

When crop transgenes wander in California, should we worry?

by Norman C. Ellstrand

The movement of transgenes into populations for which they are not intended remains a primary concern for genetically engineered crops. Such gene flow in itself is not a risk. However, we know that the transfer of genes from traditionally improved crops into wild populations has already resulted, on occasion, in the evolution of weeds more difficult to control, as well as an increased extinction risk for rare species. Just like traditional crops, genetically engineered crops could occasionally create the same problems. Currently in California, the movement of transgenes from most commercialized transgenic crops into wild plant populations is unlikely — the exception being canola. However, other transgenic plants have been field-tested in California, and if these become commercialized, in certain cases, transgenes are likely to move into the wild or into other crops of the same species. Such gene flow could result in various problems. The best containment for transgenes may involve risk assessment decisions by scientists embarking on projects to determine whether the proposed combination of organism and trait will pose any problems and if so, to determine how to create a safe product.

IN 1985, scientists published the first two papers addressing the potential environmental impacts of genetically engineered crops. California scientists played important roles in writing both. Senior personnel at CalGene, a California-based genetic engineering firm, wrote one paper (Goodman and Newell 1985), and



Scientists are studying the implications of gene flow between cultivated crops and their wild relatives, a common phenomenon between plant species. For example, studies have considered, *top left*, cultivated wine grape (*Vitis vinifera*) and its wild relative, *top right*, California wild grape (*Vitis californica*), as well as, *bottom left*, crop radish and, *bottom right*, wild radish (*Raphanus sativus*).

a UC Berkeley faculty member was senior author of the other (Colwell et al. 1985). Both articles prominently featured the possibility that hybridization could serve as an avenue for the unintended movement of engineered genes (transgenes) from transgenic crops into populations of related weeds. Such movement of genes between species or populations, called “gene flow,” in itself does not pose a risk. Gene flow by pollen and seed between cross-compatible populations is not uncommon, and often plays an important role in both evolution and plant breeding (Ellstrand 2003a).

Both papers pointed out that the presence of crop genes in wild populations has long been recognized as a stimulus for the evolution of increased

weediness or “superweeds” (Anderson 1949). Goodman and Newell (1985) stated the problem succinctly: “The sexual transfer of genes to weedy species to create a more persistent weed is probably the greatest environmental risk of planting a new variety of crop species.”

Other potential environmental problems of transgenic crops were anticipated and discussed in those articles (see sidebar, page 119), but the risks associated with the unintentional movement of engineered genes into populations for which they were not intended continue to receive the most attention in both scientific publications and the popular press. This attention may stem, in part, from the fact that the movement of unwanted crop genes into the environment poses more of a management

dilemma than unwanted nonliving “pollutants.” For example, a single molecule of DDT remains a single molecule or degrades. But a single crop allele occurs within an organism that may have the opportunity to multiply itself — and that allele — repeatedly through reproduction. The fact that unwanted genes can increase their numbers could frustrate attempts at recall or containment. Indeed, almost every general treatment of the environmental impacts of plant biotechnology gives some consideration to gene flow (Dale et al. 2002; Hails 2000; Marvier 2001; NRC 1989, 2000, 2002, 2004; Nickson and Head 1999; Rissler and Mellon 1996; Scientists’ Working Group on Biosafety 1998; Snow et al. 2005; Wolfenbarger and Phifer 2000), and a book on the topic was recently published (den Nijs et al. 2004).

Over the last 17 years, my research program has evolved to focus on the topic, addressing the following questions: How likely is it that transgenes will move into and establish in natural populations? And if transgenes do move into wild populations, is there any cause for concern? Traditionally improved crops can serve as models for the behavior of transgenic crops; indeed, the U.S. regulatory framework for transgenic plants is based on this assumption (NRC 1989, 2002). Experience with traditional crops and experiments using them can provide a tremendous amount of information for answering these questions.

Spontaneous hybridization study

In the early 1990s, the general view was that hybridization between crops and their wild relatives occurred extremely infrequently, even if they were growing in close proximity. This view was probably due to the difficulties breeders sometimes have in creating crop-wild hybrids (Fehr 1987). My research group set out to measure spontaneous hybridization between wild radish (*Raphanus sativus*), an important California weed, and cultivated radish (the same species), an important California crop (Klinger et al. 1991). (It is not unusual for a crop to be closely related to a weed of the same species.)

In 1988 and 1989, we grew the crop as if we were multiplying commercial seed and surrounded it with stands of

GLOSSARY

Allele: Type of a gene. For example, the common gene involved in human blood typing involves three alleles: A, B and O.

Allozyme: Allelic protein products of a single gene that can be visualized with starch gel electrophoresis and subsequent appropriate staining.

Cultivated and wild types: Cultivated crops may have an array of wild relatives. (See box below.)

Fitness: Reproductive success as determined by survivorship and fecundity.

Gene flow: Migration of genes among populations. In humans, this involves migration of individuals. In plants, it may also involve pollen or seed movement.

Hybridization: Crossing among individuals from genetically distinct lineages. (See box below.)

Outcrossing: Reproduction that involves crossing between two different individuals. In humans and many animals, all reproduction is due to outcrossing. In some plants and animals, self-fertilization (selfing) is also an option, as well as reproduction without fertilization (asexuality).

Phenotype: Trait or traits expressed by an organism.

Taxon (taxa): Taxonomic unit, such as species or subspecies.

A CLOSER LOOK

At hybridization . . .

Hybridization refers to crosses between individuals of different but related species. When this is the case, there are reproductive, isolating barriers that either reduce the chances of mating, such as differences in flowering, or reduce the chances of progeny passing on their genes, such as hybrid sterility (for example, the mule is a hybrid between the horse and the donkey, two separate species). Reproductive isolating barriers may be minimal or absent in the cases of hybridization between subspecies, between different varieties of the same crop, or between cultivated crops and crops that have gone wild (feral). Plant breeders often intentionally make artificial crosses to transfer beneficial traits. Spontaneous, natural hybridization is relatively rare in animal species, but is more common for plants.

. . . and cultivated versus wild type

Plants that are intentionally cultivated as crops may have an array of wild relatives. Some wild relatives may be the progenitors of the crop or other taxa whose ancestors were not cultivated. Also, some crops establish free-living (feral) populations. These populations may take off on their own evolutionary trajectory to become weeds of varying importance. In some cases, a single gene difference, such as bolting in beets, can change a crop into a weed. Finally, for many crops there are wild, weedy relatives that are descended from hybrids between cultivated plants and wild individuals without cultivated ancestors.

— N.C. Ellstrand

Right, sorghum is an important global food-grain crop, while its wild relative, far right, johnsongrass (*Sorghum halepense*), is one of U.S. agriculture's most troublesome weeds. Sorghum and johnsongrass have different numbers of chromosomes, but UC scientists found that they spontaneously hybridized when grown within 330 feet of each other.



Jack Kelly Clark

weeds at varying distances. When the plants flowered, pollinators did their job. We harvested seeds from the weeds for progeny testing. We exploited an allozyme allele that was present in the crop and absent in the weed to detect hybrids in the progeny of the weed. The experiment was repeated at the UC South Coast Research and Extension Center and at the UC Riverside Moreno Valley Field Station. At both locations, we found that many of the weed seeds analyzed at the shortest distance of 3.3 feet (1 meter) were sired by the crop (40% hybridization), and that a low level (about 2%) of hybridization was detected at the greatest distance of 0.62 mile (1 kilometer). It was clear, at least in this system, that crop alleles could enter natural populations.

But could they persist? The general view at that time was that hybrids of crops and weeds would be handicapped by crop characteristics that are agronomically favorable, but a detriment in the wild. The expectation was that crop-wild hybrids should have inferior fitness in the wild, compared to their wild parents.

We tested that view by comparing the fitness of the hybrids created in our first experiment with their nonhybrid siblings (Klinger and Ellstrand 1994). We grew them side by side under field conditions. The hybrids exhibited the huge, swollen root characteristic of the crop, but the

pure wild plants did not. The two groups did not differ significantly in germination, survival or ability for their pollen to sire seed. However, the crop-wild hybrids set about 15% more seed than the wild plants. In this system, hybrid vigor would accelerate the spread of crop alleles in a natural population.

Exception to the rule?

When I presented these results at seminars, scientists questioned the generality of the results. "Isn't radish probably an exception?" they asked. "After all, radish is outcrossing and insect-pollinated. Its wild relative is the same species. What about a more important crop? What about a more important weed?"

We decided to address these criticisms with a different combination of crop and wild relative. Sorghum (*Sorghum bicolor*) is one of the world's most important crops, and johnsongrass (*S. halepense*) is one of the world's worst weeds. The two are distinct species, even differing in chromosome number, and sorghum is largely self- and wind-pollinated. The sorghum system was about as different from radish as you could get.

We conducted experiments with sorghum that paralleled those conducted with radish. We found that sorghum and johnsongrass spontaneously hybridized, although at rates lower than the radish system, and we detected crop

alleles in seed set by wild plants growing 330 feet (100 meters) from the crop (Arriola and Ellstrand 1996). The fitness of the hybrids was not significantly different from their wild siblings (Arriola and Ellstrand 1997). The results from our sorghum-johnsongrass experiments were qualitatively the same as those from our cultivated radish-wild radish experiments.

Other labs have conducted similar experiments on crops such as sunflower (*Helianthus annuus*), rice (*Oryza sativa*), canola (*Brassica napus*) and pearl millet (*Pennisetum glaucum*) (Ellstrand 2003b; den Nijs et al. 2004). Almost all such studies obtained qualitatively similar results to those obtained by my research group. There are a few exceptions; for example, experiments have shown that potato (*Solanum tuberosum*) does not naturally mate with the wild species *S. dulcamara* and *S. nigrum* under field conditions (McPartlan and Dale 1994).

Additionally, descriptive studies conducted in my lab and others have often found crop-specific alleles in wild relatives when the two grow in proximity. In California, alleles from sugarbeets are found in populations of the wild beet *Beta macrocarpa* in the sugarbeet production region of the Imperial Valley, where the latter is a weed in and near sugar-

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Scientists evaluate potential environmental risks of transgenic crops

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A relatively small group of scientists — including some Californians — have taken a hard and thoughtful look at the potential risks of transgenic crops. These varied scientists — including ecologists, soil biologists, agronomists, geneticists, entomologists, pathologists, horticulturists, botanists and molecular biologists — realize that traditional plant improvement and agriculture have, on occasion, created problems, and those problems can serve as models for anticipating the possible downsides of transgenic crops. A set of straightforward, scientifically based concerns has evolved. The most widely discussed concerns fall into two broad categories: (1) problems created directly by growing the crops themselves, and (2) problems created by unintended descendants of those crops.

Environmental biosafety is a relatively new and rapidly developing research area. An excellent source of information on this field is the National Research Council's (NRC 1989, 2000, 2002, 2004) series of peer-reviewed reports on the potential environmental impacts of agricultural biotechnology. The most up-to-date information can be found in peer-reviewed, disciplinary journals such as *Environmental Biosafety Research*, *Ecological Applications* and *Molecular Ecology*.

Direct impacts of crops themselves

Scientific consideration of the direct impacts of transgenic crops has focused almost exclusively on the evolution of pests that are resistant to new strategies for their control, and unwanted impacts on species in associated ecosystems. Another area of concern is the unwanted impacts on surrounding plant and animal communities from the use of transgenic herbicide-resistant plants (Firbank et al. 2003). Resistance to one or more herbicides is a general feature of most crops; also, resistance to herbicides can often be obtained through nontransgenic techniques (Duke et al. 1991). The impact of herbicide-resistant

crops on surrounding community diversity depends largely on the type of herbicide, and where and how it is used.

Evolution of resistant pests. Insects, weeds and microbial pathogens often evolve resistance to controls used against them (Barrett 1983; Georgiou 1986; Green et al. 1990). When a pest evolves the ability to attack a crop, the results sometimes can be devastating. The 1970 corn leaf blight epidemic ravaged American cornfields, resulting in the loss of tens of millions of dollars to the industry (NRC 1972).

Resistance evolution is also expected to occur in pests targeted for control by or associated with transgenic crops. Although the evolution of resistance is a continuous process, the evolution of resistant pests has been considered a potential environmental hazard of transgenic crops because more environmentally damaging alternative treatments would then be needed for control. Furthermore, transgenic products at present have resulted in the use of a single, uniform control method over huge areas.

For example, most of the transgenic corn and cotton now grown in the United States, millions of acres, is engineered with a bacterial gene that allows them to manufacture their own pesticide to specifically target certain insect pests. Because the gene comes from the bacterial species known as *Bacillus thuringiensis*, these plants are commonly known as "Bt corn" and "Bt cotton." Bt cotton is the most important transgenic crop in California (Taylor et al. 2004). Because the transgenic product does not kill all insect species, it is considered relatively environmentally benign. But the evolution of resistance to Bt crops is considered inevitable (NRC 2000). The U.S. Environmental Protection Agency has issued guidelines mandating that farmers plant "refuges" of non-Bt varieties in plantations of Bt varieties to prevent or delay the evolution of resistance. Despite the commercialization of Bt crops for almost a decade, no pests have yet evolved resistance to Bt crops in the field, sug-



Genetic engineering has been used to modify all of the crops and products shown, although most are not commercially available. The environmental risks of growing transgenic crops could include pest resistance and unintended effects on nontarget species, such as increased weediness among similar wild plants.

gesting that the refuge strategy has been effective (Tabashnik et al. 2003).

Effects on nontarget species. A crop engineered to interfere with the reproduction or viability of one or more pest species might also interfere with other nonpest species. For example, Bt corn was developed to control certain moth species that damage the crop. Reports of potentially toxic effects of Bt corn pollen and flower parts eaten by monarch butterfly larvae captured widespread attention (Losey et al. 1999). A flurry of subsequent research demonstrated that the effects of Bt pollen on monarch larvae are highly variable, depending on factors such as pollen density, the crop's Bt genotype and environmental factors (Sears et al. 2001). Current commercial Bt corn varieties are not considered hazardous to monarch larvae, but one variety no longer grown would have been. This example illustrates that risk assessment research can clarify whether a putative risk is, in fact, a problem.

But is this a new environmental problem? One might ask, "Isn't it better



The National Research Council has published numerous science-based reports on the environmental risks and benefits of transgenic plants and food.

to deploy a pesticide through a plant that kills only a subset of insects than to spray one on a field that kills all insects willy-nilly?" The answer, of course, would be, "Yes, Bt in corn reduces environmental impacts relative to spraying broad-spectrum insecticides." However, prior to the advent of Bt corn, many American corn farmers did *not* spray insecticides to control the pests controlled with Bt (NRC 2000). Those farmers simply took their chances without any control, possibly because the damage from the lepidopteran pests of corn varies so much from one year to the next. In the latter comparison, the addition of Bt, if it carries adverse nontarget effects, does pose a new problem.

Unintended crop descendants

All plants — including crops — are capable of some type of reproduction. The possibility of unintended reproduction by transgenic crops has raised questions about whether their descendants might cause problems. These problems have fallen into two broad categories: first, that the direct feral descendants of the crops may prove to be new weeds or invasives, and second, that unintended hybrids between transgenic crops and other plants could lead to certain problems.

If deregulated and grown widely, these potential future crops will require further scrutiny for possible gene-flow problems.

Progeny of the transgenic crop could become a problem if the transgenic trait alters their ecological performance such that they evolve increased aggressiveness. Some crop plants — especially those with a long history of domestication (e.g., corn and soybeans) — pose little hazard because traits that make them useful to humans also reduce their ability to establish feral populations in either agroecosystems or nonagricultural habitats (NRC 1989). But other cultivated plants (e.g., certain forage grasses and turf grasses, ornamentals, rice, rye, alfalfa) often volunteer after cultivation, founding feral populations that create problems (Gressel 2005). In some cases, the tendency to found feral populations could increase as the result of acquiring new traits.

The factors that foster or limit invasiveness are not well understood (Sakai et al. 2001). Most of the current transgenic crop traits — insect, virus and herbicide resistance — are expected to confer a fitness advantage in certain environments. Empirical evolutionary-genetics studies have demonstrated that a new allele that confers a fitness advantage will usually spread rapidly through a population, but it will not necessarily result in the evolution of invasiveness (Bergelson 1994). The mere presence of a transgene that increases fitness cannot be taken as certainty that the invasiveness of a population has increased. Many crops are unlikely to become weedier by the addition of a single trait (Keeler 1989). In a few cases, however, the consequences might be obvious. The evolution of herbicide resistance in a weed population that was previously controlled by that chemical will force the consideration of new control options.

Scientifically based assessment

Genetically engineered crops are a heterogeneous group. It is no more reasonable to lump them all together to argue that, as a group, they pose an environmental danger than it is to lump them all together to argue that, as a group, they will feed the world and

cure disease. It is fair to say that just like the products of traditional plant improvement, certain products of genetic engineering will create problems. To the extent that those products can be compared to traditionally improved plants, scientifically based hazards can be identified.

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beet fields (Bartsch and Ellstrand 1999). Likewise, genetic analysis of putative spontaneous hybrids has demonstrated that cultivated grape mates with wild grape species in California (Olmo and Koyama 1980). The data from such descriptive studies and experiments provides ample evidence that if cultivated plants and their wild relatives occur in close proximity, occasional spontaneous hybridization is not unusual. This phenomenon is a general feature of most of the world's important crops, from avocado to corn, and soybean to mushrooms (Ellstrand 2003b; den Nijs et al. 2004). Even the sorghum-johnsongrass results, involving a crop so different from a wild relative that their chromosome numbers are different, have not been shown to be an exceptional case.

Impacts of natural hybridization

When I gave seminars on the results of these studies, I was met by a new question: "If gene flow from crops to their wild relatives is going to be a problem for crops improved by genetic engineering, then wouldn't such problems already have occurred for species improved by traditional, nontransgenic methods?" A good question. I conducted a thorough literature review to find out what was known about the consequences of natural hybridization between the world's most important crops and their wild relatives, a multi-year odyssey of digging through diverse literature and interviewing dozens of the world's experts on important crops and their wild relatives (Ellstrand 2003b).

I found that on occasion, crop-to-weed gene flow has created hardship through the appearance of new or more-difficult weeds. Hybridization between wild plants and their cultivated relatives has been implicated numerous times in the evolution of new weeds or the evolution of increased weediness in pre-existing weeds (Ellstrand 2003b). Especially notable is Europe's new weed beet, the spontaneous hybrids between sea beet (*Beta vulgaris* subsp. *maritima*) and sugar-beet (*B. vulgaris* subsp. *vulgaris*) and their descendants. This weed has cost Europe's sugar industry well over a billion dollars in reduced yields, damaged machinery and control costs (den



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Scott Bauer/USDA-ARS

While crop-to-wild plant gene flow has been widely documented in numerous combinations, an exception is, **bottom**, potato (*Solanum tuberosum*), which does not naturally cross with the wild related species, **top**, European bittersweet (*Solanum dulcamara*) under field conditions.

Nijs et al. 2004; Ellstrand 2003b; Parker and Bartsch 1996).

Crop-to-wild gene flow can create another problem. Theoretical models have demonstrated that hybridization between a common species and a rare one can, under the appropriate conditions, send the rare species to extinction in a few generations (Ellstrand and Elam 1993; Huxel 1999; Wolf et al. 2001). In several cases, hybridization between a crop and its wild relatives has increased the extinction risk for the wild taxon (Ellstrand 2003b). One example is the extinction of a wild subspecies of rice in Taiwan (Kiang et al. 1979). Furthermore, Ledig (1992) reported that in California, "pollen contamination from cultivated walnut may hybridize the (endangered) Hinds walnut out of existence."

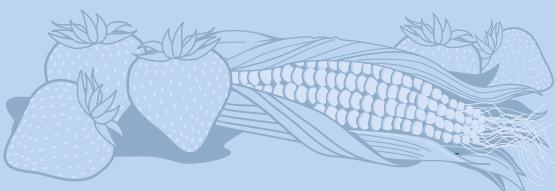
The vast majority of cases of spontaneous hybridization between cultivated plants and their wild relatives are of little consequence. But clearly gene flow from crops to wild relatives has, on occasion, had undesirable consequences.

Are transgenic crops likely to be different from traditionally improved crops? No, but that is not necessarily good news. The probability of problems due to gene flow from any individual cultivar is extremely low. But when those problems are realized, they can sometimes be costly.

New transgenic cultivars

As a group, new transgenic cultivars are no more or less likely to hybridize than their nontransgenic counterparts (Ellstrand 2003b; den Nijs et al. 2004). Whether transgenic crops are more or less likely to create gene-flow problems will depend in part on their phenotypes, the traits for which they were engineered. The majority of "first generation" transgenic crops have phenotypes — such as herbicide or pest resistance — that are apt to give a weed a fitness boost in certain environments. Although a fitness boost in itself may not lead to increased weediness, scientists engineering crops with such traits

TABLE 1. Transgenic crops approved for field-testing in California through Jan. 16, 2006*



Crop	Transgenic status	Scientific name	Among state's top 20 crops in 2003 area harvested?†	Wild or feral form of species in Calif.?	Wild species known to hybridize with crop in Calif.?	Are hybridizing plants weeds (W) or rare (R) in Calif.??
Alfalfa	Some deregulated, commercialized types	<i>Medicago sativa</i>	Yes	Yes	No	N/A
Apple	Regulated only	<i>Malus x domestica</i>	No	Yes	No§	N/A
Avocado	Regulated only	<i>Persea americana</i>	Yes	No	No	N/A
Barley	Regulated only	<i>Hordeum sativum</i>	Yes	No	No§	N/A
Beet	Some deregulated types, but none presently commercialized	<i>Beta vulgaris</i>	No	Yes	Yes	W
Brassica (broccoli, cabbage, etc.)	Regulated only	<i>Brassica oleracea</i>	Yes	No	No§	N/A
Brown mustard	Regulated only	<i>Brassica juncea</i>	No	Yes	Yes	W
Canola	Some deregulated, commercialized types	<i>Brassica napus</i>	No	Yes	Yes	W
Carrot	Regulated only	<i>Daucus carota</i>	Yes	Yes	No§	W
Chicory	Some deregulated types, but none presently commercialized	<i>Cichorium intybus</i>	No	Yes	No§	W
Chrysanthemum	Regulated only	<i>Dendranthema grandiflora</i>	No	No	No	N/A
Corn	Some deregulated, commercialized types	<i>Zea mays</i>	Yes	No	No	N/A
Cotton	Some deregulated, commercialized types	<i>Gossypium hirsutum</i>	Yes	No	No	N/A
Creeping bentgrass	Regulated only	<i>Agrostis stolonifera</i>	No	Yes	Yes	W
Cucumber	Regulated only	<i>Cucumis sativus</i>	No	No	No§	N/A
Grape	Regulated only	<i>Vitis vinifera</i>	Yes	No	Yes	No
Hybrid tea rose	Regulated only	<i>Rosa hybrida</i>	No	No	No§	N/A
Kentucky bluegrass	Regulated only	<i>Poa pratensis</i>	No	Yes	Yes	No
Lettuce	Regulated only	<i>Lactuca sativa</i>	Yes	No	Yes	W
Melon	Regulated only	<i>Cucumis melo</i>	Yes	Yes	No§	W
Onion	Regulated only	<i>Allium cepa</i>	No	No	No§	N/A
Pea	Regulated only	<i>Pisum sativum</i>	No	Yes	No	No
Pelargonium	Regulated only	<i>Pelargonium x hortorum</i>	No	Yes	No	No
Pepper	Regulated only	<i>Capsicum annuum</i>	No	No	No	N/A
Persimmon	Regulated only	<i>Diospyros kaki</i>	No	No	No	N/A
Petunia	Regulated only	<i>Petunia x hybrida</i>	No	No	No§	N/A
Potato	Some deregulated types, but none presently commercialized	<i>Solanum tuberosum</i>	No	No	No§	N/A
Raspberry	Regulated only	<i>Rubus idaeus</i>	No	No	No§	N/A
Rice	Some deregulated types, but none presently commercialized	<i>Oryza sativa</i>	Yes	Yes	No	W
Soybean	Some deregulated, commercialized types	<i>Glycine max</i>	No	No	No	N/A
Squash	Some deregulated, commercialized types	<i>Cucurbita pepo</i>	No	No	No§	No
St. Augustine grass	Regulated only	<i>Stenotaphrum secundatum</i>	No	Yes	No	No
Strawberry	Regulated only	<i>Fragaria ananassa</i>	No	No	Yes	No
Sunflower	Regulated only	<i>Helianthus annuus</i>	No	Yes	Yes	W
Tobacco	Some deregulated types, but none presently commercialized	<i>Nicotiana tabacum</i>	No	No	No§	N/A
Tomato	Some deregulated types, but none presently commercialized	<i>Lycopersicon esculentum</i>	Yes	Yes	No§	No
Walnut	Regulated only	<i>Juglans regia</i>	Yes	No	Yes	R
Watermelon	Regulated only	<i>Citrullus lanatus</i>	No	Yes	No	No
Wheat	Regulated only	<i>Triticum aestivum</i>	Yes	No	Yes	W

* Based on USDA-approved permits or acknowledged notifications (ISB 2006).

† Source: Hickman 1993.

‡ Sources: Whitson 2000; Hickman 1993.

§ One or more congeners present in California. For some of these, whether spontaneous hybridization with the crop occurs is still unknown.

should be mindful that those phenotypes might have unwanted effects in natural populations.

The crops most likely to increase the extinction risk by gene flow are those planted in new locations that bring them into the vicinity of wild relatives, thereby increasing the hybridization rate because of proximity. For example, a new variety with increased salinity tolerance might be planted within the range of an endangered salt-tolerant relative. It is clear that scientists creating new crops for field release, transgenic or otherwise, should consider the possibility of gene flow when making choices about whether it might create problems, and if so, how to create the best and safest products (NRC 2004).

Risks to California

But how likely is it that transgenes will flow to wild plants in California? At the moment, only seven different crops with genetically engineered varieties are commercially available in the United States: canola, corn, cotton, papaya, soybean, squash and tobacco. In California, five of these have no closely related wild relatives: corn, cotton (California's primary transgenic crop), papaya (which cannot be grown outdoors in California) and squash. These plants do not even establish feral populations in California. Furthermore, transgenic tobacco is not grown in California. For these six crops, gene flow into the wild in California is not possible. (As this article is going to press, genetically engineered alfalfa was deregulated for probable commercialization in California.)

Transgenic canola (*Brassica napus*) or "oilseed rape" is a different story. A tremendous amount of interspecies gene-flow research, both descriptive and experimental, has been conducted on this species in the United States, Canada, United Kingdom, France and Denmark (Ellstrand 2003b; den Nijs et al. 2004). *Brassica napus* naturally and easily intermates with wild *B. rapa* and, to a much more limited extent, with a few other mustard family species. Most of those species, including *B. rapa*, occur in California where they are known to be problematic weeds (Whitson 2000). Experiments have demonstrated that the hybrids between canola and



Due to market concerns, California rice growers are cautious about adopting transgenic crops that could cross with conventional varieties. Left, U.S. long-grain rice and, top right, rice grains. Lower right, "golden rice" is genetically engineered to accumulate pro-vitamin A in the grain, in order to help fight nutritional deficiency diseases in developing countries.

B. rapa typically have a drop in fitness relative to their parents, but that fitness is rapidly regained when those hybrids mate with one another or backcross to either parent. Only two genetically engineered types of canola are commercially available in the United States: plants engineered with resistance to the herbicide glyphosate and those engineered with resistance to the herbicide glufosinate.

Interestingly, the first and only reported case of spontaneous hybridization between a commercial transgenic crop and a wild relative involved genetically engineered glyphosate-resistant canola and *B. rapa*, in Quebec (Warwick et al. 2003). The hybrid plants were found where the wild species were growing in or adjacent to glyphosate-resistant canola.

The appearance of glyphosate resistance in *B. rapa* could present a problem if it forces farmers — who control this weed with relatively inexpensive and relatively environmentally benign glyphosate — to abandon it in favor of an alternative herbicide without those benefits. Whether or not the Quebec hybrids become a problem is currently under study by the group that discovered them. However, canola is not an impor-

tant, or even significant, California crop. Furthermore, the adoption of transgenic canola has not been nearly as enthusiastic in the United States as it has been for soybeans, corn and cotton. The majority of the U.S. canola crop remains non-transgenic. Therefore, the opportunities for the canola transgene to spread in California are much more limited than in Canada, where it is one of the most important crops.

The future of plant biotech

The face of plant biotechnology is rapidly changing. Dozens of genetically engineered crop species have been field-tested. Crops field-tested under U.S. Department of Agriculture/Animal and Plant Health Inspection Service (USDA-APHIS) notification or permit are required to be grown with some level of containment (NRC 2002). If the growers comply with those regulations, field-tests should not present an opportunity for transgene escape. Nonetheless, such crops represent the pool for new commercial transgenic crops of the next decade. As of Jan. 16, 2006, 1,215 field-test applications had been approved for 39 crops in California (table 1) (ISB 2006). The applications are for hundreds of different crop-trait combinations,

from fungal-disease-resistant avocado to pharmaceutical-producing rice.

More than half of the 39 field-tested crops have wild relatives in the California flora with which they are capable of hybridizing — either as wild plants that are same species or as closely related species known to spontaneously hybridize with the crop (table 1). In 11 cases, those wild plants are considered weeds in California. Fourteen of the field-tested crops rank among the top 20 California crops in terms of acreage harvested; 10 have cross-compatible mates in the wild flora of California. If deregulated and grown widely, these potential future crops will require further scrutiny for possible gene-flow problems. For example, wheat spontaneously hybridizes with a number of known weeds in the genus *Aegilops* that grow wild in California. Whether the movement of transgenes into the wild will create problems depends on the specific transgenic-based trait and how it is expressed in the wild populations (Ellstrand 2003b).

Compared to crop-to-wild transgene movement, crop-to-crop movement is much more likely. Different varieties of the same crop are usually fully sexually compatible. It is not unusual for adjacent and simultaneously flowering fields of the same crop to cross-pollinate. Also, gene flow by seed becomes an issue in this context. Unless very carefully segregated, seed from different varieties often becomes mixed during seed production. If a seed bank persists in the soil, individuals from last year's planting can appear within this year's crop. If a transgene moves unintended from one field of a crop to another of the same crop, a number of adverse consequences are possible, including: the loss of security for intellectual property; effects on nontarget organisms in natural or agroecosystems; and the evolution of new weeds.

Genetic pollution of crops

"Genetic pollution" may occur in crops intended to have a certain level of purity with regard to market demands — for example, crops certified as organic or intended for foreign markets that do not tolerate the presence of materials from genetically engineered plants. Health effects may be possible if genes engineered

to produce pharmaceutical or industrial compounds enter the food or feed supply. Such plants are required to be grown only under stringent field-test regulations. However, lack of compliance (NRC 2004; Taylor and Tick 2003) can create opportunities for such genes to move. Little has been written regarding the possible downsides of crop-to-crop gene flow involving transgenic plants, but recent incidents suggest that much more attention should be paid to this risk.

Herbicide resistance in canola. For example, multiple herbicide resistance developed in canola in Alberta, Canada (Hall et al. 2000). Volunteer canola plants were found to be resistant to two or more of the following herbicides: glyphosate (Roundup: Monsanto, St. Louis; Mo.), glufosinate-ammonium (Liberty: Aventis Crop Science, Research Triangle Park, N.C.) and imazethapyr (Pursuit: BASF, Research Triangle Park, N.C.). Clearly, multiple hybridization events among three different canola varieties were necessary to account for these genotypes. The alleles for resistance to glyphosate and glufosinate-ammonium are transgenes, but the allele for imazethapyr resistance is the result of mutation breeding. Although these volunteers can be managed with other herbicides, this report is signifi-

cant because it illustrates that gene flow into wild plants is not the only avenue for the evolution of plants that are increasingly difficult to manage.

Starlink corn. A better-known incident involved Starlink corn (NRC 2004; Taylor and Tick 2003). The Starlink gene and its product were approved only for animal feed, not human consumption. However, Starlink's genetically engineered protein appeared in a variety of products intended for human consumption. USDA detected the protein in over 10% of the corn samples initially screened, none of which were supposed to contain Starlink material. Although unintentional mixing of seeds during transport or storage may explain the unexpected presence of the unapproved transgenic product in the human food supply, intervarietal cross-pollination between adjacent cornfields probably played an important role as well. The story is significant because it illustrates how easy it is to lose track of transgenes. Without careful confinement and monitoring, there are plenty of opportunities for them to move from variety to variety (Christensen et al. 2005; NRC 2004).

If the two preceding incidents were the only examples of transgenes showing up where they shouldn't, they could be considered anomalous. But they are only

a tiny sample of an increasing number of such events. For a decade, more than a dozen cases of transgenes and/or their products out-of-place have been reported (Marvier and Van Acker 2005).

Gene flow in itself is not necessarily a problem, but unless specific steps are taken to isolate transgenic crops, the movement of transgenes to nontransgenic crops should not be an unusual occurrence. In fact, the frequency of these events has led some scientists to write, "the movement of transgenes beyond their intended destination is a virtual certainty" (Marvier and Van Acker 2005).

A problem for California?

Organic farming. If crop-to-crop gene flow is a "virtual certainty," which of its possible downsides are more likely to prove to be a problem in California? The issue of "coexistence" of genetically engineered crops with organic farming may be the most important (Schiemann 2003). The organic sector of California agriculture is rapidly growing. Organic crops are required to be transgene free. Presently, the onus for isolation is put on the organic grower. If transgenic crops pollinate organic crops, then their seed will bear transgenes. Of California's current major transgenic crops, this would not pose a problem for organic cotton because the seeds are removed from the lint, which is maternal tissue; on the other hand, farmers growing organic corn would have to practice some form of isolation to prevent them from being pollinated by nearby transgenic fields. Seed-source purity would be an important factor for growers of either organic cotton or organic corn. As more crops are deregulated and grown in California, the issue will continue to grow, especially for crops that are widely planted in the state.

Pharmaceutical crops. Transgenic crops that are grown to produce pharmaceutical and other industrial biochemicals pose another potential problem. These will pose special challenges for containment if we do not want those chemicals appearing in the human food supply. In the last 5 years, nine field-test applications of such plants were approved for California.

We know that it is easy to lose track of transgenic genes — if pollen moves



David Nance/USDA-ARS



Yvonne Calles/www.underthelille.com

While organic crops must be transgene-free, cross-contamination of organic cotton by transgenic varieties is not expected to be a problem because seeds (which may contain genes from an engineered pollen parent) are removed from the lint (which is pure maternal tissue). *Left*, a cotton crop; *right*, baby clothes made with organic cotton.

farther than expected, if seeds stay in the soil ungerminated or if seed are inadvertently mixed. The mixing of genes between different varieties of the same crop is a lot easier than the flow of genes into the wild, but both can have their downsides.

Weighing risks and benefits

In most cases, transgenes will not need to be contained. But sometimes containment will be helpful or necessary. New methods must be developed because present agronomic protocols are not always sufficient to do the job. New segregation procedures are being proposed (Christensen et al. 2005; Strayer 2002). Likewise, engineered constructs and other genetically based methods are being studied to effect containment (NRC 2004). All of these methods seem promising and need to be tested.

In the meantime, the creators of transgenic plants need to be as mindful of possible problems with their products as they are of potential promise. The best confinement should be up-front, with decisions made at the start of a project. In at least three cases that this author is aware of, scientists decided to stop engineering certain traits into certain crops because of anticipated problems with gene flow. But stopping a project altogether may be unnecessary. Often, a good decision will involve consideration of the safest combination of trait and organism. At one time corn was the organism of choice as a "pharm" plant. Today other plant species, often nonfood species, are being explored for this use.

The products of traditional plant improvement are not absolutely safe, and we cannot expect transgenic crops to be absolutely safe either. If we have advanced tools for creating novel agricultural products, we should use the advanced knowledge from ecology and population genetics — as well as social sciences and humanities — to make mindful choices about creating products that are best for us and our environment.

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