ENVIRONMENTAL IMPACTS OF THE RELEASE OF GENETICALLY MODIFIED ORGANISMS

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INTRODUCTION

Within a decade after recombinant DNA technology was discovered in 1973, newly formed biotechnology companies began genetically modifying crops (GMC), microorganisms (GEM), and animals for agricultural and industrial applications. Microorganisms were genetically engineered for extracting ores in deep mining operations, for degrading persistent pollutants in soil and water, and for reducing frost damage to fruits and vegetables. Crops were genetically modified for disease, pest, and herbicide resistance, as well as altered nutritional qualities. Domesticated animals and wildlife have been genetically altered for the production of food and pharmaceuticals. The release of these transgenic organisms into the environment introduces potential ecological hazards.

MICROBES

In *Diamond v. Chakrabarty* (1980), the U.S. Supreme Court approved the first patent application exclusively for a living organism. The patent described a strain of *Pseudomonas* with at least two stable extrachromosomal DNA segments that gave the organism the capacity to degrade crude oil. The patented microbe, however, was never developed beyond laboratory use in part because of the ecological uncertainties associated with releasing large quantities of the GEMs into the environment, the deficiency of a regulatory regime to evaluate and manage large scale releases, and the limited effectiveness of the organism's oil-degrading properties. In 1987, after five years of regulatory review and ecological assessment, the U.S. Environmental Protection Agency (EPA) approved the first field release of a GEM. The microorganism, known as ice minus, was constructed from the bacterium *Pseudomonas syringae* by removing genes that synthesize ice-nucleating proteins. Both in the laboratory and in preliminary field tests, ice minus was shown to inhibit frost damage to plants when the temperature drops a few degrees below freezing. Despite early signs that the genetically engineered *P. syringae* might prove successful in reducing frost damage to certain crops in the Frost Belt, no commercial product was developed from this strain (1).

GM PLANTS

From 1986 to 1994 there were more than 1500 field tests involving genetically modified organisms (GMOs) carried out throughout the world, most occurring in North America and Europe and more than 95% involving plants. In 1998 there were more than 1000 approved field tests in the United States. During the 1990s, major research and development investments were made in genetically modified seeds that conferred a pesticidal property to staple crops such as corn, soybeans, potatoes, alfalfa, peanuts, canola, barley, and cotton. The primary traits conferred to GMCs in the United States between 1987 and 1998 as a percentage of all GMCs were herbicide tolerance (29%); insect resistance (24%); product quality, such as high lysine soybeans or modified-oil soybeans (20%); and disease resistance (15%). The herbicides most frequently targeted for crop resistance are glyphosate, sulfonylurea, bromoxynil, glufosinate, and bialaphos. Glyphosate-resistant soybeans were the first of the family of herbicide resistant (or tolerant) crops (HRCs) commercialized. Genetically modified tomatoes reached consumers in 1994 while herbicide-tolerant soybeans, cotton, and canola entered commercial agriculture in 1996. By 2000 approximately one fifth of the U.S. corn acreage, over half of the soybean acreage, and three quarters of the cotton acreage,

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comprising nearly 30 million hectares, were planted with crops genetically engineered to be resistant to insects and herbicides.

ECOLOGICAL IMPACTS

Ecological concerns over the release of HRCs include the following: 1) Transgenic crops might invade natural habitats if their germination, root growth, resistance to abiotic stresses, or dispersal has been enhanced; 2) Genes transplanted to the crop for herbicide tolerance might transfer to other plants, thereby spreading herbicide tolerance in ways that are ecologically undesirable. The probability of gene flow is greatest when transgenic crops are grown in close proximity to sexually compatible wild relatives; 3) Successes of HRCs can result in the increased use of herbicides and/or compromise efforts toward incorporating integrated pest management; 4) By building herbicide resistance into a few widely used low toxicity herbicides, the rate of weed resistance is likely to increase requiring the use of more toxic herbicides.

Postulated benefits of genetically modified crops include the transition to low toxicity herbicides such as glyphosate, lower poundage of herbicide use, reduction in the use of insecticides, improvement of crops against viral diseases, and the nutritional enhancement of crops.

In a large-scale, plant-population field test to evaluate the invasiveness of a transgenic crop, Crawley et al. (2) compared conventional and genetically engineered oilseed rape (canola). No evidence was found that the transgenic rape was invasive in undisturbed natural habitats or more persistent in disturbed habitats than the nontransgenic rape. Parker and Kareiva (3) identified limitations in the study because the engineered traits were not expected to enhance plant performance or alter biotic interactions in the experimental conditions examined.

The transfer of herbicide tolerant traits by pollen has been established. Oilseed rape that was genetically engineered to be tolerant to glyphosate was planted in proximity to a closely related *Brassica campestris*. Within second-generation plants, there was a rapid spread of genes from oilseed rape to its weedy relatives. The transfer of herbicide resistance traits to feral plant populations depends on airborne dispersal rates of pollen and the distance between the GMOs and related species. Deshayes (4) reported that at 50 m from a small plot with genetically modified plants of oilseed rape containing an herbicide resistance gene, about one out of ten thousand seeds produced by the surrounding nongenetically modified oilseed rape plants showed resistance to the herbicide. While it is uncommon for pollen to be transported more than a few kilometers, it does occur during unusual weather conditions when the pollen is swept high enough in the atmosphere (5).

In field studies, Bergelson et al. (6) compared transgenic varieties of *Arabidopsis thaliana* with wild varieties on their outcrossing characteristics. The investigators found that the transgenic variety of *A. thaliana* was 20 times more likely to outcross compared to ordinary mutant strains. While no explanation could be given for the difference in this characteristic, it raises questions about the unanticipated spread of transgenic plant varieties. From growth-chamber studies Snow et al. (7) found that a transgenic variety of *Brassica nupus* with glufosinate resistance introgressed into populations of *Brassica rapa* even when herbicides were not applied. The studies also found a group of transgenic progeny that were less fit (e.g., reduced transmission and lower fecundity) than those without the transgene.

One of the world's largest selling herbicides and widely acclaimed also to be among the safest is glyphosate, marketed as Roundup. It is used in conjunction with Roundup Ready seeds. Lappé and Bailey (8) reported that glyphosate fed to animals at high levels was shown to cause liver toxicity. In a case control study in Sweden published in the journal *Cancer*, Hardel and Eriksson (9) found that exposure to glyphosate revealed increased risks for non-Hodgkin's lymphoma. The introduction of glyphosate tolerance to crops has expanded the use of this herbicide. These preliminary studies on glyphosate could be a forewarning that unsuspected hazards may accompany glyphosate resistant crops.

The primary ecological concerns of introducing insect toxins into the genomes of plants is that it would accelerate insect resistance to those toxins, that the toxins would be spread to other plants for which insect resistance is not desired, and/or that the toxins would exhibit secondary effects on beneficial insects that fed on an insect pest that fed on a pesticide-expressing plant. The first generation of crops genetically modified for insect resistance involved the transfer of the genes that synthesize delta-endotoxins of *Bacillus thuringiensis* (Bt). A major advantage of Bt over synthetic insecticides is that it is selectively toxic to certain herbivorous insects. In May 1995, the EPA approved as the first commercial release of an insecticidal transgenic crop, Bt potatoes created by Monsanto for control of the Colorado potato beetle. In August of that year commercial approval was given to Bt corn developed for control of the European Corn Borer.

Losey et al. (10) discovered that pollen from Bt corn can be hazardous to monarch butterflies. In laboratory studies, investigators dusted pollen from Bt corn on milkweeds, a food source of monarch butterflies. The monarch larvae reared on the milkweed ate less, grew more slowly, and suffered higher mortality than the controls, according to the study. Other studies have shown that Bt toxin can harm carnivorous predators of insect pests such as lacewing insects a beneficial insect that feeds on the European corn borer, and that aphids are capable of extracting toxins from agricultural crops and are using them to attack their predators—a species of beetles.

The use of transgenes to confer disease resistance to crops represents another possible ecological risk. If genes that code for viral coat proteins of pest plant viruses are transferred to crops, there is a potential that the genes will recombine in wild plants creating new plant viruses of increased disease severity and/or host range. Risk assessment studies have lagged in this area, in part, because U.S. regulatory agencies have exempted plants genetically engineered for disease resistance from government oversight (11).

TRANSGENIC FISH

Application of genetic engineering techniques to animals has expanded beyond domesticated species to marine organisms. New strains of transgenic fish are being developed for restocking natural waterways and for use in aquaculture. The traits of current interest include accelerated growth, size enhancement, disease resistance, and phenotypes suitable for survival in expanded habitats, such as tolerance for low temperatures. Whether for sport or commerce, once introduced into the environment, transgenic fish may become permanently and irreversibly established in natural aquatic communities. Mass gene transfer methods such as lipofection, particle bombardment, and electroporation of embryos and sperm cells are being developed to genetically modify large numbers of eggs (12). Potential ecological risks of releasing transgenic marine organisms into the natural environment have been widely discussed in the scientific literature (13–15). For transgenic fish, studies have found greater vulnerability to predation (16), altered foraging behavior (17), increased ability to compete for food (18), and swimming deficits (19).

To date, research on transgenic releases has concluded: case-by-case studies are important in predicting ecological outcomes (20, 21); Bt crops will increase insect resistance unless mitigating actions such as refugia (zones where non-transgenic crops are planted near the transgenic crops) are taken (22); pollen is an important source of transmission for transgenes; field tests are not likely to reveal the magnitude of risks of weed or insect resistance that may result when large areas are cultivated with transgenic crops (23), particularly when the transgenes affect biotic interactions (protection against natural enemies); release of transgenic marine organisms could destabilize aquatic ecosystems; the eggs of transgenic fish used in aquaculture could escape into local waters if confinement of the facility is not failsafe. Finally, the experience with nonindigenous species introductions can be useful in studying the release of transgenic plants, microbes, insects, and animals into the environment.

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