Field Testing Transgenic Plants

An analysis of the US Department of Agriculture's environmental assessments

R. P. Wrubel, S. Krimsky, and R. E. Wetzler

Since humans first began to cultivate the soil, crops have been improved by selecting plants with desirable characteristics. More recently, crop improvement has involved interbreeding plant varieties or closely related species. A casual glance at any seed catalogue reveals a plethora of varieties and hybrids created to maximize various genetic characteristics, such as timing and size of yield, tolerance to pests and diseases, and color, shape, and taste of fruit.

Until recently, genetic crop improvement has been limited by the degree of phenotypic variation found among plants that are sexually compatible. Through genetic engineering, however, genes can now be removed from one organism and placed into the genome of another, where they are expressed along with the recipients' own genes. The relatedness of donor and recipient is no longer a limiting factor for genetic modification. The promise of genetic engineering for crop improvement arises from this vast new reservoir of characteristics that, theoretically, can now be placed into crop plants. The development of genetically engineered plants (also called transgenic plants) is progressing rapidly. Research has moved from the laboratory and greenhouse to the field, where increasing numbers of small-scale experiments on transgenic plants are now being conducted throughout the United States and Europe (McCammon and Medley 1990; Figure 1).

The opportunity to collect important data on environmental effects is not being fully realized

In the midst of what some have called the new agricultural revolution, concerns have been raised regarding the safety of environmental releases of transgenic organisms (e.g., Colwell 1988, Hoffman 1990, Tiedje et al. 1989). The most visible of these early controversies was over the field test of a deletion mutant of *Pseudomonas syringae* (called ice minus) designed to reduce frost damage to certain crops (Krimsky and Plough 1988). Partly in response to public concerns, in 1986 the Executive Office of the President established the Coordinated Framework for Regulation of Biotechnology to "ensure the safety of biotechnology research and products" (OSTP 1986). Various executive agencies were given regulatory authority to oversee particular categories of organisms created through genetic engineering.

The set of human health and environmental issues related to the release of genetically modified organisms into the environment was placed under the aegis of the Environmental Protection Agency (EPA) and the United States Department of Agriculture (USDA; Krimsky 1991). EPA is responsible for regulating biological pesticides and various types of microorganisms used in agriculture and industry. USDA is responsible for regulating biotechnology applications in agriculture and forestry.

Within USDA, the Animal and Plant Health Inspection Service (APHIS) oversees the field testing of transgenic plants. APHIS conducts an environmental assessment, as required under the National Environmental Policy Act,[1] for each proposed release. A permit is issued allowing the release if there is a "Finding of No Significant Impact." One of the purposes of the environmental assessment review process is to assure the public that scientifically based reviews of the environmental consequences of proposed releases are being conducted before field trials are allowed. By issuing a permit for a field trial, APHIS certifies that there is no plant pest risk and no significant environmental risk associated with the release (McCammon and Medley 1990).

The goal of this article is to examine the methodology and use of ecological knowledge by USDA in con-

R. P. Wrubel is a research associate and S. Krimsky is a professor in the Department of Urban and Environmental Policy, and R. E. Wetzler is a division director in the Center for Environmental Management, Tufts University, Medford, MA 02155.

[1]U.S.C. §4321 et seq., 83 Stat. 852, Pub. L. 91-190.

ducting environmental assessments of field trials of transgenic plants and to make that information more accessible to ecologists, agronomists, molecular biologists, and others interested in the various areas of potential ecological and environmental risk addressed in these reports. We report what questions are asked by APHIS, what conclusions are drawn, what evidence is cited to support the conclusions, and whether the evidence derives from published or unpublished data, targeted experiments, general principles, or some other source.

USDA's statutory role

USDA's role in plant biotechnology is based on its statutory requirements, primarily under the Federal Plant Pest Act and the Plant Quarantine Act, to regulate any organism that constitutes a real or potential plant pest risk. Following this rationale, USDA has assumed regulatory authority over any genetically engineered organism that was created using a plant pest in any stage of its development. The plant pest may be the donor organism(s), the recipient organism, or the source of the vectors used to transfer genetic material between donor(s) and recipient. USDA has published a list of taxa containing plant pests that are therefore considered "regulated articles" (USDA 1987). Detailed descriptions and analyses of the statutory authority used by USDA to regulate the environmental release of transgenic plants and microorganisms has been presented in several sources (McCammon and Medley 1990, Mellon 1988, OSTP 1986).

None of the transgenic plants we reviewed are derived from plants that are considered plant pests (Table 1). All, however, were engineered using the bacterium *Agrobacterium tumefaciens* as the vector agent, and most used a noncoding promoter DNA sequence derived from a plant pest. Because *A. tumefaciens* causes crown gall disease, the genus is listed as a regulated article (USDA 1987). However, all environmental assessments stated that the vector has been altered by deletion of its tumor-inducing genes so that it is no longer pathogenic. Even so, USDA deems transgenic plants engineered with this disarmed vector to be regulated articles requiring permits for environmental release.

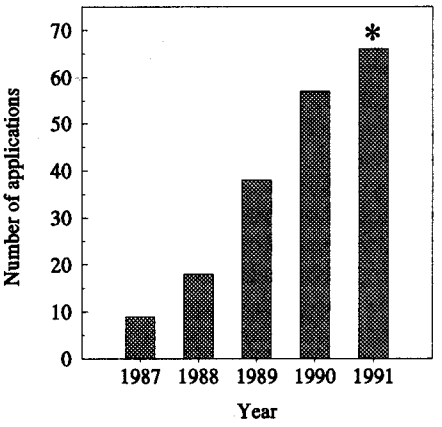

Figure 1. The number of applications submitted to USDA for field testing transgenic plants for which environmental assessments were issued or were pending through 25 September 1991. *Applications submitted 1 January to 25 September 1991.

Promoters are transferred to the target plant along with the gene of interest to enable transcription of the gene. The promoter itself is not transcribed. Promoter sequences are most often obtained from the cauliflower mosaic virus, a plant pathogen. Even though the promoters are noncoding and not themselves pathogens, the fact that they are derived from plant pathogens has been used by USDA to justify oversight.

Other techniques have been developed that do not use *A. tumefaciens* or any other plant pest as a vector. These techniques include use of a DNA-coated microprojectile to shoot the genetic material into a plant cell (Klein et al. 1988) and electroporation (Rhodes et al. 1988), in which the plant cell wall is removed and foreign genes are introduced using an electric current. It seems that applications for future field tests of transgenic plants created with these techniques and using promoters derived from nonpests would be exempt from USDA oversight.

Methods of analysis

This article examines the contents of a sample of environmental assessments issued by USDA in response to applications for permits to field test transgenic plants. Emphasis is placed on the sections of reports pertaining to ecological and environmental issues. Questions related to processes of plant transformation (i.e., questions associated with the particular donor or vector organisms and the molecular transformation methods used to add foreign genes to the plant), which are also included in USDA assessments, are beyond the scope of our study. Many of the conclusions expressed in the assessments rely on the applicants' adherence to specific procedures while conducting the field tests. Therefore, we have also examined the methods USDA used to monitor the experiments and the reporting procedures established by the agency.

We reviewed 28 of 41 environmental assessments issued by USDA, from December 1988 through March 1990, in response to university, industry, and research organization applications for permits to conduct field tests of transgenic plants. In all 41 cases, the tests were approved and permits were issued.

We chose the 28 environmental assessments in our sample (Table 1) to meet our criteria of covering all plant-gene combinations in the population of the 41 environmental assessments and to allow us to compare several assessments of the same plant-gene combination. In two cases, a single environmental assessment covered the proposed release of two plant species or plant varieties: transgenic cantaloupe (*Cucumis melo*) and squash (*Cucurbita pepo*), both engineered to resist viral diseases (EA# 89-311-01), and two transgenic potato (*Solanum tuberosum*) cultivars (Russet Burbank and Lemhi Russet) developed to test the expression of engineered marker genes (EA# 89-257-04). Thus, in effect, we reviewed 30 distinct releases.

A content analysis was carried out for each environmental assessment selected for review to determine what categories of risks were being assessed, what types of information were used to evaluate each risk factor, and what conclusions were drawn. Follow-up interviews with relevant APHIS personnel were conducted to gain a better understanding of the general approach taken in the assess-

Table 1. Environmental assessments issued by USDA-APHIS between December 1988 and March 1990 that were reviewed for this study.

Reference EA#	Engineered organism	Engineered character	Applicant
88-344-07 88-236-01 90-019-01	Tomato	Delay fruit ripening	Calgene
89-293-01	Tomato	Disease resistance, tomato and tobacco mosaic virus	Monsanto
89-030-02 88-314-05	Tomato	Insect resistance, Bt δ-endotoxin	Monsanto
88-351-12	Tomato	Herbicide resistance, glyphosate	Calgene
89-097-01	Tobacco	Insect resistance, wound-induced protease inhibitor	Iowa State University
89-074-01	Tobacco	Insect resistance, cowpea trypsin inhibitor	Calgene
88-333-02	Tobacco	Insect resistance, Bt δ-endotoxin	Rohm & Haas
89-116-20	Tobacco	Lysine biosynthesis	Biotechnica
89-065-01	Tobacco	Heavy metal sequestration	University of Kentucky
89-034-11 89-034-12 89-034-15	Soybean	Herbicide resistance, glyphosate	Monsanto
89-034-10	Cotton	Herbicide resistance, glyphosate	Monsanto
89-047-07 89-192-01	Cotton	Herbicide resistance, bromoxynil	Calgene
89-150-01	Cotton	Insect resistance, Bt δ-endotoxin and herbicide resistance, glyphosate	Monsanto
88-351-13	Cotton	Insect resistance, Bt δ-endotoxin	Agracetus
89-030-03 89-030-04	Potato (Russet Burbank)	Disease resistance, potato x and potato y, and leafroll virus	Monsanto
89-257-04	Potato (Russet Burbank and Lemhi Russett)	Antibiotic-resistant marker genes	USDA/ARS
89-038-01	Alfalfa	Herbicide resistance, glufosinate	Northrup King
89-220-01	Walnut	Insect resistance, Bt δ-endotoxin	University of California, Davis
89-109-03	Poplar	Wound-induced enzyme production	Iowa State University
89-172-01	Cucumber	Disease resistance, cucumber mosaic virus	Cornell University
89-311-01	Cantaloupe and squash	Disease resistance, cucumber mosaic virus, and papaya ring spot virus	Upjohn

ment process and to clarify issues raised in specific environmental assessments.[2]

For each ecological risk factor, supporting evidence cited in the environmental assessments was classified according to eight evidentiary categories (see Table 2). In each case, we have indicated our rationale for the category assignment. Some interpretive flexibility is required in this classification scheme. For example, when environmental assessments contain statements that appeared to be drawn from the literature, but without citation, they were still classified in the evidentiary category "empirical data: literature cited" (Table 2). In other instances, we used information in the reports to decide whether conclusions expressed in environmental assessments, unaccompanied by evidence, were based on the familiarity of the reviewer with the subject or were simply unsubstantiated statements.

Areas of ecological concern examined in the environmental assessments are detailed below, along with an analysis of how those factors were applied to specific assessments of transgenic plants. We also describe how APHIS monitors field tests and gathers data on the results of field tests.

Dissemination of genetic material

A major concern in conducting field experiments using transgenic plants is the possibility that foreign genes in modified plants might be transferred to plants outside the test site. The environmental assessments address four issues involving dissemination of genetic material: the extent of movement of genetic material to plants of the same species (intraspecific cross-pollination or outcrossing); the determination of adequate isolation distances to prevent outcrossing; the extent of dissemination of genetic material to closely related plant species (interspecific cross-pollination or hybridization); and methods to prevent the dispersal of genetic material from seeds and vegetative plant parts sur-

[2]On 22 May 1990, we conducted an interview with the following personnel from the Biotechnology Permits Unit of the Biotechnology, Biologics, and Environmental Protection Division of APHIS, Hyattsville, Maryland, involved with the preparation of the environmental assessments: Arnold Fouldin, deputy director; James Lackey, biological safety officer; and Ellen Liberman, biotechnologist.

viving field tests and emerging undetected some time after the trial ends.

Most environmental assessments contain statements that cross-pollination is the only known mechanism in nature that can result in transfer of genes between flowering plants. Thus, for intraspecific and interspecific cross-pollination, elimination of risk is based on preventing viable pollen from transgenic plants reaching receptive plants outside the test site.

Intraspecific cross-pollination

The probability of intraspecific cross-pollination is related to the extent of outcrossing typical of the plant species and the distance that pollen from the plant is likely to travel and remain viable to fertilized conspecifics. To assess the extent of outcrossing, data from the literature were cited in all cases but one (EA# 89-257-04, Lemhi Russet potatoes). In Table 3, we categorized plants according to the extent they outcross based on information provided in the environmental assessments.

Some plants do not produce viable pollen. For Russet Burbank potatoes, where the risk of outcrossing is essentially zero because males are sterile, no special precautions to prevent outcrossing were specified in the experimental designs (EA# 89-030-03 and EA# 89-030-04).

The situation is different for another potato variety, Lemhi Russet, which does produce viable pollen. Based on documentation provided by the applicant (see Table 1), the environmental assessment for this cultivar stated that it was unlikely pollen would be transferred by insects. Referring to documentation provided by the applicant, APHIS concluded that "cross-pollination of other potato plants will not occur." The nature or details of the documentation were not specified. This evidence was classified as an unsubstantiated statement in Table 2 because no basis was given for the assurances of the applicant. It should be noted that isolation distances of 1000 feet were specified in experiments involving Lemhi Russet potato, so there were provisions in the design to prevent outcrossing even though the applicant considered it unlikely.

Some cultivars generally self-pollinate, but the possibility of cross-pollination cannot be ruled out. For all plants in this category physical isolation was used to reduce the probability of cross-pollination. In some cases border rows of nontransformed conspecifics were planted surrounding the test plots. Plants in the border rows were expected to trap pollen carried from plants by insects or wind. These plants could then be destroyed after the field test.

Some plant species are mostly self-incompatible or, even if able to self-pollinate, often outcross. To prevent escape of genetic material from these transgenic plants during field tests, plots were physically isolated, border rows were planted, plants were destroyed before sexual maturity, and/or all flowers were removed from the test plants before maturation of pollen. If all flowers are removed before pollen matures, the probability of cross-pollination is zero. Flower removal was not a requirement of all experiments involving outcrossers. Trials on transgenic squash and cantaloupe, which generally outcross and are insect pollinated (Free 1970), included provisions for physical isolation from conspecifics and border rows but no requirement that flowers be removed from plants (EA# 89-311-01).

Determination of adequate isolation distances

To prevent contamination by incoming pollen, plant breeders physically isolate plots of new varieties from conspecifics (NRC 1989). Published isolation distances are cited in environmental assessments to justify the choice of minimal isolation distance to assure that pollen from transgenic plants does not fertilize nontransformed conspecifics (23 of 30 field

Table 2. Number of environmental assessments (based on 30 plant cases), including each category of evidence for different ecological risk factors.

	Evidentiary category							
	Empirical data							
Ecological risk factor	Experimental data*	Literature cited†	General principles‡	No information indicating a problem§	Familiarity of reviewer with organisms‖	Unsubstantiated statement	Procedures followed during field test¶	Factor not addressed in environmental assessment
Dissemination								
Extent of outcrossing		29				1	28	
Isolation distance	2	23						7
Extent of hybridization		23						7
Plant disposal and elimination of seed bank							30	
Weediness		13	10					9
Competitiveness	1					27		2
Susceptibility to disease				30				
Palatability to insects				30				
Impact on flora	1				29			
Impact on fauna				30				

*Experimental data provided by applicant.
†Data obtained from the literature.
‡For example, a single gene addition is not sufficient to change a nonweed into a weed.
§For example, there is no evidence to indicate the transgenic plant is more palatable to insects than nontransgenic conspecifics.
‖For example, the reviewer is familiar with the local flora.
¶Procedures were to be followed during field tests to deal with risk factor (e.g., removing flowers before pollen matures to prevent outcrossing).

Table 3. The degree to which crop plants intraspecifically cross-pollinate (outcross).

Extent of outcrossing	Plant
No outcrossing	Potato (Russet Burbank)
Mostly self-pollinate	Potato (Lemhi Russet), tomato, tobacco, cotton, soybean
Mostly outcross	Alfalfa, walnut, cucumber, cantaloupe, squash, poplar

tests). Isolation distances established for traditional plant breeding, however, do not eliminate pollen contamination but reduce it to acceptable levels (NRC 1989). A stated assumption in all the environmental assessments is that "the risks associated with the introduction of genetically engineered organisms are the same... as those associated with the introduction... of unmodified organisms and those modified by other genetic techniques." This assumption is supported by reports issued by the National Academy of Sciences (1987) and the National Research Council (1989). Thus, levels of genetic contamination acceptable to plant breeders are *ipso facto* accepted by APHIS for field tests of transgenic plants.

Arriving at a minimal isolation distance for field testing transgenic plants is not a clear-cut matter. Published isolation distances are often best estimates and sometimes are based on observation of accidental mating in the field rather than on experimental evidence (Bateman 1947, Ellstrand and Hoffman 1990). Ellstrand (1988) believes that isolation distances now in use may grossly underestimate the probability of long-distance pollen dispersal. For example, one environmental assessment (EA# 89-311-01) cites literature suggesting that 400 meters is an adequate isolation distance for field tests of squash. Kirkpatrick and Wilson (1988), using allozyme analysis, found viable hybrid progeny in wild and cultivated squash separated by 1300 meters.

Plant breeders assign seeds different levels of certification that, in part, reflect different levels of seed purity. For example, five of seven environmental assessments on transgenic tomatoes cite the certified seed distance of 30 feet as the minimal isolation distance for the field trials, whereas two environmental assessments cite the more stringent registered seed distance of 200 feet (AOSCA 1971). Both isolation distances were described in environmental assessments as "the effective distance that tomato pollen can travel under field conditions and remain viable" (cf. EA# 88-344-07 and EA# 89-293-01). In reviewing the supporting citation (Rick 1976), no data or conclusion related to isolation distances and pollen viability could be found.

Two environmental assessments on transgenic tomatoes (EA# 88-314-05 and EA# 89-293-01) contain references to experimental studies on transgenic plants to justify the choice of isolation distances. These data, provided by the applicant, are cited by APHIS to illustrate that nontransformed tomatoes were not fertilized by transgenic tomatoes planted at distances of five and eight feet. None of the other environmental assessments we reviewed included this type of experimentally derived data.

The cotton crop provides another example of the difficulties and uncertainties in establishing absolute isolation distances. Because insects are the most important source of outcrossing in cotton (Free 1970), one would expect pollen movement to vary considerably between locations due to differences in pollinator abundance and type. Many factors, including planting density and shape, nectar production, and the phenology of the plants at the time of visitation influence pollinator behavior and the distances pollinators travel (Handel 1983). Literature is cited in the environmental assessments on cotton indicating various isolation distances, ranging from 100 feet to 1 mile, depending on cotton variety, location of trials, and on the judgement of different researchers of distances sufficient to prevent insect-mediated outcrossing.

Each of the environmental assessments on cotton cited the certified (660 feet) and registered (1320 feet) seed isolation distances, with the more conservative registered seed distance used as the minimal acceptable isolation distance for the releases. But given the variability of pollen transport in cotton due to local biotic and abiotic conditions and the fact that field tests are sometimes conducted within commercial cotton fields of cooperating farmers, it is possible that some percentage of the transgenic pollen fertilized nontransgenic cotton outside the experimental site.

The environmental assessments reviewed in this study did not address this possibility and did not establish acceptable levels of gene flow. APHIS representatives said that they had a good understanding of the biology of the plants proposed for field trials.[3] In view of the extensive history of field testing of genetically modified crop varieties without apparent mishap, they were confident that the containment measures chosen were sufficient to prevent cross-pollination.

APHIS does not require applicants to determine the extent and frequency of pollen movement nor the effectiveness of border rows in limiting the transmission of pollen during field tests. In only one case (EA# 89-311-01) was an experimental study cited in the published literature on the ef-

Table 4. Information presented in environmental assessments on the ability of plants to hybridize.

Plant	Ability to hybridize
Walnut	Intrageneric hybridization possible
Poplar	Hybridizes with other *Populus* spp.
Squash	Hybridization possible with four other cultivated *Cucurbita* spp.
Alfalfa	Interspecific crosses possible with relatives outside the United States
Tomato	Can only successfully hybridize with congenerics in South America
Soybean	Can only hybridize with congenerics, none of which occur in North America
Potato	Either the variety does not produce viable pollen or the variety self-pollinates
Tobacco	No evidence of any hybrids in nature; crosses in laboratory have low viability
Cotton	No discussion in the environmental assessments of species with which cotton can hybridize
Cucumber	No discussion in the environmental assessment of species with which cucumber can hybridize
Cantaloupe	No discussion in the environmental assessment of species with which cantaloupe can hybridize

[3]See footnote 2.

fect of insect pollinator movement on the distribution of pollen among conspecifics. It should be noted, however, that some permit applications included applicant-initiated provisions to collect data on pollen flow (e.g., EA# 89-047-07). Border rows surrounding plots of transgenic plants theoretically reduce the chance that pollen will be transported outside the test site. Field trials reviewed in this study used border rows ranging from a single row to 100 feet of rows of nontransformed plants. Generally, no justification was given for choosing the particular size or configuration of border rows or for the likelihood they would have their intended effect.

Interspecific cross-pollination

The probability of interspecific cross-pollination (hybridization) is greatest when two sexually compatible species exist in close proximity (Ellstrand and Hoffman 1990). Genes might be exchanged between a crop plant and a weedy relative. Subsequent mating between the hybrid and the weed might spread the trait through the weed population, a process known as introgression. Both hybridization and introgression between cultivated sorghums (*Sorghum* spp.) and Johnsongrass (*Sorghum halapense*), are held responsible for the creation of weedy hybrids and biotypes of Johnsongrass (Baker 1972, de Wet 1966). In California, cultivated radish has become a weed due in part to introgression with a weedy relative (Baker 1972).

Twenty-three environmental assessments reviewed in this study provided a literature review of the tendency of the test plants to cross-pollinate and successfully hybridize with other plant species (Table 4). Most of the plants do not readily cross with related species without human intervention, or, if they can cross, the plants with which they are compatible do not exist in the region of the test site. For example, the only plants with which soybean is compatible are in Asia and Australia, the only plants with which tomato is known to cross are in Central America and South America, and tobacco is not known to cross naturally with any other related species. Walnut, which can hybridize with related species in the test region, was designated to have bags placed over male flowers to prevent pollen escape. Poplar, which can hybridize with other *Populus* species in the United States, was to be destroyed before reaching sexual maturity.

Two environmental assessments examined proposed field tests of cotton in Hawaii, where transgenic plants would be allowed to flower. Wild native cottons are known to inhabit the islands (Neal 1965). Neither environmental assessment presented any specific information on the potential for cultivated cotton to hybridize with these relatives.

In one assessment (EA# 89-150-01) an endemic cotton species, *Gossypium tomentosum*, was reported to exist in Hawaii. However, it was not considered a hybridization risk because it "is apparently not widely prevalent or may be extinct on Kauai Island," the site of the field test. No authority was cited for the statement. No provision was included in the field-test procedures to assure that the native cotton species was not present near the test site. Test plots were to be surrounded by an unspecified number of nontransformed cotton border rows.

In a second environmental assessment (EA# 89-192-01), several native cottons inhabiting Hawaii were listed with *G. tomentosum* the only congeneric. It was reported, with an authority cited, that *G. tomentosum* was not generally found in the area of the test site. Provision had been made in the test protocol to survey the site for *G. tomentosum*. Border rows 100 feet wide consisting of nontransformed cotton were to surround the test plot.

Most environmental assessments contained information about the likelihood of hybridization between the crop plant and wild relatives, but we found no information on the tendency of cotton, cucumber, or cantaloupe to hybridize. Some of these environmental assessments contained references to sections within the reports reputed to address hybridization, but the information was not presented (e.g., EA# 88-351-13 and EA# 89-192-01).

Disposal of plants and the elimination of the seed bank

All environmental assessments contained descriptions of the methods to be used after field trials to dispose of transgenic plants, to eliminate seeds from the seed bank, and to detect and destroy volunteer plants (Table 2). Examples of these methods for tomato and cotton (see Table 5) show that in different field trials using the same transgenic plant species, control techniques vary considerably. For example, monitoring of tomato test plots takes place for two months in one experiment, six months in another, and into the next year in a third. In one experiment, the field is disked, in another disked and irrigated, and in a third disked, fumigated, disked again, and then sprayed with herbicide. There is no explanation in the environmental assessments why a particular method is preferable or used in one instance and not in another. APHIS's stated mandate is to

Table 5. Comparison of methods used for disposal of plants and elimination of seeds from the soil after field tests.

Plant/transferred gene (environmental assessment)	Method of plant disposal and seed elimination
Tomato/TMV coat protein gene (89-293-01)	Disk plants into soil, cultivate, monitor field next spring for volunteers
Tomato/Bt δ-endotoxin gene (88-314-05)	Disk plants into soil, apply herbicide to field, and monitor field for volunteers for two months
Tomato/Bt δ-endotoxin gene (89-030-02)	Disk, irrigate field for two months, monitor for volunteers
Tomato/fruit-ripening gene (90-019-01)	Disk, fumigate field, disk, apply paraquat, and six-month fallow period
Cotton/Bt δ-endotoxin gene (88-351-13)	Monitor field one year for volunteers
Cotton/Bt δ-endotoxin gene and glyphosate resistance (89-150-01)	Cultivate for two months, monitor for volunteers
Cotton/glyphosate resistance (89-047-07)	Disk, monitor field next spring
Cotton/bromoxynil resistance (89-192-01)	Autoclave all plants, monitor field (time unspecified) for volunteers

determine whether the methods proposed in the applications are adequate to prevent escape of genetic material. Applicants are free to use more stringent methods if they desire.[4]

Determining the appropriate methods needed to eliminate seeds and volunteers requires an evaluation of the persistence and reproductive capabilities of the plants in question (NRC 1989). This information is generally not included in the environmental assessments. USDA also does not require any experimental work to be done on the cold-hardiness of seeds or the length of time seeds can remain viable in the soil. No environmental assessment we reviewed contained these data (Table 2). Thus, it is not made clear if the procedures employed will rid the seed bank of all viable seeds or remove some percentage of possible propagules.

None of the environmental assessments addressed the potential movement of seeds out of the test site by vertebrates (e.g., birds or rodents) or invertebrates (e.g., ants or beetles). If seeds were to germinate outside the test site, detection would be difficult at the level of phenotype, because all environmental assessments indicated that transgenic plants were morphologically identical to nontransformed plants. APHIS personnel acknowledged in the interview that seeds might be moved outside the test site by animals. They expected applicants to take whatever steps were necessary to minimize animal intrusion onto test sites. However, they did acknowledge it is possible that some seeds have escaped during field trials due to animal dispersal.

Other ecological factors

Weediness. Crop plants are intimately related to weed species; some crop plants are descendants of weed species, and some weed species are derived from crops (Harlan 1982). Once introduced into new regions, crop plants may become naturalized weeds. For example, guava is an aggressive weed in Hawaii (Harlan 1982). Pimentel et al. (1988) found 120 cases of agricultural and ornamental plants that were introduced into the United States and became pest weeds. There has been debate over the appropriateness of the analogy made between introduced nonnative organisms and genetically engineered organisms (Colwell 1988, NRC 1989, Tiedje et al. 1989), but concern remains that genetically altered crop plants released into the environment might become problem weeds (NAS 1987, NRC 1989).

All environmental assessments stated that the risk of transformed plants expressing plant pest characteristics such as weediness would be examined. However, in nine environmental assessments (32%) we could find no further discussion of potential weediness. In the other environmental assessments, we found little information directly addressing the risk of weediness. Thirteen environmental assessments contained some, although usually limited, information from the literature on the weed status of the plant or close relatives, or the tendency of the plants to be found as volunteers.

APHIS takes the position that if an untransformed crop plant is not considered a weed, then changing a single gene cannot transform the plant into a weed.[5] Ten environmental assessments concluded that "traits that lead to weediness in plants are polygenic traits and cannot be conferred by adding a single gene" (evidentiary category: based on general principles; Table 2). No citation was given to support this statement, and no information was presented in the environmental assessments to substantiate this conclusion.

It is true that weediness is the result of a combination of plant characteristics (Baker 1965). The question remains, what characteristics does the nonweedy plant possess that might predispose it to become a weed if conferred with additional phenotypic traits? Crop plants have been shown to have significantly fewer weedy traits, on average, than weedy plants, as well as a random sample of plants selected from a British flora (Keeler 1989). However, this observation should not lead to the conclusion that all crops cannot become weeds, because some crops have more weedy traits than some weeds (Keeler 1989).

Enhanced competitiveness. Related to the potential of a genetically altered plant to become a weed, as well as to its impact on the native flora, is the assessment of any changes in the ability of the transformed plant to compete with wild species. All environmental assessments, except one (EA# 89-257-04) on Russet Burbank and Lemhi Burbank potato, contained some statement concerning the competitive ability of transgenic plants. For 24 plants, it was concluded that neither the transferred gene nor its gene product provided any measurable selective advantage to the plant in its ability to disseminate or become established. In three environmental assessments, the reviewer concluded that there was a slight advantage for the engineered versus the nontransformed plant. Two of these (EA# 89-030-02 and EA# 88-333-02) were for plants transformed to express Bt δ-endotoxin, and the third was for herbicide-tolerant alfalfa (EA# 89-038-01). No data or discussion are provided or cited for any of the conclusions on competitive ability presented in these 27 environmental assessments. Thus, all were categorized as unsubstantiated statements in Table 2. Only one environmental assessment (cotton/Bt δ-endotoxin, EA# 88-351-13) cited explicit data from an applicant that related to plant vigor and seed production in transformed versus nontransformed plants.

Although some of the engineered characteristics, such as delayed fruit ripening and genetic markers, appear to have no obvious competitive value, others, such as tolerance to insect herbivores and disease, would seem to confer competitive advantage over plants without such characteristics. APHIS personnel[6] stated they believe that engineered plants carry an added metabolic load (the cost of having to increase protein synthesis as a result of gene additions) that put them at a competitive disadvantage compared with nontransformed conspecifics (Tiedje et al. 1989). For that reason,

[4]A. Foudin, 1991, personal communication. USDA/APHIS, Hyattsville, MD.

[5]See footnote 2.

[6]See footnote 2.

they felt that even if seeds from the transformed plants were to escape from the field site, there was little chance the resultant seedlings could compete successfully with wild plants.

Susceptibility to pathogens or changes in palatability to insects. Genetic modification of plants might unintentionally increase the susceptibility of the plant to disease or increase the palatability of the plant to herbivores. Although increased susceptibility or palatability may not be viewed as an environmental risk, it is included in environmental assessments as an important consideration when modifying crop plants. Each environmental assessment contained a statement indicating there was no reason to believe that the genetic changes conferred on the plants being considered for field testing would result in increased susceptibility to pathogens or increased palatability to herbivorous insects, compared with nontransformed conspecifics. Thus, the assessment of this ecological factor for all 30 plant cases was based on a lack of information supporting a risk. Each environmental assessment went on to explain that if there were unexpected effects they would be limited to the plants at the test site and would be detected by monitoring of the plots.

Impact on native flora. This section of the environmental assessments addressed the concern that germ plasm from transgenic plants might be transferred to the native flora. All environmental assessments stated that the transmission of genetic material from the transgenic plants to any plants in the local community was highly unlikely. This conclusion was based on two factors: first, there were no plants adjacent to the test site with which transgenic plants were sexually compatible, and, second, the measures taken to physically isolate the transformed plants within the test site were sufficient to preclude gene escape. In only one case (EA# 89-116-20) was there a species inventory of plants adjacent to the test area cited as documentation of the fact that there were no species present near the test site with which cross-pollination was possible. For the 29 other cases, the conclusion of no impact on native flora was attributed to the familiarity of the reviewer with the local flora.

Impact on native fauna. Another section of the environmental assessments addressed the potential harm that transgenic plants might cause to animals in and around the test site. Each environmental assessment concluded that there was no identifiable factor unique to the field tests that would affect any vertebrate or invertebrate animal species other than targeted herbivorous insects in some trials. No data or literature is cited for this conclusion. Consequently, the finding of no impact on native fauna for the 30 plant cases was attributed to lack of any information to the contrary. The conclusion appears to be based on an assessment of the intended effects of the genetic alteration on the plant and the assumption that no unintended effects on the fauna might occur.

Oversight of field tests and field reports

The finding of no significant impact for each environmental assessment we examined was based, in part, on the assumption that the experimental methods and monitoring procedures described in the reports would be followed by the applicant. In interviews with APHIS personnel, it was learned that the ability of the agency to detect possible problems in experiments relies largely on the company or university personnel conducting the experiments. Each environmental assessment states that an APHIS representative will visit the site at or shortly after the initiation of the field test to determine if the experimental design and any other conditions of the permit are being followed. Any follow-up visits after the initial site visit are at the discretion of the field representative. It was the responsibility of the experimenters to report to APHIS any problems encountered during the field test.

Gathering ecological and environmental data during small-scale field tests is important to assure that containment procedures are effective and could be useful in evaluating proposals for additional small-scale or large-scale releases. Researchers evaluating the results of genetic transformation understandably have a primary interest in determining the expression levels of the transferred genes and the efficacy of the new phenotypes. They are likely to be less concerned with monitoring pollen dissemination or other ecological and environmental effects of the transgenic plants.

APHIS personnel[7] indicated that they required the applicant only to collect data essential to ensure the safety of the particular field study under consideration. For example, once APHIS grants a permit for a field test, it considers the approved experimental design, including isolation distances and border rows, sufficient to prevent dissemination of genetic material. APHIS does not require the applicant to monitor gene flow during field tests to confirm that isolation distances and border rows have been effective. In interviews, APHIS representatives asserted that some additional environmental information might be useful in evaluating future field test applications; however, they could not impose conditions on an applicant doing small-scale tests based on what the applicant might propose to do in the future.

APHIS requires applicants to submit a report of the results of field tests within a year of the termination of the experiment. If we assume that field tests approved by APHIS before July 1988 would have been completed by July 1989, 19 field test reports should have been submitted to APHIS by July 1990. However, as of July 1990 only 4 reports[8] had been received. In our interview, APHIS personnel agreed that applicants had been lax in submitting reports and that more emphasis on this requirement was needed.

Copies of the four reports were reviewed for this study. The reports did not contain information pertaining to the dissemination of pollen to other plants in and around the field site, quantitative data on the effects of the transgenic plants on herbivorous insects or other organisms, or comparisons between transformed and nontransformed plants on character-

[7]See footnote 2.

[8]The field reports were for EA#s 88-314-05, 88-344-07, 89-030-04, and 89-065-01.

istics such as morphology and vigor. Each report contained a section indicating that there were no unusual problems encountered during the field test, that transgenic plants grew normally, and that no evidence of *Agrobacterium* infection was observed. Some reports indicate no seed germination during the post–field test monitoring period.

In February 1991, we reviewed three more reports[9] and found them similar in content to the four reports described above. Although APHIS personnel[10] concede that the reports still lack sufficient data and detail, they believe there has been improvement. As an example, they cite two recent reports submitted by applicants at the conclusion of field tests providing detailed data on gene movement from transgenic cotton plants into border rows.

Recommendations

On the basis of our review of the first generation of transgenic plants evaluated by USDA for small-scale release permits and subsequent interviews with regulatory officials, we make some recommendations for improving the assessment process. Containment of genetic material is the most important requirement for assuring the safety of small-scale field tests of transgenic plants. If genetic material is effectively contained and disposed at the test site, problems associated with establishment of transgenic plants in natural or agricultural habitats are eliminated. Beyond complete containment, a second line of defense is to ensure that neither the transgenic plant nor its foreign genes can adversely affect other life forms.

Our most general recommendation is that APHIS should be more explicit about justifications for its assessment conclusions. Unsubstantiated statements and conclusions based merely on the lack of information suggesting no adverse effect should be avoided. When the environmental assessments relied on empirical data, the data were almost exclusively from the extant literature. In evaluating the adequacy of containment procedures, more relevance should be given to data that are closely related to the experimental conditions.

APHIS should encourage experimental studies to quantify gene flow between conspecifics and between transformed plants and close relatives. Some may argue that the risk of dissemination of genetic material for most crop plants does not warrant delaying field tests until experimental data on gene flow can be collected. We sympathize with this view, but we recommend that APHIS require applicants to include provisions in experimental designs for determining the extent of gene flow during field tests.

Transgenic plants provide an easy mechanism for tracking pollen movement because they contain marker genes (e.g., resistance to an antibiotic) for identification. Such data would prove useful in evaluating future small-scale tests and eventually large-scale commercial release.

There appears to be almost no empirical data available to predict the outcome if transgenic plants were to escape and become established in natural or agricultural habitats. Environmental assessments tend to discount, with little evidence, the potential risk posed by the escape of weedy transgenic plants capable of competing with crops or native plants.

We believe that the potential for weediness and enhanced competitiveness of transgenic plants or any genetically novel plants should be assessed on a case-by-case basis. The information needed for evaluation includes the extent to which the crop is found in fields in subsequent growing seasons (volunteers), the weedy traits characteristic of the untransformed crop, whether the crop is now considered a weed in some habitats, the existence of weedy relatives of the crop, and the likelihood that the phenotypic character(s) transferred to the crop would influence its ability to invade and persist in arable or natural habitats. In cases where there is not adequate information from the literature or where there is significant risk of weediness, experimental studies such as those described by Crawley (1990) on the persistence and spread of novel plants should be initiated. These studies need not delay small-scale field tests but could be included in field test designs or conducted concurrently.

Because APHIS relies to a large extent on monitoring of experiments by applicants, the submission of detailed reports is important in determining if the procedures followed during the field tests were adequate to minimize or eliminate risk. Our review of a small number of field reports submitted after experiments were completed and our conversations with APHIS personnel indicate the reports do not provide sufficient detail. We therefore conclude that the opportunity to collect important data on the environmental effects of transgenic plants is not being fully realized. We recommend that APHIS require applicants to submit timely reports that provide detailed data on environmental questions, such as the effectiveness of containment measures, dormancy characteristics of transgenic seeds, and the impact of transgenic plants on other organisms and the environment.

The success of genetic engineering for crop improvement depends on public confidence that there is sufficient oversight to minimize the possibility that a transgenic plant might cause adverse environmental effects. USDA has put in place a permit unit that has reviewed and approved almost 200 permit requests to date. As far as we can determine, no environmental problems have resulted from field tests of transgenic plants that have been conducted since 1987. We now have the opportunity to evaluate and implement improvements to the oversight process as the development of plant biotechnology accelerates.

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[9]These reports were for EA#s 89-047-04, 89-320-01, and 90-044-05, which were not among the environmental assessments reviewed for this study.

[10]See footnote 4.

expressed in this work are solely those of the authors.

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