

# Going to "Great Lengths" to Prevent the Escape of Genes That Produce Specialty Chemicals<sup>1</sup>

Norman C. Ellstrand\*

Department of Genetics and Biotechnology Impacts Center, University of California, Riverside, California 92521-0124

In late 2002, the U.S. Department of Agriculture (USDA)'s Animal and Plant Health Inspection Service (APHIS), the agency that regulates field release of genetically engineered (transgenic) plants, found that the biotechnology company ProdiGene, Inc. failed to follow government regulations for growing genetically modified (GM) corn (*Zea mays*), engineered to produce a specialty pharmaceutical protein, in both Iowa and Nebraska. In Iowa, pollen from the corn designed to produce a pig vaccine might have pollinated nearby crops, leading to a government order of the incineration of 63 ha of corn growing near the experimental site. In Nebraska, engineered seed from the previous year's experiment germinated and grew as "volunteer corn" in a field of soybeans (*Glycine max*). The beans were subsequently harvested and transported to storage. Despite an APHIS inspector's request to collect/destroy the corn before the harvest of soybeans, some of those corn plants were harvested as well and ended up mixed with more than 17.5 million L (a half-million bushels) of stored soybeans. ProdiGene received a fine of \$250,000. In addition, the USDA required it to buy and destroy the soybeans containing the GM corn at an approximate cost of \$3.5 million. A penalty of this magnitude is the first of its kind for the U.S. government to levy against an agricultural biotechnology company, and it is not clear that the relatively small company will be able to pay the entire cost (Gillis, 2003).

## MUCH ADO ABOUT "PHARM" PLANTS

Plants have been used by humans for far more than food, fuel, and fiber for millennia. Historically, they have been a major source of medicines (pharmaceuticals) and remain so for millions of people today. Plants are also a source of dyes, lubricants, adhesives, and other industrial compounds. Some of these plant-derived industrial products are toxic (such as certain compounds derived from the opium poppy [*Papaver somniferum*]), and others, such as the many

industrial starches, are not (Simpson and Ogorzaly, 2001).

What's the big deal about plants grown to manufacture pharmaceuticals and other specialty chemicals (also known as "pharm crops")? Using crops for this purpose is an especially novel and promising application of agricultural biotechnology. Plants can be engineered to create chemical products that are free of animal viruses and prions. They can synthesize all kinds of proteins and store them in a stable form in seeds. Overall, it appears that plants can produce many specialty biochemicals, such as monoclonal antibodies for pharmaceutical purposes, in cleaner form, in greater quantities, and with less expense than current technologies. Theoretically, at least, a limited acreage (from a fraction of a hectare to a few dozen hectares) could produce a sufficient quantity of certain pharmaceutical products (including those for domesticated animals) to meet the annual demand (Pew Initiative on Food and Biotechnology, 2003). The prospects are exciting.

Interest in such plants is increasing. According to APHIS, "In 2002, approximately 130 acres (53 ha) of pharmaceutical producing plants were planted in experimental field tests at 34 sites. Most of these test sites were less than 5 acres (2 ha). It is anticipated, however, that the number of requests for permits for field tests, and the scale of production, will increase significantly over the next few years. Very few permits have been issued to date for plants in which the modification was made for the expressed intent of producing an industrial compound. However, as with plants engineered to produce pharmaceutical compounds, we anticipate an increase in requests for field tests of these types of plants. "Industrial" plants include those genetically engineered plants that are not intended for use as food or feed, but rather are intended to produce compounds that will be extracted for industrial uses. The range of potential uses of such substances includes, for example, applications in detergent manufacturing, paper production, mineral recovery, or in purely experimental research" (Federal Register, 2003).

However, no technology is risk-free. Some, but not all, plant-derived pharmaceutical and industrial compounds will have an impact on human or animal health if they end up, unintended, in a high enough concentration in the food or feed stream. And for

<sup>1</sup> This work was supported by the Biotechnology Risk Assessment Research Grants Program of the USDA (grant nos. 00-33120-9801 and 2002-33120-12769).

\* E-mail ellstrand@ucrac1.ucr.edu; fax 909-787-4437.

www.plantphysiol.org/cgi/doi/10.1104/pp.103.025908.

some people, the idea of corn flakes containing a drug, toxic or not, at however low concentration, is unacceptable (Fig. 1). In fact, in February 2003, the National Food Processors Association strongly urged the U.S. Food and Drug Administration "that there be no use of food or feed crops to produce plant-made pharmaceuticals or industrial chemicals without a 100% guarantee against any contamination of the food or feed supply" (National Food Processors Association, 2003).

It isn't easy to keep crop genes from wandering. For example, plant breeders trying to create corn seed of high genetic purity have recognized that the physical separation of different corn varieties by 200 m (660 feet) will still result in "contamination" due to cross-pollination at levels of about 0.1% (National Academy of Sciences, 2000). It is well known that most crops naturally mate with their wild relatives as well (Ellstrand, 2003). Seeds don't stay in place either. They can persist in the soil seed bank. They can mix in the nooks and crannies of harvesting equipment. They can bounce out of vehicles transporting them and germinate on roadsides (e. g. Pessel et al., 2001). The movement of unwanted crop genes into the environment may pose more of a management dilemma than unwanted chemicals. A single molecule of 1,1,1-trichloro-2,2-bis(*p*-chlorophenyl) ethane remains a single molecule or degrades, but a single crop allele has the opportunity to multiply itself repeatedly through reproduction, which can frustrate attempts at containment. When crop genes arrive in locations for which they were not intended, they sometimes persist and at times spread (Ellstrand, 2001, 2003). The spread of weeds between continents is a good example of how difficult it is to contain plants.

Thus, it might make sense to create pharm products from plants that are not food or feed plants.



**Figure 1.** When corn will be used to produce chemicals and pharmaceuticals, stringent regulations will be needed and will have to be enforced to make sure that the genes and the proteins they encode do not end up in the food and feed stream. Illustration by Union of Concerned Scientists.

Regrettably, that is not now the case. Recently, approximately 75% of the pharm field test applications filed in the United States were for a single crop, corn, a major source of both food and feed in both the United States and worldwide (Information Systems for Biotechnology, <http://www.n-biap.vt.edu>).

There are good reasons why corn is the organism of choice. More is known about the genetics of corn than any other crop, and it is relatively easily transferred. Its genetics are probably the best understood of all crop plants. American farmers have considerable expertise in growing and harvesting corn. Its seeds are an ideal storage location and vehicle for specialty chemicals produced by engineered genes (Pew Initiative on Food and Biotechnology, 2003).

How likely is it that corn genes will end up where they shouldn't be? Without efforts to isolate corn populations so that they don't cross-pollinate and without efforts to keep seed for different uses separate, inadvertent mixing of genetic material in corn is so likely that some mixing is a certainty. The "Starlink" GM corn incident of 2000 illustrates how easily things can get out of hand, even when some attempts are made to maintain segregation. This particular variety of GM corn was released exclusively for animal consumption before the determination of whether it was also suitable for human consumption. Nonetheless, it rapidly entered the general corn grain supply of the United States, within a single year turning up "in nearly one-tenth of 110,000 grain tests performed by U.S. federal inspectors" (Haslberger, 2001). How it spread is still a mystery. In some cases, it appears that some of the individuals handling, transporting, and processing the grain were inadequately educated or concerned about the need to keep it segregated from corn that was to be used for human consumption; in others, it appears that cross-pollination or seed dispersal accounted for the mixing. Although the Cry9C protein produced by this GM corn is unlikely to pose a hazard with a high rate of occurrence, the fact that pollen and seed moved the gene so rapidly demonstrates how quickly and extensively unintentional movement can occur.

In the United States, government regulation of field-grown transgenic pharm plants has always required efforts to prevent the escape of living transgenes. Over the last several years, plants engineered to create industrial compounds have been field tested (and in a few cases, grown commercially) under the more or less streamlined APHIS "Notification" procedure. In this case, the applicant essentially fills out a brief form and signs a statement agreeing to follow APHIS "performance standards" to confine transgenic organisms and their genes. Plants with certain special phenotypes, including those engineered to create compounds intended for pharmaceutical use, have been field tested under the more stringent "Permit" procedure, which requires a more comprehensive application and regular field visits by APHIS

personnel (National Academy of Sciences, 2002). Note that under these regulations, a compound popularly thought to be a pharmaceutical but grown without the intent to be used for that purpose might end up being grown under the less stringent Notification procedure.

### GOING TO GREAT LENGTHS

Recently, things have changed. In spring 2003, APHIS issued a set of new permit conditions for plants producing pharmaceutical and industrial compounds that include significant policy changes (Federal Register, 2003). First is the explicit mention of "industrial" compounds alongside pharmaceuticals. This mention is significant both because it acknowledges possible risks associated with specialty chemicals and because it corrects the prior possibility of field testing a plant that produces a pharmaceutical compound under Notification rather than Permit because the compound was not intended to be used for pharmaceutical purposes. This subtle change allows APHIS to scrutinize plants according to the actual compounds they produce rather than the intent for which they are being produced.

Second, the new conditions focus on explicit procedures designed to prevent the inadvertent mixture of seed. These include: (a) a perimeter fallow zone of 16 m (50 feet); (b) restricting planting food and feed crops in the same location in the following season if there is a chance for volunteer seedlings to be inadvertently harvested with the following crop; (c) dedicated planting, harvesting, and storage facilities; (d) machinery cleaning procedures; and (e) personnel training programs. These conditions are much more detailed than previous guidelines and requirements.

Third, and most important, are the field test permit conditions specific to pharmaceutical corn. These involve "great lengths" to avoid problems. APHIS requires that there will be no corn grown within 1.6 km (1 mile) of the field test site throughout the duration of any test involving open-pollinated corn. When pollen flow is controlled by placing bags over the corn tassels, APHIS requires that no other corn be grown within 800 m (2,640 feet) of the test site; in addition, the pharmaceutical corn must be planted at least 28 d before or 28 d after any corn growing in a zone extending from 800 to 1,600 m from the test site, ensuring no overlap in flowering (Federal Register, 2003).

Fourth, APHIS has made changes in compliance enforcement. Now multiple field inspections will be conducted. Furthermore, recordkeeping is required regarding how permit conditions were fulfilled. Finally, APHIS is growing and adapting. It is reviewing its regulatory system. And most importantly, APHIS has announced that it will lead a public dialogue in the near future.

### ARE THESE REQUIREMENTS ENOUGH?

Clearly, these new requirements are a big deal. Nevertheless, in some cases, industrial stewardship has already outpaced the regulators. Even a year ago, industry scientists began to create closed-loop production systems to prevent transgene escape. Some were even calling to move the sites of pharm corn production hundreds of miles from the Corn Belt (Pew Initiative on Food and Biotechnology, 2003). This extraordinary level of industrial stewardship is commendable and smart. To get a good idea of whether such strategies are excessive, prudent, or inadequate, it is worth looking at what might go wrong.

Consider what might be the "worst case scenario." One can imagine a plant transformed to produce a specialty compound is grown on limited acreage in the United States. The chosen plant is a widespread, outcrossing species, typically grown in dozens of countries and millions of hectares for human food consumption. The compound is innocuous in low concentration but has serious effects on human health if it reaches a certain concentration in food. Suppose the gene for this compound finds its way, by a rare long-distance (say, on the order of a few miles) pollination event, into a field of a variety of the same species intended for producing a food product. Let's further assume that the food-producing variety is a hybrid and, therefore, in the United States, one whose seed is a "terminal" product, that is, typically consumed or processed, but not used for replanting. That's good news at the national scale, because a low level of gene escape won't have any health effects and might not even be detected by a monitoring program.

However, a different outcome might be obtained at the global scale. Food, often in the form of living propagules (seeds or other), often moves beyond the borders of the United States—sold, sent as aid, or in the pockets of travelers. Living seeds of an American variety can end up in distant communities. For annual food crops, seeds are saved and replanted as open-pollinated landraces in most of the world. Those farmers may exchange seed with each other and experiment with seed from distant sources. Let's suppose that one or a few seeds bearing the allele for production of that specialty compound are planted into a landrace field in a different country. Finally, let's imagine that, in the landrace environment, that allele confers a substantial fitness boost to plants that bear it so that they produce more pollen, set more seed, or survive better than plants without that allele. Then, the conditions are right for the allele to increase in frequency, undetected, generation by generation. The compound also increases in frequency and in concentration in the food supply until it eventually has serious effects on the health of the humans who consume it.

Seem far-fetched? Each of the components of the scenario has a very low probability of happening. And yet, each of the steps is represented by real phenomena with a nonzero probability. For example, despite a multiyear moratorium on growing transgenic corn in Mexico, transgenes have introgressed, unintended and undetected, into remote corn landraces in that country (Alvarez Morales, 2002), likely representing the migration of those genes across international boundaries. The very limited plantings of pharm corn at this time make international leakage extremely unlikely. But the great promise of this technology and the hazard posed by the “worst case scenario” suggests that now is the time to start looking for even more effective procedures at containment.

### KEEPING CHEMICAL-PRODUCING GENES DOWN ON THE PHARM

Beyond the isolating procedures now required by APHIS, there are other tools for limiting the spread of genes that produce toxic compounds into food and feed streams. One set of tools are called “bioconfinement” mechanisms, ways of altering an organism’s biology to prevent the spread of alleles into populations for which they were unintended. Suggested methods for keeping alleles where you want them are increasing, including male sterility, triploidy, chloroplast transformation, constructs that sterilize the seeds produced by engineered plants (also known as “terminator”), and many more (compare with reviews by Gressel, 1999; Daniell, 2002). Although these techniques have great promise, each of them has its limitations. For example, male sterility will prevent pollen, but not seed, escape. Furthermore, to be effective, the techniques must not be “leaky.” I am not aware that any of them have been tested thoroughly enough to demonstrate their efficacy in a variety of environments.

Another set of tools are markers that could be used for easy and unambiguous visual identification of seeds that bear compounds not intended for consumption. Such markers would make monitoring easy, especially compared with the test kits and PCR-based methods currently in use. Imagine corn seeds that are neatly striped or phosphorescent orange. APHIS does not require monitoring, but monitoring might be advisable for certain specific products and useful in determining the efficacy of the proposed new containment methods.

Crop choice represents another tool. With its abundant pollen and seeds, corn seems a poor choice from a containment point of view. There are other food crops that might cause less concern. A potato (*Solanum tuberosum*) bouncing off a truck is less likely to establish on a roadside (and many potato varieties are both pollen and seed sterile). A sugar beet (*Beta vulgaris*) is less likely to hide in the pocket of an international traveler.

Likewise, is it always necessary to use food crops to create specialty chemicals, especially if they would have health effects if consumed by accident? There are nonfood crops that are already grown for the production of specialty chemicals—castor bean (*Ricinus communis*), opium poppy, etc. Likewise, many nonfood crops have been genetically engineered—Arabidopsis, tobacco (*Nicotiana tabacum*), *Begonia semperflorens*, belladonna (*Atropa belladonna*), petunia (*Petunia hybrida*), etc. What about insect larvae grown in vats or duckweed grown in a bioreactor? If a food crop is a necessary choice for producing a specialty chemical, is it always necessary to grow it in the field? A considerable measure of containment relative to the field could be afforded simply by growing such plants in a greenhouse. Finally, with regards to biosafety, some products just don’t make sense. A number of scientists have scrapped projects or not embarked on them because of biosafety concerns. Although APHIS sets the rules, the real regulators are mindful scientists who make the hard decisions to create the products that are the best in every sense of the word.

### ETHICAL CONSIDERATIONS

This essay explicitly and implicitly lays out many more ethical questions than it answers: Should the creators of new crops look beyond the product to how it will be used? Even though APHIS oversight is restricted to the United States, should regulators make decisions—or at least offer an opinion—about what might happen if a certain product ends up in another country where regulation is less stringent or lacking altogether? Do farmers of one crop have an obligation not to genetically contaminate the crops of other farmers who don’t want their crops contaminated? Will bioconfinement methods prove another layer of cost without attaining the level of desired containment? Is there an obligation by society to examine these questions in forums broader than the individual laboratory and the regulator’s office?

I have purposely not separated the role of science-based information from the role of nonscience-based values in risk assessment because they cannot be fully separated (National Academy of Sciences, 1996). Science can estimate the probabilities of human harm caused by a toxic compound to which people are exposed. Likewise, it can estimate the human benefits of a new drug that was impossible to create in the past. However, natural science cannot necessarily estimate the social and economic costs associated with the presence of a compound that people just don’t want in their food. Likewise, it cannot necessarily estimate the economic and societal gains associated with the new industry that creates that new drug. Natural scientists have begun to recognize that the technologies that spring from their research can have an array of impacts, good and bad,

downstream from discovery in the laboratory. In an editorial for *Science* magazine (Shalala, 2002), Donna Shalala, former Secretary of the U.S. Department of Health and Human Services, wrote, "We must open these doors carefully, never letting our science get ahead of our ethics. Science and technology are not inherently moral; the responsibility for putting them to a moral use belongs to us."

Scientists are researchers, teachers, and citizens of society. As researchers, we have a responsibility for the science that goes out of the doors of our lab. As teachers, we have a responsibility to communicate what we've learned from our science with the society that nourishes us. And as citizens of that society, we have a responsibility to listen to what the rest of society thinks about what we learn and what we teach.

To read more about pharming and pharm crops, see Ohlrogge and Chrispeels (2003) and Pew Initiative on Food and Biotechnology (2003).

#### ACKNOWLEDGMENTS

This article is partly based on a talk I presented at the Pew Initiative on Food and Biotechnology's Workshop "Pharming the Field." That talk and this manuscript benefited from feedback I received from a diverse array of individuals: Beth Burrows, Maarten Chrispeels, Phil Eppard, Mich Hein, Tracy Kahn, Alan McHughen, Margaret Mellon, Tom Nickson, Mike Pauley, and Jane Rissler. Special thanks to the Edmonds Institute's Beth Burrows for suggesting that an article on this topic would be worthwhile and to the Institute's Freida Morris for transcribing the Pew talk. I am also grateful to the Union of Concerned Scientists for sharing their illustration of "pharmaceutical" corn.

Received April 22, 2003; returned for revision April 30, 2003; accepted April 30, 2003.

#### LITERATURE CITED

- Alvarez Morales A** (2002) Transgenes in maize landraces in Oaxaca: official report on the extent and implications. The 7th International Symposium on the Biosafety of Genetically Modified Organisms: Meeting Proceedings. International Society for Biosafety Research, Beijing, p 78
- Daniell H** (2002) Molecular strategies for gene containment in transgenic crops. *Nat Biotechnol* **20**: 581–586
- Ellstrand NC** (2001) When transgenes wander, should we worry? *Plant Physiol* **125**: 1543–1545
- Ellstrand NC** (2003) *Dangerous Liaisons: When Crops Mate with their Wild Relatives*. Johns Hopkins University Press, Baltimore
- Federal Register** (2003) Field testing of plants engineered to produce pharmaceutical and industrial compounds. *Federal Register* **68**: 11337–11340
- Gillis J** (2003) U.S. will subsidize cleanup of altered corn. *Washington Post*, Wednesday, March 26, 2003, p E01
- Gressel J** (1999) Tandem constructs: preventing the rise of superweeds. *Trends Biotechnol* **17**: 361–366
- Haslberger A** (2001) GMO contamination of seeds. *Nat Biotechnol* **19**: 613
- National Academy of Sciences** (1996) *Understanding Risk: Informing Decisions in a Democratic Society*. National Academy Press, Washington, DC
- National Academy of Sciences** (2000) *Genetically Modified Pest-Protected Plants: Science and Regulation*. National Academy Press, Washington, DC
- National Academy of Sciences** (2002) *Environmental Effects of Transgenic Plants*. National Academy Press, Washington, DC
- National Food Processors Association** (2003) No use of food or feed crops for plant-made pharmaceutical production without a "100% guarantee" against any contamination, says NFPA. News release. February 5, 2003
- Ohlrogge J, Chrispeels MJ** (2003) Plants as chemical and pharmaceutical factories. In MJ Chrispeels, D Sadava D, eds, *Plants, Genes, and Crop Biotechnology*, Ed 2. Jones and Bartlett, Sudbury, MA, pp 500–529
- Pessel FD, Lecomte J, Emeriau V, Krouti M, Messean A, Gouyon PH** (2001) Persistence of oilseed rape (*Brassica napus* L.) outside of cultivated fields. *Theor Appl Genet* **102**: 841–846
- Pew Initiative on Food and Biotechnology** (2003) *Pharming the field: a look at the benefits and risks of bioengineering plants to produce pharmaceuticals*. Workshop proceedings, July 2002, Washington, DC. Pew Initiative on Food and Biotechnology. <http://pewagbiotech.org/events/0717/ConferenceReport.pdf>
- Shalala D** (2002) New directions for biomedical science. *Science* **295**: 585
- Simpson BB, Ogorzaly MC** (2001) *Economic Botany: Plants in our World*, Ed 3. McGraw-Hill, New York