Commercialization of Transgenic Plants: Potential Ecological Risks

Will evolutionary effects of engineered crops exacerbate weed and pest problems?

Allison A. Snow and Pedro Morán Palma

With the development of recombinant DNA techniques, plant breeders now have access to an astounding number of useful genes that can be inserted into the plant genome. Virtually all commercially important plants are being considered for this type of improvement, and the annual number of field tests of transgenic crop varieties is increasing exponentially (Figure 1). As of 1996, several transgenic crop plants have already been approved for commercial release in the United States, including disease-resistant squash, herbicide-resistant soybean, and insect-resistant potato and cotton. Many more crop varieties are nearly ready for commercialization. At this rate of development, the majority of all widely cultivated plants in the United States may possess genetically engineered traits within the next few decades.

Many applications of genetic engineering in agriculture and forestry will probably have neutral or beneficial environmental consequences, yet commercial-scale production of a few types of transgenic plants could lead to undesirable consequences for natural and agricultural systems. This article seeks to describe the diversity of transgenic plants that are currently being developed and to evaluate possible ecological risks associated with key species and genetically engineered traits, such as plant-produced insecticides. We have limited our review to transgenic plants that will be grown within the United States. We do not discuss genetically engineered viruses, bacteria, or fungi, even though some of these organisms will be used to improve yields of cultivated plants (e.g., insecticidal baculoviruses, nitrogen-fixing bacteria, or mycorrhizal fungi). When possible, we have restricted our focus to environmental effects that can be traced to genetic engineering per se rather than to methods used in traditional agriculture. In some cases, however, this distinction is artificial because genetic engineering is being used to speed up crop improvement that could also take place by means of methods that do not require the use of recombinant DNA.

We need to evaluate what is likely to occur in the next few decades, when many, if not most, commercially grown plants will have several highly effective transgenes

Recently, the question of whether the commercialization of transgenic crops could lead to serious environmental problems has generated considerable debate (e.g., Colwell et al. 1985, NRC 1989, Rogers and Parkes 1995, Snow and Morán Palma 1995, Tiedje et al. 1989). On one hand, agronomists often argue that the phenotypes of transgenic cultivars are similar to phenotypes that can be selected using traditional breeding methods and that these crops are therefore not inherently unfamiliar or risky (e.g., Brill 1985, Miller 1994). In contrast, some ecologists insist that access to unlimited numbers of useful genes from unrelated organisms makes genetic engineering a new and potentially dangerous technique. Their major concerns are that widespread cultivation of some transgenic crops could speed the evolution of undesirable weeds or pesticide-resistant insects, as described below (Ellstrand and Hoffman 1990, Rissler and Mellon 1993, Tiedje et al. 1989). To a large extent, these risks apply to traditionally bred crops as well, but the imminent release of transgenic plants has focused attention on this new technology and its potential consequences.

Despite continuing controversy about whether traditional and/or transgenic plants should be closely regulated, most biologists who have studied these issues agree on the

Allison A. Snow is an associate professor and Pedro Morán Palma is a doctoral candidate in the Department of Plant Biology, Ohio State University, 1735 Neil Ave., Columbus, OH 43214. They are studying gene flow and hybrid fitness in crosses between cultivated and wild species that include sunflower, radish, and squash. (Snow is also a research scientist at the University of Michigan Biological Station, Pellston, MI 49769).

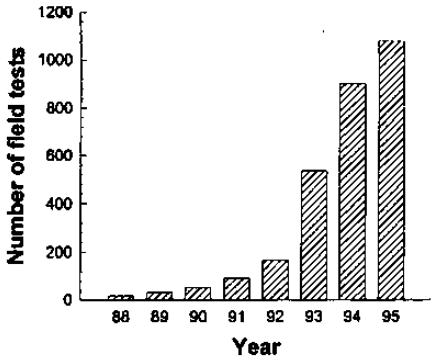

Figure 1. Numbers of field tests of genetically engineered organisms, most of which are plants. Based on total numbers of permits and notifications acknowledged by APHIS in each year. Many of these releases involve more than one site.

following points. First, it is not the molecular techniques themselves that might lead to environmental problems, but rather the phenotypic traits that result from the use of recombinant DNA. Second, the need for concern varies tremendously depending on the type of trait that is transferred and whether the transgenic organism can persist in free-living populations or hybridize with free-living relatives. Third, an informed understanding of the long-term effects of cultivating transgenic plants requires an interdisciplinary approach that encompasses ecological, evolutionary, and agricultural sciences. Finally, the hundreds of small-scale field tests that have been carried out to evaluate the performance of genetically engineered crops have not been designed to investigate the ecological risks associated with widespread commercialization (e.g., Wrubel et al. 1992).

Field releases of transgenic plants

Field releases of genetically engineered plants are monitored by the Animal and Plant Health and Inspection Service (APHIS) of the US Department of Agriculture (USDA). Information about field releases is available to the public and can be obtained over the Internet through the USDA's National Biological Impact Assessment Program (NBIAP; at http://www.nbiap.vt.edu or ftp.nbiap.vt.edu via telnet or gopher). The NBIAP database is a valuable resource, but it is not exhaustive because confidential business information is not included. Here we summarize the types of permits and notifications that have been obtained for field trials involving transgenic plants. Unless otherwise indicated, the data we discuss were obtained from APHIS documents covering 1987 through May 1996 (APHIS 1996).

Types of plants. More than 2000 small-scale field trials of 44 genetically engineered plant species have been carried out in the United States (Figure 1; Table 1). Beginning in 1993, APHIS streamlined the regulatory process for several common crops (corn, tomato, soybean, cotton, tobacco, and potato) by eliminating the need for a permit for most types of field testing and substituting a notification process. By 1994, 88% of all field trials involved these "fast-track" species, which now include carrot as well; squash, melon, rapeseed (canola), and alfalfa made up another 5% of the tests. Perennial species that have been tested include turf grass, strawberry, apple, plum, papaya, walnut, poplar, and spruce. Early success with improving these commercially important species has spurred research on a much wider range of herbaceous and woody species that are grown for food, oil, animal forage, fiber, wood, pharmaceutical use, and ornamental or landscaping value. Many of these species also occur in unmanaged natural communities.

Genetically engineered traits. Transgenic plants typically possess a marker gene, such as resistance to an antibiotic or herbicide, and genes coding for the expression of one or more traits of economic importance. The traits that are most readily manipulated using recombinant DNA techniques are those controlled by a single, well-characterized gene. The coding region of the foreign gene is fused to a promoter, usually the 35S promoter from cauliflower mosaic virus, to achieve high levels of expression. Many species that have been field tested in the United States possess transgenes for herbicide tolerance, insect resistance, disease resistance, or stress tolerance (Table 1). These field trials have demonstrated that it is possible to select for transgenic traits that are stable, heritable, and effective, with little or no decrease in plant yields.

Historically, genes coding for economically important traits have been obtained from related taxa by hybridization and several generations of backcrossing, with little knowledge of the identity of nontarget genes that "hitchhike" along due to genetic linkage. Now, however, the use of recombinant DNA techniques allows for precise transfer of only the gene(s) of interest without repeated backcrossing. Other advantages of recombinant methods are that an organism's own genome can be altered to decrease or enhance the expression of particular genes, and that genes from totally unrelated organisms are now candidates for crop improvement strategies.

Many transgenes obtained from microorganisms or animals are also found in plants (e.g., basic "housekeeping" genes and genes coding for antibacterial enzymes), and the primary reason for using foreign genes is that the genomes of these organisms have been studied more thoroughly than those of most crop plants. Thus, it is faster to take a cross-kingdom approach than to isolate the same genes from plants. In other cases, however, the absence of key traits in sexually compatible plants has been a major stimulus in the search for useful genes in unrelated organisms. For example, cold tolerance genes have been found in North Atlantic fish, and genes for potent insecticidal toxins have been isolated from bacteria. These and other novel traits have been successfully transferred from animals and microorganisms to plants.

Certain genetically engineered traits are more likely than others to require scrutiny when the plants expressing them are released commercially. These traits include those that could increase the invasiveness of sexually compatible wild plants, thereby increasing the need for weed control, as well as traits that might adversely affect animal populations and soil fertility. Another potentially troublesome trait is the production

Table 1. Plant species and transgenic phenotypes involved in field tests (from APHIS field test records as of October 1996). All phenotypes were tested in the United States unless otherwise indicated. IR = insect resistance, DR = disease resistance, HT = herbicide tolerance, and O = other traits, including stress and cold tolerance. All of these traits could be beneficial to sexually compatible wild relatives.

Family and species	Genetically altered traits
Actinidaceae	
Actinidea chinensis (kiwi)[a]	HT
Apiaceae	
Daucus carota (carrot)	DR, O
Caricaceae	
Carica papaya (papaya)	DR
Caryophyllaceae	
Dianthus caryophyllus (carnation)[a]	HT
Compositae	
Cichorium intybus (chicory, endive)	HT, O
Dendranthema grandiflora (chrysanthemum)	O
Helianthus annuus (sunflower)	IR, DR, O
Lactuca sativa (lettuce)	DR, HT
Convolvulaceae	
Ipomoea batatus (sweet potato)	HT
Cruciferae	
Arabidopsis thaliana (mouse-ear cress)	HT
Beta vulgaris (beet)	DR, HT
Brassica napus (rapeseed)	IR, DR[b], HT, O
Brassica oleracea (broccoli)	IR, O
Brassica oleracea var. botrytis (broccoli)[a]	HT
Brassica oleracea var. capitata (cabbage)[a]	IR
Cucurbitaceae	
Citrullus lanatus (watermelon)	DR
Cucumis melo (cantaloupe)	DR, HT
Cucumis sativa (cucumber)	DR
Cucurbita pepo = *C. texana* (squash, wild squash)	DR
Ericaceae	
Vaccinium macrocarpon (cranberry)	IR
Fabaceae	
Arachis hypogaea (peanut)	DR, HT
Glycine max (soybean)	DR, HT, O
Medicago sativa (alfalfa)	IR, DR, HT, O
Pisum sativum (pea)	HT, O
Trifolium spp. (clover)[a]	HT
Hamamelidaceae	
Liquidambar styraciflua (sweetgum)	DR, HT
Iridaceae	
Gladiolus spp. (gladiolus)	DR, O
Junglandaceae	
Juglans regia (walnut)	IR, DR
Liliaceae	
Allium cepa (onion)	DR
Asparagus officinalis (asparagus)[a]	O
Malvaceae	
Gossypium hirsutum (cotton)	IR, DR, HT
Pinaceae	
Picea glauca (white spruce)	IR
Poaceae	
Agrostis palustris (creeping bentgrass)	DR, HT
Hordeum vulgare (barley)	DR, HT
Oryza sativa (rice)	IR, DR, HT, O
Saccharum officinarum (sugar cane)	IR, HT
Triticum aestivum (wheat)	DR, HT, O
Zea mays (corn = maize)	IR, DR, HT, O
Rosaceae	
Amelanchier laevis (serviceberry)	IR
Fragaria sp.(strawberry)	HT[a], O, DR
Malus domestica (apple)	IR, DR
Prunus domestica (plum)	DR, O
Rosa spp. (rose)[a]	O
Rubus idaeus (raspberry)	O
Salicaceae	
Populus alba × *Populus grandidentata* (poplar)	IR, HT, O
Solanaceae	
Atropa belladonna (belladonna, nightshade)	IR
Capsicum annuum (sweet pepper)	DR[a], O
Lycopersicon esculentum (tomato)	IR, DR, HT, O
Nicotiana tabacum (tobacco)	IR, DR, HT, O
Petunia hybrida (petunia)	DR, O
Solanum melongena (eggplant)	IR, O
Solanum tuberosum (potato)	IR, DR, HT, O
Vitaceae	
Vitis sp. (grape)	HT

[a]These species/traits were tested outside of the United States and were listed in Krattiger (1994).
[b]Fungus-resistant *B. napus* was field-tested in Denmark (Jørgensen and Andersen 1995).

of pesticide; a major concern is that commercial cultivation of pesticide-producing plants will quickly select for insect pests that are resistant to these pesticides, thereby shortening the useful lifespan of environmentally "friendly" pesticides such as the toxin from the bacterium *Bacillus thuringiensis* (Bt).

Herbicide tolerance. Herbicide tolerance is a useful selectable marker as well as a trait of huge economic value to the agribusiness industry. Before the use of recombinant DNA methods, strong artificial selection sometimes resulted in herbicide-tolerant cultivars in various species (e.g., Gatehouse et al. 1992). Now, however, it is possible to choose from a variety of herbicides to create herbicide-tolerant crops (see Lal and Lal 1993). These efforts will allow nonpersistent herbicides (e.g., glyphosate) to be used more widely and will permit postemergence spraying of herbicide-resistant crops. On the negative side, transgenes for herbicide tolerance could promote greater reliance on herbicides and allow crops to be grown in soil contaminated with hazardous herbicides such as sulfonylurea. The major types of herbicide tolerance that have reached the field-testing stage in the United States are listed in Table 2.

Insect resistance. The need for alternatives to ineffective and/or toxic chemicals that are used against agricultural pests has stimulated much research on plant-produced pesticides. To date, the most common strategy is to insert various forms of the endotoxin gene from Bt into a plant's genome. Bt toxins act by damaging the membrane of the herbivore's midgut, causing massive water uptake (Gatehouse et al. 1992). A single feeding event usually causes paralysis and death in susceptible herbivores (Bt toxins have no effect on humans or other vertebrates). Purified Bt toxins are used as externally applied insecticides and are popular with organic growers. However, these biological toxins break down quickly, especially in rainy weather. Now, however, constant high-dose protection is possible with transgenic plants, and the deployment of Bt is expected to become far more widespread.

Table 2. Major herbicides and plant species for which transgenic herbicide-tolerant cultivars have been developed; commercial names of herbicides are in parentheses (data are from APHIS field test records).

Herbicide	Herbicide-tolerant plant species
Bromoxynil (Buctril)	Cotton, potato, tobacco
Glyphosate (Roundup)	Beet, corn, cotton, lettuce, poplar, rapeseed, soybean, tobacco, tomato, wheat
Phosphinothricin, glufosinate (Basta, Herbiace)	Alfalfa, *Arabidopsis*, barley, beet, corn, creeping bentgrass, melon, peanut, poplar, rapeseed, rice, soybean, sugar cane, sweet potato, tobacco, tomato, wheat
Sulfonylurea (Glean, Oust)	Corn, cotton, grape, rapeseed, tobacco, tomato
2,4-D	Cotton, potato, sweetgum

Different strains of Bt produce different crystal proteins, coded for by *Cry* genes, that are highly toxic to specific insects, mites, nematodes, flatworms, or protozoans (Fietelson et al. 1992). Among insect-specific Bt toxins, some kill only butterfly and moth larvae, whereas others are specific to weevils or beetles. Susceptibility to each class of Bt toxin is determined by the presence of specific receptors on the membrane of the insect's midgut epithelial cells. Thus, a single *Cry* transgene will protect the plant from only a limited number of pest species. This specificity can be viewed as an asset or a drawback, depending on which insects cause damage to a particular crop. To control a wider range of species, several different *Cry* transgenes can be inserted into the plant's genome (Bosch et al. 1994, van der Salm et al. 1994).

Other insect-resistant plants have been engineered to produce lectins and inhibitors of digestive enzymes (Gatehouse et al. 1992). Lectins, which are common in legume seeds, act by binding to carbohydrates and disrupting the midgut epithelial cells of many insect species. Bean and wheat germ lectins (e.g., wheat germ agglutinin) are toxic to mammals, but lectins from other species, such as pea, garlic, and snowdrop, appear to be innocuous to mammals because they are broken down during digestion (Gatehouse et al. 1992). These secondary compounds should be especially useful for protecting stored transgenic grain products from insect pests (e.g., Shade et al. 1994). Inhibitors of the digestive enzyme trypsin have been obtained from cowpea, tomato, and potato. Another type of proteinase inhibitor found in legumes, cereals, and other seeds interferes with α-amylase. This inhibitor occurs in the common bean (*Phaseolus vulgaris*), for example, and protects the seeds from bruchid beetle larvae. To date, the most effective transgenic insecticide is Bt, but eventually a wider range of insect-specific toxins should be available.

Disease resistance. Resistance to viral, bacterial, and fungal diseases has been achieved in several transgenic cultivars (Table 1). In the case of viruses, genes coding for viral coat proteins can be inserted into the cultivar's genome, often resulting in "immunity" to specific viral pathogens (e.g., Grumet 1994). For reasons that are not fully understood, the expression of low levels of a viral coat protein in the plant prevents disease symptoms from developing. Many viruses infect a range of host species, so the same coat protein gene can be transferred to several species. However, a given coat protein is only effective against one virus or its close relatives, so different genetic constructs are needed to ensure protection against different pathogens.

More than 20 plant species have been field tested for transgenic viral resistance, all involving coat protein genes. A few researchers have expressed concerns about the risks of new pathogens evolving due to transgenic viral coat proteins (e.g., AIBS 1995, Grumet 1994), a topic that is beyond the scope of this article. In the future, it is likely that genes for broader-spectrum viral resistance will also be developed, and work in this area is progressing rapidly. For example, resistance mediated by transgenic movement proteins, which affect the cell-to-cell spread of viruses through a plant's plasmodesmata, could provide more general protection to viral pathogens than viral coat protein strategies (e.g., Lapidot et al. 1993).

In contrast to the specificity of transgenic viral coat proteins, genetically engineered resistance to bacterial and fungal diseases typically involves single genes that are effective against many diseases. This is an active area of research, with a variety of strategies under development and few that have progressed to the field-testing stage. In many organisms, antibacterial responses can be attributed to cecropins, attacins, magainins, and lysozymes (Gatehouse et al. 1992). For example, the cecropin B gene from the giant silk moth (*Hyalophora cecropia*) has been transferred to tobacco, potato, and apple to reduce bacterial infections (APHIS 1996). Likewise, a lysozyme gene from chicken (*Gallus domesticus*) was transferred to apple (APHIS 1996). Plant species also possess antibacterial genes, but the cross-kingdom approach has been used more often because it affords easier access to well-characterized genes.

Fungi are responsible for diseases such as rusts, wilts, and mildews and are notoriously difficult to control (e.g., Broglie et al. 1991). Plants naturally resistant to these diseases often exhibit coordinated inducible gene activation during the onset of infection, resulting in the production of hydrolytic enzymes that degrade fungal cell walls. Chitinase and glucanase break down chitin and carbohydrates, respectively, and genes that code for these enzymes have been introduced into tobacco, tomato, petunia, corn, potato, lettuce, squash, cucumber, and melon (APHIS 1996; Gatehouse et al. 1992, Lal and Lal 1993). Genes coding for phytoalexin production are also used, because these low molecular weight secondary compounds have antimicrobial properties.

Stress tolerance and other transgenic traits. Plant breeders have tra-

ditionally assumed that many types of stress tolerance are multifaceted and polygenic, but single genes do appear to alleviate some types of physiological stress (Bartels and Nelson 1994, McKersie et al. 1993). A gene from winter flounder (*Pseudopleuronectus americanus*) was found to increase cold tolerance when expressed in potato and tomato (APHIS 1996), and overproduction of superoxide dismutase protects tobacco plants from ozone and other stresses (van Camp et al. 1994). Genetically engineered tolerance of drought stress is also being investigated (APHIS 1996).

Other transgenic traits fall under the categories of "product quality" or "agronomic properties" and affect ease of harvesting, appearance, taste, shelf life, and nutritional or processing characteristics of plant products. In addition, transgenic plants may someday be used as biochemical "factories" for producing pharmaceutical and industrial compounds (e.g., Haq et al. 1995, Topfer et al. 1995).

Crop-to-wild hybridization

Commercialization of genetically engineered plants will allow transgenes coding for beneficial traits to be transferred to wild or weedy populations of these taxa and their close relatives (e.g., Ellstrand and Hoffman 1990, Raybould and Gray 1993, 1994, Rogers and Parkes 1995; references in Seidler and Levin 1994). Genetic exchange between crops and their wild relatives is known to have occurred in the past, but most often the focus of such studies has been on how crop cultivars are affected by wild-type genes rather than the converse. Little is known about the long-term persistence of crop genes in wild populations or about the impact of fitness-related crop genes on the population dynamics of weedy relatives.

The first attempts to introduce fitness-related traits into crop plants involved one or two genes at a time, but the current trend is to continue to insert additional traits that improve crop yields. Within the next decade or two, it is likely that genetically engineered crops will possess many yield-enhancing traits that are absent or rare in populations of free-living relatives. For US agriculture, a potential risk of escaped transgenes is that hybridization with populations of free-living relatives will make these plants increasingly difficult to control, especially if they are already recognized as agricultural weeds and if they acquire resis-

Table 3. Examples of commercially important species that can hybridize with wild relatives in the United States. Genetically engineered species from Table 1 are underlined. Wild relatives that have been recognized as weeds (i.e., unwanted species in agricultural or natural habitats) are also underlined. This list is not exhaustive, especially with regard to commercially important grasses and woody species, which often occur in unmanaged populations. Also, for many cultivars the extent of hybridization with wild relatives has not been studied.

Family and cultivated species	Wild relative
Apiaceae	
Apium graveolens (celery)	Same species
Daucus carota (carrot)	Same species (wild carrot; Wijnheijmer et al. 1989)
Chenopodiaceae	
Chenopodium quinoa (quinoa—a grain)	*Chenopodium berlandieri* (Wilson and Manhart 1993)
Compositae	
Chicorium intybus (chicory)	Same species
Helianthus annuus (sunflower)	Same species
Lactuca sativa (lettuce)	*Lactuca serriola* (wild lettuce; Anderson 1949)
Cruciferae	
Beta vulgaris (beet)	*B. vulgaris var. maritima* (hybrid is a weed; Boudry et al. 1992)
Brassica napus (oilseed rape; canola)	Same species, *Brassica campestris*, *Brassica juncea* (Jørgensen and Andersen 1995)
Brassica rapa (turnip)	Same species (*B. campestris*)
Raphanus sativus (radish)	Same species, *Raphanus raphanistrum* (Panetsos and Baker 1967)
Cucurbitaceae	
Cucurbita pepo (squash)	Same species (*Cucurbita texana*, wild squash; Decker 1988)
Ericaceae	
Vaccinium macrocarpon (cranberry)	Same species
Vaccinium angustifolium (blueberry)	Same species
Fabaceae	
Trifolium spp. (clover)	Same species
Medicago sativa (alfalfa)	Same species
Hamamelidaceae	
Liquidambar styraciflua (sweetgum)	Same species
Junglandaceae	
Juglans regia (walnut)	*Juglans hindsii*
Liliaceae	
Asparagus officinalis (asparagus)	Same species
Pinaceae	
Picea glauca (spruce)	Same species
Poaceae	
Avena sativa (oat)	*Avena fatua* (wild oats; Baum 1977)
Cynodon dactylon (bermuda grass)	Same species
Oryza sativa (rice)	Same species (red rice; Langevin et al. 1990)
Sorghum bicolor (sorghum)	*Sorghum halepense* (Johnson grass; Arriola and Ellstrand 1996)
Rosaceae	
Amelanchier laevis (serviceberry)	Same species
Fragaria sp. (strawberry)	*Fragaria virginiana*
Rubus spp. (raspberry, blackberry)	Same species
Salicaceae	
Populus alba × *Populus grandidentata* (poplar)	*Populus* spp.
Solanaceae	
Nicotiana tabacum (tobacco)	Same species (escapes cultivation)
Vitaceae	
Vitis vinifera (grape)	*Vitis* spp. (wild grape)

tance to widely used herbicides. Another risk that is harder to evaluate in economic or ecological terms is that weedy populations will become more invasive in nonagricultural areas (e.g., roadsides, recreational areas, state and national forests, and preserves) and may contribute to declines in populations of native species.

Some ecologists have argued that rare plant species might be threatened by hybridization with transgenic plants (e.g., Rissler and Mellon 1993). However, this type of genetic "pollution" is unlikely to differ from existing levels of crop–wild gene flow. It is difficult to imagine how a few transgenes, in and of themselves, would negatively affect the genetic diversity of native plants.

Gene flow to wild relatives. The escape and persistence of transgenes in free-living populations poses possible risks only when the crop itself can survive without cultivation or when the crop spontaneously hybridizes with closely related wild taxa (see examples in Table 2). On this basis, it is possible to rank transgenic crops into risk categories of high, medium, and low. High-risk species are those that also occur as free-living populations or that hybridize easily with wild relatives. Holm et al. (1977) noted that 11 out of 18 of the most serious weed species worldwide are also grown as crops. Canola (*Brassica rapa*) often escapes from cultivation and can sometimes persist as a weed (Adler et al. 1993), and cultivars of squash, sunflower, and radish are sexually compatible with wild and weedy populations in both cultivated and noncultivated habitats. When free-living populations of the latter three species occurred within approximately 500–1000 m of the crop, gene flow via insect pollinators resulted in hybrid progeny (Arias and Rieseberg 1994, Kirkpatrick and Wilson 1988, Klinger et al. 1992, 1994). Likewise, wind pollination between cultivated and weedy wild rice resulted in hybrid progeny in Louisiana (Langevin et al. 1990). Hybridization is not necessary for the persistence of crop genes in naturalizing species that self-pollinate or spread vegetatively (e.g., poplar). Crop genes can also spread via seeds that persist in soil seed banks or disperse over wide geographic areas (Linder and Schmitt 1994). These examples show that commercialization of certain transgenic crops will eventually allow transgenes to escape into free-living populations of wild relatives. Containment of genes from commercially grown crops will be difficult if not impossible after transgenic plants are available to the general public.

Medium-risk crop species are those in the same genus and sharing similar chromosome numbers as wild relatives, thereby increasing the chance that at least a portion of the interspecific hybrid progeny would be viable and fertile. (Depending on the taxa, crosses between genera can also yield fertile hybrids.) Interspecific hybridization is common in certain taxa, such as among squash species (*Cucurbita*), which all have the same number of chromosomes (n = 14; Wilson 1990). Even if only a small proportion of the hybrid progeny from a given pair of taxa are viable, strong selection (e.g., frequent herbicide applications in fields where herbicide-tolerant hybrid genotypes occur) could favor the persistence of progeny that carry escaped transgenes. Unfortunately, published literature on the range of wild relatives that can interbreed with commercially grown cultivars in the United States is spotty, and studies that identify which wild species are capable of full or limited crossing with cultivated plants are needed. Species that are difficult to cross by means of hand-pollination may nonetheless hybridize in the field, as was demonstrated in experimental plantings of canola (*Brassica napus*) and field mustard (*Brassica campestris*; Jørgensen and Andersen 1995). Although these two species have different chromosome numbers (n = 19 and 10, respectively), the movement of transgenic herbicide resistance into free-living *B. campestris* has been detected under field conditions (Mikkleson et al. 1996).

The number of low-risk crop species is probably substantial, but until further studies are conducted on a case-by-case basis it may be premature to assume that a given species does not hybridize with free-living plants. Some crops, such as corn, soybean, tomato, and potato, do not appear to interbreed with wild species in the continental United States, and close wild relatives of cotton are restricted to Hawaii. However, it is possible that new weeds could evolve due to crop–wild gene flow in other regions of the world and then be transported to North America. Alien species constitute a large and increasing fraction of the flora in many regions of the United States (e.g., Myers and Henry 1979, Ruesink et al. 1995), and it would be naive to assume that weeds evolving in other temperate and subtropical regions of the world are incapable of being transported to the United States.

Potential for increased weediness in wild relatives. If genetic exchange between transgenic crops and wild relatives has the potential to create more serious weed problems, there should be evidence that this process has also occurred in nontransgenic crop–weed complexes. Indeed, such exchanges have occurred. In California, for example, a new weed, known as wild radish, originated in the nineteenth century due to hybridization between cultivated radish and an introduced weed, *Raphanus raphanistrum* (Panetsos and Baker 1968). Likewise, in Africa a harmful weed of pearl millet (*Pennisetum glaucum*) arose from hybridization between this crop and a wild relative, *Pennisetum americanum* (Brunken et al. 1987). Johnson grass (*Sorghum halepense*), considered one of the most noxious weeds worldwide, appears to be an interspecific hybrid between cultivated sorghum (*Sorghum bicolor*) and the wild *Sorghum propinquum* of southeast Asia (Paterson et al. 1995). And in the past few decades, a new weed evolved in France due to contamination of seed sources of sugar beet (*Beta vulgaris*) with pollen from a Mediterranean subspecies (ssp. *maritima*; Boudry et al. 1993). These examples demonstrate the potential for new weeds to evolve quickly when different species come into contact.

In general, however, there are few examples of weeds benefiting from specific fitness-related crop genes. This could be due to several fac-

tors—the lack of attention to the phenomenon, the absence of crop genes that confer strong fitness advantages to wild relatives, or simply the fact that the impact of beneficial genes is not dramatic. Despite a shortage of relevant empirical studies, we believe that gene flow from crops to wild and weedy relatives may have greater consequences in the future than in the recent past. Recombinant DNA methods are faster, more precise, and allow access to a vastly greater array of economically desirable genes than traditional breeding methods. Therefore, the frequency with which highly beneficial genes move into wild populations is expected to increase.

Whether escaped transgenes persist and spread in free-living populations depends largely on the viability of wild–crop hybrids and on phenotypic traits conferred by the transgenes. Some traits, such as delayed fruit ripening, production of pharmaceutical chemicals, and modified seed oil composition, are unlikely to be beneficial to free-living plants (but see Linder and Schmitt 1994). However, resistance to disease, herbivory, environmental stress, or herbicides is likely to enhance the fitness of weedy relatives. Nonweedy plant species could also become a problem if plants carrying escaped transgenes are subject to "ecological release" from biotic and/or abiotic factors that limit current populations of these species (Schmitt and Linder 1994).

The long-term persistence of fitness-related genes depends on the balance between the cost of expressing the phenotype, if any (e.g., due to pleiotropic genetic effects or reallocation of limiting nutrients), and the strength of selection favoring the trait. Preliminary studies show that the costs associated with fitness-related transgenic traits appear to be negligible (Crawley et al. 1993, references in Raybould and Gray 1993, but see Bergelson 1994), probably because of the precision of recombinant DNA techniques and careful choice of vigorous recombinant genotypes for further propagation. In contrast to the processes of natural selection or traditional plant breeding, which often lead to inadvertent selection for deleterious alleles that are linked to genes coding for beneficial traits, recombinant methods allow single genes to be inserted into the genome without the accompaniment of unwanted genes.

Even if transgenic traits do incur a cost, they could still be favored in the field if their benefits are great enough. For example, May Berenbaum and her colleagues studied the costs and benefits of natural variation in furanocoumarins in populations of a common weed, *Pastinaca sativa* (cow parsnip; Berenbaum et al. 1986). This variation affected levels of damage by a specialist herbivore, the parsnip webworm. When plants were grown in an insect-free greenhouse, resistance was negatively correlated with growth and reproduction, suggesting a cost of producing these secondary compounds. In the field, however, where webworms were ubiquitous, individuals resistant to herbivores had higher flower and seed production than those lacking specific furanocoumarins. In a similar vein, evaluating the costs and benefits of transgenic traits requires an understanding of how the new phenotype affects the organism in its natural environment.

Ultimately, we need to know whether beneficial transgenes will affect the invasiveness of weedy species. This will be difficult to study under natural conditions, but as a starting point one could test for greater vegetative biomass and seed production in transgenic versus nontransgenic wild plants. We also need to know which life history stages (e.g., seeds, seedlings, juveniles, adults) suffer enough mortality or damage to limit population growth rates, and whether transgenic traits that alleviate problems at these key life history stages would allow weedy populations to increase.

Evolution of resistant pests

Constant exposure to pesticides and herbicides has often led to the evolution of resistant pests, and the cultivation of some types of transgenic plants will likely facilitate this process. This concern applies to all plant pests, including insects, pathogens, and weeds, but most discussion has focused on the rapid evolution of pesticide-resistant insects (e.g., Gould 1988, 1991, Raffa 1989, Van Rie 1991).

Many traditionally bred cultivars require repeated pesticide applications to achieve high yields, a practice that is often expensive, ineffective, and/or damaging to humans and the environment. To alleviate some of these problems, companies such as Monsanto and Mycogen have developed Bt transgenic crops that are intrinsically toxic to herbivorous insects. Field trials have shown that these plants produce Bt toxins at high enough levels to have a dramatic impact on local pest populations. However, commercial-scale cultivation of pesticide-producing plants will lead to strong selective pressures in a given habitat, and resistant biotypes are likely to evolve within three to five years of constant exposure (Gould 1988, 1991).

Hundreds of arthropod species have evolved various types of insecticide resistance in the past few decades, leading Raffa (1989, p. 255) to conclude that "there is no physiological mode of insecticidal action, if applied with sufficient intensity, that cannot be overcome by insect populations." Resistance can evolve whenever selective forces are strong enough, as documented in diamondback moths exposed to externally applied Bt (Tabashnik 1994). A further concern is that selection for resistance to one type of pesticide sometimes confers cross-resistance to other pesticides (e.g., Gould et al. 1982). For example, when the tobacco budworm (*Heliothis virescens*) was exposed to the *Cry* IA(c) Bt toxin in the laboratory for 20 generations, it evolved resistance to not only this Bt toxin but also other forms of Bt (Gould et al. 1992, 1995). Therefore, if cross-resistance is common, multiple Bt toxins may not provide adequate protection from evolving pests.

The evolution of pesticide resistance will proceed more slowly if selective pressures are variable in space and time, allowing susceptible insects to be maintained in natural populations (Gould 1988, Raffa 1989). In general, the goal should be to suppress insect populations to levels that result in economic benefits but still allow susceptible insects to survive and reproduce. This level of

control could be achieved by cultivating mixtures of protected and unprotected host plants. To be successful, however, this approach relies on the cooperation of knowledgeable growers and a certain amount of luck, because the ideal frequencies and sizes of non-Bt refuges depend on the local movements of pest species. Even with refuges, resistant genotypes might be able to mate with each other, rather than with susceptible insects, such that many surviving insects would be homozygous for resistance to the pesticide (which is often a recessive trait).

An alternative strategy is to design plants that produce insect-deterring chemicals only in specific tissues, such as fruits, seeds, or young leaves, and to engineer plants with more than one type of resistance. In addition, each toxin should be produced in concentrations that are much higher than needed to kill the target pest. This strategy is important because partially resistant insects are more likely to survive and reproduce at low toxin levels, allowing resistance to evolve more quickly.

Without preventive measures, avoiding the rapid evolution of resistance will be a major challenge whenever pesticide-producing plants are cultivated on large areas of land. This issue is being taken seriously by the US Environmental Protection Agency (EPA) and various genetic engineering companies, which will require growers to maintain non-Bt refuges to prolong the effectiveness of Bt. Unfortunately, however, the first pesticide-producing crops to be released in the United States are producing only one Bt toxin, and local selection for resistant insects seems likely (see NBIAP 1995 for more information and a range of opinions).

There are several reasons to be concerned about the evolution of resistant pest biotypes. First and foremost, the loss of an effective means of controlling insect populations is clearly undesirable and may promote the use of more environmentally damaging methods of pest control (if alternative methods exist). Many entomologists regard Bt as an unusually benign pesticide that warrants extremely careful management, given the lack of acceptable alternatives at present. In addition, past studies of resistance and cross-resistance demonstrate that unintended selection can result in pest problems that are greater than those that existed before deployment of novel insecticides (Raffa 1989). For example, insects that have evolved pesticide resistance may be able to feed on a wider variety of formerly unpalatable plant species and may be more difficult to control than previous biotypes (Raffa 1989). Therefore, losing the efficacy of Bt toxins represents one of the most urgent ecological risks associated with transgenic plants.

Ecological effects of insect-resistant plants

Predicting the ecological effects of more thorough pest control on target and nontarget organisms is difficult and will require a case-by-case approach to identify possible unintended side effects. As with externally applied pesticides, the economic value of using transgenic insect-resistant crops will depend on direct and indirect effects on many co-occurring insect species (susceptible pests, resistant pests, and beneficial species, such as predators, parasitoids, and pollinators). High mortality in target insect populations might reduce competition with naturally resistant pest species, causing formerly minor pests to become more abundant. In some situations, targeted pest populations could shift to other host plants, decline in numbers, or evolve resistance to plant-produced pesticides. A complete discussion of this problem is beyond the scope of this article, but we present some general issues below.

Some of the first transgenic plants to be ready for commercial release possess genes coding for Bt endotoxins. The specificity of different Bt genes limits the numbers of nontarget insect species killed, but little is known about which insect species within each of these broad categories (e.g., lepidoptera) are susceptible to the toxin. Many nontarget species that cause little economic loss are probably susceptible, and in some cases one or more pest species may be naturally resistant (e.g., Bosch et al. 1994). Because of these complex ecological and evolutionary factors, the long-term efficacy of toxin production by plants is likely to vary under different ecological situations. A yield increase that can be demonstrated under highly controlled experimental conditions might not occur when the plants are grown commercially in a wide range of environments. On the other hand, when the primary pests of a given species are susceptible to the plant-produced toxin(s), dramatic effects could be realized.

Sharp declines in herbivore populations might affect predators or parasites that feed on the target insects. For example, commercially important tree species are prime candidates for improvement through genetic engineering, and beneficial traits such as insect resistance are likely to spread to noncommercial populations as well. Thus, large tracts of forest could become unavailable to insect herbivores if the dominant tree species have transgenes for resistance. Reduced insect populations could then lead to declines in insectivorous birds and other predators that often regulate populations of leaf-chewing forest insects (e.g., Holmes et al. 1979, Marquis and Whelan 1994). In an agricultural setting, there is the worry that populations of beneficial predators and parasitoids that kill crop pests would plummet if pests are eradicated completely. Artificial reintroduction of beneficial insects would likely be costly and difficult. In the broader context for risk assessment, these considerations suggest that the ecological consequences of pesticide-producing plants are likely to be more problematic than abstaining from pesticide-intensive management but much less serious than the impact of conventional, broad-spectrum pesticides.

Effects on soil biota and fertility

The ecological impact of commercial-scale use of transgenic plants on below-ground processes is also difficult to predict, as noted in several recent reviews (see references in Seidler and Levin 1994). Assessing possible risks is complicated by the fact that standard agricultural and forestry practices, such as frequent tilling, clear-cutting, and heavy pes-

ticide use, have detrimental effects on soil fertility. Direct effects of genetically engineered plants on soil biota may be relatively small because proteins—the products of recombinant DNA—are quickly broken down in the environment. Indirect effects, both positive and negative, will depend on how the use of transgenic cultivars affects the amounts of pesticides, herbicides, fertilizers, and water needed to maximize economic returns.

Soil fertility could be reduced if crop leachates inhibit the activity of soil biota and slow down natural rates of decomposition and nutrient release. Plants that are now being field tested probably pose little risk to soil fertility, but if widespread and continuous cultivation of certain transgenic cultivars is found to be detrimental to beneficial soil organisms such as mycorrhizal fungi or earthworms, this would be cause for concern (Donegan et al. 1995). Standard toxicological studies should be carried out when there is a scientifically based reason to suspect that plant residues could be detrimental to key groups of organisms, such as bacteria, fungi, nematodes, and other microinvertebrates and macroinvertebrates. Negative effects on individual species or strains are of less concern because of the great amount of functional redundancy in healthy soil ecosystems (Jepson et al. 1994).

Ecological information from small-scale field tests

Hundreds of small-scale field tests have been carried out by private companies, federal labs, and academic researchers to assess the performance of transgenic cultivars under different field conditions. Results from these small-scale tests are sometimes presented as evidence that transgenic plants pose no significant ecological risks at any scale of cultivation, for example, when APHIS decided to deregulate crops such as disease-resistant squash or insect-resistant cotton. However, there are several reasons to suspect that such evidence can be inadequate. First, to avoid possible criticisms regarding safety of the tests themselves, the tests are usually conducted so that escape of pollen, seeds, and vegetative propagules is unlikely (Wrubel 1992). Gene flow via pollen is often minimized by early harvests, bagging the flowers, or planting border rows to intercept transgenic pollen. Applicants for new field test permits are required to describe the chance of hybridization with related species, but empirical studies of gene flow are not required. Applicants are also required to explain what efforts will be made to dispose of the plants, their seeds, and any vegetative propagules after the experiment is completed. Thus, a major risk associated with commercial production—the escape of fitness-related transgenes via pollen, propagules, or seeds—is not addressed in small-scale tests.

Second, the scale at which the tests are conducted is so small (often less than 100 acres) and short (one to two growing seasons) that undesirable effects on nontarget organisms such as beneficial insects are unlikely to be observed. Furthermore, the possibility that microbes, insects, and weeds will quickly evolve resistance to plant-produced antibiotics, toxins, and herbicides cannot be addressed in these tests due to their short duration and limited acreage. Ecological and evolutionary responses to novel transgenic traits are more likely to occur when hundreds of thousands of acres are dominated by transgenic plants year after year.

Finally, field trial reports submitted to APHIS often include statements such as "no characteristics associated with weediness were detected" or "no effects were seen in nontarget organisms" when little attention was paid to these effects. Thus, the fact that "nothing happened" in the field trials is not useful in evaluating ecological risks unless these questions are the focus of carefully designed long-term experiments (Mellon and Rissler 1995, Rissler and Mellon 1993, Wrubel et al. 1992).

Small-scale field trials do provide some ecologically relevant information, however. In particular, these tests illustrate the extent to which transgenes have their intended effects on plant phenotypes and whether there is any change in yield—positive or negative—associated with a given transgenic trait. In addition, some tests have involved planting border rows around the test plot to examine the extent of local gene flow via pollen. A few companies, such as Calgene, have encouraged collaboration with academic ecologists. When such collaborations have resulted in peer-reviewed scientific publications (e.g., Morris et al. 1994), useful and reliable ecological information is available to the public. In most cases, however, the data contained in field test reports to APHIS are too sketchy and incomplete to be useful in assessments of ecological risks. Potential risks associated with commercial-scale production are not considered when permits for small-scale testing are requested from APHIS or institutional biosafety committees. Thus, little attention has been paid to the ecological and evolutionary consequences of deregulating market-ready transgenic plants.

Future research

Rather than take a short-term view of how small-scale plantings of cultivars will affect biological communities, we need to evaluate what is likely to occur in the next few decades, when many, if not most, commercially grown plants will have several highly effective transgenes. Ecologists can provide valuable input in the planning and evaluation of high-risk genetically engineered plants, but at present federal support for ecological research in this area is minimal. The USDA is in the fifth year of spending 1% of the funds allocated to biotechnology research on risk assessment, which amounts to only $1–2 million per year for studies of transgenic microorganisms, plants, and animals combined. Other potential funding is limited, for example, from the Weed Science Program at the USDA.

Further empirical studies of the ecological impact of commercial-scale cultivation of transgenic plants are clearly needed, particularly with regard to the following questions:

- Which cultivated plants have sexually compatible wild relatives that could become troublesome weeds after inheriting fitness-related transgenes, and to what extent will this conversion to weediness occur?

- Will the propagation of certain transgenic plants result in the evolution of newly resistant plant pests (microbial pathogens, insects, and weeds), and if so, how can the evolution of these resistant biotypes be delayed or avoided?
- What effects will plant-produced pesticides have on the population dynamics of nontarget organisms, especially beneficial predators, parasitoids, pollinators, components of soil food webs, and endangered species? (We assume that foods consumed by humans will be monitored for possible health risks by the Food and Drug Administration).

In addition, we recommend that workshops and conferences be organized to address these questions and solicit advice from panels of knowledgeable ecologists and population geneticists. Although several such workshops have been convened in the past few years, rapid progress is being made in applications of biotechnology to agriculture and forestry, and scientifically based risk assessment has not kept pace with emerging questions. As novel types of transgenic phenotypic traits are incorporated into commercially grown plants, every effort should be made to objectively determine whether undesirable ecological and/or evolutionary consequences are likely to ensue. Some of the possible consequences we describe could be alleviated after the problem arises (e.g., declines in nontarget insect populations), whereas other effects, such as the evolution of new weeds or highly resistant insect pests, have the potential to spread and persist indefinitely.

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