Genetically Engineered Plants in the Environment--Applications and Issues

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ABSTRACT

Almost 20 years have past since the first report of genetic engineering of plants. During that time, significant technical advances have been made in plant transformation, in gene isolation and design and in the regulation of gene expression. Increasing numbers of food, fiber and horticultural species can be engineered with a broad range of engineered traits of potential value for agricultural, human health and environmental clean up applications. The majority of early commercial product candidates have been herbaceous crop plants engineered for resistance to agronomic pests or to herbicides. Field testing of genetically engineered plants has occurred in numerous countries. However, many unresolved environmental, regulatory, proprietary and public acceptance issues remain. An overview of the types of engineered plant products that are being developed is presented. Reported non-target effects of genetically engineered plants on plant, microbial and invertebrate populations are summarized. Research to assess the potential long term non-target ecological and health effects of engineered plants is proposed. Technical and non-technical points to consider in developing and releasing genetically engineered plants are also discussed.

key words: genetically engineered plants, non-target ecological effects, risk assessment, transgenic plants

INTRODUCTION

Selection of natural variants and specific breeding of plants for given agronomic, horticultural or silvicultural applications are commonly used crop improvement practices. In conjunction with fertilizers, weed and pest control, plant breeding efforts have been very successful in producing plants with improved yields and desired crop quality characteristics. For the most part, planned introductions of herbaceous and woody plants, even in geographies outside their native ranges, have proceeded without adverse ecological or health effects. Many of our major crop species, such as wheat, corn, rice, potatoes, and soybeans, have been successfully introduced world-wide (80). However, numerous escapes from cultivation of non-engineered agronomic, horticultural and tree species are causing unwanted ecological effects. Examples include kudzu, johnson grass, purple loosestrife and *Melaleuca* (64, 111, 112, 131, 163, 172, 177). With genetic engineering, traditional breeding barriers between plants can be overcome, thereby making possible the creation of truly novel plants. Genes from other species or genera of plants, and even genes from microbes and animals, are being introduced into an increasingly broad array of herbaceous and woody, food, fiber, ornamental and specialty crop (e. g., nut and vine) species to create engineered plants having desired characteristics (44, 48, 50, 77, 126, 146). As these novel plants rapidly progress from laboratory culture to greenhouse and field testing (86, 149) and into commercial production, tests of their efficacy and yields tend to be well addressed. However, the degree of evaluation of their potential ecological impacts and human biosafety may vary, depending in part on the existence and rigor of regulations (104). Typically, assessment for ecological and human safety consists of short term, single species, toxicity tests done under laboratory conditions. The objectives of this review are to (a) provide a brief summary of the major proposed applications of engineered plants, (b) highlight published results on their potential non-target effects, (c) suggest points to consider in developing and releasing engineered plants, (d) propose research needed to identify potential long term effects of releasing engineered plants and (e) briefly discuss potential impacts of regulations, intellectual property rights and public acceptance on the development, safety evaluation and commercialization of engineered plant products.

CREATING ENGINEERED PLANTS

Agrobacterium, particle guns and electroporation represent three major, not necessarily mutually exclusive or equally effective, approaches for introducing genes into plants (48, 50. 77. 126, 146). In spite of the relative ease of transformation of many plant species, some species. particularly legumes, cereals, and woody species, often remain recalcitrant to transformation simply by treatment of tissue surfaces, cells or protoplasts with engineered *Agrobacterium* (106. 126, 162). Higher rates of transformation may sometimes be achieved by using physical approaches such as mechanical energy or electrostatic forces to introduce organisms, plasmids, transposable elements or nucleic acids containing sequences of interest (24, 25, 26, 68, 90, 146). In ballistics-based approaches, gold or other non-biological particles coated with *Agrobacterium*, plasmids or nucleic acid sequences are literally explosively propelled into plant host tissues, embryos or cells. In electroporation, charge differences are applied to facilitate entrance of engineered plasmids or nucleic acids into recipient cells or protoplasts. Preliminary estimates of transformation success may be obtained in each of these systems by using selective marker genes, typically for antibiotic resistance, which allow transformed survivors to grow on selective media. Reporter genes such as the *GUS*, Green Flourescent Protein and *lux* systems, which produce visible stains or emit visible or flourescent light respectively, also may be used to quantify gene expression and study gene regulation in transformants (69, 107, 115, 128, 151). Various nucleic acid-based amplification and hybridization methods and protein- based immunological techniques may additionally or alternatively be used to detect and quantify gene expression, and to select candidates for advanced testing and breeding efforts (57, 65, 135). Ideally, transformants having desired expression levels can be regenerated into whole plants and used in subsequent seed increase and traditional breeding efforts to produce plants which stably express the desired gene in progeny which are agronomically fit (17, 85). To help protect proprietary interests, additional engineering steps may be taken to introduce genes for seed sterility. Use of this controversial seed sterilizing technology to prevent farmers and growers from saving seed for replanting and breeding purposes respectively, has been termed "terminator" technology in the public press (28, 81, 139).

PROPOSED USES OF GENETICALLY ENGINEERED PLANTS

In addition to numerous journal articles and reviews (19, 49, 171), various academic. trade and activist newsletters provide periodic updates on the kinds of engineered plants which are being developed and field tested (54, 66, 156).

Three categories which broadly describe the proposed applications of engineered plants are crop protection, crop quality and specialty uses. The crop protection category includes plants designed for resistance to insect pests, to plant diseases and to herbicides. Examples of plants designed for protection against lepidopteran pests such as hornworms, ear worms, budworms and bollworms, include tomatoes, corn and cotton that express pesticidal delta-endotoxin or *cry* genes from *Bacillus thuringiensis* var. *kurstaki (B t.k.),* (34, 47, 122). Addition of genes to express serine protease inhibitors also has been explored as a means of increasing *B.t.k.* activity (51, 93). Crystal toxin genes from *Bacillus thuringiensis* var. *tenebrionis (B.t.k.).* have been used to confer resistance to a coleopteran insect, the Colorado potato beetle (103).

Disease resistance strategies have focused primarily on control of viral and fungal diseases of plants. Incorporation of viral coat protein genes, anti-sense or ribozyme sequences has been explored as means of conferring resistance to viruses such as TMV and ToMV in tobacco and tomato, CMV in cucurbit crops and tomatoes, and PVX and PVY in potatoes (9, 21, 29, 60, 73, 89, 161). To achieve resistance to fungal pathogens, sequences encoding hydrolytic enzymes such as chitinases, gluconases and phosphatases have been inserted into plants such as tobacco, potatoes, corn and roses (18, 96, 152, 167). Other disease resistance strategies include insertion of sequences to express anti-bacterial cysteine-rich peptides such as thionins and defensins (20), or production of more broadly active ribosome inactivating proteins (92, 169). Insertion of glucose oxidase genes has been postulated to result in increased production of hydrogen peroxide, which may be directly toxic to some pathogens; the hydrogen peroxide in turn, has been suggested to activate or enhance the protective response of plant systemic aquired resistance (SAR) mechanisms (152). Use of genetic engineering to increase production of secondary metabolites such as chalcones also has been proposed as a mechanism to confer plant disease resistance (109). Genetically engineered plants are also being developed as tools to better understand, induce and regulate SAR responses (22, 37).

Mechanisms to confer herbicide resistance include insertion of plant or bacterial genes which encode enzymes for herbicide inactivation or degradation, or for inactivation of target sites (27, 63, 76, 120). For example, bromoxynil resistant cotton and canola have been produced by introducing microbial genes for the enzyme nitrilase, which can degrade bromoxynil (147), and resistance to the herbicide glufosinate has been achieved by cloning the gene for phosphinothricin acetyl transferase from an actinomycete into crop plants (63). Introduction of plant or microbial sequences encoding modified EPSP (5-enolpyruvl-3phosphoshikimate) synthases, has been used to produce soybean, cotton and canola plants tolerant to the herbicide glyphosate (76).

The major objectives of crop quality improvements are the modification of traits to enhance nutritional benefits to consumers or economic benefits to growers and food processing companies. Approaches to increase levels of the antioxidant, vitamin E, have been described (113, 141). To produce oils more suitable for cosmetic uses or for human consumption respectively, the degree of saturation of lipids can be increased or decreased using anti-sense technology (82, 83). Anti-sense technology also has potential applications in controlling shelf life of produce by inhibiting expression of enzymes involved in cell wall degradation. Using altered plant or microbial genes for enzymes involved in sugar and starch biosynthesis, starch levels can be increased in corn and potato cultivars used as starch or ethanol sources; they also can be used to decrease starch and associated oil absorption in potato cultivars used for French fries (49, 140). Genetic engineering may also be used to either increase the gluten content of wheat flour used in making bread or decrease it in flour used for making pastries (15).

A major proposed specialty application of engineered plants includes production of pharmaceuticals. Plants have been engineered to produce vaccines, antibodies and peptides (e. g., enkaphalins and inteferons), for veterinary and human therapeutic uses (10, 61, 99, 119, 144, 165, 183). Plants also have been designed to produce industrial enzymes including bacterial alpha-amylase, which may be useful in food and beverage processing and in stain removal, and to produce fungal lignin-peroxidase to degrade wastes from pulp mills (6). They also have been proposed as production sources for plastics and pigments (70, 123, 154).

Phytoremediation is attractive as a lower cost, *in situ* alternative to transporting contaminated soils for clean up by extraction or incineration methodologies (4, 46, 134, 136).

Engineering of plants to selectively develop rhizosphere flora capable of degrading specific xenobiotic compounds has been proposed (117). Pesticide and heavy metal tolerant plants for use in clean up of polluted soils, for treatment of industrial waste streams and as environmental biosensors also have been proposed (84, 110, 166). Mechanisms for phytoremediation include enzyme secretion by roots, to degrade xenobiotic chemicals in soil, and binding of metals to introduced metallothioneins or peptides (108, 148).

Strategies to produce plants tolerant to natural stressors such as freezing temperatures and salts, have been described which utilize gene sources ranging from bacteria to fish (7, 55, 62, 102, 155, 181, 182). Availability of plants able to grow in physically and chemically demanding environments could result in a redefinition of current concepts of arable soils. Coupled with increases in photosynthetic efficiency and modifications in patterns of carbon allocation (75, 95), improved stress tolerance could lead to the development of plants customized for optimal growth and yield even in areas which have short growing seasons and suboptimal growing conditions.

POTENTIAL NON-TARGET ECOLOGICAL AND HEALTH EFFECTS OF ENGINEERED PLANTS

Numerous international symposia have been held to discuss proposed applications and biosafety considerations for the release of engineered plants (Table 1). In part because of the newness of the technology, relatively little is available in the peer-reviewed literature on observed non-target ecological effects of engineered plants. Most of the ecological risk concerns have focused on three areas: (a) the potential for gene flow and outcrossing of herbicide resistance genes, with the resultant development of crop-weed hybrids (b) the development of resistance to *B. t. k.* delta-endotoxins in lepidopteran insect pest populations and (c) the effects of pesticidal plants on soil foodweb biota.

Many of the early and continuing concerns regarding the release of engineered plants have centered on the potential for escape of herbicide resistant plants or their genes, to crop and non-crop relatives (5, 11, 16, 30, 31, 32, 33, 45, 53, 58, 71, 74, 78, 79, 97, 129, 130, 138, 145, 157, 158, 159, 179). Publications on gene flow between engineered and non-engineered plants in greenhouse or field situations are becoming increasingly available (12, 23, 36, 91, 127). Accordingly, strategies to reduce and manage the risks of gene flow from engineered plants are of interest, and have been discussed (72, 133). For cotton, soybeans and corn, which at least in the major growing areas in the continental U.S., do not have closely related wild relatives, outcrossing has not been a significant concern (145). However in the U.S., where sunflowers, cucurbits and radish have wild relatives; in Canada and the northern U.S., where canola may coexist with wild mustards; and in Europe, where wild beets may coexist in proximity to cultivated sugarbeet crops; herbicide resistant gene flow to wild relatives could result in the creation of crop-weed hybrids. A recent report suggesting enhanced outcrossing of transgenic plants (12) is of particular ecological concern; it highlights a need to carefully monitor the outcrossing rates of genetically engineered plants. Additional factors which need to be looked at in longer term studies of potential non-target effects of gene flow from transgenic plants are viable seed production, spread and persistence of crop-weed hybrids.

Preventing or decreasing resistance development to *B. t. k.* delta endotoxins in target and non-target susceptible lepidopteran insect populations, has received much attention from entomologists (2, 59, 67, 100, 101, 132, 153, 164). Recently, a major agricultural biotechnology company announced that growers should plant areas adjacent to fields planted with insect resistant engineered corn, with non-insecticidal cultivars (173). These areas would serve as refugia, in which target pest populations would not be exposed to the pesticidal proteins. Entomologists have additionally recommended that "pyramiding", the use of multiple engineered and non-engineered genes to confer resistance to target pests, should also be considered as part of an over all strategy to slow down resistance development in target pest populations (132).

Several studies are available on the short term ecological effects of engineered plants containing insecticidal genes on soil foodweb components. Using a broad array of techniques, changes in the size and diversity of bacterial, fungal and plant feeding nematode populations and in soil enzyme actvities, have been found in soil exposed to leaf litter from cotton expressing the *B.t.k.* delta-endotoxin gene and potato plants expressing the *B.t. t.* crystal protein gene (39, 40). Similarly, soil incorporation of tobacco leaves expressing an insecticidal protease inhibitor, resulted in changes in soil respiration and in populations of nematodes, protozoans and microarthopods (42). Using immunological methods, the persistence of *B.t.k.* delta-endotoxin and protease inhibitor in engineered leaves of cotton and tobacco respectively, was observed for several months following incorporation into soil (42, 121). Using molecular methods, antibiotic resistance marker sequences in plasmid DNA and in potato leaf litter, could be detected for months following incorporation into field soils and soil microcosms, respectively (175, 176). Non-target effects of alfalfa plants designed for industrial enzyme production include changes in the community composition and substrate utilization patterns of microbial populations, decreases in plant biomass and changes in nutrient content of both greenhouse grown and field grown engineered alfalfa plants (35, 41, 170). Differences have been noted in endophytic and rhizosphere microbial communities between nonengineered canola cultivars and those engineered to be herbicide resistant (143). Delays and decreases in arbuscular mycorrhizal infection have been observed in some tobacco transformants engineered to express phosphatases for fungal disease resistance (167). In contrast, tobacco engineered for disease resistance with defensin genes had no inhibitory effect on arbuscular mycorrhizal infection (13). In alfalfa containing a fungal lignin-peroxidase gene, a trend toward decreased arbuscular mycorrhizal infection was observed in plants grown in greenhouses (170). An excellent review is available which summarizes the potential and reported effects of transgenic plants producing anti-bacterial and antifungal proteins on saprophytic soil microflora (56). A summary of reported short term non-target effects of engineered plants expressing traits for insect, disease or herbicide resistance and for production of specialty chemicals is presented in Table 2.

The possibility exists that non-target or unintended effects may be the result of somaclonal variation. For example, in a study of non-transformed cotton plants regenerated from tissue culture, significant differences in boll and seed number and in fiber quality were attributed to somaclonal variation (3). It is thus conceivable that some non-target or unintended effects of engineered plants may not be due to direct or indirect activities of engineered gene products *per se*, but to somaclonal variation. Additional possibilities include positional and pleiotropic effects related to where introduced genes have inserted into the host plant genome. The generally random nature of gene insertion may result in activation or inactivation of genes having functions which differ from those of the inserted gene. Whether introduced genes, somaclonal variation, positional or pleotropic effects result in unwanted agronomic, health or ecological effects or potentially yield and crop quality benefits, are areas deserving of careful screening, selection and monitoring (39, 105, 180).

Few reports are available in the peer reviewed literature on evaluation of potential effects of engineered plants on human health (52, 114). Development of allergenicity to proteins in engineered plants is a potential concern, since it may not be apparent with short term, single acute exposures. Over a long period of cumulative dietary or contact exposure however, susceptible individuals may develop allergic responses to these proteins. Interest in modifying seed storage proteins, such as those found in soybeans or Brazil nuts (87) appears to have waned, in large part due to allergenic concerns (116). Another human health concern which has been raised and debated, and which is more of a concern in some parts of the world than in others, is transfer of antibiotic resistance from genes in ingested plant tissues to human gut flora.

Because of their common use as selective markers to facilitate the detection of transformants. DNA encoding resistance to antibiotics such as streptomycin and kanamycin, is present in many engineered plant tissues. Potential ways to minimize concerns about the transfer of antibiotic resistance genes from plant tissue to intestinal flora are utilization of transformation strategies which can avoid the use of antibiotic resistance markers, removal of antibiotic resistance genes by ge : and biochemical means, or determination of acceptable levels of risk.

REGULATORY AND ECONOMIC POINTS TO CONSIDER IN DEVELOPING GENETICIALLY ENGINEERED PLANTS

It is understandably difficult to propose universally applicable testing requirements for the broad spectrum of engineered gene and plant possibilities. Arguments for case by case regulation or no regulation, have thus sometimes been brought forward. Both national and international efforts continue to harmonize regulations for environmental testing permits and for commercial use registrations (168). International entities such as the Organization for Economic and Community Development (OECD), the United Nations Industrial Development Organization and national Departments, Agencies and Ministries of Agriculture, Food Safety, the Environment and Forestry of individual countries, may each provide varying degrees of oversight and regulatory guidance. Based on the nature of specific crops and traits, either engineered plants, or their active (engineered) ingredients may be regulated. For example, in the U.S., shipment and field tests of engineered plants are regulated by the U.S. Department of Agriculture (U.S.D.A.), the active engineered pesticidal ingredients expressed in engineered pestididal plants are regulated by the U.S. Environmental Protection Agency (U.S.E.P.A.), and food additives are regulated by the Food and Drug Administration (F.D.A.). An overview of the coordination of the various roles of U.S.D.A., F.D.A. and E.P.A., under their respective statutory authorities (Plant Pest Act; Federal Insecicide, Fungicide and Rodenticide Act and the Toxic Substances Control Act; Federal Food, Drug and Cosmetic Act), have recently been summarized (8). In addition to federal regulations, individual states may have notification, permitting or other regulatory requirements. Points that may be useful to consider as engineered plants are developed, tested and considered for commercialization, are presented in Table 3. These include the market need for that type of plant product, efficacy, economic returns and short and long term non-target ecological and health effects.

RESEARCH NEEDS FOR ASSESSMENT OF NON-TARGET ECOLOGICAL EFFECTS OF ENGINEERED PLANTS

Given the published observations on outcrossing potential of genes to weeds, effects on the size and diversity of soil foodweb populations and on host plants, a need for longer term ecological monitoring seems apparent. For example, if plants designed for phytoremediation, crop protection or specialty chemical production result in accumulation of toxic compounds in their shoots or rhizospheres, potential impacts on herbivores, pollinators, pathogens, pests, symbionts, detritovores and saprophytes might be anticipated. Downstream effects on rates of litter decomposition and nutrient cycling could also develop. Similarly, escape, persistence and reproduction of herbicide tolerant plants or crop-weed hybrids, could bring about changes in plant community composition, much in the same way that exotic weeds have invaded and altered rangelands, grasslands, wetlands and forests.

The effects of potential changes in agonomic practices necessitated by the use of engineered crops, also need to be addressed. If the types and rates of agricultural chemical application for the engineered crop differ from those of non-engineered cultivars, the associated spectrum of pests and pathogens may change on each. The pest and pathogen spectrum may also change on adjacent fields of other crops and on non-crop plant species. If modified crop chemical recommendations are needed for engineered cultivars, the impacts of both crop and chemicals on subsequent crop rotation and chemical options also will need to be considered.

Reliance on methods used to monitor the fâte, transport and persistence of chemicals may not be sufficient or even appropriate, for novel biologicals produced by some kinds of engineered plants. Similarly, use of single species, short term test systems may not be appropriate or sufficient for some types of engineered plant products. Increased attention should be given to utilizing and developing methods to look at both short and long term soil foodweb, trophic and community level responses, as alternatives or supplements to single species acute toxicity tests. Studies are needed to determine if long term dietary or contact exposure to engineered products may lead to toxicity in wildlife and humans and development of allergenicity to humans.

Use of modern molecular and immunological methods, often similar or identical to those used in gene isolation and in quantification of gene expression, may facilitate detection, tracking and fate of engineered genes and gene products (121, 125, 142, 175, 176). Knowledge of the degree of spread of transgenic genes and genetically engineered plants can then be used to design and implement control strategies appropriate (i. e., if needed and in accordance with determined risks), for escaped and persistent genetically engineered crops and crop-weed hybrids. Fig. 1 summarizes the major types of targeted applications of engineered plants; it also highlights areas where research may be needed to identify and mitigate potential long term non-target ecological and health effects of genetically engineered plants.

NON-ECOLOGICAL ISSUES REGARDING THE RELEASE AND COMMERCIALIZATION OF GENETICALLY ENGINEERED PLANTS

It is clear that many different kinds of plants can and have been genetically engineered for diverse, novel and potentially useful agronomic and specialty chemical applications. Issues that are unclear and which continue to be debated in the media, court rooms, board rooms and in the court of public opinion, are who if anyone, should own or manipulate genes or life forms initially found in nature (38, 150). In many countries, regulatory paths remain unclear, or are not yet in place. Even as commercial products have begun to be marketed, difficult questions regarding regulation, ownership and public acceptance persist (137, 139). Cost of access to engineered seed and biological, marketing and contractual strategies to limit growers attempts to save and replant seed progeny, can become challenging public relations issues (81, 174). Patents may barely be granted and licensing agreements signed, before they are challenged in court (1, 14, 43, 98, 118, 124). Licensing and royalty fees may be paid simply as costs of doing business, to avoid lengthy and costly legal battles over patent rights.

CONCLUSIONS

Technical advances now permit introduction of genes from diverse sources into a broad array of herbaceous and woody food and fiber crops. Engineered plants have begun entering commerce, particularly in the U.S., and are being tested in numerous countries. Gene flow studies have documented transfer of engineered genes to crop and weed species; soil foodweb studies have demonstrated effects of several types of engineered plants on microbial and invertebrate populations in soil. Consequently, there is a need for ecological and health effects studies to be performed both prior to and after broad scale release. In addition, monitoring and mitigation plans are needed to help ensure the long term environmental and human safety of releasing and using engineered plant products.

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TABLE 1. Examples of meetings on biosafety and risk assessment of engineered plants

Meeting/Title	Location/Year Held	Editor/Publisher/Date
1st International Symposium on the Biosafety Results of Field Tests of Genetically Modified Plants and Microorganisms	Kiawah Island, SC, 1990	MacKenzie, D.R., Henry, S.C. (eds.). Agricultural Research Institute, 1991.
Pesticidal Transgenic Plants: Product Development, Risk Assessment and Data Needs	Annapolis, MD, 1990	US EPA, Office of Pesticide Programs, 1991
Workshop on Safeguards for Planned Introduction of Transgenic Oilseed	Ithaca, NY, 1990	USDA, Animal and Plant Health Inspection Service, 1990
Symposium on Ecological Implications of Transgenic Plant Release	College Park, MD, 1992	Levin, M. and R.J. Seidler (eds.), Blackwell Scientific Publ., Oxford, UK. Molecular Ecol. 3:1-90, 1994
2nd International Symposium on the Biosafety Results of Field Tests of Genetically Modified Plants and Microorganisms	Goslar, Germany, 1992	Casper, R., Landsmann, J. (eds.). Biologische Bundesanstalt fur Land-und Forstwirtschaft, 1992
Toward Enhanced and Sustainable Agricultural Productivity in the 2000's: Breeding Research and Biotechnology	Taipei, Taiwan, 1993	Academia Sinica, Nankang, Taichung District Agricultural Improvement Station, 1994
3rd International Symposium on the Biosafety Results of Field Tests of Genetically Modified Plants and Microorganisms	Monterey, CA, 1994	Jones, D. D. (ed.), University of California, 1994
OECD Workshop on Ecological Implications of Transgenic Crop Plants Containing *Bacillus thuringiensis* Toxin Genes	Queenstown, New Zealand, 1994	Hokkanen, H.M.T. (ed.), University of Helsinki, Finland, 1994
Herbicide-resistant Crops: a Bitter or Better Harvest?	Memphis, TN, 1995	Southern Weed Science Society, Champaign, IL, 1995
Dialogue on Risk Assessment of Transgenic Plants: Scientific, Technological and Societal Perspectives	Dornach, Switzerland, 1997	Heaf, D. (coordinator), Ifene, UK, 1997
4th International Symposium on the Biosafety Results of Field Tests of Genetically Modified Plants and Microorganisms	Tsukuba-machi, Japan, 1997	Matsui, S., Miyasaki, S., Kasamo, K. (eds.). Japan International Research Center for Agricultural Sciences, 1997
Virus-resistant Transgenic Plants: Potential Ecological Impact	Godollo, Hungary, 1997	Tepfer, M. (ed.), Springer Verlag, Berlin, Germany, 1997
5th International Symposium on The Biosafety Results of Field Tests of Genetically Modified Plants and Microorganisms	Braunschweig, Germany, 1998	Biologische Bundesanstalt fur Land-und Forstwirtschaft

TABLE 2. Examples of non-target and unintended effects of engineered plants

Trait	Plant	Effects	References
Insect resistance	cotton and potato	Changes in size and diversity of soil microbial, nematode and microarthopod populations; changes in soil enzyme activity	Donegan *et al.*, 1995 Donegan *et al.*, 1996
	tobacco	Changes in soil respiration; changes in size and diversity of protozoa, nematode and microarthopod populations	Donegan et al., 1997
Disease Resistance	tobacco	Decrease and delay in arbuscular mycorrhizal infection	Vierhelig *et al.*, 1995
Herbicide Resistance	*Arabidopsis*	Gene outcrossing	Bergelson *et al.*, 1998
	beets	Gene outcrossing	Dietz-Pfeilstetter and Kirchner, 1998
	canola	Gene outcrossing	Chèvre *et al.*, 1997; Lefol *et al.*, 1991; Purrington & Bergelson, 1995
	canola	Change in endophytic and rhizosphere microbial populations	Siciliano *et al.*, 1998
Specialty Uses: Lignin-Peroxidase	alfalfa	Changes in rhizosphere and soil microbial populations	Di Giovanni *et al.*, 1999; Donegan *et al.*, 1999
	alfalfa	Reduced shoot biomass and changes in shoot macronutrient content	Donegan *et al.*, 1999
	alfalfa	Reduced shoot biomass; changes in macronutrient and micronutrient content; decreased mycorrhizal infection	Watrud *et al.*, 1998
Auxin, Enzymes	aspen	Altered wood anatomy and shoot growth; change in lignin structure	Tuominen *et al.*, 1995; Lapierre *et al.*, 1999
Pigments	petunia	Loss of color	Mackenzie, 1990

TABLE 3. Points to consider in developing genetically engineered plants

Criteria	Questions
Market Need/Fit	Are effective products currently available
Technical Feasiblity	Are transformation systems and genes available for crop and trait of interest
Efficacy	Will it work better, faster, more safely than existing products
Agronomic Impacts	Will herbicide, insecticide and fungicide recommendations for current crop differ from recommendations for non-engineered cultivars Will modified chemical recommendations affect future crop rotations and chemical selections
Economics	Who owns gene sources and modified genes Do farmers have rights to save seeds What are anticipated returns to developers and growers
Ecological and Health Effects	Are there potential adverse effects to crop and non-crop plants Are there potential adverse effects to humans, wildlife, beneficial microbes and invertebrates
Mitigation	Are monitoring and control methods available
Regulations	Is a regulatory framework in place; are regulatory requirements known
Public Acceptance	Is proposed product perceived to be beneficial, safe, ethical

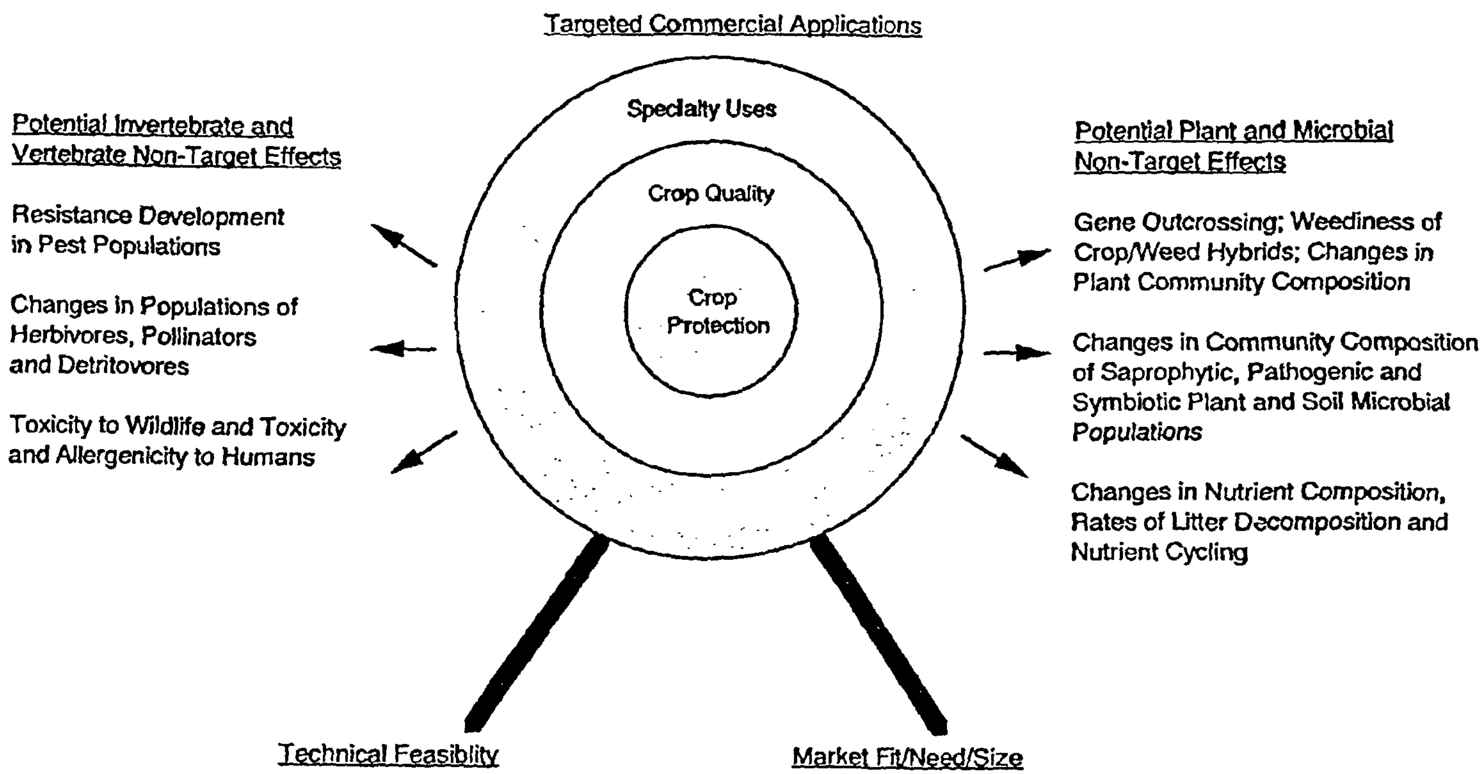
Targeted Commercial Applications
Specialty Uses
Crop Quality
Crop Protection
Potential Invertebrate and Vertebrate Non-Target Effects
Resistance Development in Pest Populations
Changes in Populations of Herbivores, Pollinators and Detritovores
Toxicity to Wildlife and Toxicity and Allergenicity to Humans
Potential Plant and Microbial Non-Target Effects
Gene Outcrossing; Weediness of Crop/Weed Hybrids; Changes in Plant Community Composition
Changes in Community Composition of Saprophytic, Pathogenic and Symbiotic Plant and Soil Microbial Populations
Changes in Nutrient Composition, Rates of Litter Decomposition and Nutrient Cycling
Technical Feasiblity
Market Fit/Need/Size

FIG. 1. Rationale and commercial applications for genetically engineered plants are represented by the supports and central target areas, respectively. Arrows identify areas of concern where research is needed to identify and mitigate potential short-term and long-term non-target and unintended ecological and health effects of genetically engineered plants.