GMO's and Gene Flow: A Plant Breeding Perspective*

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*This manuscript was originally prepared for the Agricultural Summit, Purdue University, Indianapolis, IN, September 13, 2002

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INTRODUCTION

Plant breeding is the art and science of plant improvement via genetic modification. What constitutes plant improvement has to be defined relative to the breeder's objectives or what the grower, processor, or end-user desires. Growers typically want increased yield, better standability, and other traits that maximize profit. Processors and end-users typically are looking for modifications in the physical or chemical properties of the grain or forage farmers produce that maximize their profits. The desires of producers and processors and end-users sometimes overlap when they result in an increase in value for all of those involved in the production and distribution system.

The improvement of plants themselves has rarely caused controversy or provoked a need to regulate plant breeding or the products of plant breeding. Despite the lack of controversy, however, plant breeding has not been environmentally benign (NCR, 2002). There are several examples where cultivars developed via plant breeding have had negative ecological effects. The transfer of genes between sexually incompatible species, called transformation, has provoked controversy and has resulted in regulation of plants containing transgenes. There is also now concern about the movement of transgenes from crop-to-crop, crop-to-weed, and from crop-to-wild and the environmental, social, and economic impact of this movement.

The so called third wave of agricultural biotechnology has created new concerns among the public and the commodity growers themselves. This third wave has been called "plant molecular farming" (Felsot, 2002) and refers to "the cultivation of plants for industrially, medically, or scientifically useful biomolecules, rather than for traditional uses of food, feed, or fibre" (Felsot, 2002). The concern of course, is that these biomolecules are being produced in food and feed crops and may find there way into the commodity crops via gene flow. This third wave of biotechnology has produced a renewed interest in gene flow via pollen movement.

The objective of this paper is to review the issue of pollen movement and gene flow from a plant breeding perspective. This paper will concentrate on the crop-to-crop movement of transgenes via pollen movement, provide a comparison of conventional breeding of crop plants versus the introduction of novel genes via transformation, and provide some recommendations for controlling the movement of transgenes. There are a multitude of social, cultural, legal, and economic issues surrounding the use of transgenes – these issues will not be covered in this paper. Smyth et. al. (2002) provides an overview of some of these issues.

CONVENTIONAL VERSUS TRANSGENIC CROP IMPROVEMENT:

A recent National Research Council report (NRC, 2002) on the environmental effects of transgenic plants provided an excellent comparison of conventional and transgenic approaches to crop improvement. Humans, either knowingly or unknowingly, have been genetically modifying plants for thousands of years. As humans have become more sophisticated and our knowledge in science has increased, sophisticated plant breeding methodologies have been developed to genetically improve plants (Hallauer and Miranda, 1988; Fehr, 1991). The majority of these methods are focused on improving complexly inherited traits such as grain or forage yield, standability, grain moisture content, etc. It is generally assumed that these traits are controlled by many genes and the environmental effect on the trait is large. These are called quantitative traits in the vernacular of plant breeding. When plant breeders select for these traits they have no control over what genotype is selected. They select for the phenotype and take the genotype that comes with the phenotype. There is no direct selection on genotype (Mayr, 1997)

This can be contrasted with resistance to some diseases, grain color, or in some cases plant stature, which are traits that are controlled by one or two genes with little or no environmental effect. There is frequently little molecular information known about the gene, but plant breeders can exercise direct selection on genotype because there is a one-to-one relationship between phenotype and genotype. These genes are frequently transferred from a donor to a recipient via a method called backcrossing.

Despite the recent advances in molecular biology and the genome sequencing projects, virtually nothing is known about what happens at the DNA level when plant breeders select for quantitative traits. The kind of changes that occur and why these changes occur has not yet been discovered. Labate et al. (1999) used neutral genetic markers to assess genome wide changes following 50 years of selection in corn, but specific conclusions about how plant breeding affects the genome are difficult to make.

Transgenes are typically inserted plants via one of several methods (NCR, 2002). The method of insertion itself is not important in that they all result in one or more copies of the transgene being inserted more or less at random in the genome. Plants are then screened to have usually only one functioning copy of the transgene. The transgene is then moved from the genetic background it was inserted into to a more agronomically elite background via backcrossing, the same method used to move genes controlling qualitative traits. In fact, the transgene is moved to new cultivars and inbred lines in most species via backcrossing as if it were a normal part of the plants complement of genes. In practice, the process is a little more complicated than described, but general features are accurate.

Plant genomes are fluid and dynamic, not static and stable as might be expected given the stability of phenotype. For this reason, it is biologically and scientifically difficult to conclude that a transgenic event is fundamentally different from what is normally going on in a plants genome. The big difference is in the origin of the gene that is inserted. Plants do not normally incorporate genes from species or genus with which they are sexually incompatible. It is true that transgenes have been placed into a new context (the recipient genome), but it is generally thought that this is not fundamentally different than when a plant's native genes moved using conventional hybridization techniques. On an evolutionary time scale, however, genes and even whole genomes have been moved between species (Martin et al., 2002). For example, the chloroplasts of modern plants are the result of a symbiotic relationship between early plants and oxygen producing cyanobacteria (McFadden, 2001). For these reasons and others it is difficult to make a clear distinction between a transgene insertion event and the dynamic processes that occur during the conventional breeding of a crop plant.

Transgenes can clearly result in a plant producing novel phenotypes or traits, but the recent NRC (2002) concluded that "the transgenic process presents no new categories of risks compared to conventional methods of crop improvement, but specific traits introduced by both approaches can pose unique risks." There are several examples of where conventional plant breeding has resulted in genotypes with unexpected and novel phenotypes that were not predicted (NCR, 2002 p. 43). The novel phenotype of a specific transgene can usually be predicted, but its effect on other plant traits and characters cannot be predicted. The changes in traits and characters that occur from developing a cultivar via conventional or from the insertion of a transgene into an existing cultivar must be measured by growing the plants in the environments it was developed to be grown in. The NRC (2002) committee recommended and I agree that we should be more concerned about the **products** of genetic modifications rather than the **processes** used to create the genetic modifications.

GENE FLOW

In the field of population genetics, the phrase **gene flow** is used specifically to refer to movement between groups that result in genetic exchange (Hedrick, 2000). Gene flow is an important concept in evolution. High levels of gene flow between groups or populations results in the homogenization of groups so that genetically they become alike. Low levels of gene flow coupled with forces like selection leads to genetic differentiation. The evolutionary importance of gene flow and the amount of gene flow that occurs is under debate because gene flow is difficult to measure. Recent results with gene flow, however, suggest that the amount of gene flow that occurs has been underestimated (Ellstrand, et al., 1999)

From a plant breeding and crop production perspective, the main concern of gene flow is contamination. Genetic exchange is only important for producers if they save their own seed – in which case they can potentially propagate the transgene and be contaminated indefinitely. Genetic exchange is important to plant breeders when breeding stocks become unintentionally contaminated with a transgene. Contamination and gene flow are separate processes that have similar implications. For example, the mixing of seeds of GMO and non-GMO crops results in contamination, but will not result in gene flow unless the GMO and non-GMO crops in the mixture exchange genes. For example, it is possible in a highly self-pollinated crop to have a mixture of GMO and non-GMO crops growing in the field, but if there is 100% self pollination (this is not a likely event), there will not be gene flow. If this crop mixture is harvested, however, there will be contamination. Gene flow, then, always results in contamination but contamination does not always result in gene flow. The issues of gene flow and contamination are separate processes that have similar implications for the producer who purchases new seed each year.

Gene flow or more specifically the movement of transgenes that results in genetic exchange will occur by one of three mechanisms – seed dispersal, horizontal transfer, and pollen movement (NRC, 2002). The common types of seed dispersal events will be spillage (either pre- or post-harvest), seed shattering and related mechanisms that result in the direct transfer of seeds to the environment, or mechanical mixing events related to machinery. Horizontal transfer is the nonsexual transfer of genetic material from one organism to another (NCR, 2002). Horizontal transfer of genetic material appears to be important on an evolutionary scale (McFadden, 2001), but it is probably not important on the time scale of a plant breeder. Movement of pollen carrying transgenes will always result in genetic exchange if viable pollen finds receptive stigmas (female reproductive organs of plants). Seed dispersal and pollen movement are the most important concerns from a plant breeding perspective.

The primary issues with pollen movement and seed dispersal are crop-to-wild, crop-to-weed, and crop-to-crop gene flow and contamination. A great deal has been written about crop-to-wild and crop-to-weed gene flow (NRC, 2002). The concerns here are primarily related to altering the fitness (ability to reproduce and leave viable offspring) of wild and weedy populations by an infusion of genes from domesticated crops. None of the major grain crops has wild relatives in the U.S. so gene flow to wild relatives is typically not a concern. It can be a concern, however, as has been recently highlighted by the transgenic maize issue in Mexico as transgenic crops are moved to and become adopted in other parts of the world. The major grain crops also have no sexually compatible weed species to be concerned with either (there are important exceptions). Crop-to-wild and crop-to-weed gene flow is a major concern, however, and the risks need to be assessed on a crop-by-crop basis. The recent NRC (2002) report provides a good review of these issues and the associated hazards and risks and I will not address them further.

From a plant breeding perspective and from a crop production perspective, the primary concern is crop-to-crop gene flow and contamination. Gene flow via pollen movement is primarily an issue with cross-pollinated crops. Cross-pollinated crops can be broadly broken into two categories on the basis of how the pollen moves: insect pollinated crops and wind pollinated crops. None of the major grain crops (corn, sorghum, wheat, rice) are pollinated via insects, but many forage crops, such as alfalfa, depend on insects for pollination (Fehr and Hadley, 1980). Preventing the long distance movement of pollen in an insect-pollinated crop would appear to be a difficult if not impossible task, since insects have the potential to travel long distances. Pollen movement for wind-pollinated crops, such as corn and sorghum, is dependent on other factors such as the environmental conditions during pollination and on the physical characteristics of the pollen, which determine how far it is likely to move with the wind.

Seed dispersal is often overlooked as a mechanism of gene flow, but it may be a significant source of gene flow, especially in areas of continuous cropping. The harvesting process leaves a certain amount of grain in the field – the exact amount can only be determined on a case-by-case basis and is dependent on many factors. Grain left behind in the field has the potential to germinate the following crop year. If the field is planted to the same crop, any volunteer crop from the previous year will almost certainly cross pollinate with the current years crop. If the field is planted to another crop, the volunteers will still come up, but it may be possible to mechanically or chemically control the volunteers prior to pollen shed. Even if the volunteers emerge in another crop, they also have the potential to out-cross with nearby fields of the same species.

To understand the issue of pollen movement I will examine pollen movement in corn, a wind-pollinated crop.

POLLEN MOVEMENT IN CORN

One of the features of a wind pollinated plant is the production of an enormous amount of pollen. Estimates of the amount of pollen produced for open-pollinated varieties ranges from 18,000,000 to 25,000,000 pollen grains per plant (Kiesselbach, 1999). Modern hybrids have smaller tassels than open-pollinated varieties, but still produce an enormous amount of pollen. The average plant will have less than 1000 silks, which means that one corn plant potentially has enough pollen to pollinate about an acre of corn. Of course, the pollen must be distributed perfectly for this to happen and in a real maize field this would not happen. When the one plant is multiplied by the 25,000 in one acre and that is multiplied by the size of the field, it is easy to see that the pollen load created in a corn field is enormous.

There have only been a few published studies of pollen movement in corn. This has been unfortunate, because it leads to speculation about what can or could happen and reinforces what people have heard has happened. Scientists at Pioneer Hi-Bred International, Inc. have published two studies on pollen movement in corn. The first study was designed assess the likelihood of contamination resulting from the use of common breeding practices to produce the relatively small quantities of seed required for breeding purposes (Garcia et al., 1998). They tracked pollen movement by taking advantage of the fact that yellow-seeded corn is dominant to white-seeded corn. If white-seeded corn plants are pollinated by yellow-seeded corn plants, the white-seeded corn plants will have yellow kernels interspersed among the white kernels, so pollen movement is easily detected. Garcia et al. (1998) evaluated two breeding techniques but the one that has the most applicability to a farmer production system was the use of an isolated crossing block to produce seed. An isolated crossing block is a planting arrangement that resembles what is used for commercial seed production in corn. White-seeded corn was used as the pollen source and the tassels were removed from the yellow-seeded corn to simulate what would typically be done with a transgenic pollen source. If yellow kernels were found on ears of white-seeded plants, then either tassel removal was not done effectively or pollen from another source contaminated the field. This experiment was conducted twice, once by spatial isolation (the nearest corn was 184 m from the field) on once by spatial and temporal isolation.

Garcia et al. (1998) found low levels of pollen movement. With spatial isolation only they found 17 yellow seeds from the examination of 607 ears of the white-seeded pollen source giving 0.01% contamination. The authors felt that they got complete removal of tassels from the yellow-seed plants in the study and concluded that the low level of contamination came from yellow-seeded fields that were more than 184 m from their isolation field. When the study was repeated and isolated in both space and time, they found no contamination from pollen of yellow-seeded corn.

Their results from the isolated crossing block experiment and from their triplet experiments (which I did not discuss) indicated that it will be more difficult to control transgenic pollen movement when the transgenic is used as a male. This of course makes sense, because if the tassels are removed from the transgenic plants prior to pollen shed, they should not produce any pollen if the tassel removal was complete. Garcia et al. (1998) make the following recommendations to aid in the reduction/elimination of transgenic pollen movement: a) use transgenic plants as females and remove their tassels prior to pollen shed; b) spatial isolation of >185 m; c) temporally isolate the transgenic plants from surrounding corn – this would involve planting 1 to 2 weeks later than the last corn plantings in the area of pollen movement; and d) scouting and destroying sexually compatible plants that are the same reproductive age as the transgenic plants in the area of presumed pollen movement. This study by Garcia et al. (1998) was conducted in Puerto Vallarta, Mexico and the results regarding the distance that pollen moves may not be generally applicable to the continental U.S. Their general conclusions on controlling pollen movement, however, are applicable.

Another way of studying pollen movement and its potential for movement is to study the physical and biological properties of the pollen itself to see how long it may survive in the environment and if it is physically able to move long distances. Luna et al. (2001) conducted such a study at the same research location as the study by Garcia et al. (1998). Their first experiment examined pollen longevity as measured by seed set after the pollen was exposed to ambient environmental conditions and then was used to pollinate fresh silks. They found a 58 to 96% reduction in pollen viability after 1 hour of exposure, the large range being due to differences in ambient humidity when the experiment was conducted. After 2 hours of exposure there was 100% reduction in pollen viability. They also conducted experiments to measure the distance pollen would travel by planting a 4000 m^2 inbred pollen source block (the inbred was yellow-seeded and also contained other genetic markers so that it could be distinguished from other sources of yellow pollen) and surrounding it in the cardinal directions with a white-seeded hybrid planted 100, 150, 200, 300, and 400 m from the pollen source. The furthest distance that out-crossing was detected was 200 m and the incidence was very low (only 2 kernels). These results are important in managing pollen movement, but we need to be careful in extrapolating these results to other locations that will have a different set of environmental conditions. Also, as the authors pointed out, their results apply only to research scale plantings.

Felsot (2002) reports on two unpublished studies on pollen movement in corn conducted in Missouri with results that are qualitatively similar to those of Garcia et al. (1998) and Luna et al. (2001). Basically most of the corn pollen stays close to the source. The studies that Felsot (2002) reported on used a 10 acre block of pollen donor, which may or may not be reflective of the size of those that will be used commercially for the production of plant-made pharmaceuticals. What these studies do indicate, however, is that if your neighbor is growing a standard GMO hybrid in open field conditions the chances of your crop being contaminated by your neighbor's crop is quite probable, since there is frequently no isolation distance between neighbor's fields.

The studies Felsot (2002) describe found 0.0301% contamination at a distance of 660 feet from the pollen source. To put this in perspective, Felsot (2002) says this is just 3 kernels out of every 10,000. When the tassels were removed on 90% of the pollen source the contamination level at 660 feet the contamination rate was only 1.3 kernels for every 10,000. On the face of it, these numbers sound very small. Let's look at it from a different perspective. On the average one pound of corn contains about 1800 kernels, which means a 56 lb bushel contains 100,800 kernels. If we assume the average semi load of corn is 750 bushels, then this semi is hauling 75,600,000 kernels. At a contamination rate of 0.03% the average semi load contains 22,680 kernels that don't belong, which is roughly equal to 1/5 of a bushel. The question of whether a 1/5 of a bushel contamination in a 750 bushel load is a lot or not depends on the buyer's tolerance and the ability to sample a truck load of corn.

None of the studies I have reported on address the critical issue of long distance gene flow. The studies did not look for pollen movement beyond 900 feet from the pollen source. The absence of pollen at 900 feet is not evidence of the absence of pollen at greater distances.

WHAT CAN BE DONE?

There have been numerous reports written about gene flow containment. I would like to outline and discuss what I feel are the major considerations to preventing gene flow and discuss them in the context of what has been published. Producers and regulators need to deal with the following barriers to gene flow:

- Physical barriers to gene flow
- Biological barriers to gene flow
- Mechanical barriers to gene flow
- Spatial barriers to gene flow
- Temporal barriers to gene flow

Physical barriers to gene flow: Physical barriers are barriers that will physically prevent pollen from moving out of the zone of production. An obvious physical barrier is a glass house. If a crop is grown inside of a glass house, then pollen movement is severely restricted, but there is still a finite possibility of escape. Under field conditions, I could imagine an 80 acre field surrounded by poplar trees or other fast growing species that are taller than corn and would provide both a wind break and a physical barrier to the movement of pollen from the field.

Biological barriers to gene flow: There have been several reports written about biological barriers to gene flow. The most comprehensive is the report by Daniell (2002) who outlines eight molecular techniques for transgene containment. Some are only in the development stages and may never have practical applications for a variety of reasons. The “terminator technology” is an example. Terminator technology was originally developed to protect intellectual property and prevent farmers from saving seed of protected varieties (Smyth et al., 2002). Because the seeds produced on plants with the technology are sterile it would have also functioned as a mechanism to prevent the proliferation of the transgenic crop. The “terminator technology” has not been well received, however, by all segments of society (Shand, 2002). Male sterility, particularly cytoplasmic male sterility, is an example of a technology that could be used to biologically suppress gene flow (Feil and Stamp, 2002). The use of this technology has already been demonstrated in corn with the TopCross® high-oil production method (Thomison et al., 2001). Essentially the transgene would be introduced into a male sterile hybrid which would be pollinated by a non-transgenic pollinator. There are also transgenes that are known to render the pollen grains in which they are expressed unviable. These transgenes could then be linked to the transgene that is of interest to effectively block unwanted gene flow (M. P. Scott, 2002, Personnel communication).

Mechanical barriers to gene flow: A mechanical barrier in the case of corn would be removal of the tassel prior to pollen shed. If done properly and in a timely fashion, tassel removal is very effective. For crops with perfect flowers, however, mechanical barriers are not an option.

Spatial barriers to gene flow: Spatial barriers are the most commonly prescribed treatment to prevent gene flow. The rationale is that if enough distance is placed between the unwanted pollen source and the plants you don’t want the pollen to move into that there will be 0 or very small levels of contamination. Unfortunately, it is also the method over which we have the least control. Crop certification requirements are frequently cited as justification for the use of spatial barriers. Crop certification requirements, however, have built in levels of acceptable contamination. In fact, rather high levels (1 to 5%) of contamination can be found in seed production fields. These levels can be tolerated because it is very difficult for producers to identify the contamination.

Temporal barriers to gene flow: Temporal barriers to gene flow can be quite effective. In order for there to be gene flow via pollen movement there must be receptive and un-pollinated stigmas. By waiting until neighboring crops have been pollinated or planting well before the neighboring crops are planted, the whole issue of crop-to-crop gene flow can be avoided.

Taken individually, none of the five methods I have listed are sufficient to prevent gene flow via pollen movement. Therefore, I recommend that some aspect of all five methods be employed. Stacking of these barriers would most likely reduce the probability of pollen movement by the product of each independent barrier. Thus, stacking individual barriers would rapidly reduce the probability of pollen movement to negligible levels. APHIS currently has the following requirements for the field testing of corn for the production of pharmaceuticals:

- Transgenic corn must be planted at sites that are at least 1 mile away from corn seed production
- Corn from previous seasons must be harvested and removed in a radius of 0.25 miles of the transgenic corn plot, before the transgenic corn is sown
- The land within 25 feet of the transgenic plant area must remain fallow during the test.
- No other corn plants are grown in within a radius of 0.5 miles (0.25 miles if a buffer is used) of the transgenic test plants, at anytime during the field test.

- Transgenic corn must be planted no less than 21 days before or 21 days (14 days with a buffer) after the planting dates of any other corn that is growing within a zone extending from 0.5 to 1.0 miles (0.25 to 0.5 miles with a buffer) of the transgenic test plants.

These regulations only encompass two of the five recommended barriers to pollen movement, spatial and temporal. There are no requirements that the applicants also use mechanical and biological means of controlling pollen movement. Adding these mechanisms as an additional requirement would prudent given that the tolerance to the products of these transgenes in the environment is currently zero.

The reality, however, as pointed out by Smyth et al. (2002) is even if pollen movement is effectively kept to zero, there are other ways that the GMO crops can become co-mingled with non-GMO crops or other GMO crops producing other products. Volunteer plants are an issue the following season as there is no perfect method of harvesting agronomic crops. The technology could be misappropriated in a variety of ways and contaminate the crop. Human errors and accidents during transportation and handling of the crop could also lead to contamination events that effectively would have the same result as contamination via pollen movement.

In this regard, we support grower and distribution handler certification programs using ISO standards. Maier (2002, http://www.agcom.purdue.edu/AgCom/Pubs/GQ/GQ-47.pdf) has pointed out the concerns of contamination from the point of view of grain producers and grain handlers. In particular, he has point out the lack of federal oversight, the need to federally regulated tolerance levels in food and feed crops, and the need for reliable and inexpensive strip test kits.

CONCLUSIONS

Gene flow via pollen movement will be difficult to contain in any crop, but it will be particularly difficult in wind-pollinated crops. I have recommended that to prevent crop-to-crop gene flow that four of the five listed barriers to pollen movement should be required. This will probably not eliminate pollen movement, but it should reduce the probability of pollen movement events. If these barriers are then coupled with the appropriate identity preservation process, there should be minimum unwanted contamination via pollen movement.

The Food and Drug Administration in a recent call for public comment on a document entitled "Guidance for industry: Drugs, biologics, and medical devices derived from bioengineered plants for use in humans and animals" (http://www.fda.gov/cber/gdlns/bioplant.htm) is strongly recommending that tests be made available that can detect the presence of the transgene and the protein produced by the transgene in the commodity crop. This recommendation should be a requirement, because these tests will be needed to not only test for contamination but to also verify any identify preservation process that is put into place. In addition, it would be beneficial if the transgenes were engineered so that they could be traced directly to the lab of origin so the owner of the gene can be identified in the event that there are multiple genes producing the same product.

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