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GENETICALLY ENGINEERED ORGANISMS AND THE ENVIRONMENT: CURRENT STATUS AND RECOMMENDATIONS[1]

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Abstract. The Ecological Society of America has evaluated the ecological effects of current and potential uses of field-released genetically engineered organisms (GEOs), as described in this Position Paper. Some GEOs could play a positive role in sustainable agriculture, forestry, aquaculture, bioremediation, and environmental management, both in developed and developing countries. However, deliberate or inadvertent releases of GEOs into the environment could have negative ecological effects under certain circumstances.

Possible risks of GEOs could include: (1) creating new or more vigorous pests and pathogens; (2) exacerbating the effects of existing pests through hybridization with related transgenic organisms; (3) harm to nontarget species, such as soil organisms, non-pest insects, birds, and other animals; (4) disruption of biotic communities, including agroecosystems; and (5) irreparable loss or changes in species diversity or genetic diversity within species. Many potential applications of genetic engineering extend beyond traditional breeding, encompassing viruses, bacteria, algae, fungi, grasses, trees, insects, fish, and shellfish. GEOs that present novel traits will need special scrutiny with regard to their environmental effects.

The Ecological Society of America supports the following recommendations. (1) GEOs should be designed to reduce environmental risks. (2) More extensive studies of the environmental benefits and risks associated with GEOs are needed. (3) These effects should be evaluated relative to appropriate baseline scenarios. (4) Environmental release of GEOs should be prevented if scientific knowledge about possible risks is clearly inadequate. (5) In some cases, post-release monitoring will be needed to identify, manage, and mitigate environmental risks. (6) Science-based regulation should subject all transgenic organisms to a similar risk assessment framework and should incorporate a cautious approach, recognizing that many environmental effects are GEO- and site-specific. (7) Ecologists, agricultural scientists, molecular biologists, and others need broader training and wider collaboration to address these recommendations.

In summary, GEOs should be evaluated and used within the context of a scientifically based regulatory policy that encourages innovation without compromising sound environmental management. The Ecological Society of America is committed to providing scientific expertise for evaluating and predicting the ecological effects of field-released transgenic organisms.

Key words: *agriculture; aquaculture; benefit and risk assessment; biosafety; ecology; environmental risks/benefits of genetic engineering; genetically modified organisms (GMO); monitoring; risk management; transgenic organisms.*

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EXECUTIVE SUMMARY

The Ecological Society of America has evaluated the ecological effects of current and future uses of field-released genetically engineered organisms (GEOs), as described in this position paper. GEOs have the potential to play a positive role in sustainable agriculture, forestry, aquaculture, bioremediation, and environmental management, both in developed and developing countries. However, deliberate or inadvertent releases of GEOs into the environment could have negative ecological impacts under some circumstances. For example, fast-growing transgenic salmon that escape from aquaculture net pens might jeopardize native fish populations. Ecological knowledge about potential environmental effects of transgenic organisms is crucial for understanding and avoiding these types of risks.

We reaffirm that risk evaluations of GEOs should focus on the phenotype or product rather the process of genetic engineering (e.g., NRC 1987, 2000, 2002*a*, Tiedje et al. 1989), but we also recognize that some GEOs possess novel characteristics that require greater scrutiny than organisms produced by traditional techniques of plant and animal breeding. Also, unlike commercialized crops or farm-raised fish, some GEOs are organisms for which there is little previous experience with breeding, release, and monitoring. Future applications of genetic engineering extend far beyond traditional breeding, encompassing transgenic viruses, bacteria, algae, fungi, grasses, trees, insects, fish, shellfish, and many other nondomesticated species that occur in both managed and unmanaged habitats.

The environmental benefits and risks associated with GEOs should be evaluated relative to appropriate baseline scenarios (e.g., transgenic vs. conventional crops), with due consideration of the ecology of the organism receiving the trait, the trait itself, and the environment(s) into which the organism will be introduced. Long-term ecological impacts of new types of GEOs may be difficult to predict or study prior to commercialization, and we strongly recommend a cautious approach to releasing such GEOs into the environment. Engineered organisms that may pose some risk to the environment include cases where:

- there is little prior experience with the trait and host combination;
- the GEO may proliferate and persist without human intervention;
- genetic exchange is possible between a transformed organism and nondomesticated organisms; or
- the trait confers an advantage to the GEO over native species in a given environment.

An assessment of environmental risk is needed to minimize the likelihood of negative ecological effects such as:

- creating new or more vigorous pests and pathogens;
- exacerbating the effects of existing pests through hybridization with related transgenic organisms;
- harm to nontarget species, such as soil organisms, nonpest insects, birds, and other animals;
- disruptive effects on biotic communities; and
- irreparable loss or changes in species diversity or genetic diversity within species.

GEOs should be evaluated and used within the context of a scientifically based regulatory policy that encourages innovation without compromising sound environmental management. The process by which this occurs should be open to public scrutiny and broad-based scientific debate. In addition, current regulatory policies should be evaluated and modified over time to accommodate new applications of genetic engineering and improved ecological science.

In light of these points, we offer the following recommendations regarding the development, evaluation, and use of GEOs in the environment.

1) *Early planning in GEO development.*—GEOs should be designed to reduce unwanted environmental risks by incorporating specific genetic features, which might include sterility, reduced fitness, inducible rather than constitutive gene expression, and the absence of undesirable selectable markers.

2) *Analyses of environmental benefits and risks.*—Rigorous, well-designed studies of the benefits and risks associated with GEOs are needed.

a) Ecologists, evolutionary biologists, and a wide range of other disciplinary specialists should become more actively involved in research aimed at quantifying benefits and risks posed by GEOs in the environment.

b) Because of the inherent complexity of ecological systems, this research should be carried out over a range of spatial and temporal scales.

c) We further recommend that the government and commercial sectors expand their support for environmental risk assessment (including environmental benefits) and risk management research.

3) *Preventing the release of unwanted GEOs.*—Strict confinement of GEOs is often impossible after large-scale field releases have occurred. Therefore, we recommend that large-scale or commercial release of GEOs be prevented if scientific knowledge about possible risks is inadequate or if existing knowledge suggests the potential for serious unwanted environmental (or human health) effects.

4) *Monitoring of commercial GEOs.*—Well-designed monitoring will be crucial to identify, manage, and mitigate environmental risks when there are reasons to suspect possible problems. In some cases, post-release monitoring may detect environmental risks that were not evident in small-scale, pre-commercial risk

evaluations. Because environmental monitoring is expensive, a clear system of adaptive management is needed so that monitoring data can be used effectively in environmental and regulatory decision-making.

5) *Regulatory considerations.*—Science-based regulation should: (a) subject all transgenic organisms to a similar risk assessment framework, (b) recognize that many environmental risks are GEO- and site-specific, and therefore that risk analysis should be tailored to particular applications, and (c) incorporate a cautious approach to environmental risk analysis.

6) *Multidisciplinary training.*—Ecologists, agricultural scientists, molecular biologists, and others need broader training to address the above recommendations. We strongly encourage greater multidisciplinary training and collaborative, multidisciplinary research on the environmental risks and benefits of GEOs.

In summary, we urge scientifically based assessment of the benefits and risks of GEOs that are proposed for release into the environment, and scientifically based monitoring and management for environmental effects that may occur over large spatial scales and long time frames. GEOs that are phenotypically similar to conventionally bred organisms raise few new environmental concerns, but many novel types of GEOs are being considered for future development. These include baculoviruses that are engineered for more effective biological control, microorganisms that promote carbon storage, fast-growing fish, and fast-growing plants that tolerate cold, drought, or salinity. The Ecological Society of America is committed to providing scientific expertise for evaluating and predicting ecological benefits and risks posed by field-released transgenic organisms.

INTRODUCTION

Advances in genomics and genetic engineering are progressing rapidly, and innovative applications of this knowledge are just beginning to be imagined and understood. Genes that have been artificially moved into an organism's genome, i.e., transgenes, make it possible to create organisms with traits that cannot be obtained through normal sexual reproduction. Transgenes are novel, synthetic genes that have never existed in nature; minimally, they are composed of a target gene sequence flanked by a promoter and other elements that may come from different organisms. Transgenic organisms are often called ''genetically modified organisms'' (GMOs), despite the fact that plants and animals have been genetically modified by selective breeding since the beginning of agriculture. It is the engineering aspect of transgenic organisms that distinguishes them from previous varieties (e.g., Snow 2003). In this report, we use the terms genetic engineering, genetic modification, transgenics, and recombinant DNA technology interchangeably.

The goal of predicting how genetic engineering will affect organisms that live and disperse outdoors under variable biotic and abiotic conditions is a major challenge. Phenotypic characteristics, such as an organism's size, health, and reproductive capacity, are determined by complex interactions among its genes and its surroundings. It is important to ask how the phenotypes of transgenic organisms differ from those of their non-transgenic counterparts, and whether these phenotypes can be characterized adequately in small-scale experiments. Also, how will receiving populations, ecological communities, and ecological processes be affected when vast numbers of genetically engineered organisms (GEOs) enter managed and unmanaged habitats? This type of ecological knowledge is crucial for reaching defensible decisions about how to regulate and monitor transgenic organisms.

Here we discuss possible risks and benefits of GEOs from the perspective of ecological science. Building on an earlier paper by Tiedje et al. (1989), we present the consensus viewpoint of the Ecological Society of America, as formulated by an authoring committee with input from the ESA Governing Board and reviewers with expertise in ecology, evolution, genetics, agricultural sciences, and microbiology. The topics we cover can be evaluated from several perspectives—scientific, political, socioeconomic, or ethical. The ratio of benefits to risks that are attributed to GEOs can be perceived very differently by developing vs. industrialized nations, and by different stakeholders within each nation. Even within the context of science, there is a great deal of debate about how transgenic organisms should be developed, regulated, and deployed. Our goal is to provide ecological insights that should be considered prior to release, and recommendations for post-release evaluation of GEOs. An assessment of possible human health effects of GEOs is beyond the scope of this paper.

Because the commercialization of GEOs is relatively recent and is limited to only a few types of crops, many of the ecological questions we raise have yet to be examined empirically (e.g., Wolfenbarger and Phifer 2000, Dale et al. 2002, NRC 2002*a*, *b*). Some GEOs are expected to provide environmental benefits, as outlined in Box 1. However, in this report, we focus more on potential environmental risks of GEOs than on their benefits for two reasons: risks are a more immediate concern for ecologists, regulatory agencies, and the public, and many environmental benefits have yet to be developed or rigorously documented. We begin with an introduction to the current status of transgenic organisms, followed by a discussion of potential ecological effects and regulatory challenges. We emphasize

Box 1. Examples of GEOs with potential environmental benefits

See text for details and note that most of these GEOs are still under development. The caveats listed below do not include environmental risks, which are discussed in the text. Moreover, the benefits listed below do not include human health or socioeconomic benefits:

1) Transgenic, glyphosate-tolerant crop plants (e.g., Roundup Ready soybean)

Environmental benefits:
- These crops can facilitate no-tillage or low-tillage weed management, which
  - ○ conserves topsoil and soil moisture; reduces erosion
  - ○ allows greater carbon sequestration in soil organic matter
- Glyphosate breaks down more quickly and is more ‘‘environmentally friendly’’ than many other herbicides

Caveats:
- Glyphosate is the most widely used herbicide in the United States and
  - ○ its use may not be sustainable if weed shifts occur to favor glyphosate-tolerant weeds and/or if certain weeds evolve resistance to glyphosate†
  - ○ new alternatives to glyphosate-tolerant crops may be too expensive or difficult for chemical companies to develop and commercialize

2) Crop plants with transgenic resistance to lepidopteran or coleopteran pests (e.g., Bt cotton, Bt corn)

Environmental benefits:
- Can reduce the use of broad-spectrum insecticides

Caveats:
- May not be sustainable if secondary pests become more problematic and/or if target pests evolve resistance to the Bt crop†
- Above ground, broad-spectrum insecticides are not applied to most of the U.S. corn acreage

3) Crop plants with transgenic resistance to common diseases (e.g., fungal disease of fruit crops)

Environmental benefits:
- Could reduce the use of fungicides and insecticides that currently are used to kill disease vectors or disease-causing organisms

Caveats:
- May not be sustainable if pathogen evolves resistance to transgenic varieties†
- Few crops with transgenic disease resistance have been deregulated to date (exceptions include virus-resistant squash, papaya, and potato)

4) Crop plants with higher yields due to one or more transgenic traits

Environmental benefits:
- Higher yield per area could reduce pressure on natural areas because less area needs to be cultivated for a given amount of yield

Caveats:
- Higher yield per area could be an incentive to cultivate transgenic crops on larger areas (assuming profits increase with area)
- Evidence for yield gains above current production levels is not well documented as yet; more evidence of higher yields is needed

5) Decreased lignin production in transgenic, commercial tree plantations

Environmental benefits:
- Cleaner paper milling and less pollution of waterways

Caveats:
- Early in development, not known if economically viable

6) Bioremediation of polluted soils using transgenic plants or bacteria

Environmental benefits:
- More effective and/or less expensive cleanup of toxic waste sites than with current methods

Caveats:
- Early in development, not known if effective in the field or economically viable (see Box 4)

7) Use of transgenic plants, fish, or microbes as biomonitors to detect pollutants

Possible environmental benefits:
- More effective and/or less expensive than current methods, thereby enhancing detection capabilities

Caveats:
- Early in development, not known if effective in the field or economically viable

8) Transgenic pigs with enhanced salivary phytase

Environmental benefits:
- Enhanced ability to utilize phytate in plant-derived feeds
  - ○ decreases phosphorus in animal wastes and reduces pollution from manure, which is often used as fertilizer in row crops

Caveats:
- Early in development, not known if economically viable

† Caveats about the evolution of resistance also apply to the efficacy of pesticides that are used to manage weeds, insects, and diseases of crop plants.

the present generation of transgenic crop plants because they have been studied most intensively, and we supplement this with discussions of genetically engineered microorganisms, invertebrates, and fish.

CURRENT STATUS OF GEOs

Techniques for creating transgenic organisms

What is genetic engineering and what distinguishes it from classical breeding? Plant, animal, and microbial breeding consist of three major phases: (1) assembling or generating new genetic diversity, (2) selecting and testing different genotypes to identify superior varieties, and (3) the release, distribution, and commercialization of new progeny (e.g., Gepts 2002). The difference between genetic engineering and classical breeding is in the first phase. In classical breeding, genetic diversity can be enhanced by sexual crosses. For example, when plant breeders want to combine the high-yielding characteristics of line *A* with disease resistance genes from line *B*, they cross the two lines and from the progeny they select individuals with both high yield and disease resistance. Only a small fraction of the progeny is likely to exhibit high yield and disease resistance, without any extraneous unwanted traits. Moreover, breeding efforts can be jeopardized at the outset by a lack of appropriate genes from sexually compatible relatives. Genetic engineering provides a powerful alternative because specific genes from any source (microbe, plant, animal, or synthetic) can be introduced into recipient cells and integrated directly into the organism's genome. For example, genes for disease resistance would be incorporated into a transgene and introduced directly into the genome of the high-yielding line, avoiding the potential limitations of sexual crosses.

There are several ways in which a transgene can be inserted into an organism's genome. In dicotyledonous plants, the method used most often relies on the normal disease-causing mechanism of a soil pathogen, *Agrobacterium tumefaciens*. This bacterium carries a modified plasmid (a small circle of DNA) that integrates new, recombinant DNA into the plant genome and does not cause disease. Another widely used method of transforming plants is by particle bombardment, which is especially useful in monocots, such as corn and rice (e.g., Brettschneider et al. 1997). Additional transformation methods include the introduction of transgenes by electrical (electroporation), physical (silicon fibers, microinjection), or chemical (polyethylene glycol) techniques. Related approaches can be used to transform microbes and animals, but animals are often more challenging to transform and regenerate than other organisms (NRC 2002*b*). With any of these methods, the transformation process is very inefficient, and only a small fraction of the cultured cells incorporate the transgene (e.g., Birch 1997). Therefore, the transgene usually is linked to a selectable marker gene, typically a gene encoding herbicide or antibiotic resistance, to allow rapid selection of the few cells that incorporate the transgene. These cells and tissues are cultured to produce whole organisms.

Methods for creating transgenic organisms are constantly becoming more sophisticated, and today's GEOs will soon be viewed as comparatively primitive. Also, new molecular techniques are expected to blur the distinction between what is considered to be transgenic and what is not. For example, the strategy of silencing native genes by transgenic methods falls into this gray area. Another intermediate technique is ''directed evolution,'' in which new microbial genes are selected from mixtures of DNA fragments in laboratory cultures. Directed evolution has been used to breed bacteria that are better at degrading pesticides and other environmental pollutants and to breed viruses for biological control (e.g., Soong et al. 2000, Raillard et al. 2001, Sakamoto et al. 2001). Since organisms modified by this method use only natural recombination, albeit under artificial conditions, regulatory agencies do not consider them to be ''genetically engineered.''

Types of transgenic organisms

Plants.—Crops with transgenic resistance to certain herbicides, insects, or diseases are planted widely, especially in the United States (Box 2). Future transgenic plants may offer a much wider array of products, including applications in rangelands, forests, landscaping, nutrition, pharmacology, biological control, production of industrial chemicals, and bioremediation (Table 1, Boxes 2 and 6; Dunwell 1999). Transgenic plants with enhanced tolerance to abiotic stresses such as salinity, drought, or freezing, to biotic stresses such as pathogens, and to anthropogenic stresses such as heavy metal contamination are under development. Work is progressing on the use of genetically engineered (GE) plants for phytoremediation of contaminated soils, sometimes using natural or altered transgenes from bacteria (e.g., Rosser et al. 2001, Chaudhry et al. 2002). Transgenic tree plantations potentially could have trees with lower lignin content (for cleaner paper milling), higher yields, and sterile reproductive structures (Strauss et al. 2001, Pilate et al. 2002). Transgenic plants that produce pharmacologically active proteins, vitamins, industrial polymers, and those that improve qualities of animal feed have reached the field-testing stage of development. Food plants with reduced allergenicity also are under development. One type of commercially produced transgenic corn manufactures avidin, a chemical used in medical testing (Hood et al. 1997).

BOX 2. Food plants with transgenic resistance traits

The first transgenic organisms to be widely used on a regional scale were developed by large agrochemical companies (e.g., Monsanto, Syngenta, Dow, DuPont) and, independently, by the People's Republic of China. In 2002, four countries (United States, Argentina, Canada, and China) accounted for 99% of the global area planted with transgenic crops (see James 2002). By 2003, an estimated 80% of the soybean acreage in the United States had transgenic herbicide tolerance (USDA 2003). At least 120 transgenic varieties of sugar beet, chicory, corn, cotton, flax, melon, papaya, potato, oilseed rape, rice, soybean, squash, and tomato have been approved for commercial use, mostly in the United States (see ISB 2004*b*).

Four crops, soybean, corn, cotton, and oilseed rape, predominate the acreage planted to date. These ‘‘first generation'' transgenic crops have specific traits to improve the efficiency of agricultural production. Most commercialized transgenic crops have a single transgene for either herbicide tolerance or certain types of insect resistance, while a few varieties have more than one transgenic trait. Transgenic traits in commercialized crops also include altered fruit ripening, male sterility (for hybrid seed markets), and other characteristics. Many major food crops have been genetically engineered for virus resistance, although relatively few of these transgenic crops have been released commercially. Three examples of commercialized transgenic crops are presented here.

Virus-resistant papaya.—Papaya (*Carica papaya*) is a small, short-lived tree originating in Central and South America and grown throughout the tropics. Papaya ringspot virus (PRSV) is a virus that can decimate papaya plantations, and cultivation of papaya in Hawaii was severely threatened by an epidemic of PRSV in the 1980s. The virus is transmitted by aphids, but insecticide treatments against aphids are not effective. Resistance to either the virus or its insect vector has not been found in papaya germplasm, so transgenic methods were used to breed for resistance to the virus. In the late 1980s, Beachy et al. (1990) discovered the general principle that the transgenic coat protein of a virus could provide plants with resistance to the same virus. Subsequently, researchers were able to transform papaya with the coat protein gene of PRSV to obtain a line that was highly resistant to the local strain of the virus in Hawaii (Fig. 1; Gonsalves 1998). Following regulatory approval by the EPA and USDA (APHIS) and consultation with the FDA, licenses were obtained from the holders of patents on technology to develop the transgenic papaya varieties, which are currently grown in Hawaiian plantations.

Roundup Ready soybean.—In the United States, herbicides account for >90% of the pesticides farmers use on the major field crops (corn, soybeans, wheat, and cotton). The introduction of soybean cultivars with transgenic resistance to glyphosate (commercially known as Roundup Ready soybean) allows growers to replace multiple herbicide treatments spread out over the growing season by one or two treatments of this broad-spectrum herbicide (Reddy and Whiting 2000). Furthermore, the herbicide can be applied after the soybean plants have emerged, allowing more efficient weed control and less dependence on crop rotation and/or tillage as ways to manage broad-leaved weeds. To date, few weed species have evolved resistance to this effective and widely used herbicide.

Bt corn.—*Bacillus thuringiensis* (Bt) is a soil bacterium with many strains that produce a variety of crystalline protein endotoxins, each of which affects specific groups of insects. Thus, different *B. thuringiensis* strains are toxic to lepidopterans (butterflies and moths), coleopterans (beetles), and dipterans (mosquitoes, black flies, and fungus gnats). In these insects, small amounts of the toxin (parts per billion) damage the intestinal system, and the insects typically die within days of a single feeding. One Bt strain is commonly used in both organic and conventional agriculture as an insecticidal spray, and is not toxic to humans or most other organisms, including honeybees and plants. Genes coding for Bt toxins have been isolated and transformed into the genome of several crop plants, including corn, cotton, and potato. Several of these so-called *cry* genes (for crystal protein) have been used, including *cry1Ab*, *cry1Ac*, *cry1F*, and *cry9C* (against moth larvae); *cry3A* and *cry3C* (against beetles and beetle larvae); and *cry3Bb* and a combination of *cry34* and *cry35* (against corn rootworms). Some of these Bt proteins differ in their stability characteristics. For example, the protein from *cry9C* is more resistant to degradation in conditions simulating those in the human gut. Hence, this protein was deemed to have a potential for allergenicity and was authorized by the EPA in the Starlink variety of Bt corn for animal use only. Nevertheless, it ended up in corn chips and other food products, which had to be recalled. A possible health benefit of other Bt corn is lower levels of mycotoxin in regions where damage from the eastern corn borer is extensive (e.g., Clements et al. 2003).

TABLE 1. Examples of current and planned GEOs.

Host organism	Function or product of introduced gene	Intended use
Microbes		
Pseudomonas syringae, P. fluorescens	deletion of ice-nucleating cell membrane protein	‘‘ice minus bacteria’’ sprayed on crops to protect from frost
Pseudomonas fluorescens	several genes for hydrocarbon degradation and light production	detect and degrade pollutant (polycyclic aromatic hydrocarbons); fluorescent marker
Pseudomonas putida	4-ethylbenzoate-degrading enzyme	degradation of pollutant (benzene derivatives)
Clavibacter xyli	*Bt* crystal protein toxin	colonize plant vascular tissue to protect plant from insect pests
Baculoviruses	scorpion neurotoxin; proteases from rat, human, and flesh fly†	biological control of specific insects (see Box 3)
Plants		
Corn, cotton, potato	insect-specific toxin	kill or deter target insects eating plant tissues (many lines deregulated)
Soybean, cotton, corn, oilseed rape, wheat, turfgrass, poplar	glyphosate resistance	ability to withstand application of glyphosate herbicide (many lines deregulated)
Squash, papaya	viral coat protein	provide resistance to specific viruses (some lines deregulated)
Corn	Pharmaceutical and industrial compounds, e.g., avidin and many others	purify as inputs into other commercial products
Rice	provitamin A	provide vitamin A precursor for better nutrition
Rice	ferritin	increase iron content of rice to reduce anemia
Strawberry	antifreeze polypeptide	resistance to freezing
Tomato	anti-sense polygalacturonase	delay ripening when red, allowing more time on-vine (Flavr Savr brand was deregulated, but is no longer produced commercially)
Poplar	modified lignin	enhanced paper-making qualities; less pollution during milling
Animals		
Pink bollworm	marker and sterility genes	research method for tracking dispersal of adult moths; reduce moth populations by suppressing mating
Mouse	virus-neutralizing monoclonal antibody	model system for testing protection against viral encephalitis
Atlantic salmon	growth hormone	accelerated growth rate, improved feed conversion efficiency (now under regulatory review)
Zebra fish	fluorescent protein‡	pet fish that fluoresce under natural and ultraviolet light (sold commercially)
Pig	insulin-like growth factor I	accelerated growth rate, improved feed conversion efficiency, leaner carcass composition
Pig	phytase	ability to utilize phytate in plant-derived feeds, decreasing phosphorus in wastes
Pig	human factor VIII	secrete blood clotting factor in milk, to be administered to hemophiliacs
Goat	human tissue plasminogen activator	production of anti-clotting agent
Sheep	human α1 anti-trypsin	production of agent for treatment of asthma and emphysema

Notes: Examples have been modified from Hallerman (2002) and, where applicable, ISB (2004*a*). This is not a comprehensive list, but rather a sampling of the types of traits under consideration. A few of these organisms have been approved in the United States for commercial production, as noted (see intended use).

† Source: Harrison and Bonning (2001).

‡ Source: ⟨http://www.glofish.com/⟩.

BOX 3. Biological control of insects using GE pathogens

Biological control has provided a safe and effective alternative to the use of pesticides for the control of many agricultural pests. Recently, genetic modification has been used to enhance the effectiveness of several insect pathogens, including bacteria, a variety of baculoviruses, nematodes, and fungi (Lacey et al. 2001). In general, most efforts have focused on increasing the speed of action of these biocontrol agents, since most pathogens need time to develop a fatal infection. To date, the most advanced work has been carried out with *Bacillus thuringiensis* (Bt) and the baculoviruses (Cory 2000, Harrison and Bonning 2001), although genetic modification of insect-killing fungi (St. Leger and Roberts 1997) and nematodes (Gaugler et al. 1997) is receiving growing attention. Baculoviruses are already used successfully as biological control agents in some systems. For example, the nuclear polyhedrosis virus of the soybean caterpillar, *Anticarsia gemmatalis*, is used on approximately 1 million ha of soybeans in Brazil (Moscardi 1999). Recently, there has been considerable effort to increase baculovirus efficiency by inserting genes that express toxic proteins, such as insect specific scorpion toxins, mite neurotoxin, diuretic hormone, and juvenile hormone esterase (Moscardi 1999). Field tests have shown some success in increasing the speed of action for some constructs, but relatively few large-scale tests have been carried out, and no recombinant baculoviruses have been registered for commercial use. Possible risks associated with GE viruses are discussed in the text.

Viruses, microorganisms, and algae.—Most genetic modification of viruses intended for environmental release has focused on baculoviruses (Cory 2000). Nontransgenic baculoviruses are already used for biological control of insects (Box 3), and researchers have tried to increase their efficiency by inserting genes that express toxic proteins, such as insect-specific scorpion toxins (Maeda et al. 1991). To date, work with transgenic baculoviruses has taken place under contained conditions or in small-scale field tests, and no baculoviruses have been released commercially.

Transgenic bacteria and yeasts are used widely to manufacture biologically based products in medicine, food processing, and agriculture (e.g., pesticides), but most of these GE products are produced indoors. For example, transgenic *Escherichia coli* that produce human insulin have replaced animal-derived insulin for medical uses; also, genetically engineered bacteria produce rennet, which is used commonly by cheese processors in the United States and elsewhere. Bovine growth hormone produced by transgenic bacteria has been used to increase milk production in many U.S. dairy farms. Transgenic microorganisms are being developed for bioremediation of toxic compounds (Box 4), and for removing excess carbon dioxide from the atmosphere. Experimental field tests of microorganisms intended for release into the environment include bacteria with visual markers, biosensors of toxic chemicals, insecticidal properties, and reduced virulence.

FIG. 1. Field plot in Hawaii showing papaya trees with and without transgenic disease resistance (photo courtesy of D. Gonsalves). The small, yellowish trees on the left have the ringspot virus, whereas the larger, transgenic trees on the right do not (see Box 2).

BOX 4. Bioremediation using GE microbes

Twenty years ago, microbial GEOs were projected to be among the most extensive GEO products for environmental release (e.g., references in Tiedje et al. 1989). Their previous use in bioremediation, biocontrol, and waste treatment, along with their relative ease of genetic modification and potential for rapid growth, led to this perception. Concern over environmental releases of GE microbes was high because their small size could lead to rapid dispersal and there would likely be no means to recall such small, numerous organisms if problems emerged. However, it is very difficult to manage microbial populations in the field for a reliable economic benefit, whether transgenic or not. This fundamental barrier has diverted interest from microbial GEOs to other approaches, and earlier expectations have not been met. Nonetheless, some interest remains in microbial GEOs, but with more consideration of the unique advantages and limitations of microbes.

The development of microbial GEOs for bioremediation remains an active field of research because cleanup of toxic sites is so difficult and costly, and because these sites are often isolated, restricted in access, and altered from their native condition. GE strategies have involved construction of biosensors to monitor pollutant concentrations; production of biosurfactants to increase pollutant uptake by other microbes; adding missing enzymes to complete biodegradation pathways; improving those enzymes, often by directed evolution; altering the regulation of biodegradation gene expression (e.g., to achieve constitutive, over-expression); and placing the biodegradation genes in a more suitable host. Primary targets are microbes that can be made to grow on PCBs, chlorinated ethylenes (PCE, TCE), explosives (TNT), and polynuclear aromatic hydrocarbons (PAHs), which are the most problematic environmental pollutants in the industrialized world.

Production of micro- and macroalgae is an important sector of world aquaculture, and there is increasing interest in developing transgenic algae for applications such as food, animal feed, polysaccharides (e.g., carrageenan), pharmaceuticals, fuel, and bioremediation (e.g., Stevens and Purton 1997, Ask and Azanza 2002). Transgenic unicellular algae are being developed as a way to deliver vitamins, vaccines, and growth hormones to shrimp, fish, chicken, and other animals (R. Sayre, *personal communication*).

Animals.—Animals have been genetically engineered to grow faster, resist diseases, tolerate cold, produce organs for transplants, or produce biologically active, therapeutic proteins (NRC 2002*b*; Table 1). Much of this research is still exploratory. Fast-growing salmon that will increase production of farm-raised fish

FIG. 2. Non-transgenic and transgenic coho salmon (*Oncorhynchus kisutch*) at one year of age (photo courtesy of R. Devlin). The larger fish have a transgene for faster growth rate.

Box 5. Reducing insect-borne disease

Vector-borne diseases are among the most intractable of human diseases (Beatty 2000). Diseases such as malaria, dengue, leishmania, trypanosomiasis, West Nile encephalitis, and yellow fever are resurgent throughout much of their traditional geographic range and are emerging in many new areas. Hundreds of millions of people are infected with these diseases annually and millions die each year, especially in economically disadvantaged populations. The resurgence of diseases that were previously controlled is due in part to the development of insecticide resistance in vector populations, the development of drug resistance in parasites, the shrinkage of public health programs for vector control, and the lack of effective vaccines for many diseases (Beatty 2000).

The resurgence of vector-borne diseases has led to interest in the genetic manipulation of vectors to reduce transmission competence. Most research has focused on three major objectives: (1) the development of efficient transformation of the targeted vector species; (2) the identification of parasite-specific molecules that impair competence; and (3) the development of a mechanism to drive the competence-reducing molecules through the vector population (Beatty 2000). Proposed drive mechanisms include transposable elements, densoviruses, and bacterial symbionts of the vectors such as *Wolbachia*. In some disease systems, such as Chagas disease, researchers have focused on genetic engineering of the bacterial symbionts themselves to express and release transgene products that are deleterious to the disease agent into insect tissues (Durvasula et al. 1997). In the Chagas system, the most promising drive mechanism relies simply on the feces-eating behavior of the insect vector to disperse the recombinant symbiont, *Rhodococcus rhodnii* (Durvasula et al. 1999). The disease system receiving the most attention is undoubtedly malaria, but success in this complex arena may prove to be elusive (Spielman et al. 2001, Enserink 2002). Although mosquitoes have been transformed to express antiparasitic genes that makes them inefficient vectors (Ito et al. 2002), such genetic modification often reduces their fitness (Catteruccia et al. 2003).

are currently being considered for commercial release (Fig. 2). Expression of introduced growth hormone genes, usually from the same species, results in several-fold faster growth rates in salmon, tilapia, and mud loach (Devlin et al. 1994, Rahman and Maclean 1999, Nam et al. 2001). Several transgenic applications have more novel goals, such as creating medaka and mummichog that can be used as biomonitors to detect mutagens in confined aquatic environments (Winn et al. 2000). For shellfish, significant technical challenges have yet to be overcome before transgenic improvement is feasible. Research on transgenic invertebrates has focused on biological control methods for insect pest management and on public health applications, such as the production of vector insects that are incapable of transmitting disease (Box 5; Hoy 2003, Pew Initiative on Food and Biotechnology 2003). Other innovative applications under investigation include industrial production of spider silk by transgenic goats and reduced phosphorus output in manure from genetically engineered pigs, which addresses a key issue for the management of confined animal feed units (Golovan et al. 2001).

In summary, opportunities for genetically engineering many forms of life are likely to increase as potentially useful genes are identified and as the techniques to achieve integration and reliable inheritance of transgenes are developed for a broader range of species. Transgenesis is used widely as a research tool by academic groups, government agencies, and biotechnology companies in many developed and developing countries. The pace at which new GEOs are released into the environment and the types of GEOs that are developed will depend largely on economic and political incentives, as well as improved scientific methods for achieving desired results. Here we focus primarily on the diversity of GEOs that are currently available or known to be under development.

Unintended phenotypes

Recombinant DNA methods have been viewed as particularly precise because the inserted gene sequences can be characterized and monitored. Nonetheless, the process of transformation can result in unintended effects that may be difficult to detect under laboratory conditions. A major cause of unintended phenotypes, known as position effects, stems from the fact that transgenes often are inserted into random chromosomal locations, often at multiple sites in the genome. While targeted insertion is possible in viruses, bacteria, and yeasts, this goal has been elusive in more complex organisms (e.g., Puchta 2002). The specific locations of transgenic insertions can influence the level and consistency of gene expression, and the magnitude of these position effects can range from minor to lethal. Transgenic DNA sequences may interrupt native genes or the promoter sequences that regulate them. Furthermore, the insertion of transgenic DNA may bring about small-scale rearrangements of the transgene and native DNA sequences at the insertion site (e.g., Pawlowski and Somers 1998, Svitashev et al. 2000, 2002, Windels et al. 2001), and multiple copies of the transgenes are often inserted unintentionally. If individuals with multiple insertions are retained for breeding, redundant copies of transgenic DNA can interact with each other to cause gene silencing. These types of unintended effects will continue to occur until more sophisticated methods are available for inserting transgenes into predetermined locations on the genome.

Another cause of unintended phenotypes is interactions among the transgene and native genes. Few examples have been identified so far, but these epistatic interactions are widespread among nontransformed organisms and are expected to be common in GEOs as well. In yeast, for example, experimental studies showed that individual genes interacted with 3–8 other genes (Hartman et al. 2001). Other causes of unintended phenotypes include pleiotropic effects of transgenes (i.e., transgenes affecting multiple traits) and mutations that occur when small, undifferentiated masses of transformed cells are regenerated into whole organisms in the laboratory (e.g., Dale and McPartlan 1992). Often, the causes of unintended phenotypes are difficult to identify. For example, Saxena and Stotzky (2001) reported that several commercial varieties of transgenic corn expressing the Bt toxin from the bacterium *Bacillus thurigenenesis* had higher lignin content than non-transgenic corn, perhaps due to pleiotropy. In *Arabidopsis thaliana*, which is mainly self-pollinating, Bergelson et al. (1999) found that plants with different insertions of the same transgene exhibited a tenfold difference in their outcrossing rates.

Of course, unintended phenotypes can occur during non-transgenic breeding as well, often because of genes that are closely linked to the primary gene of interest. For example, unwanted health and plant disease risks have arisen in conventionally bred celery, potato, and corn, through the appearance of toxic compounds (NRC 2000). So far, we are not aware of health or environmental problems that have occurred due to unintended phenotypes of commercially produced GEOs. Like their non-transgenic counterparts, GEOs with obvious abnormalities are not used in commercial lines. Abnormal individuals or their progeny can be eliminated during extensive screening among locations and years, which is an integral part of any breeding program. However, small unintended effects may remain undetected because they may depend on cumulative action, specific environmental conditions, or introgression into different genetic backgrounds.

Molecular methods for mitigating unwanted effects

Recommendation 1

***Early planning in GEO development*.—GEOs should be designed to reduce unwanted environmental risks by incorporating specific genetic features, which might include sterility, reduced fitness, inducible rather than constitutive gene expression, and the absence of undesirable selectable markers.**

Here, we describe a few of the many strategies by which transgenic approaches to breeding could be improved to reduce environmental risks. One concern pertains to crop plants in which the selectable marker is a gene for antibiotic or herbicide resistance. These genes are not needed in the final product, and several alternatives have been proposed, such as selection in a high osmolite (sugar) medium (Todd and Tague 2001). Alternatively, it is possible to excise the selectable marker genes using a variety of methods (Hare and Chua 2002). Some biologists have proposed genetic mechanisms for excising the transgenes themselves before an organism reproduces, but this concept is probably years away from becoming practical (Keenan and Stemmer 2002).

In some cases, it is desirable to prevent GEOs from proliferating and interbreeding with native populations. This problem could be lessened by transgenic methods to limit gene flow (e.g., Daniell 2002, NRC 2004). Another goal is to prevent harm to other organisms from plant-produced toxins. Nontarget effects of toxins such as Bt proteins could be reduced if the expression of transgenes was controlled by tissue-specific or developmental stage-specific promoters rather than by constitutive promoters. Alternatively, the transgenes could be regulated by inducible promoters that are activated by external conditions, such as heat, chemical sprays, or biotic factors (e.g., insect damage). It may be possible to delay the evolution of resistance in insects by combining several types of high-dose Bt genes in a single transgene construct (e.g., Gould 1998). In summary, a wide array of environmental goals potentially could be addressed during the early planning and design of GEOs.

ECOLOGICAL EFFECTS OF GEOS

Recommendation 2

***Analyses of environmental benefits and risks*.—Rigorous, well-designed studies of the benefits and risks associated with GEOs are needed.**

a) Ecologists, evolutionary biologists, and a wide range of other disciplinary specialists should become more actively involved in research

TABLE 2. Major environmental concerns regarding transgenic organisms.

Process	Potential ecological consequences
Transgenic organisms persist without cultivation	Transgenic organisms that are able to spread and maintain self-sustaining populations could disrupt biotic communities and ecosystems, leading to a loss of biological diversity.
Transgenic organisms interbreed with related taxa	Incorporation of transgenes could result in greater invasiveness or loss of biodiversity, depending upon the amount of gene flow from generation to generation and the transgenic trait(s).
Horizontal gene flow	The transfer of genes through nonsexual means is common in some microbes but rare in plants and animals. Ecological consequences would depend on amount of gene flow and the transgenic trait(s).
Changes in viral disease	In transgenic virus-resistant organisms, recombination between viral transgenes and invading viruses could lead to increased virulence of a disease and undesirable effects on wild hosts in natural habitats.
Nontarget and indirect effects	Loss of biodiversity, including species of conservation concern, may occur, as well as altered community or ecosystem function, including reduced biological pest control, reduced pollination, altered soil carbon and nitrogen cycling, and secondary pest outbreaks.
Evolution of resistance	Resistance to pesticides (including pesticide-producing plants) can lead to greater reliance on chemicals and other pest control methods that are damaging to the environment, including unregistered pesticides under emergency exemptions. This applies to insects, weeds, and other pests.

Note: Note that few types of transgenic organisms have been released into the environment, and therefore few of the potential ecological consequences listed have been documented to date (see *Ecological effects of GEOs* for details).

aimed at quantifying benefits and risks posed by GEOs in the environment.

b) Because of the inherent complexity of ecological systems, this research should be carried out over a range of spatial and temporal scales.

c) We further recommend that the government and commercial sectors expand their support for environmental risk assessment (including environmental benefits) and risk management research.

Here, we evaluate some of the benefits and concerns associated with GEOs that are already in use or under development (Table 2). Greater attention has been paid to risk assessment, which involves identifying possible unwanted effects (hazards) and the likelihood that these hazards will occur, as compared to benefits of GEOs (e.g., Boxes 1–6). Tiedje et al. (1989) provide a useful summary of how ecological risks of novel GE traits can be evaluated for different taxa and for the environments into which GEOs are released. Other broad reviews of this topic include Rissler and Mellon (1996), Snow and Moran-Palma (1997), Ammann et al. (1999), the National Research Council (e.g., 2000, 2002*a*, *b*, 2004), CAST (2002), Dale et al. (2002), Letourneau and Burrows (2002), and Conner et al. (2003). Below we discuss the types of ecological knowledge that can contribute to risk assessment of GEOs.

Gene flow

Recommendation 3

***Preventing the release of unwanted GEOs*.—Strict confinement of GEOs is often impossible after large-scale field releases have occurred. Therefore, we recommend that the large-scale or commercial release of GEOs be prevented if scientific knowledge about possible risks is inadequate or existing knowledge suggests the potential for serious unwanted effects.**

Gene flow among crops and their relatives.—Because transgenes are inherited in the same way as naturally occurring genes, they have the potential to persist indefinitely in cultivated or free-living populations. Transgenic pollen and seeds can disperse into seed nurseries, commercial fields, and local landraces (e.g., Quist and Chapela 2001, 2002, Beckie et al. 2003). Subsequently, these transgenes can continue to spread among other plants of the same species, especially if they confer traits that are favored by artificial or natural selection (e.g., traits that increase tolerances to abiotic or biotic stresses) or if transgene flow is maintained from a large source population. The ecological and evolutionary consequences of crop-to-crop gene flow are just beginning to be investigated. One possible consequence is that exposure of nontarget organisms (including humans) to novel proteins could be greater than expected based on known acreages of a particular transgenic crop.

Crop-to-wild gene flow occurs when transgenic plants become established in unmanaged populations and/or when they interbreed with related species. Gene flow between crops and free-living, noncultivated plants occurs when pollen moves from a crop to its wild or feral relative (or vice versa) and genes from their offspring spread further via the dispersal of pollen and seeds. Many crops hybridize spontaneously with

BOX 6. Restoring the American chestnut

Eventually it may be possible to use biotechnology to propagate rare and endangered species. Whether these species could ever become reestablished as wild populations is uncertain, but this has not prevented researchers from trying. For example, transgenic methods might help species that are threatened by a primary overriding factor, such as single disease. In North America, the introduction of exotic pathogens has severely threatened populations of American chestnut (*Castanea dentata*), American elm (*Ulmus americana*), and butternut (*Juglans cinerea*). Prior to the arrival of a fungal blight (*Cryphonectria parasitica*) in the early 1900s, the American chestnut was a dominant species in eastern hardwood forests. Now, stump sprouts are common but the sprouts typically die from chestnut blight before they reach sexual maturity. Native trees with resistance genes have not been found, but a related species from Asia is resistant to the blight. In Chinese chestnut (*C. mollisima*), resistance is conferred by two or three incompletely dominant genes that have been backcrossed into surviving American chestnuts. Only a portion of backcrossed progeny from these lines are expected to be resistant, however, because the resistance genes are additive and it appears that at least two must be homozygous to obtain resistance (Mann and Plummer 2002; W. A. Powell, *personal communication*). To augment this backcrossing program, several research teams are working on transgenic methods to introduce dominant resistance genes into native chestnut trees (Mann and Plummer 2002). They have made progress in refining transformation methods, screening candidate resistance genes, and developing strategies to address environmental and social concerns (W. A. Powell, *personal communication*). If this effort is successful, other pests such as the chestnut gall wasp (*Dryocosmus kuriphilus*) still may be a problem, but managed and horticultural populations of chestnut probably could be maintained.

wild or weedy relatives that occur nearby (e.g., Ellstrand et al. 1999, Messeguer 2003). In the United States, for example, these include rice, squash, oilseed rape, sunflower, sorghum, wheat, sugar beet, strawberry, radish, lettuce, poplar, and many grasses and horticultural plants (NRC 2000*b*, Ellstrand 2003). Some crops, such as oats, radish, carrot, and oilseed rape, also can proliferate as weeds. On the other hand, corn, cotton, soybean, potato, and many other species do not have wild or weedy relatives in the United States. Thus, the extent of gene flow between crops and weeds is expected to vary among crops and geographic regions, and should be examined carefully wherever transgenic crops are cultivated.

Currently, it is not possible to prevent gene flow between sexually compatible species that inhabit the same region because pollen and seeds disperse too easily and too far to make complete reproductive confinement practical (NRC 2004). Therefore, it is important to determine which types of transgenic crops have novel traits that might persist and cause problems. A first step is to determine how widely the transgenes will be dispersed, and whether new transgenic traits are likely to have positive or negative effects on the fitness of wild or weedy organisms. Deleterious transgenic traits might persist if very high rates of gene flow from the crop allow these transgenes to become fixed in adjacent populations, but other situations will allow these traits to be purged by natural selection. In the few studies that address the question of ''fitness costs,'' transgenes that conferred resistance to herbicide, insects, or disease in crop plants were not associated with a decrease in survival, yield, or fecundity of wild relatives (e.g., Snow et al. 1999, 2003, Burke and Rieseberg 2003). The ''fitness benefits'' associated with transgenic traits depend on the type of trait and the ecology of recipient populations. With regard to herbivores, studies of both native and exotic species suggest that herbivores can have a dramatic impact on plant population dynamics (e.g., Guretzky and Louda 1997, Rees and Paynter 1997). Also, several recent studies have reported negative impacts of microbes on the growth, survivorship, and reproduction of plants in natural populations (e.g., Power 2002, Mitchell and Power 2003, Callaway et al. 2004). Snow et al. (2003) reported up to 55% greater seed production in wild sunflowers due to the expression of a Bt transgene for resistance to lepidopterans. This suggests that fitness benefits of individual transgenes could be surprisingly large. In successive generations, these types of transgenic traits could become common in natural populations.

If fitness-enhancing transgenes become established in natural populations, the populations may or may not become larger, more widespread, or more difficult to manage, depending on ecological factors that limit population growth. In the short term, the spread of transgenic herbicide resistance to weedy relatives of crop plants could favor larger weed populations, creating logistical and economic problems for farmers. Delaying increases in populations of herbicide-resistant weeds is a basic goal of sustainable agricultural practices. Over the longer term, certain weeds may benefit from transgenes that confer faster growth and resistance to herbivores, diseases, or harsh growing conditions. Initially, the effects of one or a few transgenes may be difficult to detect unless weed populations are released from strongly limiting factors (e.g., drought stress or salinity). For most weeds, little is known about the extent to which various ecological factors limit the weed's abundance, competitive ability, or geographic range. These data gaps present challenges for predicting whether transgenic weeds could become more difficult to manage than those that lack novel transgenes.

Gene flow in aquatic GEOs.—Genetically engineered fish and other aquatic species have the potential to disperse and proliferate in natural ecosystems. Commercial aquaculture operations have routine and often significant escape of their stock due to problems with equipment, handling or transport operations, predator intrusion, storms, and other factors (CEQ and OSTP 2000). Because of the possibility of escape, we anticipate possible ecological harm to conspecific populations and other species with which aquatic GEOs interact (Kapuscinski and Hallerman 1990, Hallerman and Kapuscinski 1992, Muir and Howard 1999, 2002, Hedrick 2001, NRC 2002*b*). These effects could include heightened predation or competition, colonization by GEOs of ecosystems outside the native range of the species, and alteration of population or community dynamics due to activities of the GEO. In extreme cases, these effects might endanger or eliminate non-transgenic conspecifics, competitors, prey, or predators.

Once they are released, fertile GE fish could interbreed with natural populations. Genetic or evolutionary impacts of interbreeding will depend on the fitness of transgenic genotypes in the wild (Muir and Howard 1999, Hedrick 2001). Fertile transgenic Atlantic salmon might interbreed with endangered stocks in the northeastern United States, but it is difficult to predict the fitness of these transgenic fish (CEQ and OSTP 2000, Devlin et al. 2004). Transgenic salmon expressing a growth hormone consume more food and develop faster than control fish (Stevens and Sutterlin 1999), but these traits could increase their susceptibility to predation and stressful environments. Given that the findings to date reveal contradictory effects of enhanced growth on different components of fitness, it is difficult to assess the likely ecological outcome of transgenic salmon escape (NRC 2002*b*).

Looking toward future aquatic GEOs, it is useful to consider the dispersal dynamics of crustaceans and mollusks. Many freshwater crustaceans such as crayfishes are capable of overland dispersal; also, they are produced in large, outdoor ponds where confinement is difficult. Marine crustaceans have planktonic larvae that drift in the water column with great potential for dispersal, thereby complicating confinement options (ABRAC 1995). Possible environmental hazards posed by escape of transgenic shellfish into natural ecosystems have not yet been thoroughly considered, and little research has focused on this topic (NRC 2002*b*).

A framework has been developed for identifying and managing risks posed by genetically modified fish and shellfish (ABRAC 1995). Production in indoor, recirculating aquaculture systems would be preferable to that in the floating net-pen systems that dominate production of salmonids, as net pens do not provide consistently reliable physical confinement (Hallerman and Kapuscinski 1992, CEQ and OSTP 2000). Where physical confinement is not strict, reproductive confinement is needed. Proponents of transgenic salmon intend to produce all-female triploid stocks, but 100% triploid induction may be difficult to achieve at a commercial scale (NRC 2004). Other methods for achieving reliable reproductive confinement of aquatic GEOs need to be developed (Devlin and Donaldson 1992).

Horizontal gene flow.—Horizontal (nonsexual) gene flow between species is extremely rare in plants and animals, except over long evolutionary time frames, but ongoing horizontal gene flow can be common in microbes. Complete genome sequences of free-living bacteria and archaea show that from 1% to 20% of an organism's genome derives from foreign DNA, mostly from other prokaryotes but some from eukaryotes such as metazoa (Ochman et al. 2000, Koonin et al. 2001). This foreign DNA is now recognized as a major source of innovation in microbial evolution. Horizontally transferred genes usually carry nonessential but highly selectable traits, such as antibiotic resistance, pathogenicity, and enzymes to metabolize new resources—in essence, providing a means for the species to explore new environments. Genes added by transgenic methods may transfer to other bacterial species in the same manner as other genes.

Horizontal gene transfer, however, is dependent on microbial density, and its occurrence is less frequent among more distantly related taxa. Hence, horizontal transfer of a transgene would be most likely in more dense, non-starved microbial communities such as intestinal tracts, biofilms, rhizospheres, plant nodules, fresh detritus, and pathogen lesions (cf., Bertolla and Simonet 1999). In general, there is a reasonable probability of genetic exchange between recombinant microbes and indigenous microbes, but possible hazards of such genetic exchange will depend on the traits involved.

Questions also have arisen as to whether microbes, including those in human intestines, could acquire transgenes from commercial GE plants. The movement of transgenes from plants to microbes has been demonstrated, but this transfer depends on there being homologous regions in the plant's transgene and the microbe's DNA, as well as a means to release the plant's DNA in close proximity to a microbe with the capacity to take up the DNA (Gebhard and Smalla 1998, Nielsen et al. 1998, Bertolla and Simonet 1999). For example, Kay et al. (2002) showed that antibiotic resistance transgenes in a plant's chloroplast could be transferred to a microbe as a result of a co-infection of the plant by a plant pathogen and another bacterium with homologous DNA sequences and competency for DNA uptake. So far, such studies demonstrate that inter-kingdom transfer can occur, but several idealized conditions are needed for the transfer to be detected. The combination of these conditions in the field should be rare, although this process can be important on an evolutionary time scale.

Viruses

GE baculoviruses.—Non-transgenic baculoviruses (BVs) are used for biological control of insect pests, and researchers are exploring transgenic methods to make them more effective (Box 3). The risks associated with recombinant viruses include possible effects of gene flow and negative effects on nontarget organisms. Baculoviruses can exchange or acquire new genetic material from other BVs via several mechanisms. When recombinant and wild-type BVs are replicating in an insect host, transgenes can be transferred through recombination (Merryweather-Clarke et al. 1994). In addition, genetic material is commonly transferred by transposable elements (Friesen 1993). These processes suggest that transgene movement from transgenic BVs to wild-type BVs is likely, but the ecological effects of this type of gene flow are not well understood.

Another concern that has been raised about recombinant BVs is the potential for persistence in the environment, which could influence gene flow as well as nontarget organisms. BVs infect insects and are not pathogenic to plants or vertebrates. The viral particles of baculoviruses are embedded in a protein matrix, and they can persist outside the host for years if they are not subject to ultraviolet radiation. Recombinant BVs may be particularly persistent in soils, due to slower decomposition of the insect cadaver (Fuxa et al. 1998). Such pathogen reservoirs may play important roles in transmission dynamics and therefore may tend to amplify the persistence of recombinant BVs, allowing them more opportunities for recombination and impacts on nontarget organisms (Richards et al. 1998).

Studies of nontarget hosts of the virus have shown that the effects of recombinant BVs may differ substantially from the effects of wild-type BVs. Virus productivity, distribution, and the timing of virus release all may vary, such that the effects of recombinant BVs on a suite of nontarget hosts may be difficult to predict (Richards et al. 1998, Hernandez-Crespo et al. 1999). Moreover, effects on predators and parasitoids also vary substantially. While most generalist predators do not seem to be negatively impacted by eating prey infected with recombinant BVs (Smith et al. 2000), there is some evidence of lower fitness of parasitoids developing in host larvae infected with recombinant BVs compared to wild-type BVs (McCutchen et al. 1996). In addition, several studies have shown that recombinant BVs survive in some predators, and this may provide a pathway for the persistence and dispersal of the genetically engineered viruses (Smith et al. 2000). To summarize, it is too early to know whether GE baculoviruses can be used as environmentally benign substitutes for their non-transgenic counterparts.

Effects of virus-resistant crops.—Potential ecological risks associated with the widespread adoption of transgenic virus resistance in plants fall into three categories: (1) possible benefits of transgenic virus resistance to weedy relatives of the crop (discussed above); (2) recombination between viral transgenes and invading viruses; and (3) interactions between transgene products and invading viruses, such as synergies or transcapsidation (Power 2002, Tepfer 2002). Recent molecular strategies for minimizing risks of recombination or other transgene–virus interactions through the careful selection of gene constructs are promising, but they cannot prevent all ecological risks discussed here (Power 2002).

Recent studies suggest that recombination between an invading virus and the viral RNA encoded by the transgenic plant is highly probable (Miller et al. 1997, Aaziz and Tepfer 1999, Hammond et al. 1999, Tepfer 2002). Several hazards may be associated with virus–transgene recombination. First, increased virulence could lead to greater damage to hosts of the virus, including any wild hosts in natural habitats. Such increased plant damage could, in turn, lead to changes in competitive relations between plants, which could have profound effects on natural plant communities. Second, if recombination were to lead to alterations of host range, similar changes in plant competitive relations would be expected. Moreover, any changes in transmission characteristics resulting from recombination could allow the recombinant virus to colonize hosts that were previously unavailable to the parental virus. Again, this could lead to significant changes in species interactions within natural plant communities. Recent laboratory studies have demonstrated increased virulence, increased competitiveness, and expansions of viral host range due to virus–transgene recombination (Schoelz and Wintermantel 1993, Kiraly et al. 1998, Borja et al. 1999). These studies illustrate the potential for significant impacts of transgenic virus resistance on the population biology of viruses in nature, as well as impacts on plant communities.

Another hazard of releasing crops with engineered virus resistance is the enhancement of virus spread through interactions between transgene products and invading viruses (de Zoeten 1991, Miller et al. 1997). One type of interaction that may pose relatively low ecological risks is transcapsidation (encapsidation of viral RNA of one virus by the coat protein of another virus in mixed infections). Many authors have reported transcapsidation of invading viruses in transgenic plants producing coat proteins for a wide range of viruses (for review see Power 2002, Tepfer 2002), although field studies indicate that such transcapsidation occurs at low rates (Thomas et al. 1998, Fuchs et al. 1999). Transcapsidation can result in the one-time colonization of new hosts, but may be unlikely to lead to long-term changes in transmission patterns because virus replication would produce the original parental virus.

A second type of interaction between transgene products and invading virus may present more significant risks. Synergistic interactions between viruses in mixed infections can result in increased virus replication and disease that is much more severe than that caused by either virus alone (Miller et al. 1997). When synergy between invading viruses and transgene products results in higher rates of virus replication, an increase in virus population levels in transgenic hosts could lead to greater disease pressure on nearby plantings of nontransgenic varieties or wild hosts. The combination of synergy and transgene flow may also pose significant risks in wild hosts if synergy results in increased disease severity.

Evolution of resistance in pests

The evolution of resistance in insects, weeds, and pathogens to pest control methods has become a serious problem worldwide, especially in regions with modern, industrialized agriculture. Resistance occurs quickly when there is strong, uniform selection on a pest population for long periods of time over large geographic areas. Modern intensive agriculture, with its reliance on pesticides, monoculture, and uniform production practices, provides these conditions, and resistant insects, weeds, viruses, fungi, and other pathogens have proliferated. In the United States, the cost of resistance to insecticides was estimated at about U.S. $133 million annually in extra insecticide applications, measured in 1980 dollars (Pimentel et al. 1980). For some pests, such as the Colorado potato beetle and diamondback moth, resistance is so extensive that few effective pest control alternatives remain.

To illustrate ongoing problems associated with resistant pests in general, we focus on crops with Bt toxins for insect resistance (Box 2). Resistance to Bt toxins in sprays has been documented in >17 insect species (Tabashnik 1994, Huang et al. 1997), so it is widely assumed that resistance to transgenic Bt crops will occur as well (Gould 1998, Andow 2001, Tabashnik et al. 2003). Indeed, resistance in pink bollworm to Bt cotton crops was reported in 1997 (Tabashnik et al. 2000), but during the past seven years frequencies of resistant insects have remained very low, perhaps due to fitness costs and resistance management strategies (Carriere et al. 2003, Tabashnik et al. 2003). The goal of resistance management is to delay or prevent the evolution of resistance in the target pests. The producers and users of Bt crops are the major beneficiaries of resistance management, and they would pay the costs of poor stewardship and resistance failures. Two additional reasons have compelled government agencies to take an active role in ensuring that effective resistance management is implemented. First, other farmers who depend on Bt-based insecticides and do not use Bt crops are concerned about resistant insects. Under present federal guidelines, Bt sprays, but not transgenic Bt crops, can be used as a part of organic agricultural production, and it is important to prolong the efficacy of Bt sprays. Second, resistance management of transgenic Bt crops preserves a pest control method that results in less harm to the environment and human health than many other insecticides. Bt toxins have a relatively narrow range of nontarget species effects, very low mammalian toxicity, and no record of carcinogenicity. Loss of Bt-based controls because of the evolution of resistance would probably increase use of insecticides that are more harmful to the environment or human health in some crops.

Similar concerns apply to the overuse of herbicides and the rapid evolution of herbicide-resistant weeds. More than 286 weed biotypes have evolved resistance to various herbicides during the past 30 years (see e.g., International Survey of Herbicide Resistant Weeds [*available online*]).[9] Widespread adoption of a limited number of herbicide-resistant crops could aggravate problems with resistance, especially if the same herbicides are used repeatedly in crop rotations. Herbicide resistance also can spread from transgenic crops to feral crop plants and hybridizing weeds via pollen and seed dispersal, as mentioned above.

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[9] ⟨http://www.weedscience.org⟩

Nontarget effects

In agriculture, nontarget organisms are species that are not the direct target of pest control methods. These species can be grouped into several overlapping categories: (a) beneficial species, including natural enemies of pests (lacewings, ladybird beetles, parasitic wasps, and microbial parasites) and pollinators (bees, flies, beetles, butterflies and moths, birds and bats); (b) nontarget herbivores; (c) soil organisms; (d) species of conservation concern, including endangered species and popular, charismatic species (e.g., the monarch butterfly); and (e) species that contribute to local biodiversity.

Effects of GEOs on nontarget organisms will range from positive to negative and will depend on a suite of biological, physical, and geographical factors. Many scientists and regulatory agencies have highlighted the need for a case-by-case analysis of ecological effects that integrates laboratory and field studies, and that includes data on spatial and temporal variability. Lethal or sublethal effects of GEOs on nontarget species can occur directly due to exposure to a GEO or its products, or indirectly if a GEO alters the physical or biological environment on which a nontarget species depends. Indirect effects could arise from changes in food supply or in habitat quality (e.g., changes in soil properties, plant communities). Although analysis of indirect hazards is complex, in some cases they may be as important or more important than direct hazards. Positive nontarget effects would be expected when GE products replace ecologically damaging practices, such as the use of agrochemical pesticides.

Nontarget effects of transgenic crops with insecticidal properties (i.e., Bt corn, Bt cotton) have received the greatest attention for obvious reasons: if a Bt toxin kills pest insects, it also has the potential to kill other insects. The U.S. Environmental Protection Agency requires data on nontarget effects for a standard group of soil organisms and beneficial insects, but these short-term studies often involve sample sizes that are too small for meaningful statistical analysis (Marvier 2002). Studies in the peer-reviewed literature have largely focused on the effects of Bt corn on a small number of insect species, including monarch and swallowtail butterflies (see Losey et al. 2001, Obrycki et al. 2001, Sears et al. 2001, Zangerl et al. 2001). Butterfly larvae inadvertently ingest corn pollen that is deposited on their food plants. Recent studies indicated that survival and larval growth rates vary according to the transformation event (type of Bt corn) and butterfly species, and that current exposure levels appear to be minor (Sears et al. 2001). Unlike DDT and other persistent pesticides, Bt toxins are unlikely to accumulate in vertebrates because most of these toxins are readily digested by vertebrates. Negative tritrophic level effects of transgenic Bt corn pollen and Bt sprays have been reported for the green lacewing (*Chrysoperla carnea*) in the laboratory (reviewed by Hilbeck 2001, Dutton et al. 2002, 2003). Recent studies indicate that lacewing larvae are not directly affected by Bt toxin, but that differences in prey quality between treatments may explain negative tritrophic effects detected previously (Romeis et al. 2004). Plot-level studies detected no significant effects of Bt corn on the abundance of green lacewings, although the authors point out the need for studies on larger fields because of high between-year variability and small plot sizes (Pilcher et al. 1997). Prey insects vary in how much Bt toxin they assimilate (Head et al. 2001); therefore, the abundance and diversity of prey insects as well as an insect predator's foraging preferences will affect the results of Bt studies carried out under field conditions. Other types of transgenic insecticidal toxins such as lectins and protease inhibitors will require more scrutiny than Bt, but biotechnology companies have largely steered clear of these strategies for insect control.

Single-species studies of nontarget effects represent a narrow approach to assessing the positive and negative ecological impacts of nontarget effects. Understanding the ecological consequences of nontarget effects also depends on accurately identifying what physical and biological processes a transgenic organism may alter, and understanding what impacts these alterations have on ecosystems. Much of the focus of nontarget studies has relied on measuring changes in survival and reproduction of a limited number of focal species in laboratory and small-scale field studies, without addressing the potential for community and ecosystem level effects after large-scale introductions. Negative nontarget effects on one species or a group of species may cause a cascade of ecological changes that result in the disruption of biotic communities or in the loss of species diversity or genetic diversity within species (Rissler and Mellon 1996), or they may have no repercussions, especially in communities with high redundancy of ecological function. Alternatively, positive effects of GEOs may enhance the diversity, complexity, and function of biotic communities.

Studies of nontarget effects of one Bt toxin (*Cry1Ab*) in soils and its consequences have begun to assess the potential for community-level, nontarget effects (reviewed by Stotzky 2001). Under certain soil conditions the *Cry1Ab* protein can remain active for months, but no effects of the *Cry1Ab* protein were observed on earthworm mass or mortality or on total numbers of nematodes, protozoa, bacteria, or fungi in soils over a 45-day period (Stotzky 2001). Some genetically engineered crops and microorganisms have been shown to affect soil ecosystems (e.g., Dunfield and Germida

2001), but the ecological significance of these changes is unclear. In summary, ecological studies can provide information to assess the significance and relative impacts of nontarget effects on communities and ecosystems if they are designed to compare transgenic and relevant alternatives. These studies should rely on integrating effects on single species with those on community and ecosystem levels, such as species diversity and ecological function (e.g., nutrient cycling).

Effects of GEOs on agricultural practices

All agricultural practices can have significant impacts on ecological systems, within crop fields as well as on adjacent areas. Depending on the case-specific details, transgenic crops may mitigate, exacerbate, or not affect existing ecological consequences of current agricultural practices, and evidence suggesting environmental benefits as well as environmental risks exists. Here we focus on the impacts of current transgenic crops on agricultural practices. Current crops are intricately linked to changes in pesticide use that may include shifts in the types of pesticides used, what quantities are used, and the timing of applications.

Globally and in the United States, herbicide-tolerant crops represent the largest acreage of GE crops currently used (James 2003). Large-scale adoption of herbicide-tolerant crops has been correlated with changes in herbicide use practices. Not surprisingly, the introduction of glyphosate-tolerant soybeans in the United States is associated with increases in glyphosate usage (Wolfenbarger and Phifer 2000, Carpenter et al. 2002), and decreases in the number of herbicide applications per acre (Carpenter et al. 2002). Glyphosate has low toxicity to vertebrates and invertebrates, as well as soil-binding and degradation characteristics that result in less movement or transport within the environment, compared to many other herbicides when standardized by mass. Quantifying nontarget effects of herbicides on ecological communities within and near cropland will contribute greatly to comparing the ecological effects of transgenic and conventional agricultural practices.

In the United Kingdom, the indirect effects of using herbicide-tolerant crops were examined experimentally in the Farm Scale Evaluations Project. Researchers reported significant changes in abundances and diversity of invertebrates associated with the management of genetically engineered herbicide-tolerant beets, oilseed rape, and corn, both within cropland and in habitats adjacent to fields (Andow 2003*b*, Brooks et al. 2003, Haughton et al. 2003, Roy et al. 2003). These studies underscore the value of a case-by-case approach because the direction and magnitude of effects on invertebrates varied among the crops studied. For example, most decreases in invertebrate taxa were associated with transgenic herbicide-tolerant beet and oilseed rape, and most increases were associated with transgenic herbicide-tolerant corn. Changes in invertebrate abundance were associated with more effective weed control in fields planted with genetically engineered beets and oilseed rape (Heard et al. 2003). In particular, timing of herbicide spraying affected weed control and therefore the associated invertebrate communities. Invertebrate detritivores increased in fields of all three genetically engineered herbicide-tolerant crops, which was attributed to greater biomass of dead weeds in these fields (Hawes et al. 2003). The design of the Farm Scale Evaluations most likely underestimated ecological effects because cumulative effects were not included and because a split-plot design could reduce the possibility of detecting scale effects. These results highlight earlier discussions about the impacts of ‘‘clean’’ agricultural fields and field margins on habitat that supports other organisms, including birds (e.g., Watkinson et al. 2000) and concerns about energy contents of seeds available to game and nongame species (Krapu et al. 2004).

Transgenic herbicide-tolerant crops may promote conservation tillage practices, although they are not required for low- or no-till agriculture. Conservation tillage increases soil quality, decreases erosion, decreases nutrient leaching, and inhibits the build-up of weed seeds in the soil (e.g., Carpenter et al. 2002; Box 1). Another environmental benefit of no-till agriculture is greater sequestration of atmospheric carbon in the form of soil organic matter. Surveys in the United States indicate that conservation tillage in soybean production has increased substantially between 1989 and 2000 with the largest increases occurring between 1991 and 1993 (prior to the commercialization of Roundup Ready soybean; see Core4 Conservation Alliance table of conservation tillage trends [*available online*]).[10] The Conservation Tillage Information Center reports a correlation between no-till conservation practices and the use of herbicide-tolerant soybeans (see Fawcett and Towery 2003). How other factors, such as farm subsidies or other soil conservation programs, may also correlate was not discussed, but these may be important causal factors in explaining trends in no-till conservation.

Insecticide use is expected also to change with transgenic crops having insecticidal properties, such as those engineered with Bt genes (e.g., Box 1), but other factors can cause these shifts too (e.g., economic forces, new machinery, and new landowner farming preferences). Significant reductions in insecticide use for Bt cotton have been reported (e.g., Ortman 2001, Pray et al. 2002), as well as reduced pest populations (Car-

[10] ⟨http://www.ctic.purdue.edu/Core4/CT/CTSurvey/NationalData8902.html⟩

BOX 7. Common definitions used in risk assessment

- Harm—An unwanted effect, for example, on the state of a gene pool, population, species, ecological community, or ecosystem processes.
- Hazard—An action, substance, or phenomenon that has the potential to cause harm.
- Risk—The likelihood of a hazard being realized. Risk typically is expressed as the product of two probabilities: the probability of exposure to the hazard and the probability of the hazard causing harm.
- Risk assessment—The science and process of estimating risk.
- Risk management—The process of considering alternative courses of action, and selecting the most appropriate option after integrating the results of risk assessment with engineering, social, economic, and political concerns in order to reach a decision.
- Risk analysis—The process including risk assessment, risk management, and risk communication.

riere et al. 2003). Although not widely adopted, Bt sweet corn may also reduce insecticide use associated with controlling corn earworm (U.S. EPA 2001). However, depending on the pest species targeted, a Bt crop may or may not replace insecticide use. For example, prior to the introduction of Bt corn that targets the European corn borer, only a small percentage of corn was treated with insecticides to control corn borer infestations (Obrycki et al. 2001). Environmental benefits associated with virus-resistant crops are also uncertain. Some virus management strategies rely on insecticides to control the insect vector of the virus (e.g., aphids), but this practice is not common because vector control is often ineffective. Thus, environmental benefits from using less insecticide with virus-resistant crops are likely to be limited, except when a real or perceived need to use insecticides for controlling vectors can be alleviated.

As reviewed above, much of the effort to document environmental benefits has focused on the correlation between adoption of GE crops and concomitant alterations in management practices. These findings often rely on surveys of farmers and data from the U.S. Department of Agriculture that are aggregated at large spatial scales (i.e., by state). The interpretation of such correlations should be strengthened by critically evaluating alternative hypotheses and explanations for observed trends. Although a hypothesis-driven approach will improve the interpretation of these data, this is not a sufficient means for documenting environmental effects. The collective information that we have on the adoption of herbicide-tolerant crops illustrates the limitations of assessing risks and benefits using only information on how management practices change. Herbicide-tolerant crops can have positive environmental effects relative to previous herbicide practices, as mentioned above, but their long-term effects on local biodiversity are not yet known (e.g., Firbank 2003). Single measures of environmental risk or of environmental benefits will give only partial information to evaluate the suite of environmental impacts. Well-designed, scientific studies that include ecological indicators, as well as the management changes associated with the adoption of genetically engineered crops, are needed to document positive as well as negative environmental impacts.

RISK ASSESSMENT, REGULATION, AND FUTURE RESEARCH

Risk assessment terminology

Risk assessment methodology assigns specific and fairly technical meanings to commonly used words. Without going into detail, Box 7 provides a guide as to how these terms typically are applied in the context of GEOs (e.g., NRC 1996, 2002*a*). Although this terminology may be more useful for evaluating the effects of toxic chemicals, radioactivity, faulty machinery, and other hazards, as opposed to those discussed in this report, it is helpful to understand how specific terms are interpreted and used in discussions of biosafety issues.

Uncertainty, monitoring, and adaptive management

Risk assessment that is carried out prior to commercialization has several inherent weaknesses. In general, small-scale, pre-commercial field experiments are not sufficiently sensitive enough to detect small or moderate effects of a GEO. Small-scale studies will readily detect order-of-magnitude differences in an ecological effect, but less dramatic effects will be difficult to document due to variability among replicates (e.g., Andow 2003*a*). Adding more replicates can address this problem, but pre-commercial field studies are not likely to include the large amount of replication needed to identify small but important effects. To illustrate this type of problem, we conducted a simple statistical analysis of some published insect density data (cf., Oelhert 2000, Marvier 2002). Andow and Ostlie (1990) measured the population density of a target pest of Bt corn, the European corn borer, in three environments replicated four times each. A power analysis of their data shows that 10 replicates would be needed to detect a statistically significant difference of 200% among environments, given the observed variation among replicates. To detect a 10% difference in population density, they would have had to examine 134 replicates for each environment! Thus, many small-scale field experiments involving a Bt crop, for instance, are unlikely to detect 10% increases or decreases in the abundance of nontarget species unless a power analysis indicates that replication levels are sufficient. Yet ecological effects of 10% could be highly biologically significant.

Recommendation 4

***Monitoring of commercial GEOs*.—Well-designed monitoring will be crucial to identify, manage, and mitigate environmental risks when there are reasons to suspect possible problems. In some cases, post-release monitoring may detect environmental risks that were not evident in small-scale, pre-commercial risk evaluations. Because environmental monitoring is expensive, a clear system of adaptive management is needed so that monitoring data can be used effectively in environmental and regulatory decision-making.**

If the pre-commercialization risk analysis process only identifies large, order-of-magnitude ecological effects, it should be evident that low probability events and low magnitude effects will not be detected (Fig. 3). These include both infrequent effects and small effects. As the GEO is commercialized over larger spatial and temporal scales, however, it may become possible to observe smaller and less frequent ecological risks. Rigorous monitoring may be the only realistic way to detect such effects. Such monitoring should not, of course, substitute for rigorous pre-commercialization testing at logistically feasible scales, nor should the inherent limitations of monitoring be overlooked. By the time a problem is common enough to be detected by a monitoring program, it may be too late to mitigate or reverse the problem. Even if this is the case, however, detection of irreversible environmental problems can allow for actions to prevent similar situations from occurring in the future.

A second reason that ecological monitoring may be needed after commercialization is that ecosystems are complex. This complexity stems from year-to-year variation, spatial variation, and indirect biotic effects. Because laboratory and small-scale field experiments do not adequately replicate all of the interactions that occur in an ecosystem, the only way to observe the full range of ecological effects of a GEO is to observe it in actual ecosystems. Some of these effects cannot be predicted beforehand, in which case ecological monitoring will be necessary to detect any adverse ecological effects.

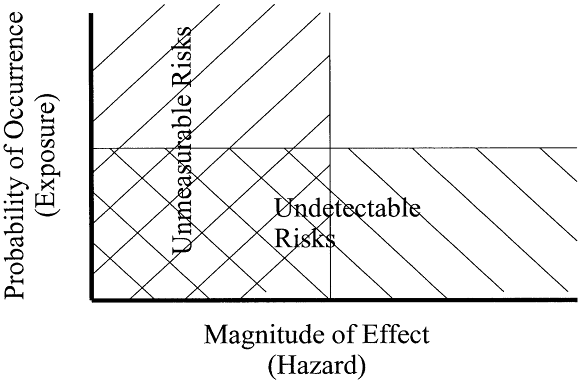

FIG. 3. Diagram illustrating challenges of using small-scale field experiments to detect environmental risks that are infrequent or relatively small. Risk is a combination of hazard (*x*-axis) and exposure (*y*-axis). Risks that have a low probability of occurrence (low exposure, less frequent effects) are unlikely to occur during a small-scale experiment and are therefore unlikely to be detected. Risks that have a low environmental effect (low hazard, small effects) are unlikely to be measurable in a small-scale experiment because of the high experimental variability as discussed following Recommendation 4.

For example, one concern about transgenic plants is their potential to invade neighboring ecosystems. However, short-term, spatially limited field trials are poor predictors for environmental impact of invasions (Kareiva et al. 1996). Small-scale field trials conducted as part of a permit or petition for deregulation of a transgenic crop will ultimately have little predictive power regarding potential ecosystem effects or potential for invasiveness. Kareiva et al. (1996) explored the predictive power of a large data set comparing potential invasiveness of transgenic and non-transgenic canola over three years in the United Kingdom (Crawley et al. 1993). They found that predictive power varied depending upon how many sites and years were incorporated in the analyses, and the magnitude of errors often exceeded 100%. These authors stated ''we have so little faith in models and short-term experiments regarding predictions about invasions, that we advocate extensive monitoring of any introduced [transgenic crop] with any ecologically relevant traits (such as disease resistance, herbivore tolerance, and so forth).'' We do not mean to imply that small-scale studies are useless, but rather that they may be insufficient and misleading, depending on the questions being asked and the statistical power of the data analysis.

If risk management practices are implemented at the time of commercialization, due to specific environmental concerns, ecological monitoring will be needed to document whether these management practices have been successful. For example, it may be possible to delay the evolution of resistance to Bt crops by maintaining large enough refuges (areas where non-Bt varieties of the crop are grown) so that selection for resistance is reduced (Gould 1998, Andow and Ives 2002, Ives and Andow 2002). Monitoring is needed to evaluate the success of resistance management strategies and to adjust them if resistance arises. In a similar vein, monitoring could be used to determine whether herbicide-resistance transgenes have spread to weedy relatives of a particular crop. An obvious challenge, however, is to decide when monitoring is needed in the first place, and how to design monitoring programs that are both practical to carry out and scientifically rigorous.

Environmental monitoring is expensive, so information from monitoring activities should be used within a clear system of adaptive management (NRC 2002*a*). Adaptive management involves repeated cycles of goal-setting, program design, implementation, and evaluation, in a deliberate ''learn-as-you-go'' fashion. One problem with many adaptive management programs is that they are not well-suited for dealing with long-term ecosystem responses (Moir and Block 2001). A more immediate problem for monitoring GEOs is that adaptive management systems have not been developed specifically for this purpose (but see Kapuscinski et al. 1999). Moreover, monitoring standards and action triggers have not been established. For example, to manage resistance evolution, the frequency of resistance should be monitored in the field, but additional research is needed to set monitoring standards, implement action triggers, and determine appropriate management responses that could be taken to mitigate the problem (Andow and Ives 2002).

Monitoring for potential adverse effects of specific GEOs should follow four complementary strategies (NRC 2002*a*). First, the *spatiotemporal distribution of the GEO* should be monitored. This will provide an historical record of GEO use, which can then be correlated with other patterns to examine and test possible environmental effects of a particular GEO. Because many environmental effects are localized to small areas on the order of the dispersal ranges of the species involved, the spatial distribution of GEOs should be characterized on as fine a spatial scale as feasible. For some GEOs, such as corn and soybean in the United States, this will be a simple exercise of reporting sales and associated use statistics. For other GEOs, such as fishes or canola, where organisms or genes escape into wild or feral populations, this will be a greater challenge.

Second, existing technical personnel in agriculture and natural areas management should receive supplemental training so that they can detect unexpected environmental effects of GEOs (NRC 2002*a*). These *trained-observer networks* are already functioning informally. For example, many agricultural extension workers are hearing from farmers about some potential effects of GEOs, and if they had additional training, they would be better prepared to separate pure fiction from possible or plausible fact. By using existing networks of observers, the cost of this monitoring is minimized. Information gained could feed into research, so that the reported effects can be rigorously tested.

This leads to a third approach, which is hypothesis-driven *monitoring research.* In the context of monitoring, research can test the observations from the trained observers or from spatiotemporal correlations. As a case in point, several extension workers from the U.S. Corn Belt reported that Bt corn stalks were tougher than non-Bt corn, resulting in less lodging, lower preference by livestock, slower decomposition, and even damage to tractors. Following these reports, Saxena and Stotzky (2001) found that Bt corn is in fact tougher than non-Bt corn due to higher lignin content. Although the ecological consequences of these findings remain to be evaluated, this illustrates informal linkages between observer networks and research. Research also can be used to develop the appropriate monitoring methods. For example, to monitor the changing frequency of rare, nearly recessive resistance genes in natural insect populations, Andow and Alstad (1998) developed an F_2 screen, which improves monitoring efficiency by several orders of magnitude (Andow and Ives 2002). Furthermore, research can confirm or refute uncertain risk assessments. Initially, laboratory and small-scale field experiments might indicate that certain nontarget effects might occur at larger spatial scales. If this can be articulated as a testable hypothesis, monitoring research can be developed for its evaluation.

Finally, a deliberative process involving discussions with stakeholders should be initiated to *identify indicators* for monitoring environmental effects of GEOs (NRC 2002*a*). Deliberative processes are by their nature adaptive, and by involving stakeholders effectively, a clear consensus for how to proceed with long-term environmental monitoring of GEOs can be developed. Although we do not presume to identify these indicators at this time, ecological research on monitoring (e.g., Marvier et al. 2001) suggests that these indicators should be targeted to specific effects, and should focus on areas at greatest risk to minimize cost and maximize usefulness.

Regulation of GEOs in the United States

Recommendation 5

***Regulatory considerations*.—Science-based regulation should: (1) subject all transgenic organisms to a similar risk assessment framework, (2) recognize that many environmental risks are GEO- and site-specific, and therefore that risk analysis should be tailored to particular applications, and (3) incorporate a cautious approach to environmental risk analysis.**

Although the U.S. regulatory system for GEOs has worked reasonably well up to now for GE crops, federal regulatory policies are often imperfect. The current system needs to be improved, especially as new types of GEOs are proposed (NRC 2002*a*). Here we summarize salient features of how GEOs are regulated at present, with the caveat that some of these regulations are in the process of being modified as this paper goes to press. A thorough discussion of how GEOs are regulated is beyond the scope of this paper.

In the United States, transgenic organisms are regulated under the ''Coordinated Framework'', which extends the authority of previously existing legislation to regulate the use of transgenic organisms (OSTP 1986, 1992). The goal of the framework is to provide adequate regulation to ensure human health and environmental safety, while maintaining sufficient regulatory flexibility to avoid impeding the growth of the biotechnology industry. Three federal executive agencies and 10 federal statutes were identified to regulate GEOs. Environmental risk assessment falls under three agencies, the U.S. Department of Agriculture (USDA), the Environmental Protection Agency (EPA), and the Food and Drug Administration (FDA), which primarily regulate transgenic organisms as potential plant pests, pesticides, and food or drugs, respectively. USDA and FDA also are required to examine the consequences of any agency decision on the environment through the National Environmental Policy Act (NEPA). These agencies review the safety of GEOs to be used in the United States, but they do not consider the environmental or human health effects of the dispersal of GEOs to other countries.

Many transgenic crop varieties have been approved for experimental and commercial use under the Coordinated Framework. As of February 2004, $>$10 000 permits and notifications have been approved by USDA for experimental field trials of GE crop varieties (ISB 2004*a*). After the field-testing stage, many GEOs are further evaluated for commercialization. Several transgenic lines of 12 plant species have been deregulated by USDA as of this writing (ISB 2004*b*). In conjunction with these deregulation decisions, the EPA registered several transgenic insecticidal crop lines (seven corn, with four still commercially available, two cotton, and one potato) and a potato line with transgenic resistance to a virus. EPA also determines food tolerance limits (maximum levels of residues) or grants exemptions on food tolerance limits for insecticidal transgenic plants. (As of this writing, the EPA has granted tolerance exemptions for registered, transgenic microbial pesticides and insecticidal plants.) The FDA's primary role is to oversee food and feed safety, which are not discussed here, but some of this agency's responsibility involves environmental assessments. For example, transgenic Atlantic salmon expressing an introduced growth hormone gene are regulated by FDA under the Federal Food, Drug, and Cosmetics Act and are subject to the National Environmental Policy Act. Transgenic salmon are regulated by the FDA as new animal drugs because the transgene is effectively a hormone delivery system, and the potential adverse environmental effects associated with its decision on transgenic salmon will be assessed by FDA. For all other organisms not mentioned above, such as transgenic bacteria for bioremediation, federal oversight is authorized by EPA under the Toxic Substances Control Act.

The Coordinated Framework was controversial during the earliest field tests of genetically modified organisms (e.g., Rissler and Mellon 1996.), and some issues remain unresolved. The Framework has some significant lapses (NRC 2000, 2002*a*, *b*); for example, the quality of the research used for regulatory approval has been criticized as scientifically inadequate in some cases (NRC 2000, 2002*a*). Another concern is that some transgenic organisms are inadequately assessed, in part due to language in the rules of the regulation that emphasizes the intended use of the transgenic organism as a criterion for oversight, as opposed to using scientific considerations of harm (NRC 2002*a*). Therefore, the producer's stated use for the transgenic organisms influences what roles FDA, EPA, and USDA have in regulation.

Also, by utilizing only a subset of the laws available, the Framework does not consistently involve all the relevant federal agencies in oversight of particular classes of GEOs. For example, authority for oversight of transgenic fishes under the Endangered Species Act or the Lacey Act could bring to bear the expertise of the U.S. Fish and Wildlife Service and the National Marine Fisheries Service (CEQ and OSTP 2000). Finally, the Coordinated Framework does not authorize post-commercial monitoring uniformly. USDA cannot require monitoring for unwanted effects of transgenic plants that have been deregulated; EPA can require monitoring under its statutory authority, but this is restricted to plants with insecticidal properties.

In the European Union and elsewhere, the regulation of GEOs places a greater emphasis on a precautionary approach to risk assessment. The role of precaution in the regulation of transgenic organisms is highly contentious and, to some people, support for precaution symbolizes complete opposition to the use of transgenic organisms. This sorry state of affairs has come about in part because the term ''precaution'' has taken on many meanings, and the regulatory and trade implications of its interpretation can be great. However, the scientific rationale for a precautionary approach to regulation should not be ignored amidst this controversy. Precaution is considered in a wide range of environmental laws, treaties, and protocols (e.g., Cartagena Protocol on Biosafety 2000, New Zealand Royal Commission 2001). Simply put, precautionary actions have been justified even in the absence of clear scientific evidence that a hazard is likely to occur. In other words, these actions involve ''scientific evidentiary standards that err on the side of preventing serious and irreversible health and environmental effects'' (NRC 2002*a*). This simple statement belies considerable subjective ambiguity, however. What kinds of precautions are justified, how little evidence is tolerable, and how small a hazard is significant? When a risk is irreversible and is imposed on unwilling parties, individuals and society usually take a precautionary approach toward that risk (NRC 1996). As discussed above, several environmental risks associated with gene flow, viral recombination, evolution of resistance, and some nontarget effects are essentially irreversible. In specific cases, however, even these risks can prove to be reversible. Hence, additional research is needed to evaluate the circumstances under which environmental risks are irreversible, and if reversible, the costs for undoing the effects.

Need for broadly trained scientists

Recommendation 6

***Multidisciplinary training*.—Ecologists, agricultural scientists, molecular biologists, and others need broader training to address the above recommendations. We strongly encourage greater multidisciplinary training and collaborative, multidisciplinary research on the environmental risks and benefits of GEOs.**

We have argued that application of ecological expertise and knowledge is essential during all stages of the development of GEOs that are to be released into the environment, from the earliest planning to post-release monitoring and management. Active involvement of professionals with an understanding of relevant ecological and evolutionary processes can help avert environmental problems and facilitate promising applications of GEOs. New types of GEOs will need to be evaluated by even broader groups of scientists, including foresters, range scientists, aquatic ecologists, entomologists, and pathologists. In the future, scientific and technological advances will continue to expand the possibilities for the artificial design and manufacture of living organisms. Already, the young fields of genomics and bioinformatics have made it much easier to identify commercially important genes that potentially can be transferred among species. Ecologists have much to contribute to the broader public debate about how society and the environment can avoid risks and gain benefits from these innovations.

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