., Ed., Volume 2, National Cotton Council, Memphis, TN.

Brown, D. G. 1988. The cost of plant defense: an experimental analysis with inducible proteinase inhibitors in tomato. *Oecologia* **76**: 467–470.

Canedo, V., Benavides, J., Golmirzaie, A., Cisneros, F., Ghislain, M., and Lagnaoui, A. 1999. Assessing Bt-transformed potatoes for potato tuber moth, *Phthorimaea operculella* (Zeller), management. **In**: *Impact on a Changing World. International Potato Center Program Report 1997–1998*, pp. 161–169. International Potato Center (CIP), Lima, Peru.

Cao, J., Tang, J. D., Strizhov, N., Shelton, A. M., and Earle, E. D. 1999. Transgenic broccoli with high levels of *Bacillus thuringiensis* Cry1C protein control diamondback moth larvae resistant to Cry1A or Cry1C. *Mol. Breed.* **5**: 131–141.

Carbonero, P., Royo, J., Diaz, I., Garcia-Maroto, F., Gonzalez-Hidalgo, E., Gutierez, C., and Casanera, P. 1993. Cereal inhibitors of insect hydrolases (α-amylases and trypsin): genetic control, transgenic expression and insect pests. **In**: *Workshop on Engineering Plants Against Pests and Pathogens,* 1–13 Jan 1993. Bruening, G. J., Garcia-Olmedo, F., and Ponz, F. J., Eds., Instituto Juan March de Estudios Investigacions, Madrid, Spain.

Chakrabarti, S. K., Mandaokar, A. D., Kumar, P. A., and Sharma, R. P. 1998. Synergistic effect of Cry1A(c) and Cry1F delta-endotoxins of *Bacillus thuringiensis* on cotton bollworm, *Helicoverpa armigera*. *Curr. Sci.* **75**: 663–664.

Charity, J. A., Anderson, M. A., Bittisnich, D. J., Whitecross, M., and Higgins, T. J. V. 1999. Transgenic tobacco and peas expressing a proteinase inhibitor from *Nicotiana alata* have increased resistance. *Mol. Breed.* **5**: 357–365.

Chevre, A. M., Eberm, F., Baranger, A., and Renard, M. 1997. Gene flow from transgenic crops. *Nature* **389**: 924.

Chilton, M. D. 1983. A vector for introducing new genes into plants. *Sci. Am.* **248**: 50–59.

Cho, H., Choi, K., Yamashita, M., Morikawa, H., and Murooka, Y. 1995. Introduction and expression of the *Streptomyces* cholesterol oxidase gene (choA), a potent insecticidal protein active against boll weevil larvae, into tobacco cells. *Appl. Microbiol. Biotechnol.* **44**: 133–138.

Chrispeels, M. J., Grossi-de-Sa, M. F., and Higgins, T. J. V. 1998. Genetic engineering with $\propto$-amylase inhibitors seeds resistant to bruchids. *Seed Sci. Res.* **8**: 257–263.

Clark, T. L., Foster, J. E., Kamble, S. T., and Heinrichs, E. A. 2000. Comparison of Bt (*Bacillus thuringiensis* Berliner) maize and conventional measures for control of the European corn borer (Lepidoptera: Crambidae). *J. Entomol. Sci.* **35**: 118–128.

Cloutier, C., Jean, C., Fournier, M., Yelle, S., and Michaud, D. 2000. Adult Colorado potato beetles, *Leptinotarsa decemlineata* compensate for nutritional stress on oryzacystatin I-transgenic potato plants by hypertrophic behavior and over-production of insensitive proteases. *Arch. Insect Biochem. Physiol.* **44**: 69–81.

Cohen, M. B., Alinia, F., Ghareyazie, B., Rubia, L., and Bennett, J. 2000. Effect of plant age, larval age, and fertilizer treatment on resistance of a Cry1A(b)-transformed aromatic rice to lepidopterous stem borers and foliage feeders. *J. Econ. Entomol.* **93**: 484–493.

Cook, R. J. 2000. Science-based risk assessment for the approval and use of plants in agricultural and other environments. **In**: *Agricultural Biotechnology and the Poor*, pp. 123–130. Persley, G. J. and Lnatin, M.M., Eds., Consultative Group on International Agricultural Research, Washington DC.

Corbin, D. R., Greenplate, J. T., Wong, E. Y., and Purcell, J. P. 1994. Cloning of an insecticidal cholesterol oxidase gene and its expression in bacteria and plant protoplasts. *Appl. Environ. Microbiol.* **60**: 4239–4244.

Cosa De, B., Moar, W., Lee, S. B., Miller, M., and Daniell, H. 2002. Overexpression of the *Bt cry2Aa2* operon in chloroplasts leads to formation of insecticidal crystals. *Nature Biotechnol.* **19**: 71–74.

Costa, J., Ojembarrena, A., and Alcalde, E. 2002. Biotechnology and sustainability. *Agric. Rev. Agropec.* **71**, 102–105.

Cotsaftis, O., Sallaud, C., Breitler, J. C., Meynard, D., Greco, R., Pereira, A., and Guiderdoni, E. 2002. Transposon-mediated generation of T-DNA- and marker-free rice plants expressing a Bt endotoxin gene. *Mol. Breed.* **10**: 165–180.

Crecchio, C. and Stotzky, G. 1998. Insecticidal activity and biodegradation of the toxin from *Bacillus thuringiensis* subsp. *kurstaki* bound to humic acids from soil. *Soil Biol. Biochem.* **30**: 463–470.

Crickmore, N., Ziegler, D. R., Fietelson, J., Schnepf, E., Van Rie, J., Lereclus, D., Baum, J., and Dean, D.H. 1998. Revision of the nomenclature for *Bacillus thuringiensis* pesticidal crystal proteins. *Microbiol. Mol. Biol. Rev.* **62**: 807–813.

Cui, J. J. and Xia, J. Y. 1999. Effects of transgenic Bt cotton on development and reproduction of cotton bollworm. *Acta Agric. Univ., Henan* **33**: 20–24.

Czapla, T. H. and Lang, B. A. 1990. Effects of plant lectins on the larval development of European corn borer (Lepidoptera: Pyralidae) and Southern corn rootworm (Coleoptera: Chysomelidae). *J. Econ. Entomol.* **83**: 2480–2485.

Dale, E. C. and Ow, D. W. 1991. Gene transfer with subsequent removal of the selection gene from the host genome. *Proc. Natl. Acad. Sci. USA* **88**: 10558–10562.

Dale, P. J., Irwin, J. A., and Scheffler, J. A. 1993. The experimental and commercial release of transgenic crop plants. *Plant Breed.* **111**: 1–22.

Daniels, N. E. 1981. *Migration of Greenbugs in the Texas Panhandle in Relation to their Biotypes*. Texas Agriculture Experiment Station Miscellaneous Publication, MP-1487, College Station, Texas.

Datta, K., Vasquez, A., Tu, J., Torrizo, L., Alam, M. F., Oliva, N., Abrigo, E., Khush, G. S., and Datta, S. K. 1998. Constitutive and tissue-specific differential expression of the cryIA(b) gene in transgenic rice plants conferring resistance to rice insect pests. *Theor. Appl. Genet.* **97**: 20–30.

Davidson, M. M., Jacobs, J. M. E., Reader, J. K., Butler, R. C., Frater, C. M., Markwick, N. P., Wratten, S. D., and Conner, A. J. 2002. Development and evaluation of potatoes transgenic for a cry1Ac9 gene conferring resistance to potato tuber moth. *J. Am. Soc. Hort. Sci.* **127**: 590–596.

Delannay, X., LaVallee, B. J., Proksch, R. K., Fuchs, R. L., Sims, S. K., Greenplate, J. T., Marrone, P. G., Dodson, R. B., Augustine, J. J., Layton, J. G., and Fischhoff, D. A. 1989. Field performance of transgenic tomato plants expressing *Bacillus thuringiensis* var *kurstaki* insect control protein. *Bio/Technology* **7**: 1265–1269.

De Leo, F., Bonade-Bottino, M. A., Ceci, L. R., Gallerani, R., and Jouanin, L. 1998. Opposite effects on *Spodoptera littoralis* larvae of high expression level of a trypsin inhibitor in transgenic plants. *Plant Physiol.* **118**: 997–1004.

Dhankher, O. P. and Gatehouse, J. A. 2003. Non-systemic induction of polyphenol oxidase in pea and chickpea after wounding. *Physiol. Mol. Biol. Plants* **9**: 125–129.

Diao, Y. P. and Xie, F. L. 1997. Bollworm resistant gene Bt examined and appraised by experts. *China Cottons* **24**(8): 27.

Ding, X., Gopalakrishnan, B., Johnson, L. B., White, F. F., Wang, X., Morgan, T. D., Kramer, K. J., and Muthukrishnan, S. 1998. Insect resistance of transgenic tobacco expressing an insect chitinase gene. *Transgenic Res.* **7**: 77–84.

Dogan, E. B., Berry, R. E., Reed, G. L., and Rossignol, P. A. 1996. Biological parameters of convergent lady beetle (Coleoptera: Coccinellidae) feeding on aphids (Homoptera: Aphididae) on transgenic potato. *J. Econ. Entomol.* **89**: 1105–1108.

Dombrowski, J. E., Pearce, G., and Ryan, C. A. 1999. Proteinase inhibitor-inducing activity of the prohormone prosystemin resides exclusively in the C-terminal systemin domain. *Proc. Natl. Acad. Sci. USA* **96**: 12947–12952.

Doolittle, W. F. 1999. Phytogenic classification and the universal tree. *Science* **284**: 2124–2127.

Duan, X., Li, X., Xue, Q., Abo el Saad, M., Xu, D., and Wu, R. 1996. Transgenic rice plants harboring an introduced potato proteinase inhibitor II gene are insect resistant. *Nature Biotechnol.* **14**: 494–498.

Duggan, P. S., Chambers, P. A., Heritage, J., and Forbes, J. M. 2003. Fate of genetically modified maize DNA in the oral cavity and rumen of sheep. *British J. Nutr.* **89**: 159–166.

Dulmage, H. T. 1981. Insecticidal activity of isolates of *Bacillus thuringiensis* and their potential for pest control. **In**: *Microbial Control of Pests and Plant Diseases*, pp. 129–141. Burges, H. D., Ed., Academic Press, London.

Dutton, A., Klein, H., Romies, J., and Bigler, F. 2002. Uptake of Bt-toxin by herbivore feeding on transgenic maize and consequences for the predator *Chrysoperla carnea*. *Ecol. Entomol.* **27**: 441–447.

Ebel, J. 1986. Phytoalexin synthesis: the biochemical analysis of the induction process. *Annu. Rev. Phytopath.* **24**: 235–264.

Ebinuma, H., Sugita, K., Matsunaga, E., and Yamakado, M. 1997. Selection of marker-free transgenic plants using the isopentenyl transferase gene. *Proc. Natl. Acad. Sci. USA* **94**: 2117–2121.

Edmonds, H. S., Gatehouse, L. N., Hilder, V. A., and Gatehouse, J. A. 1996. The inhibitory effects of the cysteine protease inhibitor, oryzacystatin, on digestive proteases and on larval survival and development of the southern corn rootworm (*Diabrotica undecimpunctata* Howard). *Entomol. Exp. Applic.* **78**: 83–94.

Estruch, J. J., Carrozzi, N. B., Desai, N., Duck, N. B., Warren, G. W., and Koziel, M. G. 1997. Tramsgenic plants: an emerging approach to pest control. *Nature Biotechnol.* **15**: 137–141.

Estruch, J. J., Warren, G. W., Mullins, M. A., Nye, G. J., Craig, J. A., and Koziel, M. G. 1996. Vip3A, a novel *Bacillus thuringiensis* vegetative insecticidal protein with a wide spectrum of activities against lepidopteran insects. *Proc. Natl. Acad. Sci. USA* **93**: 5389–5394.

Fang, H. J., Li, D. L., Wang, G. L., and Li, Y. H. 1997. An insect-resistant transgenic cabbage plant with the cowpea trypsin inhibitor (CpTi) gene. *Acta Bot. Sinica* **39**: 940–945.

Fearing, P. L., Brown, D., Vlachos, D., Meghji, M., and Privalle, L. 1997. Quantitative analysis of CryIA(b) expression in Bt maize plants, tissues and silage, and stability of expression over successive generations. *Mol. Breed.* **3**: 169–176.

Federici, B. A. 1998. Broad-scale leaf pest-killing plants to be true test. *Calif. Agric.* **52**: 14–20.

Fischhoff, D. A., Bowdish, K. S., Perlak, F. J., Marrone, P. G., MvCormick, S. M., Niedermeyer, J. G., Dean, D. A., Kusano-Kretzmer, K., Mayer, E. J., Rochester, D. E., Rogers, S. G., and Fraley, R. T. 1987. Insect tolerant tomato plants. *Bio/Technology* **5**: 807–812.

Fitches, E., Gatehouse, A. M. R., and Gatehouse, J. A. 1997. Effects of snowdrop lectin (GNA) delivered via artificial diet and transgenic plants on the development of tomato moth (*Lacanobia oleracea*) larvae in laboratory and glasshouse trials. *J. Insect Physiol.* **43**: 727–739.

Fitter, A., Perrins, J., and Williamson, M. 1990. Weed probability challenged. *Bio/Technology* **8**: 473.

Foissac, X., Nguyen, T. L., Christou, P., Gatehouse, A. M. R., and Gatehouse, J. A. 2000. Resistance to green leafhopper (*Nephotettix virescens*) and brown planthopper (*Nilaparvata lugens*) in transgenic rice expressing snowdrop lectin (*Galanthus nivalis* agglutinin; GNA). *J. Insect Physiol.* **46**: 573–583.

Foster, J. E., Ohim, H. W., Patterson, F. L., and Taylor, P. L. 1991. Effectiveness of deploying single gene resistances in wheat for controlling the damage by Hessian fly (Diptera: Cecidomyiidae). *Environ. Entomol.* **20**: 964–969.

Franck-Oberaspach, S. L. and Keller, B. 1997. Consequences of classical and biotechnological resistance breeding for food toxicology and allergenicity. *Plant Breed.* **116**: 1–17.

Fujimoto, H., Itoh, K., Yamamoto, M., Kayozuka, J., and Shimamoto, K. 1993. Insect resistant rice generated by a modified delta endotoxin genes of *Bacillus thuringiensis*. *Bio/Technology* **11**: 1151–1155.

Gatehouse, A. M. R., Davison, G. M., Stewart, J. N., Gatehouse, L. N., Kumar, A., Geoghegan, I. E., Birch, A. N. E., and Gatehouse, J. A. 1999. Concanavalin A inhibits development of tomato moth (*Lacanobia oleracea*) and peach potato aphid (*Myzus persicae*) when expressed in transgenic potato plants. *Mol. Breed.* **5**: 153–165.

Gatehouse, A. M. R., Down, R. E., Powell, K. S., Sauvion, N., Bahbe, Y., Newell, C. A., Merryweather, A., Hamilton, W.D.O., and Gatehouse, J. A. 1996. Transgenic potato plants with enhanced resistance to the peach-potato aphid *Myzus persicae*. *Entomol. Exp. Applic.* **79**: 295–307.

Gatehouse, A. M. R. and Gatehouse, J. A. 1998. Identifying proteins with insecticidal activity: use of encoding genes to produce insect-resistant transgenic crops. *Pest. Sci.* **52**: 165–175.

Gatehouse, A. M. R., Powell, K. S., Peumans, W. J., Van Damme, E. J. M., and Gatehouse, J. A. 1995. Insecticidal properties of plant lectins: Their potential in plant protection. **In**: *Lectins, Biomedical Perspectives*, pp. 35–57. Pusztai, A., and Bardocz, S., Eds., Taylor and Francis, London.

Gatehouse, A. M. R., Shi, Y., Powell, K. S., Brough, C., Hilder, V. A., Hamilton, W. D. O., Newell, C., Merryweather, A., Butler, D., and Gatehouse, J. A. 1993. Approaches to insect resistance using transgenic plants. *Philos. Trans. R. Soc. Lond. Biol. Sci.* **B342**: 279–286.

Gebhard, F. and Smalla, K. 1998. Transformation of *Acinetobactor* sp. strain BD 413 by transgenic sugarbeet DNA. *Appl. Environ. Microbiol.* **64**: 1550–1554.

Ghareyazie, B., Alinia, F., Menguito, C. A., Rubia, L. G., Palma, J. M. de., Liwanag, E. A., Cohen, M. B., Khush, G. S., and Bennett, J. 1997. Enhanced resistance to two stem borers in an aromatic rice containing a synthetic CryIA(b) gene. *Mol. Breed.* **3**: 401–414.

Gibson, D. M., Gallo, L. G., Krasnoff, S. B., and Ketchum, R. E. B. 1995. Increased efficiency of *Bacillus thuringiensis* subsp. *kurstaki* in combination with tannic acid. *J. Econ. Entomol.* **88**: 270–277.

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

Gillard, M. S., Flynn, L., and Rowell, A. 1999. Food scandal exposed. *The Gaurdian* **12.2.99**: 1.

Girard, C., Le-Metayer, M., Zaccomer, B., Bartlet, E., Williams, I., Bonade-Bottino, M., Pham-Delegue, M. H., and Juanin, L. 1998a. Growth stimulation of beetle larvae reared on a transgenic oilseed rape expressing a cysteine proteinase inhibitor. *J. Insect Physiol.* **44**: 263–270.

Girard, C., Picard-Nizou, A. L., Grallien, E., Zaccomer, B., Jouanin, L., and Pham-Delegue, M. H. 1998b. Effects of proteinase inhibitor ingestion on survival, learning abilities and digestive proteinases of the honeybees. *Transgenic Res.* **7**: 239–246.

Giri, A. P. and Kachole, M. S. 1998. Amylase inhibitors of pigeonpea (*Cajanus cajan*) seeds. *Phytochemistry* **47**: 197–202.

Gore, J., Leonard, B. T., and Adamczyk, J. J. 2001. Bollworm (Lepidoptera: Noctuidae) survival on Bollgard and Bollgard II cotton flower bud and flower components. *J. Econ. Entomol.* **94**: 1445–1451.

Greene, J. K., Turnipseed, S. G., and Sullivan, M. J. 1997. Treatment thresholds for stink bugs in transgenic B.t. cotton. **In**: *Proceedings, Beltwide Cotton Production and Research Conference*, pp. 895–898. 6–10 January 1997, New Orleans, LA, USA, Volume 2, National Cotton Council, Memphis, TN.

Greenplate, J. T. 1999. Quantification of *Bacillus thuringiensis* insect control protein Cry1A(c) over time in Bollgard cotton fruit and terminals. *J. Econ. Entomol.* **92**: 1377–1383.

Gregorius, H. R. and Steiner, W. 1993. Gene transfer in plants as a potential agent of introgression. **In**: *Transgenic Organisms*, pp. 83–107. Workman, K., and Tommie, J., Eds., Birkhauser, Verlag, Basel, Switzerland.

Griffiths, W. 1998. Will genetically modified crops replace agrochemicals in modern agriculture? *Pesticide Outlook* **9**: 6–8.

Guo, S. D., Cui, H. Z., Xia, L. Q., Wu, D. L., Ni, W. C., Zhang, Z. L., Zhang, B. L., and Xu, Y. J. 1999. Development of bivalent insect-resistant transgenic cotton plants. *Sci. Agric. Sini*. **32**: 1–7.

Habibi, J., Backus, E. A., and Czapla, T. H. 1992. Effect of plant lectins on survival of potato leafhopper, pp. 373. **In**: *Proceedings, XIX International Congress of Entomology*, China Scientific Book Service, Beijing, China.

Hadi, M. Z., McMullen, M. D., and Finer, J. J. 1996. Transformation of 12 different plasmids into soybean via particle bombardment. *Plant Cell Rep*. **15**: 500–505.

Hallahan, D. L., Pickett, J. A., Wadham, L. J., Wallsgrove, R. M., and Woodcock, C. M. 1992. Potential of secondary metabolites in genetic engineering of crops for resistance. **In**: *Plant Genetic Manipulation for Crop Protection*, pp. 215–248. Gatehouse, A. M. R., Hilder, V. A. and Boulter, D., Eds., CAB International, Wallingford.

Harshavardhan, D., Rani, T. S., Sharma, H. C., Richa, A., and Seetharama, N. 2002. Development and testing of *Bt* transgenic sorghum. **In**: *International Symposium on Molecular Approaches to Improve Crop Productivity and Quality,* 22–24 May 2002. Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu, India.

Harris, A. 1991. Comparison of costs and returns associated with *Heliothis* resistant Bt cotton to non-resistant varieties. **In**: *Proceedings, Beltwide Cotton Production and Research Conference, 1991*, pp. 249–297. National Cotton Council, Memphis, TN, USA.

Herman, R. A., Scherer, P. N., Young, D. L., Mihaliak, C. A., Meade, T., Woodsworth, A. T., Stockhoff, B. A., and Narva, K. E. 2002. Binary insecticidal crystal protein from *Bacillus thuringiensis*, strain PS149B1: effects of individual protein components and mixtures in laboratory bioassays. *J. Econ. Entomol*. **95**: 635–639.

Hilbeck, A., Baumgartner, M., Fried, P. M., and Bigler, F. 1998. Effects of transgenic *Bacillus thuringiensis* corn-fed prey on mortality and development time of immature *Chrysoperla carnea* (Neuroptera: Chrysopidae). *Environ. Entomol*. **27**: 480–487.

Hilder, V. A. and Boulter, D. 1999. Genetic engineering of crop plants for insect resistance—a critical review. *Crop Prot*. **18**: 177–191.

Hilder, V. A. and Gatehouse, A. M. R. 1991. Phenotypic costs to plants of an extra gene. *Transgenic Res*. **1**: 54–60.

Hilder, V. A., Gatehouse, A. M. R., Sheerman, S. E., Baker, R. F., and Boulter, D. 1987. A novel mechanism of insect resistance engineered into tobacco. *Nature* **330**: 160–163.

Hilder, V. A., Powell, K. S., Gatehouse, A. M. R., Gatehouse, J. A., Gatehouse, L. N., Shi, Y., Hamilton, W. D. O., Merryweather, A., Newell, C. A., Timans, J. C., Peumans, W. J., Van Damme, E., and Boulter, D. 1995. Expression of snowdrop lectin in transgenic tobacco plants results in added protection against aphids. *Transgenic Res*. **4**: 18–25..

Hoffmann, M. P., Zalom, F. G., Wilson, L. T., Smilanick, J. M., Malyj, L. D., Kisen, J., Hilder, V. A., and Barnes, W. M. 1992. Field evaluation of transgenic tobacco containing genes encoding *Bacillus thuringiensis* δ-endotoxin or cowpea trypsin inhibitor: efficacy against *Helicoverpa zea* (Lepidoptera: Noctuidae). *J. Econ. Entomol*. **85**: 2516–2522.

Hofte, H. and Whiteley, H. R. 1989. Insecticidal crystal proteins of *Bacillus thuringiensis*. *Microbiol. Rev.* **53**: 242–255.

Hoy, C. W. 1999. Colorado potato beetle resistance management strategies for transgenic potatoes. *Am. J. Potato Res*. **76**: 215–219.

Huang, F., Buschman, L. L., Higgins, R. A., and McGaughey, W. H. 1999. Inheritance of resistance to *Bacillus thuringiensis* toxin (Dipel ES) in the European corn borer. *Science* **284**: 965–967.

Hudspeth, R. L. and Grula, J. W. 1989. Structure and expression of maize gene encoding the phosphoenolpyruvate carboxylase isozyme involved in C4 photosynthesis. *Plant Mol. Biol*. **12**: 579–589.

Husnain, T., Asad, J., Mqbool, S. B., Datta, S. K., and Raizuddin, S. 2002. Variability in expression of cry1Ab gene in indica basmati rice. *Euphytica* **128**: 121–128.

Hyde, J., Martin, M. A., Preckel, P. V., and Edwards, C. R. 1999. The economics of Bt corn: valuing protection from the European corn borer. *Rev. Agric. Econ*. **21**: 442–454.

Innacone, R., Grieco, P. D., and Cellini, F. 1997. Specific sequence modifications of a Cry3B endotoxin gene result in high levels of expression and insect resistance. *Plant Mol. Biol*. **34**: 485–496.

Ishimoto, M. and Chrispeels, M. J. 1996. Protective mechanism of the Mexican bean weevil against high levels of alpha-amylase inhibitor in the common bean. *Plant Physiol*. **111**: 393–401.

Ishimoto, M., Sato, T., Chrispeels, M. J., and Kitamura, K. 1996. Bruchid resistance of transgenic azuki bean expressing seed alpha amylase inhibitor of common bean. *Entomol. Exp. Applic*. **79**: 309–315.

James, C. 2002a. Global review of commercialized transgenic crops: 2002. International Service for the Acquisition of Agri-biotech Applications, Ithaca, New York. http://www.isaaa.org/Publications/briefs_27.htm

James, C. 2002b. Global review of commercialized transgenic crops: 2001 (Feature Bt cotton). International Service for the Acquisition of Agri-biotech Applications, Ithaca, New York, USA. http://www.isaaa.org/Publications/briefs_26.htm.

Jansens, S., Cornelissen, M., Clercq, R. de, Reynaerts, A., and Peferoen, M. 1995. *Phthorimaea operculella* (Lepidoptera: Gelechiidae) resistance in potato by expression of *Bacillus thuringiensis* Cry IA(b) insecticidal crystal protein. *J. Econ. Entomol*. **88**: 1469–1476.

Jansens, S., Vliet, A. van, Dickburt, C., Buysse, L., Piens, C., Saey, B., Wulf, A. de, Gossele, V., Paez, A., and Gobel, E. 1997. Transgenic corn expressing a Cry9C insecticidal protein from *Bacillus thuringiensis* protected from European corn borer damage. *Crop Sci*. **37**: 1616–1624.

Jayanand, B., Sudarsanam, G., and Sharma, K. K. 2003. An efficient protocol for regeneration of whole plant of chickpea (*Cicer arietinum* L.) by using axillary meristem explant derived from in vitro germinated seedlings. *In Vitro Cell. Dev. Biol. Plant* **39**: 171–179.

Jiang, H., Zhu, Y. X., and Chen Z. L. 1996. Insect resistance of transformed tobacco plants with a gene of a spider insecticidal peptide. *Acta Bot. Sin*. **38**: 95–99.

Johnson, M. T. 1997. Interaction of resistant plants and wasp parasitoids of tobacco budworm (Lepidoptera: Noctuidae). *Environ. Entomol*. **26**: 207–214.

Johnson, M. T. and Gould, R. 1992. Interaction of genetically engineered host plant resistance and natural enemies of *Heliothis virescens* (Lepidoptera: Noctuidae) in tobacco. *Environ. Entomol*. **21**: 586–597.

Johnson, M. T., Gould, F., and Kennedy, G.G. 1997. Effects of natural enemies on relative fitness of *Heliothis virescens* genotypes adapted and not adapted to resistant host plants. *Entomol. Exp. Applic*. **82**: 219–230.

Johnson, R., Narvaez, J., An, G., and Ryan, C. 1989. Expression of proteinase inhibitors I and II in transgenic tobacco plants: effects on natural defense against *Manduca sexta* larvae. *Proc. Natl. Acad. Sci., USA* **86**: 9871–9875.

Johnston, K. A., Gatehouse, J. A., and Anstee, J. H. 1993. Effect of soybean protease inhibitors on growth and development of larval *Helicoverpa armigera*. *J. Insect Physiol*. **39**: 657–664.

Jongsma, M. A., Bakker, P. L., Peters, J., Bosch, D., and Stiekema, W. J. 1995. Adaptation of *Spodoptera exigua* larvae to plant proteinase inhibitors by induction of proteinase activity insensitive to inhibition. *Proc. Natl. Acad. Sci. USA* **92**: 8041–8045.

Jouanin, L., Girard, C., Bonade-Bottino, M., Metayer, M. le, Nizou A. L. P., Lerin, J., and Delegue, M. H. P. 1998. Impact of transgenic oilseed rape expressing proteinase inhibitors on coleopteran pests and honeybees. *Cah./Agric*. **7**: 531–536.

Kaiser, J. 1996. Pests overwhelm Bt cotton crop. *Nature* **273**: 423.

Kanrar, S., Venkateswari, J., Kirti, P. B., and Chopra, V. L. 2002. Transgenic Indian mustard (*Brassica juncea*) with resistance to the mustard aphid (*Lipaphis erysimi* Kalt.). *Plant Cell Rep*. **20**: 976–981.

Kar, S., Basu, D., Das, S., Ramkrishnan, N. A., Mukherjee, P., Nayak, P., and Sen, S. K. 1997. Expression of CryIA(c) gene of *Bacillus thuringiensis* in transgenic chickpea plants inhibits development of pod borer (*Heliothis armigera*) larvae. *Transgenic Res*. **6**: 177–185.

Karim, S., Riazuddin, S., and Dean, D. H. 1999. Interaction of *Bacillus thuringiensis* delta-endotoxins with midgut brush border membrane vesicles of *Helicoverpa armigera*. *J. Asia Pacific Entomol*. **2**: 153–162.

Khanna, H. K. and Raina, S. K. 2002. Elite indica transgenic rice plants expressing modified Cry1Ac endotoxin of *Bacillus thuringiensis* show enhanced resistance to yellow stem borer (*Scirpophaga incertulas*). *Transgenic Res*. **11**: 411–423.

Klein, T. M., Wolf, E. D., Wu, R., and Sanford, J. C. 1987. High-velocity microprojectiles for delivering nucleic acids into living cells. *Nature* **327**: 70–73.

Knipling, E. F. 1979. The basic principles of insect population suppression. *Bull. Entomol. Soc. Am*. **12**: 7–15.

Konarev, A. V. 1996. Interaction of insect digestive enzymes with plant protein inhibitors and host-parasite co-evolution. *Euphytica* **92**: 89–94.

Koziel, M. G., Beland, G. L., Bowman, C., Carozzi, N. B., Crenshaw, R., Crossland, L., Dawson, J., Desai, N., Hill, M., Kadwell, S., Launis, K., Lewis, K., Maddox, D., McPherson, K., Meghji, M. R., Merlin, E., Rhodes, R., Warren, G. W., Wright, M., and Evola, S. V. 1993. Field performance of elite transgenic maize plants expressing an insecticidal protein derived from *Bacillus thuringiensis*. *Bio/Technology* **11**: 194–200.

Kuiper, H. A. and Noteborn, H. J. M. 1994. Food safety assessment of transgenic insect-resistant Bt tomatoes. Food safety evaluation. **In**: *Proceedings of an OECD-sponsored Workshop*, pp. 50–57, 12–15 September 1994, Oxford, UK. Organization for Economic Cooperation and Development (OECD), Paris, France.

Kumar, P. A., Mandaokar, A., Sreenivasu, K., Chakrabarti, S. K., Bisaria, S., Sharma, S. R., Kaur, S., and Sharma, R. P. 1998. Insect-resistant transgenic brinjal plants. *Mol. Breed*. **4**: 33–37.

Lang, B. A., Moellenbeck, D. J., Isenhour, D. J., and Wall, S. J. 1996. Evaluating resistance to CryIA(b) in European corn borer (Lepidoptera: Pyralidae) with artificial diet. *Resistant Pest Manage. Newsl*. **8**: 29–31.

Lauer, J. and Wedberg, J. 1999. Grain yield of initial Bt corn hybrid introductions to farmers in the Northern Corn Belt. *J. Prod. Agric*. **12**: 373–376.

Lawrence, P. K. and Koundal, K. R. 2002. Plant protease inhibitors in control of phytophagous insects. *Electron. J. Biotechnol*. http//:www.ejb.org/content/vol5/issue1/full/3/htm

Lecardonnel, A., Chauvin, L., Jouanin, L., Beaujean, A., Prevost, G., and Sangwan Norreel, B. 1999. Effects of rice cystatin I expression in transgenic potato on Colorado potato beetle larvae. *Plant Sci*. **140**: 71–79.

Lee, M. K., Curtis, A., Alcantora, E., and Dean, D. H. 1996. Synergistic effect of *Bacillus thuringiensis* toxins Cry1Aa and Cry 1Ac on the gypsy moth, *Lymnatria dispar*. *Appl. Environ. Microbiol*. **62**: 583–586.

Lee, S. I., Lee, S. H., Koo, J. C., Chun, H. J., Lim, C. O., Mun, J. H., Song, Y. H., and Cho, M. J. 1999. Soybean Kunitz trypsin inhibitor (SKTI) confers resistance to the brown planthopper (*Nilaparvata lugens* Stal.) in transgenic rice. *Mol. Breed*. **5**: 1–9.

Lehrer, S. B. 2000. Potential health risks of genetically modified organisms: how can allergens be assessed and minimized. **In**: *Agricultural Biotechnology and the Poor*, pp. 149–155. Persley, G. J. and Lantin, M. M., Eds., Consultative Group on International Agricultural Research, Washington, DC.

Li, Y. E., Zhu, Z., Chen, Z. X., Wu, X., Wang, W., and Li, S. J. 1998. Obtaining transgenic cotton plants with cowpea trypsin inhibitor. *Acta Gossypii Sin*. **10**: 237–243.

Llewellyn, D. and Fitt, G. 1996. Pollen dispersal from two field trials of transgenic cotton in the Namoi Valley, Australia. *Mol. Breed*. **2**: 157–166.

Loc, N. T., Tinjuangjun, P., Gatehouse, A. M. R., Christou, P., and Gatehouse, J. A. 2002. Linear transgene constructs lacking vector backbone sequences generate transgenic rice plants which accumulate higher levels of proteins conferring insect resistance. *Mol. Breed*. **9**: 231–244.

Losey, J. E., Rayor, L. S., and Carter, M. E. 1999. Transgenic pollen harms monarch larvae. *Nature* **399**: 214.

Lozzia, G. C., Furlanis, C., Manachini, B., and Rigamonti, I. E. 1998. Effects of Bt corn on *Rhopalosiphum padi* L. (Rhynchota: Aphididae) and on its predator *Chrysoperla carnea* Stephen (Neuroptera: Chrysopidae). *Bull. Zool. Agr. Bachic. Ser. II* **30**: 153–164.

Luginbill, P., Jr. and Knipling, E. F. 1969. Suppression of wheat stem fly with resistant wheat. United States Department of Agriculture/Agriculture Research Service (USDA/ARS). *Prod. Res. Rep*. **107**: 1–9.

Lynch, R. E., Wiseman, B. R., Plaisted, D., and Warnick, D. 1999a. Evaluation of transgenic sweet corn hybrids expressing CryIA(b) toxin for resistance to corn earworm and fall armyworm (Lepidoptera: Noctuidae). *J. Econ. Entomol*. **92**: 246–252.

Lynch, R. E., Wiseman, B. R., Sumner, H. R., Plaisted, D., and Warnick, D. 1999b. Management of corn earworm and fall armyworm (Lepidoptera: Noctuidae) injury on a sweet corn hybrid expressing a CryIA (b) gene. *J. Econ. Entomol*. **92**: 1217–1222.

MacIntosh, S. C., Kishore, G. M., Perlak, F. J., Marrone, P. G., Stone, T. B., Sims, S. R., and Fuchs, R. L. 1990. Potentiation of *Bacillus thuringiensis* insecticidal activity by serine protease inhibitors. *J. Agric. Food Chem*. **38**: 1145–1152.

MacIntosh, S. C., Stone, T. B., Jokerst, R. S., and Fuchs, R. L. 1991. Binding of *Bacillus thuringiensis* proteins to a laboratory selected strain of *Heliothis virescens*. *Proc. Natl. Acad. Sci. USA* **188**: 8930–8933.

Maddock, S. E., Hufman, G., Isenhour, D. J., Roth, B. A., Raikhel, N. V., Howard, J. A., and Czapla, T. H. 1991. Expression in maize plants of wheatgerm agglutinin, a novel source of insect resistance. **In**: *3rd International Congress of Plant Molecular Biology.* Tucson, Arizona, USA.

Malone, L. A., Giacon, H. A., Burgess, E. P. J., Maxwell, J. Z., Christeller, J. T., and Laing, W. A. 1995. Toxicity of trypsin endopeptidase inhibitors to honey bees (Hymenoptera: Apidae). *J. Econ. Entomol*. **88**: 46–50.

Mandal, S., Kundu, P., Roy, B., and Mandal, R. K. 2002. Precursor of the inactive 2S seed storage protein from the Indian mustard *Brassica juncea* is a novel trypsin inhibitor: characterization, post-translational processing studies, and transgenic expression to develop insect-resistant plants. *J. Biol. Chem*. **277**: 37161–37168.

Mandaokar, A. D., Goyal, R. K., Shukla, A., Bisaria, S., Bhalla, R., Reddy, V. S., Chaurasia, A., Sharma, R. P., Altosaar, I., and Kumar, P. A. 2000. Transgenic tomato plants resistant to fruit borer (*Helicoverpa armigera* Hubner). *Crop Prot*. **19**: 307–312.

Marchetti, S., Delledonne, M., Fogher, C., Chiaba, C., Chiesa, F., Savazzini, F., and Giordano, A. 2000. Soybean Kunitz, C-II and PI-IV inhibitor genes confer different levels of insect resistance to tobacco and potato transgenic plants. *Theor. Appl. Genet*. **101**: 519–526.

Mascarenhas, R. N., Boethel, D. J., Leonard, B. R., Boyd, M. L., and Clemens, C. G. 1998. Resistance monitoring to *Bacillus thuringiensis* insecticides for soybean loopers (Lepidoptera: Noctuidae) collected from soybean and transgenic Bt-cotton. *J. Econ. Entomol*. **91**: 1044–1050.

Maxwell, F. G., Jenkins, J. N., and Parrot, W. L. 1973. Resistance of plants to insects. *Adv. Agron*. **24**: 187–265.

McBride, K. E., Svab, Z., Schaaf, D. J., Hogan, P. S., Stalker, D. M., and Maliga, P. 1995. Application of a chimeric *Bacillus* gene in chloroplasts leads to extraordinary level of an insecticidal protein in tobacco. *Bio/Technology* **13**: 362–365.

McCaskill, D. and Croteau, R. 1998. Some caveats for bioengineering terpenoid metabolism in plants. *Trends Biotechnol*. **16**: 349–355.

McElroy, D., Rothenberg, M., and Wu., R. 1990. Structural characterization of a rice actin gene. *Plant Mol. Biol*. **14**: 163–171.

McGaughey, W. H. 1985. Insect resistance to the biological insecticide *Bacillus thuringiensis*. *Science* **229**: 193–194.

McLaren, J. S. 1998. The success of transgenic crops in the USA. *Pesticide Outlook* **9**: 36–41.

McManus, M. T., Burgess, E. P. J., Philip, B., Watson, L. M., Laing, W. A., Voisey, C. R., and White, D. W. R. 1999. Expression of the soybean (Kunitz) trypsin inhibitor in transgenic tobacco: effects on larval development of *Spodoptera litura*. *Transgenic Res*. **8**: 383–395.

Meenakshisundaram, K. S. and Gujar, G. T. 1998. Proteolysis of *Bacillus thuringiensis* subspecies *kurstaki* endotoxin with midgut proteases of some important lepidopterous species. *Indian J. Exp. Biol*. **36**: 593–598.

Mochizuki, A., Nishizawa, Y., Onodera, H., Tabei, Y., Toki, S., Habu, Y., Ugaki, M., and Ohashi, Y. 1999. Transgenic rice plants expressing a trypsin inhibitor are resistant against rice stem borers, *Chilo suppressalis*. *Entomol. Exp. Applic*. **93**: 173–178.

Mohammed, A., Douches, D. S., Pett, W., Grafius, E., Coombs, J., Liswidowati, W. L., and Madkour, M. A. 2000. Evaluation of potato tuber moth (Lepidoptera: Gelechiidae) resistance in tubers of Bt-Cry5 transgenic potato lines. *J. Econ. Entomol.* **93**: 472–476.

Morton, R. L., Schroeder, H. E., Bateman K. S., Chrispeels, M. J., Armstrong, E., and Higgins, T. J. V. 2000. Bean alpha-amylase inhibitor 1 in transgenic peas (*Pisum sativum*) provides complete protection from pea weevil (*Bruchus pisorum*) under field conditions. *Proc. Natl. Acad. Sci. USA* **97**: 3820–3825.

Mqbool, S. B., Husnain, T., Raizuddin, S., and Christou, P. 1998. Effective control of yellow rice stem borer and rice leaf folder in transgenic rice *indica* varieties Basmati 370 and M 7 using novel δ-endotoxin Cry2A *Bacillus thuringiensis* gene. *Mol. Breed.* **4**: 501–507.

Munkvold, G. P., Hellmich, R. L., and Rice, L. G. 1999. Comparison of fumonisin concentrations in kernels of transgenic Bt maize hybrids and nontransgenic hybrids. *Plant Dis.* **83**: 130–138.

Nandi, A. K., Basu, D., Das, S., and Sen, S. K. 1999. High level expression of soybean trypsin inhibitor gene in transgenic tobacco plants failed to confer resistance against damage caused by *Helicoverpa armigera*. *J. Bioscience* **24**: 445–452.

National Academy of Sciences (NAS). 1987. *Introduction of Recombinant DNA-Engineered Organisms into the Environment: Key Issues*. National Academy of Sciences, Washington, DC.

Nayak, P., Basu, D., Das, S., Basu, A., Ghosh, D., Ramakrishnan, N. A., Ghosh, M., and Sen, S. K. 1997. Transgenic elite *indica* rice plants expressing CryIAc delta-endotoxin of *Bacillus thuringiensis* are resistant against yellow stem borer (*Scirpophaga incertulas*). *Proc. Natl. Acad. Sci. USA* **94**: 2111–2116.

Neuhaus, G., Spangenberg, G., Scheid, O. M., and Schweiger, H. G. 1987. Transgenic rapeseed plants obtained by the injection of DNA into microscope derived embryoids. *Theor. Appl. Genet.* **75**: 30–36.

Ni, W. C., Huang, J. Q., Guo, S. D., Shu, C. G., Wu, J. Y., Wang, W. G., Zhang, Z. L., Chen, S., Mao, L. Q., Wang, Y., Xu, Y. J., Gu, L. M., Zhou, B. L., Shen, X. L., and Xiao, S. H. 1996. Transgenic bollworm-resistant cotton plants containing the synthetic gene coding *Bacillus thuringiensis* insecticidal protein. *Jiangsu J. Agric. Sci.* **12**: 1–6.

Noteborn, H. P. J. M., Bienenmann Ploum, M. E., Alink, G. M., Zolla, L., Reynaerts, A., Pensa, M., Kuiper, H. A., and Fenwick, G. R. 1996. Safety assessment of the *Bacillus thuringiensis* insecticidal crystal protein Cry IA(b) expressed in transgenic tomatoes. **In**: *Agri Food Quality: An Interdisciplinary Approach*, pp. 23–26. Hedley, C., Richards, R. L., and Khokhar, S., Eds., Special Publication No. 179, Royal Society of Chemistry, Cambridge, UK.

Nutt, K. A., Allsopp, P. G., McGhie, T. K., Shepherd, K. M., Joyce, P. A., Taylo, G. O., McQualter, R. B., Smith, G. R., and Ogarth, D. M. 1999. Transgenic sugarcane with increased resistance to canegrubs. **In**: *Proceedings of the 1999 Conference of the Australian Society of Sugarcane Technologists*, pp. 171–176, 27–30 April 1999, Townsville, Brisbane, Australia.

Oberhauser, K. S., Prysby, M. D., Mattila, H. R., Stanley-Horn, D. E., Sears, M. K., Dively, G., Pleasants J. M., Lam, W. F., and Hellmich, R. L. 2001. Temporal and spatial overlap between monarch larvae and corn pollen. *Proc. Natl. Acad. Sci. USA* **98**: 11913–11918.

Oerke, E. C., Dehne, H. W., Schonbeck, F., and Weber, A. 1994. *Crop Production and Crop Protection: Estimated Losses in Major Food and Cash Crops*. Elsevier, Amsterdam, The Netherlands.

Orozco-Cardenas, M., McGurl, B., and Ryan, C. A. 1993. Expression of an antisense prosystemin gene in tomato plants reduces resistance toward *Manduca sexta* larvae. *Proc. Natl. Acad. Sci. USA* **90**: 8273–8276.

Orr, D. B., and Landis, D. A. 1997. Oviposition of European corn borer (Lepidoptera: Pyralidae) and impact of natural enemy populations in transgenic versus isogenic corn. *J. Econ. Entomol.* **90**: 905–909.

Parker, C. D., Jr., Mascarenhas, V. J., Luttrell, R. G., and Knighten, K. 2000. Survival rates of tobacco budworm (Lepidoptera: Noctuidae) larvae exposed to transgenic cottons expressing insecticidal protein of *Bacillus thuringiensis* Berliner. *J. Entomol. Sci.* **35**: 105–117.

Parker, R. D. and Huffman, R. L. 1997. Evaluation of insecticides for boll weevil control and impact on non-target arthropods on non-transgenic and transgenic B.t. cotton cultivars. **In**: *Proceedings, Beltwide Cotton Production and Research Conference*, pp. 1216–1221, 6–10 January 1997, New Orleans, LA, USA. Volume 2, National Cotton Council, Memphis, TN.

Patankar, A. G., Harshulkar, A. M., Giri, A. P., Gupta, V. S., Sainani, A. M., Ranjekar, P. K., and Deshpande, V. V. 1999. Diversity in inhibitors of trypsin and *Helicoverpa armigera* gut proteinases in chickpea (*Cicer arietinum*) and its wild relatives. *Theor. Appl. Genet.* **99**: 719–726.

Peng, J. H. and Black, L. L. 1976. Increased proteinase inhibitor activity in response to infection of resistant tomato plants by *Phytophthora infestans*. *Phytopathology* **66**: 958–963.

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

Pham-Delegue, M. H. and Jouanin, L. 1997. The honey bee and transgenic plants. *Rev. Fr. Apic.* **574**: 250–251.

Picard-Nizou, A. L., Grison, R., Olsen, L., Pioche, C., Arnold, G., and Pham-Delegue, M. H. 1997. Impact on proteins used in plant genetic engineering toxicity and behavioral study in the honeybee. *J. Econ. Entomol.* **90**: 1710–1716.

Pilcher, C. D., Rice, M. E., Higgins, R. A., Steffey, K. L., Hellmich, R. L., Witkowski, J., Calvin, D., Ostlie, K. R., and Gray, M. 2002. Biotechnology and the European corn borer: measuring historical farmer perceptions and adoption of transgenic Bt corn as a pest management strategy. *J. Econ. Entomol.* **95**: 878–892.

Potrykus, I. 1991. Gene transfer to plants: assessment of published approaches and results. *Annu. Rev. Pl. Physiol. Mol. Biol.* **42**: 205–225.

Powell, K. S., Gatehouse, A. M. R., Hilder, V. A., and Gatehouse, J. A. 1993. Antimetabolic effects of plant lectins and plant and fungal enzymes on the nymphal stages of two important rice pests, *Nilaparvata lugens* and *Nephotettix cinciteps*. *Entomol. Exp. Applic.* **66**: 119–126.

Powell, K. S., Gatehouse, A. M. R., Hilder, V. A., and Gatehouse, J. A. 1995. Antifeedant effects of plant lectins and an enzyme on the adult stage of the rice brown planthopper, *Nilaparvata lugens*. *Entomol. Exp. Applic.* **75**: 51–59.

Purcell, J. P., Greenplate, J. T., Jennings, M. G., Ryers, J. S., Pershing, J. C., Sims, S. R., Prinsen, M. J., Corbin, D. R., Tran, M., Sammons, R. D., and Stonard, R. J. 1993. Cholesterol oxidase: a potent insecticidal protein active against boll weevil larvae. *Biochem. Biophys. Res. Comm.* **196**: 1406–1413.

Pusztai, A. 1972. Metabolism of trypsin-inhibitory proteins in the germinating seeds of kidney bean (*Phaseolus vulgaris*). *Planta* **107**: 121–129.

Pusztai, A., Ewen, S. W. B., Grant, G., Brown, D. S., Stewart, J. C., Peumans, W. J., Damme, E. J. M., and Bardocz, S. 1993. Antinutritive effects of wheatgerm agglutinin and other N-acetylglucosamine-specific lectins. *Br. J. Nutr.* **70**: 313–321.

Pusztai, A., Grant, G., Brown, D. J., Stewart, J. C., Bardocz, S., Ewen, S. W. B., Gatehouse, A. M. R., and Hilder, V. A. 1992. Nutritional evaluation of the trypsin (EC3.4.21.4) inhibitor from cowpea (*Vigna unguiculata*). *Br. J. Nutr.* **68**: 783–791.

Pusztai, A., Koninkx, J., Hendriks, H., Kok, W., Hulscher, S., Damme, E. J. M. van, Peumans, W. J., Grant, G., and Bardocz, S. 1996. Effect of the insecticidal *Galanthus nivalis* agglutinin on metabolism and the activities of brush border enzymes in the rat small intestine. *J. Nutr. Biochem.* **7**: 677–682.

Qaim, M. and Zilberman, D. 2003. Yield effects of genetically modified crops in developing countries. *Science* **299**: 900–902.

Quist, D. and Chapela, I. H. 2001. Transgenic DNA introgressed into traditional maize landraces in Oxaca, Mexico. *Nature* **414**: 541–543.

Raybould, A. F. and Gray, A. J. C. 1993. Genetically modified crops and hybridization with wild relatives: a UK perspective. *J. Appl. Ecol.* **30**: 119–219.

Reddy, V. S., Sadhu, L., Selvapandiyan, A., Raman, R., Giovanni, F., Shukla, V., and Bhatnagar, R. K. 2002. Analysis of chloroplast transformed tobacco plants with cry1Ia5 under rice psbA transcriptional elements reveal high level expression of Bt toxin without imposing yield penalty and stable inheritance of transplastome. *Mol. Breed.* **9**: 259–269.

Rensburg van, J. B. J. 1999. Evaluation of Bt-transgenic maize for resistance to the stem borers *Busseola fusca* (Fuller) and *Chilo partellus* (Swinhoe) in South Africa. *South Afr. J. Plant Soil* **16**: 38–43.

Richardson, M. 1977. The proteinase inhibitors of plants and microorganisms. *Phytochemistry* **16**: 159–169.

Rickauer, M., Fournier, J., and Esquerre-tugaye, M. T. 1989. Induction of proteinase inhibitors in tobacco cell suspension culture by elicitors of *Phytophtora parasitica var. nocotianae*. *Plant Physiol*. **90**: 1065–1070.

Riddick, E. W., Dively, G., and Barbosa, P. 1998. Effect of a seed-mix deployment of Cry3A-transgenic and nontransgenic potato on the abundance of *Lebia grandis* (Coleoptera: Carabidae) and *Coleomegilla maculata* (Coleoptera: Coccinellidae). *Ann. Entomol. Soc. Am.* **91**: 647–653.

Rigev, A., Keller, M., Strizhov, M., Sneh, B., Prudovsky, E., Chet, I., Ginzberg, I., Koncz, Z., Schell, J., and Zilberstein, A. 1996. Synergistic activity of a *Bacillus thuringiensis* δ-endotoxin and bacterial endochitinase against *Spodoptera littoralis* larvae. *Appl. Environ. Microbiol*. **62**: 3581–3586.

Riggin Bucci, T. M. and Gould, F. 1997. Impact of intraplot mixtures of toxic and nontoxic plants on population dynamics of diamondback moth (Lepidoptera: Plutellidae) and its natural enemies. *J. Econ. Entomol.* **90**: 241–251.

Romeis, J., Babendreier, D., and Wackers, F. L. 2003. Consumption of snowdrop lectin (*Galanthus nivalis* agglutinin) caused direct effects on parasitic wasps. *Oecologia* **134**: 528–536.

Rosso, M. N., Schooten, A., Roosien, J., Borst Vrenssen, T., Hussey, R. S., Gommers, F. J., Bakker, J., Scho, A., and Abad, P. 1996. Expression and functional characterization of a single chain antibody directed against secretions involve a plant nematode infection process. *Biochem. Biophys. Res. Comm.* **220**: 255–263.

Ryan, C. 1990. Protease inhibitors in plants: genes for improving defenses against insects and pathogens. *Annu. Rev. Phytopath.* **28**: 425–449.

Ryan, C. A. and Pearce, G. 1998. Systemin: a polypeptide signal for plant defensive genes. *Annu. Rev. Cell Dev. Boil*. **14**: 1–17.

Sachs, E. S., Benedict, J. H., Stelly, D. M., Taylor, J. F., Altman, D. W., Berberich, S. A., and Davis, S. K. 1998. Expression and segregation of genes encoding CryIA insecticidal proteins in cotton. *Crop Sci*. **38**: 1–11.

Sanford, J. C. 1990. Biolistic plant transformation. *Physiol. Plant*. **79**: 206–209.

Satyendra, R., Stewart, J. M., and Wilkins, T. 1998. Assessment of resistance of cotton transformed with lectin genes to tobacco budworm. **In**: *Special Report*, pp. 95–98. Arkansas Agricultural Experiment Station, University of Arkansas, Fayetteville, USA.

Saxena, D., Flores, S., and Stotzy, G. 2002a. Vertical movement in soil of Cry 1Ab protein from *Bacillus thuringiensis*. *Soil. Biol. Biochem* **34**: 111–120.

Saxena, D., Flores, S., and Stotzy, G. 2002b. Bt toxin is released in root exudates from 12 transgenic corn hybrids representing three transformation events. *Soil. Biol. Biochem* **34**: 133–137.

Schell, J. 1997. Cotton carrying the recombinant insect poison Bt toxin: no case to doubt the benefits of plant biotechnology. *Curr. Opin. Biotechnol*. **8**: 235–236.

Schnepf, H. E. and Whiteley, H. R. 1981. Cloning and expression of *Bacillus thuringiensis* crystal protein gene in *Escherichia coli*. *Proc. Natl. Acad. Sci., USA* **78**: 2893–2897.

Schroeder, H. E., Gollasch, S., Moore, A., Tabe, L. M., Craig, S., Hardie, D. C., Chrispeels, M. J., Spencer, D., and Higgins, T. J. V. 1995. Bean alpha-amylase inhibitor confers resistance to pea weevil (*Bruchus pisorum*) in transgenic peas (*Pisum sativum* L.). *Plant Physiol*. **107**: 1233–1239.

Seetharam, A., Kuiper, G., Visser, P. B., Kogel de, W. J., Udayakumar, M., and Jongsma, M. A. 2002. Expression of potato multicystatin in florets of chrysanthemum and assessment of resistance to western flower thrips, *Frankliniella occidentalis*. *Acta Hort*. **572**: 121–129.

Selvapandian, A., Reddy, V. S., Ananda Kumar, P., Tiwari, K. K., and Bhatnagar, R.K. 1998. Transformation of *Nicotiana tabaccum* with a native cry1Ia5 gene confers complete protection against *Heliothis armigera*. *Mol. Breed*. **4**: 473–478.

Serratos, J. A., Willcox, M. C., Castillo-Gonzalez, F. (Eds.). 1997. *Gene Flow Among Maize Landraces, Improved Maize Varieties, and Teosinte: Implications for Transgenic Maize*. International Wheat and Maize Research Institute. México DF, México. 122 pp.

Setamou, M., Bernal, J. S., Legaspi, J. C., Mirkov, T. E., and Legaspi, Jr., B. C. 2002. Evaluation of lectin-expressing transgenic sugarcane against stalk borers (Lepidoptera: Pyralidae): effects on life history parameters. *J. Econ. Entomol*. **95**: 469–477.

Shade, R. E., Schroeder, H. E., Pueyo, J. J., Tabe, L. M., Murdock, L. L., Higgins, T. J. V., and Chrispeels, M. J. 1994. Transgenic pea seeds expressing α-amylase inhibitor of the common bean are resistant to bruchid beetles. *Bio/Technology* **12**: 793–796.

Sharma, H. C. 1993. Host plant resistance to insects in sorghum and its role in integrated pest management. *Crop Prot*. **12**: 11–34.

Sharma, H. C. and Agarwal, R. A. 1983. Role of some chemical components and leaf hairs in varietal resistance in cotton to jassid, *Amrasca biguttula buguttula* Ishida. *J. Entomol. Res*. **7**: 145–149.

Sharma, H. C., Ananda Kumar, P., Seetharama, N., Hari Prasad, K. V., and Singh, B. U. 1999. Role of transgenic plants in pest management in sorghum. **In**: *Symposium on Tissue Culture and Genetic Transformation of Sorghum*, 23–28 Feb 1999. International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Patancheru 502 324, Andhra Pradesh, India.

Sharma, H. C., Crouch, J. H., Sharma, K. K., Seetharama, N., and Hash, C. T. 2002a. Applications of biotechnology for crop improvement: prospects and constraints. *Plant Sci*. **163**: 381–395.

Sharma, H. C. and Norris, D. M. 1991. Chemical basis of resistance in soybean to cabbage looper, *Trichoplusia ni*. *J. Sci. Food Agric*. **55**: 353–364.

Sharma, H. C. and Ortiz, R. 2000a. Transgenics, pest management, and the environment. *Curr. Sci*. **79**: 421–437.

Sharma, H. C. and Ortiz, R. 2002. Host plant resistance to insects: an ecofriendly approach for pest management and environment conservation. *J. Environ. Biol*. **23**: 111–135.

Sharma, H. C., Sharma, K. K., Seetharama, N., and Ortiz, R. 2000. Prospects for transgenic resistance to insects. *Elec. J. Biotechnol. www.ejbiotechnology.info/content/vol5/issue2/full/htm.pp25*.

Sharma, H. C., Sharma, K. K., Seetharama, N., and Ortiz, R. 2001. Genetic transformation of crop plants: risks and opportunities for the rural poor. *Curr. Sci*. **80**: 1495–1508.

Sharma, K. K. and Ortiz, R. 2000b. Program for the application of genetic engineering for crop improvement in the semi-arid tropics. *In Vitro Cell. Dev. Biol. Plant* **36**: 83–92.

Sharma, K. K., Sharma, H. C., Seetharama, N., and Ortiz, R. 2002b. Development and deployment of transgenic plants: biosafety considerations. *In Vitro Cell. Dev. Biol. Plant*. **38**: 106–115.

Shimamoto, K., Terada, R., Iwaza, T., and Fujimoto, H. 1989. Fertile transgenic rice plants regenerated from transformed protoplasts. *Nature* **338**: 274–276.

Shukle, R. H. and Murdock, L. L. 1983. Lipoxygenase, trypsin inhibitor, and lectin from soybeans: effect on larval growth of *Manduca sexta* (Lepidoptera: Sphingidae). *Environ. Entomol*. **12**: 787–791.

Sijmons, P. C. 1993. Plant-nematode interactions. *Plant Mol. Biol*. **23**: 917–931.

Sims, S. R. and Berberich, S. A. 1996. *Bacillus thuringiensis* Cry IA protein levels in raw and processed seed on transgenic cotton: determination using insect bioassay and ELISA. *J. Econ. Entomol*. **89**: 247–251.

Sims, S. R. and Holden, L. R. 1996. Insect bioassay for determining soil degradation of *Bacillus thuringiensis* subsp. *kurstaki* CryIA(b) protein in corn tissue. *Environ. Entomol*. **25**: 659–664.

Singsit, C., Adang, M. J., Lynch, R. E., Anderson, W. F., Aiming, Wang, Cardineau, G., and Ozias-Akins, P. 1997. Expression of a *Bacillus thuringiensis* CryIA(c) gene in transgenic peanut plants and its efficacy against lesser cornstalk borer. *Transgenic Res*. **6**: 169–176.

Smigocki, A., Neal, J. W., Jr., McCanna, I., and Douglass, L. 1993. Cytokinin-mediated insect resistance in *Nicotiana* plants transformed with the ipt gene. *Plant Mol. Biol*. **23**: 325–335.

Stewart, J. G., Feldman, J., and LeBlanc, D. A. 1999. Resistance of transgenic potatoes to attack by *Epitrix cucumeris* (Coleoptera: Chrysomelidae). *Canadian Entomol*. **131**: 423–431.

Stewart, L. M. D., Hirst, M., Ferber, M. L., Merryweather, A. T., Cayley, P. J., and Possee R. D. 1991. Construction of an improved baculovirus insecticide containing an insect-specific toxin gene. *Nature* **352**: 85–88.

Stoger, E., Williams, S., Christou, P., Down, R. E., and Gatehouse, J. A. 1999. Expression of the insecticidal lectin from snowdrop (*Galanthus nivalis*

agglutinin; GNA) in transgenic wheat plants: effects on predation by the grain aphid *Sitobion avenae*. *Mol. Breed.* **5**: 65–73.

Sun, J., Tang, C. M., Zhu, X. F., Guo, W. Z., Zhang, T. Z., Zhou, W. J., Meng, F. X., and Sheng, J. L. 2002. Characterization of resistance to *Helicoverpa armigera* in three lines of transgenic Bt upland cotton. *Euphytica* **123**: 343–351.

Svab, Z. and Maliga, P. 1993. High frequency plastid transformation in tobacco by selection for a *acd* A gene. *Proc. Natl. Acad. Sci. USA* **90**: 913–917.

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

Tabashnik, B. E., Dennehy, T. J., Sims, M. A., Larkin, K., Head, G. P., Moar, W. J., and Carriere, Y. 2002. Control of resistant pink bollworm (*Pectinophora gossypiella*) by transgenic cotton that produces *Bacillus thuringiensis* toxin Cry2Ab. *Appl. Environ. Microbiol.* **68**: 3790–3794.

Tabashnik, B. E., Finson, N., and Johanson, M. W. 1991. Managing resistance to *Bacillus thuringiensis*: lessons from the diamondback moth (Lepidoptera: Plutellidae). *J. Econ. Entomol.* **84**: 49–55.

Tabashnik, B. E., Liu, Y. B., Dennehy, T. J., Sims, M. A., Sisterson, M. S., Biggs, R. W., and Carriere, Y. 2002. Inheritance of resistance to Bt toxin Cry1Ac in a field-derived strain of pink bollworm (Lepidoptera: Gelechiidae). *J. Econ. Entomol.* **95**: 1018–1026.

Tailor, R., Tippett, J., Gibb, G., Pells, S., Pike, D., Jordan, L., and Ely, S. 1992. Identification and characterization of a novel *Bacillus thuringiensis*-endotoxin entomocidal to coleopteran and lepidopteran larvae. *Mol. Microbiol.* **7**: 1211–1217.

Tan-Wilson, A. L., Hartl, P. M., Delfel, N. E., and Wilson, K. A. 1985. Differential expression of Kunitz and Bowman-Birk soybean proteinase inhibitors in plant and callus tissues. *Plant Physiol.* **78**: 310–314.

Tang, J. D., Collins, H. L., Roush, R. T., Metz, T. D., Earle, E. D., and Shelton, A. M. 1999. Survival, weight gain, and oviposition of resistant and susceptible *Plutella xylostella* (Lepidoptera: Plutellidae) on broccoli expressing Cry1A(c) toxin of *Bacillus thuringiensis*. *J. Econ. Entomol.* **92**: 47–55.

Tomalski, M. D. and Miller, L. K. 1991. Insect paralysis by baculovirus-mediated expression of a mite neurotoxin gene. *Nature* **352**: 82–85.

Thomas, D. J. I., Morgan, J. A. W., Whipps, J. M., and Saunders, J. R. 1997. Plasmid transfer by the insect pathogen *Bacillus thuringiensis* in the environment. **In**: *Microbial Insecticides: Novelty or Necessity? Proceedings of a Symposium held at the University of Warwick*, April 1997, pp. 261–265, 16–18, Coventry, British Crop Protection Council, Farnham, UK.

Thomas, J. C., Adams, D. G., Keppenne, V. D., Wasmann, C. C., Brown, J. K., Kanost, M. R., and Bohnert, H. J. 1995a. *Manduca sexta* encoded protease inhibitors expressed in *Nicotiana tabacum* provide protection against insects: *Plant Physiol. Biochem.* **33**: 611–614.

Thomas, J. C., Adams, D. G., Keppenne, V. D., Wasmann, C. C., Brown, J. K., Kanosh, M. R., and Bohnert, H. J. 1995b. Proteinase inhibitors of *Manduca sexta* expressed in transgenic cotton. *Plant Cell Rep.* **14**: 758–762.

Thomas, J. C., Wasmann, C. C., Echt, C., Dunn, R. L., Bohnert, H. J., and McCoy, T. J. 1994. Introduction and expression of an insect proteinase inhibitor in alfalfa (*Medicago sativa* L.). *Plant Cell Rep.* **14**: 31–36.

Tortiglione, C., Malva, C., Pennacchio, F., Rao, R., and Scarascia Mugnozza, G. T. 1999. New genes for pest control. **In**: *Genetics and Breeding for Crop Quality and Resistance. Proceedings of the XV EUCARPIA Congress*, 20–25 September, 1998, Viterbo, Italy, pp. 159–163. Porceddu, E. and Pagnotta, M. A., Eds., Kluwer Academic Publishers, Dordrecht, Germany.

Urwin, P. E., Atkins, H. J., Waller, D. A., and McPherson, M. J. 1995. Engineered oryzacystatin-I expressed in transgenic hairy roots confers resistance to *Globodera pallida*. *Plant J.* **8**: 121–131.

Vaeck, M., Reynaerts, A., Hofte, H., Jansens, S., DeBeuckleer, M., Dean, C., Zabeau, M., Van Montagu, M., and Leemans, J. 1987. Transgenic plants protected from insect attack. *Nature* **327**: 33–37.

Van der Salm, T., Bosch, D., Honee, G., Feng, I., Munsterman, E., Bakker, P., Stiekema, W. J., and Visser, B. 1994. Insect resistance of transgenic plants that express modified Cry1A(b) and Cry1C genes: a resistance management strategy. *Plant Mol. Biol.* **26**: 51–59.

Van Engelen, F. A., Schouen, A., Molthoff, J. W., Roosien, J., Salinas, J., Dirkse, W. G., Schots, A., Bakker, P., Gommers, F., Jongsma, M. A., Bosch, D., and Stiekma, W. J. 1994. Coordinate expression of antibody subunit genes yields high levels of functional antibodies in roots of transgenic tobacco. *Plant Mol. Biol.* **26**: 1701–1710

Vrieling, K., van Wijk, C. A. M., and Swa, Y. J. 1991. Costs assessment of the production of pyrrolizidine alkaloids in ragwort (*Senecio jacobaca* L.). *Oecologia* **97**: 541–546.

Walker, D., Boerma, H. R., All, J., and Parrott, W. 2002. Combining cry1Ac with QTL alleles from PI 229358 to improve soybean resistance to lepidopteran pests. *Mol. Breed.* **9**: 43–51.

Wang, C. Y. and Xia, J. Y. 1997. Differences of population dynamics of bollworms and of population dynamics of major natural enemies between Bt transgenic cotton and conventional cotton. *China Cottons* **24**: 13–15.

Wang, C. Z. and Qin, J. D. 1996. Effect of soybean trypsin inhibitor, gossypol and tannic acid on the midgut protease activities and growth of *Helicoverpa armigera* larvae. *Acta Entomol. Sín.* **39(4):** 337–341.

Watson, J. D., Hopkins, N. H., Roberts, J. W., Steitz, J. A., and Weiner, A. M. 1987. *Molecular Biology of the Gene*. Benjamin/Cummings Publishing Co., Menlo Park, CA. 1163 pp.

Williams, W. P., Sagers, J. B., Hanten, J. A., Davis, F. M., and Buckley, P. M. 1997. Transgenic corn evaluated for resistance to fall armyworm and Southwestern corn borer. *Crop Sci.* **37**: 957–962.

Wilmink, A., van de Ven, B. C. E., and Dons, J. J. M. 1995. Activity of constitutive promoters in various species from the Liliaceae. *Plant Mol. Biol.* **28**: 949–955.

Wilson, W. D., Flint, H. M., Deaton, R. W., Fischhoff, D. A., Perlak, F. J., Armstrong, T. A., Fuchs, R. L., Berberich, S. A., Parks, N. J., and Stapp, B. R. 1992. Resistance of cotton lines containing a *Bacillus thuringiensis* toxin to pink bollworm (Lepidoptera: Gelechiidae) and other insects. *J. Econ. Entomol.* **85**: 1516–1521.

Wiseman, B. R., Lynch, R. E., Plaisted, D., and Warnick, D. 1999. Evaluation of Bt transgenic sweet corn hybrids for resistance to corn earworm and fall armyworm (Lepidoptera: Noctuidae) using a meridic diet bioassay. *J. Entomol. Sci.* **34**: 415–425.

Wong, E. Y., Hironaka, C. M., and Fischhoff, D. A. 1992. *Arabidopsis thialiana* small subunit leader and transit peptide enhance expression of *Bacillus thuringiensis* proteins in transgenic plants. *Plant Mol. Biol.* **20**: 81–93.

Wood, E. A., Jr. 1971. Designation and reaction of three biotypes of the greenbug cultured on resistant and susceptible cultivars of sorghum. *J. Econ. Entomol.* **64**: 183–185.

Wraight, C. L., Zangerl, A. R., Carroll, M. J., and Berenbaum, M. R. 2000. Absence of toxicity of *Bacillus thuringiensis* pollen to black swallowtails under field conditions. *Proc. Natl. Acad. Sci. USA* **97**: 7700–7703.

Wu, G., Cui, H. R., Shu, Q. Y., Ye, G. Y., and Xia, Y. W. 2000. GUS histochemical assay: a rapid way to screen stripped stem borer (*Chilo suppressalis*) resistant transgenic rice with a Cry1A(b) gene from Bt (*Bacillus thuringiensis*). *J. Zhejiang Uni. Agric. Life Sci.* **26**: 141–143.

Wu, G., Cui, H., Ye, G., Xia, Y., Sardana, R., Cheng, X., Li, Y., Altosaar, I., and Shu, Q. 2002. Inheritance and expression of the Cry1A(b) gene in Bt (*Bacillus thuringiensis*) transgenic rice. *Theor. Appl. Genet.* **104**: 727–734.

Wu, J. Y., He, X. L., Shu, C., Chen, S., Fu, C. X., and Huang, J. Q. 1997a. Influence of water logging on the bollworm resistance of Bt cotton. *Jiangsu J. Agric. Sci.* **13**: 231–233.

Wu, L. Q., Wang, X. F., Song, C. P., and Jia, Q. 1999. A comparative study on transgenic cotton varieties resisting insect pests and traditional cotton. *J. Hebei Agric. Uni.* **22**: 13–15.

Wu, Y. R., Llewellyn, D., Mathews, A., and Dennis, E.S. 1997b. Adaptation of *Helicoverpa armigera* (Lepidoptera: Noctuidae) to a proteinase inhibitor expressed in transgenic tobacco. *Mol. Breed.* **3**: 371–380.

Xiang, Y., Wong, W. K. R., Ma, M. C., and Wong, R. S. C. 2000. *Agrobacterium*-mediated transformation of *Brassica campestris* ssp. *parachinensis* with synthetic *Bacillus thuringiensis* Cry1A(b) and Cry1A(c) genes. *Plant Cell Rep.* **19**: 251–256.

Xu, D., McElroy, D., Thoraburg, R. W., and Wu, R. 1993. Systemic induction of a potato pin 2 promoter by wounding methyl jasmonate and abscisic acid in transgenic rice plants. *Plant Mol. Biol.* **22**: 573–588.

Xu, D. P., Xue, Q. Z., McElroy, D., Mawal, Y., Hilder, V. A., and Wu, R. 1996. Constitutive expression of a cowpea trypsin inhibitor gene, CpTi, in transgenic rice plants confers resistance to two major rice insect pests. *Mol. Breed.* **2**: 167–173.

Xue, M., Dong, J., and Zhang, C. S. 2002. Effect of feeding Bt cotton and other plants on the changes of development and insecticide susceptibilities of lesser armyworm *Spodoptera exigua* (Hubner). *Acta Phytopath. Sin.* **29**: 13–18.

Yang, C. D., Tang, K. X., Wu, L. B., Li, Y., Zhao, C. Z., Liu, G. J., and Shen, D.L. 1998. Transformation of haploid rice shoots with snowdrop lectin gene (GNA) by *Agrobacterium*-mediated transformation. *Chinese J. Rice Sci.* **12**: 129–133.

Yang, G. D., Zhu, Z., Li, Y., Zhu, Z. J., Xiao, G. F., and Wei, X. L. 2002. Obtaining transgenic plants of Chinese cabbage resistant to *Pieris rapae* L. with modified CpTI gene (sck). *Acta Hort. Sin.* **29**: 224–228.

Yao, B., Wu, C. J., Zhao, R. M., and Fan, Y. L. 1996. Application of insect-specific neurotoxin AaIT gene in baculovirus and plant genetic engineering. *Rice Biotech. Quart.* **26**: 24.

Yao, Q., Lai, F. X., and Zhang, Z. T. 2002. Detached-rice appraisement method for rice resistance to striped stem borer. *Chinese J. Rice Sci.* **16**(4): 385–386.

Ye, G. Y., Shu, Q. Y., Cui, H. R., Hu, C., Gao, M. W., Xia, Y. W., Cheng, X., and Altosaar, I. 2000. A leaf-section bioassay for evaluating rice stem borer resistance in transgenic rice containing a synthetic cry1Ab gene from *Bacillus thuringiensis* Berliner. *Bull. Entomol. Res.* **90**: 179–182.

Yeh, K. W., Lin, M. L., Tuan, S. J., Chen, Y. M., Lin, C. Y., and Kao, S. S. 1997. Sweet potato (*Ipomoea batatas*) trypsin inhibitors expressed in transgenic tobacco plants confer resistance against *Spodoptera litura*. *Plant Cell Rep.* **16**: 696–699.

Yoder, J. I. and Goldsbrough, A. P. 1994. Transformation systems for generating marker-free transgenic plants. *Bio/Technology* **12**: 263–267.

Zambryski, P. C. 1992. Chronicles from the *Agrobacterium*-plant cell DNA transfer story. *Annu. Rev. Pl. Physiol. Plant Mol. Biol.* **43**: 465–490.

Zambryski, P. C., Joss, H., Genetello, C., Leemans, J., VanMontagu, L. M., and Schell, J. 1983. Ti-plasmid vector for the introduction of DNA into plant cells without alteration of their normal regeneration capacity. *EMBO J.* **2**: 2143–2150.

Zhang, F. P., Yin, W., Yu, C., Lan, L. B., Zhen, Z., and Hui, L. X. 1998. On the resistance of CpTI transgenic tobacco plants to cotton bollworm. *Acta Phytopath. Sin.* **24**: 331–335.

Zhang, W. and Wu, R. 1988. Efficient regeneration of transgenic plants from rice protoplasts and correctly regulated expression of the foreign gene in plants. *Theor. Appl. Genet.* **76**: 835–840.

Zhao, J. Z., Fan, X. L., Shi, X. P., Zhao, R. M., and Fan, Y. L. 1997. Gene pyramiding: an effective strategy of resistance management for *Helicoverpa armigera* and *Bacillus thuringiensis*. *Resist. Pest Manage.* **9(2)**: 19–21.

Zhao, J. Z., Shi, X. P., Fan, X. L., Zhang, C. Y., Zhao, R. M., and Fan, Y. L. 1998a. Insecticidal activity of transgenic tobacco co-expressing Bt and CpTI genes on *Helicoverpa armigera* and its role in delaying the development of pest resistance. *Rice Biotech. Quart.* **34**: 9–10.

Zhao, J. Z., Zhao, K. J., Lu, M. G., Fan, X. L., and Guo, S. D. 1998b. Interactions between *Helicoverpa armigera* and transgenic Bt cotton in North China. *Sci. Agric. Sin.* **31**: 1–6.