12-2004

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Abstract
Seed production is one of the least visible yet most important aspects of food and feed production. This part of the system is often taken for granted, even by the farmers who plant the seed. One of the reasons is that much of the seed in the United States is provided by the private sector, particularly for corn and soybean. Also, seed production is a technical process that requires in-depth knowledge of the reproductive mechanisms in plants.

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Chapter 4
Seed Production in Corn and Soybean

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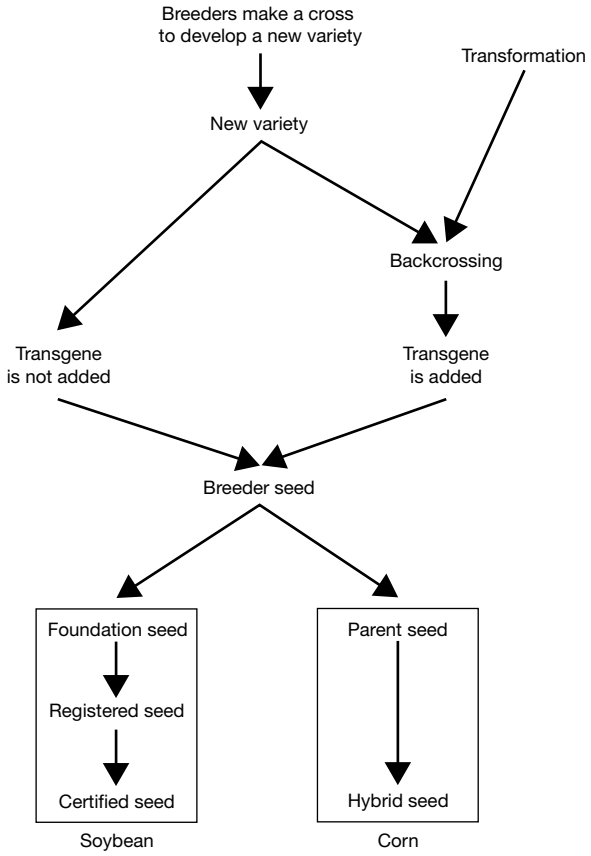
Seed production is one of the least visible yet most important aspects of food and feed production. This part of the system is often taken for granted, even by the farmers who plant the seed. One of the reasons is that much of the seed in the United States is provided by the private sector, particularly for corn and soybean. Also, seed production is a technical process that requires in-depth knowledge of the reproductive mechanisms in plants.

For the purposes of this chapter, seed production has been defined broadly as the series of steps that begins with the breeding of a new variety and ends with seed that is sold to farmers (Figure 4-1). The seed production process is usually conducted by three or four specialized departments in a seed company. Plant breeders develop varieties[20] and produce breeder seed—the source seed from which all varieties are propagated.

Some companies now have separate departments that do all breeder seed production. In the case of corn, breeders will also be responsible for adding male sterility if needed. Parent or foundation seed production is usually done by a separate department. The production of hybrid or certified seed—the seed that will be sold to farmers—is done by yet another department.

Seed producers have many goals, but this chapter focuses on a detailed description and analysis of the steps depicted in Figure 4-1 with regard to maintaining genetic purity. Genetic purity is important because gardeners and farmers expect varieties with the same names to be identical from one year to the next.

Figure 4-1 **The Seed Production Process**



We are concerned with two sources of contamination: impurity resulting from physical mixing of seed and impurity resulting from the movement of pollen. The seed production process is described in sufficient detail to identify points

20 A variety is a subgroup of plants within a species whose genetic makeup and characteristics distinguish it from other varieties of the species. Crop varieties are often called cultivars, especially by agricultural scientists.

where such contamination may occur. The goal is to determine if seed of pharma crops can be produced in a way that ensures virtually zero contamination of the food system.

This chapter also makes recommendations for achieving virtually zero contamination of the food system by pharma crops.

PRODUCING COMMODITY SEED TO BE GROWN BY FARMERS

Seed production practices for corn and soybean have evolved to provide farmers with genetically pure varieties. The Association of Official Seed Certifying Agencies (AOSCA) has developed and published genetic purity standards, and many seed companies have developed best management practices for maintaining acceptable levels of genetic purity.[21] The standards, however, do not specify or guarantee 100 percent genetic purity. It is assumed that most hybrids and varieties on the market contain some low level of contamination.

AOSCA consists of 44 state certification agencies in the United States and seven national agencies. Among other things, AOSCA is responsible for setting and monitoring genetic purity standards for the various classes of certified seed. AOSCA standards are at least partly responsible for the high seed quality that U.S. farmers have come to expect from seed suppliers.

Four classes of seed are officially recognized in the United States: breeder, foundation, registered, and certified. A brief description of each, as taken from the AOSCA genetic and crop standards handbook, follows. Understanding these classes is important to understanding the U.S. seed production system.

1. **Breeder seed** is seed directly controlled by the originator or sponsoring plant-breeding institution or person. This is the class of seed from which all other classes of seed are derived.

2. **Foundation seed** is developed from breeder or foundation seed produced under control of the originator or sponsoring plant-breeding institution or person. Foundation seed is a class of certified seed produced under procedures established by the certifying agency.

3. **Registered seed** is developed from breeder or foundation seed and is produced and handled under procedures acceptable to the certifying agency.

4. **Certified seed** is developed from breeder, foundation, or registered seed and is produced and handled under procedures acceptable to the certifying agency.

Not all classes of seed are used in all crops, and it should be noted that the United States has no laws or regulations requiring that only certified seed be sold to farmers. Certification of seed production is optional for seed sold in this country but is usually required for seed sold in other parts of the world, especially Europe. The requirements for each class of certified seed vary from crop to crop.

The AOSCA certification requirements for corn and soybeans are reproduced in Table 4-1 (p. 56). These are minimum standards and apply only to those segments of the flow chart in Figure 4-1 that are enclosed in boxes.

21 The Genetic and Crop Standards of AOSCA manual can be downloaded from the AOSCA website (*ftp://www.aosca.org/geneticstandards.pdf*), as can the Operational Procedures of AOSCA manual (*ftp://www.aosca.org/operationalprocedures.pdf*). These manuals outline the procedures that must be followed by the state and national agencies that certify seed.

Table 4-1 **Minimum Standards for Certification of Plant Materials under the AOSCA System**[1]

Crop kind	Foundation				Registered				Certified			
	Land[2]	Isolation[3]	Field[4]	Seed[5]	Land[2]	Isolation[3]	Field[4]	Seed[5]	Land[2]	Isolation[3]	Field[4]	Seed[5]
Corn												
Inbred lines	0	660[6,7]	1,000[8,9]	0.1[10]								
Foundation												
Single-cross	0	660[6,7]	1,000[8,9]	0.1[10]								
Backcross	0	660[6,7]	1,000[8,9]	0.1[10]								
Hybrid									0	660[7,11]	1,000	0.5
Open-pollinated									0	660[7,11]	200	0.5
Sweet									0	660[7,12]	---	0.5
Soybean	1[13]	0[14]	1,000	0.1	1[13]	0[14]	500	0.2	1[13]	0[14]	200	0.5

1 Extracted and reformatted from the Genetics and Crop Standards of AOSCA manual (*ftp://www.aosca.org/geneticstandards.pdf*).
2 Number of years that must elapse between the planting of different classes of seed. A certification agency may grant a variance in land cropping history in specific circumstances where cultural practices have proven adequate to maintain varietal purity.
3 Distance in feet from any contaminating source.
4 Minimum number of plants or heads in which one plant or head of another variety or off-type is permitted.
5 Maximum percentage of seed of other varieties or off-types permitted.
6 No isolation is required for the production of hand-pollinated seed.
7 When the contaminant is of the same color and texture, the isolation distance may be modified by adequate natural barriers or differential maturity dates, provided there are no receptive silks in the seed parent at the time the contaminant is shedding pollen. In addition, dent sterile popcorn requires no isolation from dent corn.
8 Refers to off-type plants in the pollen parent that have shed pollen, or to the off-type plants in the seed parent at the time of the last inspection.
9 Detasseling, cutting, or pulling of cytoplasmic male-sterile seed parent is permitted.
10 Refers to off-type ears. Ears with off-colored or different-textured kernels are limited to 0.5% or a total of 25 off-colored seeds or different-textured kernels per 1,000 ears.
11 Where the contaminating source is corn of the same color and texture as that of the field-inspected or white endosperm corn, optically sorted, the isolation distance is 410 feet and may be modified by the planting of pollen parent border rows according to the table below:

Minimum distance from contaminant (feet)	Minimum number of border rows required	
	Field size up to 20 acres	Field size 20 acres or more
410	0	0
370	2	1
330	4	2
290	6	3
245	8	4
205	10	5
165	12	6
125	14	7
85	16	8
0	Not permitted	10

12 The required minimum isolation distance for sweet corn is 660 feet from the contaminating source, plus 4 border rows when the field to be inspected is 10 acres or fewer in size. This distance may be decreased by 15 feet for each increment of 4 acres in the size of the field to a maximum of 40 acres, and further decreased 40 feet for each additional border row to a maximum of 16 rows. These border rows are for pollen-shedding purposes only.
13 Unless the preceding crop was another kind, or unless the preceding soybean crop was planted with a class of certified seed of the same variety, or unless the preceding soybean crop and the variety being planted have an identifiable character difference (in which case, no time need elapse).
14 Distance adequate to prevent mechanical mixture is necessary.

Variety Types

The type of variety grown in a crop is determined primarily by the crop's mode of reproduction (self-pollinated in soybean versus cross-pollinated in corn). These are the major determinants of the steps involved in seed production. Seed production in corn is more complex than in soybean.

Corn

Of the many possible variety types, we will restrict ourselves to three: inbred lines, open-pollinated varieties (OPVs), and single-cross hybrids. An inbred (or pure) line is a strain that breeds true to type when individual plants of the strain pollinate themselves (self-pollination). An inbred line can be developed from repeated generations of self-pollination or from repeated generations of crossing to a common parent (backcrossing).

An OPV can be a landrace[22] developed by farmers, landraces improved by breeders or farmers, or synthetics created by crossing landraces, synthetics, or inbred lines together. OPVs are genetically heterogeneous and are maintained by allowing individual plants to cross-pollinate in isolation. A single-cross hybrid is the first generation cross between two inbred lines.

22 A landrace is a variety developed and maintained locally by farmers.

OPVs were the dominant variety type in the United States before the introduction of hybrids in the mid-1930s. OPVs were gradually replaced by hybrids, and single-cross hybrids now account for at least 99 percent of the hybrid seed sold in this country (Wych 1988). OPVs are no longer grown on large acreages, but when they are, seed production is usually done on the farm by the farmer.

Soybean

The most popular variety type grown by farmers in soybean is a pure line. Pure lines of soybean are equivalent to inbred lines of corn. Soybean is predominantly self-pollinated, so inbred lines of soybean can be maintained by just planting and harvesting them. Seed mixtures (blends) of two or more soybean varieties are also a possible variety type (Fehr 1987). Hybrids have been proposed and have been evaluated extensively in soybean (Burton 1987), but they are not widely used by farmers because of the difficulty and expense in producing hybrid seed (Palmer et al. 2001).

Variety Development, Transformation, and Backcrossing

The variety development process in corn and soybean will not be described in detail because the conversion to a transgenic and/or pharma crop usually occurs after a new variety has been developed (though there are some exceptions in soybean that will be discussed). Since transgenic varieties are not usually crossed together to initiate the breeding process, there is a virtually zero chance of a pharma crop contaminating the food system during the breeding phase as outlined in Figure 4-1 (p. 54). We are therefore assuming that transgenes producing pharma crop traits will be backcrossed into existing varieties and not used in the breeding process when developing new varieties.

Transformation consists of three main phases: introducing the gene into plant cells; regeneration, the process by which a plant develops from the cells into which the gene was introduced; and maturation, the process of inducing the regenerated plants to produce seed.[23] Backcrossing is the breeding procedure used to move the transgene from the regenerated plants into other more desirable plants. The maturation phase of transformation and backcrossing is of primary concern for contamination.

Corn

Before the seed of a commercial single-cross hybrid can be produced, the inbred lines used to produce the hybrid must be developed. The methodology for developing inbred lines varies widely from breeder to breeder and from company to company. Hallauer (1990) has outlined a standard inbred line and hybrid development program. During the entire developmental phase, controlled pollinations are made either by hand or by wind in isolated crossing blocks similar to what is done in commercial seed production fields. The final products are inbred lines that can be used to produce a commercial hybrid.

The first step in generating a transgenic plant is to introduce a transgene into plant cells and have it become integrated into a cell's DNA. This is most commonly done in corn using a procedure called particle bombardment. Once cells contain the transgene, a plant is regenerated from the cells in cell culture. Regenerated plants are transplanted to a greenhouse so that when they flower, they can either be self-pollinated or crossed to

23 Personal communication with Kan Wang, director, Center for Plant Transformation, Iowa State University, 2003.

another plant. (It is common to do both when possible.)

The most difficult step in transformation is regenerating plants from cell culture. Because not all varieties can be used for this purpose, cell culture is said to be genotype-dependent (Armstrong et al. 1991; Armstrong 1994). Most corn transformation labs use the same basic genetic material for plant regeneration (Armstrong 1994). This germplasm[24] from which plants are regenerated is not commercially viable, so a backcrossing procedure is initiated immediately after plant regeneration to move transgenes of interest into commercially viable inbred lines.

After plants containing transgenes (transformed plants) have been regenerated from tissue culture, molecular screens are conducted to identify individuals that carry only one or two copies of the transgene. Individual plant(s) meeting these criteria will usually be both self-pollinated and crossed to an elite line. These plants will be evaluated to determine if the transgene is inherited genetically and to verify that the transgene is producing the protein it was designed to produce.

Regenerated plants are self-pollinated, backcrossed to an elite inbred line, and crossed to a hybrid. The purpose of self-pollination is to obtain additional seed of the original plant that contains the transgene; the purpose of the backcross is to transfer the transgene into an elite inbred line. The subsequent cross to the hybrid enables the production of enough grain to begin preliminary protein extraction (in the case of pharma crops). All of these crosses occur in the greenhouse; all crosses in subsequent generations are made in the field.

The geographic area where these crosses are made is important. Breeding and backcrossing nurseries may be conducted on land rented or leased from farmers or on land owned by companies or universities. Some companies conduct their field breeding with experimental transgenes in dedicated off-season nurseries in other parts of the world. The use of such nurseries makes it easier to obtain isolation from other crops and minimizes the probability of transgenes entering non-transgenic products outside the nursery.[25]

Backcrossing is a breeding scheme designed to move single transgenes from one inbred line into another inbred line (Figure 4-2). The inbred line that the transgene will be transferred to is called the recipient. The recipient is typically an elite inbred already used in commercial hybrids. The inbred line or plant donating the transgene is called the donor.

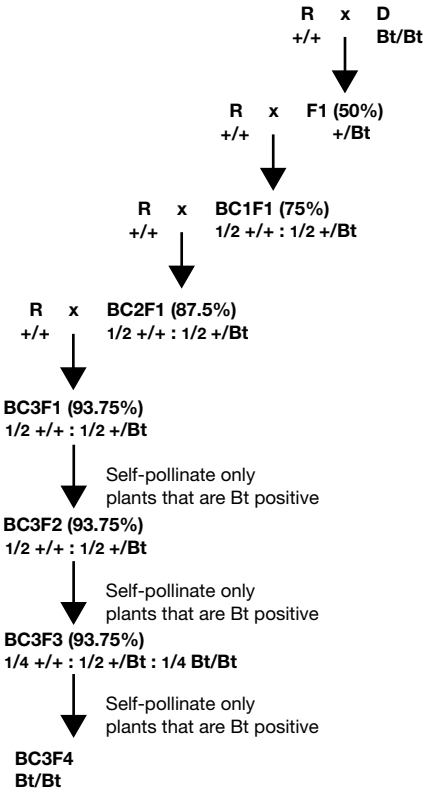
The goal of backcrossing is to transfer the gene of interest from the donor into the recipient without otherwise genetically changing the recipient. Backcrossing starts by making a cross between the recipient and donor. The progeny produced from this and later generations are repeatedly crossed with the recipient inbred line. With each generation of backcrossing, the amount of donor genome remaining is reduced by about one half. Each round is called a backcross.

The number of backcrosses used varies from two to seven with an average of about five. Because the recipient inbred line is already used to produce commercial hybrids in corn, there is typically no field testing for performance until the desired number of backcrosses has been completed. The decision to conduct field tests for performance

24 In general terms, germplasm refers to the genetic material that is the physical basis of heredity and is passed from one generation to the next. When applied to plants, germplasm refers to the seed or other structures by which plants are propagated.

25 The use of off-season nurseries varies from company to company and university to university. A cursory look at U.S. Department of Agriculture field testing permits for transgenic crops suggests that experimental events of corn are frequently grown in off-season nurseries, while soybean is grown in both off-season and summer season nurseries (ISB 2004).

Figure 4-2 **A Typical Backcrossing Scheme in Plants**



In this example, a gene called Bt is being backcrossed from a donor inbred (D) into a recipient inbred line (R). The genetic contribution of each generation is listed under the generation. The +/+ genotype is wildtype and the Bt/Bt genotype is homozygous for Bt. BC followed by a number is the backcross generation number. F followed by a number is the filial generation. For example, BC1F1 is the first backcross generation and the first generation cross. BC3F2 means the backcross 3F1 has been selfed once.

during the backcrossing procedure will depend on the amount of prior experience with the particular transgenic event.

After backcrossing is completed, the newly developed transgenic inbred line is crossed with another inbred to produce a hybrid for evaluation. The numbers of locations, replications per location, and years used for testing vary depending on prior knowledge of performance of the converted inbred, the quality of the conversion, and known problems with modifier genes associated with backcrossing a particular event.

Because a commercial hybrid receives extensive testing prior to commercialization, it is not necessary to re-prove the performance of the hybrid. Following backcrossing of a transgene it is necessary to demonstrate that the backcrossing process itself did not disrupt the performance of the hybrid. Breeders are most interested in the comparison of the transgenic hybrid with its non-transgenic counterpart.

The amount of testing required to do this will depend on the protocols in place at specific companies and the amount of risk they are willing to take. The time lag between the development of a new inbred line and its sale to farmers as a transgenic hybrid is three to four years, depending on how long it takes to backcross the transgene, to obtain adequate quantities of parent seed, and to produce the hybrid. Typical testing of a transgenic hybrid following backcrossing would involve 25 to 40 locations and one to three replications per location. The yield of pharma corn will not be such a major concern and less testing will need to be done.

Comparison of a transgenic hybrid with its non-transgenic counterpart would continue until a decision was made to either drop the hybrid or use it commercially. If the hybrid will be used commercially, up to three years of data would likely be obtained on the hybrid before it is actually planted by farmers.[26]

The backcrossing procedure is conducted by making hand pollinations. If the transgene of interest (such as Roundup Ready™) has been approved (that is, allowed on the market by the federal government), the necessary crosses are

26 The information and procedures in this paragraph are based on the author's conversations with private breeders who conduct backcrossing programs. What is done at a specific company may vary considerably from what has been described.

conducted in the field in summer or winter breeding nurseries (such as Hawaii or Puerto Rico) used by the companies. Most companies have extensive protocols in place to keep approved transgenes from entering their non-transgenic breeding germplasm. Corn breeders at most breeding companies are required to use separate nurseries for GMO (genetically modified organism) and non-GMO breeding activities. The author is unaware of any public sector corn breeding programs that use inbred lines containing approved transgenes to develop new breeding populations.

If the transgene is experimental and has not been approved, different procedures are used. Public sector scientists will conduct as much of the backcrossing as possible in the greenhouse, but it is also frequently done in the field using protocols developed by government regulators. Private sector scientists will usually do all of the backcrossing with experimental transgenes in a winter nursery. There are two advantages to this strategy: many winter nursery locations are tropical, enabling two or three breeding generations to be obtained in a year; and in many of these locations, such as Hawaii, it is easy to obtain long-distance (greater than one mile) isolation from other corn.[27]

Breeder seed of both transgenic and non-transgenic inbreds is established and maintained by the originating breeder or breeding company by hand pollinations. Breeder seed of non-transgenic inbreds and inbreds that contain approved transgenes is maintained in summer or winter breeding nurseries as needed. It is the breeder's responsibility to ensure that the inbred is homozygous[28] for the desired transgene, has uniform plant type, and adequately represents the genetic constitution of the inbred (Wych 1988).

This description of corn variety development, transformation, and backcrossing is a generic description. There are numerous major and minor variations in the execution of all three activities.

Soybean

Several breeding procedures are used to develop soybean varieties. Fehr (1987) lists five commonly used methods. The methods all involve self-pollination in every generation following the development of a breeding population. They differ primarily in the number of generations of self-pollination that are conducted before the lines are tested in replicated trials for their potential as new varieties. Single-seed descent is the most popular method of soybean variety development (Fehr 1987). Soybean is naturally self-fertilizing and hand pollinations are only used to produce hybrids for developing breeding populations.

The variety development phase for soybean, like corn, is not a source of contamination of the food supply by pharma crops. As in corn, the experimental transgenes and transgenes used in pharma crops are introduced into established varieties. Developing breeding populations that contain experimental or pharma crop transgenes would be too risky and difficult to control.

The transformation phase for soybean is similar to that used in corn except that a different protocol may be used to introduce the transgene.[29] If

27 The information in this paragraph was gleaned from informal conversations with commercial plant breeders and cannot be attributed to any single company. The author's impression is that most multinational seed companies practice good stewardship and do everything possible to minimize the escape of an experimental transgene into the food supply. There are many ways this could be accomplished, but restricting breeding activities for experimental transgenes to one location helps minimize the probability of escape.

28 Homozygous means that all sex cells of the inbred carry the transgene.

29 For more information on plant transformation, see the Iowa State University Plant Transformation Center website (*http://www.agron.iastate.edu/ptf/web/mainframe.htm*).

backcrossing is used to move the transgene into soybean inbred lines, the backcrossing procedure used would be nearly identical to that described above for corn (Figure 4-2, p. 59).

Commercial soybean breeders use a procedure called forward breeding to incorporate transgenes (usually herbicide tolerance) into new varieties. This basically involves initiating new breeding crosses with inbred line(s) that already contain the transgene. If both inbred lines contain the transgene, all progeny resulting from the cross will carry it. If only one inbred carries the transgene, then only some progeny will carry it, and the breeders will have to select for the trait to keep it in plants that are self-pollinated. For herbicide resistance, selection is easy. The breeder sprays the soybean with the herbicide and keeps those that survive.

There are three reasons why soybean breeders take this approach: 1) backcrossing is difficult in soybean because soybean is generally difficult to cross and backcrossing involves repeated rounds of hand crossing; 2) there is no time lag between the development of a superior variety and converting it to herbicide resistance; and 3) Roundup Ready™ soybean has been widely accepted and grown by farmers. No transgene has achieved such a wide acceptance in corn.

Since forward breeding is used primarily to incorporate herbicide tolerance into new soybean varieties, these breeding activities take place at standard soybean breeding facilities. Experimental transgenes are also often field tested in standard soybean production areas, judging by applications to the U.S. Department of Agriculture for field test releases (ISB 2004).[30] Many of these tests are conducted in Hawaii and Puerto Rico, but a number are also conducted in states where soybean is commonly grown.

Breeder seed is produced and maintained in a manner similar to that in corn. The primary difference is that in soybean, controlled pollinations are not used to produce seed. Fehr (1987) gives an excellent description of the production of breeder seed. Breeder seed is produced by planting the soybean inbred line in the field and allowing it to self-pollinate. Off-type plants, that is, plants that have different physical characteristics from the majority of the plants in the inbred line, are removed when they are identified.

Foundation Seed Production

Foundation seed increase is the next step in the process of developing a new variety (Figure 4-1, p. 54). Foundation seed production is also called parent seed production in corn because the foundation seeds of inbred lines of corn are used as parents to produce the hybrid corn seed sold to farmers.

Cytoplasmic male sterility (CMS) is a sterility mechanism frequently used in corn to reduce or eliminate the need to emasculate corn plants (remove the male part, or tassel) in the production of hybrid seed. Because CMS complicates the production of foundation seed, this section discusses male-fertile female parents and male-sterile female parents separately.

Corn

Male-fertile parents. Large quantities of seed are required of each inbred line (the male and female parents of the hybrid) to produce enough seed for sale to farmers (Table 4-2, p. 62). This phase of seed production is usually referred to as foundation seed production. Although procedures vary from company to company, Wych (1988) outlined the typical steps, the two most important of which for our purposes are establishing and maintaining a supply of breeder seed and producing foundation inbred seed.

30 Field testing must follow protocols established by the U.S. Department of Agriculture and other regulatory agencies that oversee field trials of transgenic plants.

Table 4-2 **Quantities of Production Required for Hybrid Corn Seed Production**

Hybrid			Female Inbred Parent			Male Inbred Parent		
Final Hybrid Acreage	Units of Hybrid Seed Required[1]	Acres of Hybrid Seed Production[2]	Number of Kernels of Foundation Female Seed Required[3]	Acres of Female Parent Seed Production Needed[4]	Number of Selfed Plants If Seed Is Produced by Hand[5]	Number of Kernels of Foundation Male Seed Required[3]	Acres of Male Parent Seed Production Needed[4]	Number of Selfed Plants If Seed Is Produced by Hand[5]
50	16	0.3	6,760	0.0017	27	1,690	0.0004	7
100	33	0.7	13,520	0.0034	54	3,380	0.0008	14
500	163	3.3	67,600	0.0169	270	16,900	0.0042	68
1,000	325	6.5	135,200	0.0338	541	33,800	0.0085	135
2,000	650	13.0	270,400	0.0676	1,082	67,600	0.0169	270
3,000	975	19.5	405,600	0.1014	1,622	101,400	0.0254	406
5,000	1,625	32.5	676,000	0.1690	2,704	169,000	0.0423	676
10,000	3,250	65.0	1,352,000	0.3380	5,408	338,000	0.0845	1,352
75,000,000	24,375,000	487,500.0	10,140,000,000	2,535.0000	40,560,000	2,535,000,000	633.7500	10,140,000

1 A commercial unit of hybrid seeds is assumed to contain 80,000 kernels and will plant approximately 3.1 acres, assuming a planting rate of 26,000 plants per acre.
2 The acres of hybrid seed production that need to be planted to produce a given quantity of hybrid seed, assuming the female yields 50 units of hybrid seed per acre of total production (including the acreage occupied by the males). The author assumed a planting ratio of 4 female to 1 male.
3 Number of kernels of foundation female or male seed required to plant the hybrid production field was calculated assuming a hybrid seed field planted in a ratio of 4 females to 1 male, and that the male and female parents were planted in the hybrid production field at the rate of 26,000 plants per acre.
4 Acres of female and male production required to produce a given number of kernels was calculated assuming the male and female parents yield 50 units of seed per acre and that each unit contains 80,000 kernels.
5 The number of plants selfed if seed is produced by hand, assuming that 250 kernels are produced with each self-pollination.

Foundation inbred seed production fields are planted with the initial supply of breeder seed. The foundation inbred seed increases are produced in isolation (via wind pollination) where the inbred line is allowed to sib-mate[31] or open-pollinate. Since all plants in the inbred line are theoretically identical, sib-mating or open pollination is equivalent to self-pollination. This initial inbred increase produces the first crop of foundation seed, which is then used to plant subsequent inbred increases and produce larger quantities of foundation seed for hybrid seed production.

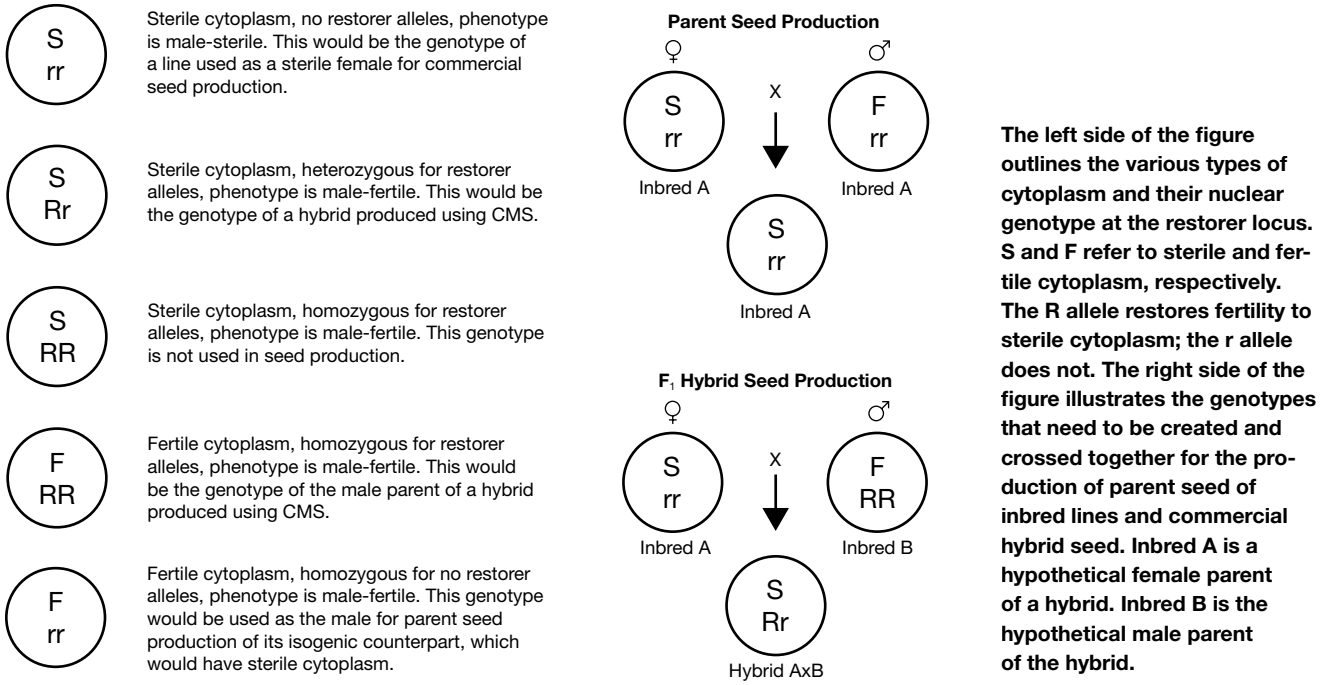
Foundation inbred seed increase fields are isolated from external pollen sources to maintain genetic purity. Only 1 in 1,000 off-type plants is allowed for certification of foundation inbred seed (Table 4-1, p. 56). Commercial companies certify most foundation seed that will be used for export or production of seed for export, but certification is not required for foundation seed produced for use in the United States. There is no registered or certified class of seed for inbred lines because this seed is not sold directly to producers.

Although larger companies produce their own foundation seed of inbred lines, smaller companies may contract this production to another company or simply purchase their foundation seed from one of the industry's genetic suppliers. Foundation inbred seed production has typically occurred in the primary corn production areas on a contract basis with farmers or on land leased from farmers. To increase purity, foundation seed production has recently been moving to areas with no commercial corn production.

Male-sterile parents. If CMS is used to produce commercial hybrid seed, then parent seed production becomes slightly more complicated. The use of CMS involves an interaction between nuclear

31 Sib-mating is a term breeders use to describe the mating of individual plants. Sib-mating is usually done by hand and is similar to open pollination except that self-pollination is not allowed to occur.

Figure 4-3 **The CMS System in Corn and Its Use in Seed Production**



The left side of the figure outlines the various types of cytoplasm and their nuclear genotype at the restorer locus. S and F refer to sterile and fertile cytoplasm, respectively. The R allele restores fertility to sterile cytoplasm; the r allele does not. The right side of the figure illustrates the genotypes that need to be created and crossed together for the production of parent seed of inbred lines and commercial hybrid seed. Inbred A is a hypothetical female parent of a hybrid. Inbred B is the hypothetical male parent of the hybrid.

genes and genes inherited only through the cytoplasm. This system takes advantage of the fact that a plant can only inherit genes in the cytoplasm from the plant that served as its female parent.

There are two types of cytoplasm in a CMS system, which we will simply refer to as sterile (S) and fertile (F). There is also a dominant nuclear gene (R) that restores the male fertility of plants that contain sterile cytoplasm. This dominant gene is usually called the restorer gene, and one copy is enough to restore complete male fertility. Inbred lines are converted to CMS using the same backcrossing procedure used to incorporate transgenes into inbred lines. In the case of CMS conversion, however, the objective is to substitute the whole genome of an inbred line into the sterile cytoplasm.

The production of parent seed from a male-sterile inbred requires what is known as a maintainer line. A maintainer line is isogenic[32] to the sterile inbred, carries non-restorer alleles, and has fertile cytoplasm. It is therefore male-fertile and will be used as the pollen parent in a seed production field similar to those used to produce hybrids, with the female being the sterile isogenic version of the same line (Figure 4-3). This is a slightly more complex process for producing parent seed than what is required if CMS is not used, but it must be done in isolation as well.

Soybean

Production of foundation seed to be grown by farmers is much simpler in soybean than in corn. Once breeder seed is available, foundation seed

32 Two inbred lines are said to be isogenic if they are genetically identical at all loci except one. In practice, breeders refer to these lines as near-isogenic lines because a block of genes around the gene of interest is moved, rather than a single gene (Fehr, 1991).

production can begin. Foundation seed is produced in the same manner as breeder seed—by letting plants self-pollinate. The major difference between producing foundation seed in soybean and corn is that there is no isolation requirement in soybean beyond that required to prevent physical mixing. Although there is a registered class of soybean seed, it is not widely used and not recognized in some states (Tekrony, Egli, and White 1987). Soybean foundation seed is usually produced near the area where the commercial seed is expected to be sold.

Commercial Seed Production

Although corn and soybean have very different modes of reproduction, commercial seed production involves many of the same steps. The basic steps are seed packaging, planting, crop maintenance, controlling pollen movement (corn only), harvesting, transporting, drying, shelling (corn only), seed conditioning, bagging, and storing.

Each of these steps often has several sub-steps and some of the processes differ slightly between corn and soybean. For example, there is no control for pollen movement in soybean, but isolation is used as insurance against physical mixing. Corn is harvested, transported, and dried on the ear, whereas soybean seed is removed from the pod by a combine in the field, then transported and dried. Corn seed production is technically more difficult and involves more steps.

Corn

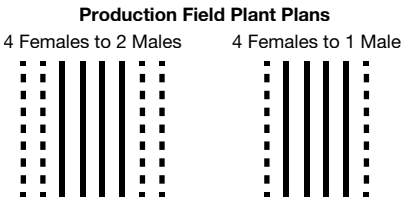
Once the seed of a hybrid's inbred parents has been produced in adequate quantities, commercial production of the hybrid can begin. The first step is selecting the production area and the growers (Wych 1988). The majority of hybrid seed corn sold here is produced in the Corn Belt region of the United States, but the seed of new hybrids is also produced in the Southern Hemisphere. One of the most important factors in selecting the growing area and grower is isolation of the seed fields from other corn fields. The minimum isolation distances and contamination levels are shown in Table 4-1 (p. 56).

Once the fields have been selected, the next step is planting. Production fields are planted using a predetermined ratio of male to female rows (Figure 4-4). Depending on the ability of the male to produce pollen and the difference in maturity between the female and male inbred lines, either one or two rows of male parent are used for every four to six rows of female. The male and female parents may be planted at different times depending on the difference in their flowering times. In many production fields, once the male has finished shedding pollen it is mowed down to prevent accidental mixing at harvest and the theft of male inbred seed.

Standard equipment and production and pest control practices are used at this stage. Most cultural and management practices are designed to improve the yield of hybrid seed per acre.

The primary concern in a seed corn production field is pollen control. Female parents of hybrids are prevented from shedding pollen either by emasculation or CMS. CMS may or may not require emasculation depending on the cytoplasm

Figure 4-4 **Typical Planting Arrangements Used in Hybrid Seed Corn Production Fields**



The dashed lines represent male rows and the solid lines represent female rows. This is repeated across the entire field; the extra male rows in the figure are part of the repeated design.

used, the genotype of the female inbred, and the environmental conditions during flowering (Duvick 1959a, b, 1965). Neither emasculation nor CMS prevents contamination from pollen in nearby fields; this is accomplished entirely through spatial and sometimes temporal isolation. Ensuring an abundant supply of pollen from the male parent during the emergence of silks from the female also reduces contamination from nearby fields (Carcova et al. 2000).

Once the grain in a seed production field has reached physiological maturity, the seed crop will be harvested on the ear to minimize damage to the kernels. Harvesting is done with special machines that minimize ear damage. The seed is then hauled to a seed production facility where is it dried, shelled, cleaned, sized, treated, and placed into bags. These operations are accompanied by a series of quality assurance operations to determine the physical quality, purity, and germination of the seed. The seed is then stored until its distribution to dealers and farmers.

Soybean

Certified seed is the class of soybean seed sold directly to farmers. It is either produced from registered seed or, more commonly, from foundation seed, and is usually produced near the area where it is expected to be planted. AOSCA requirements for certified seed production are listed in Table 4-1 (p. 56), but certification is not mandatory for soybean seed in the United States and the use of certification agencies varies from company to company. The processing of soybean seed for shipment to farmers is similar to what is done with corn, though there are subtle differences.

VULNERABILITY OF SEED PRODUCTION TO PHARMA CROP CONTAMINATION

There are major differences in scale between seed production for commodity crops and pharma crops. On average, corn and soybean are each planted on more than 70 million U.S. acres annually (USDA NASS 2004). Predicting expected production acreage for pharma crops would be conjecture, but seed production needs will be far less than for commodity crops. This difference in scale implies that standard production practices may not necessarily be used for pharma crop seed production, which would therefore more closely resemble commodity foundation seed production. All seed production, however, no matter what the scale, shares some common steps and points of vulnerability (Table 4-3, p. 66) that will be explored below.

The differences in scale are illustrated in Tables 4-2 and 4-4. Table 4-2 (p. 62) lists the seed production requirements for corn given an expected acreage to be planted with hybrids. For example, the planting of 1,000 acres of pharma crop would require about 325 bags of hybrid seed corn (the typical bag contains about 80,000 kernels), which would take 6.5 acres to produce. In turn, less than 0.1 acre would be required to produce enough seed of the male and female inbred parents to plant 6.5 acres of production. According to these calculations, the amount of seed needed to plant the entire U.S. commodity corn crop would require approximately 487,000 acres of hybrid seed production.

The situation is similar for soybean seed production, except that it generally takes more acres to produce enough seed to plant an acre of soybean. For example, planting 1,000 acres of soybeans would require 60,000 pounds of seed, which would take 25 acres to produce (Table 4-4, p. 67). Twenty-five acres of seed production would require about 0.6 acre of foundation seed production. In other words, producing enough seed to plant 1,000 acres requires about four times as much land for soybean as it does for corn.

Table 4-3 **Points of Vulnerability in the Seed Production Process**

VARIETY DEVELOPMENT
Seed packaging and preparation
- ☐ Spillage
- ☐ Seed mixing
- ☐ Mislabeling of seed

Planting breeding nursery
Maintaining crop
- ☐ Cultivating
- ☐ Spraying

Making controlled pollinations
- ☐ Pollinations made by hand
- ☐ Pollinations made by wind
- ☐ Pollen movement

Harvesting breeding nursery
- ☐ Seed on plants not harvested
- ☐ Disposal of unwanted grain
- ☐ Disposal of unwanted plants
- ☐ Cleanout of machine used for gleaning field
- ☐ Disposal of seed gleaned from field
- ☐ Spilled grain
- ☐ Volunteer plants emerge in field the following year

Transporting grain to shelling facility
Shelling/threshing and seed processing
- ☐ Accidental mixing of seed
- ☐ Mixing during shelling
- ☐ Mislabeling of seed during seed processing
- ☐ Improper discarding of seed

Field testing of new varieties
- ☐ Seed packaging and preparation
- ☐ Planting field test
- ☐ Crop maintenance
  - Cultivating
  - Spraying
- ☐ Field testing on land rented from farmers
  - Farmers could accidentally harvest test plots
  - Seed may be spilled
- ☐ Pollen movement
- ☐ Harvest
  - Cleanout of machine used for harvesting
  - Disposal of harvested seed
- ☐ Volunteer plants emerge the following year

Discarding seed of varieties that are not productive
- ☐ Seed may be mixed with other varieties
- ☐ Seed may accidentally grow (resulting in pollen movement)

TRANSFORMATION
Bombardment
Regeneration
Maturation
- ☐ Pollen movement
- ☐ Physical mixing

BACKCROSSING
Seed packaging and preparation
- ☐ Spillage
- ☐ Seed mixing
- ☐ Mislabeling of seed

Planting breeding nursery
Maintaining crop
- ☐ Cultivating
- ☐ Spraying

Making controlled pollinations
- ☐ Pollinations made by hand
- ☐ Pollinations made by wind
- ☐ Pollen movement

Harvesting breeding nursery
- ☐ Seed on plants not harvested
- ☐ Disposal of unwanted grain
- ☐ Disposal of unwanted plants
- ☐ Cleanout of machine used for gleaning field
- ☐ Disposal of seed gleaned from field
- ☐ Spilled grain
- ☐ Volunteer plants emerge in field the following year

Transporting grain to shelling facility
Shelling/threshing and seed processing
- ☐ Accidental mixing of seed
- ☐ Mixing during shelling
- ☐ Mislabeling of seed during seed processing
- ☐ Improper discarding of seed

Field testing new varieties
- ☐ Seed packaging and preparation
- ☐ Planting field test
- ☐ Crop maintenance
  - Cultivating
  - Spraying
- ☐ Field testing on land rented from farmers
  - Farmers could accidentally harvest test plots
  - Seed may be spilled
- ☐ Pollen movement
- ☐ Harvest
  - Cleanout of machine used for harvesting
  - Disposal of harvested seed
- ☐ Volunteer plants emerge the following year

Discarding seed of varieties that are not productive
- ☐ Seed may be mixed with other varieties
- ☐ Seed may accidentally grow (resulting in pollen movement)

BREEDER SEED PRODUCTION
Seed packaging and preparation
Planting breeding nursery
Maintaining crop
Making controlled pollinations
Harvesting breeding nursery
Transporting grain to shelling facility
Shelling/threshing
Seed processing and conditioning

FOUNDATION SEED PRODUCTION
Seed packaging
Seed planting
Crop maintenance
Pollen movement
Harvest
Transportation
Drying
Shelling
Conditioning
Storage

COMMERCIAL SEED PRODUCTION
Seed packaging
Seed planting
Crop maintenance
Pollen movement
Harvest
Transportation
Drying
Shelling
Conditioning
Storage

The frequency of seed production can also be a problem. For example, if only one pharma crop is to be produced on 1,000 acres, then seed production is greatly simplified. If, however, 100 different pharma crops are to be planted on 1,000 acres each, then the frequency of production becomes a greater concern than the scale of production.

The major points of vulnerability to contamination in the food system are outlined in Table 4-3. The seed production system has been divided into six major steps (as shown in Figure 4-1, p. 54): variety development, transformation, backcrossing, breeder seed production, parent seed production, and commercial seed production. Each of these steps is then analyzed for points of vulnerability. Corn and soybean often share the same points, but the degree of vulnerability may differ.

Corn

Variety Development. As stated earlier, the variety development stage of seed production is not a point of vulnerability because pharma crop transgenes are currently introduced only into established varieties. If, however, pharma crop transgenes were introduced at the variety development stage, and if the program were conducted in parallel with conventional breeding programs, then avoiding contamination entirely would become highly improbable if not impossible. Because the points of vulnerability for variety development are the same as those for backcrossing, we will discuss the reasons for contamination in that section.

Transformation. Transformation is required to introduce all new transgenes into plants. The sources of potential contamination during bombardment and regeneration come from plant tissue. Most transformation labs destroy all unused biological material by autoclaving, and the biological material is confined to a laboratory or greenhouse. As long as this process is followed, contamination of the food supply from biological material should not occur. The main concern with the transformation phase arises after a plant has been regenerated from cell culture and produces flowers.

The first plants after regeneration are grown and pollinated in the greenhouse, where, without appropriate precautions, contamination of other corn plants with the new transgene via pollen movement or seed mixing is likely to occur. The contaminated plants would most likely be other plants that already contain experimental transgenes. If the greenhouse is not a confined facility, pollen may escape into the atmosphere and contaminate plants outside the greenhouse.

Detection of a contaminant by an outside third party at this stage would be difficult because the transgene and its product are usually regarded as confidential business information, which means that only the transgene developer could identify the contaminant gene. Detecting compounds produced by the contaminant gene is less likely to involve confidential business information, but the ease with which that can be done will vary.

Backcrossing. The vulnerability of working with transgenes in the field is illustrated by backcrossing. The primary use of backcrossing is to move a transgene into a more desirable inbred line, but all field-breeding activities, no matter their objective, have points at which food crops are vulnerable to contamination.

The points of vulnerability are: seed packaging and preparation; planting the breeding nursery; making controlled pollinations; harvesting the breeding nursery; transporting, drying, shelling, and processing seed; field testing new varieties; and discarding the seed of unproductive varieties. Some of these activities occur simultaneously in a breeding program. For example, the planting of the 2005 breeding nursery will be done simultaneously with the planting of field tests of varieties developed in the 2004 breeding nursery.

Table 4-4 **Quantities of Production Required for a Given Acreage of Soybean Production**

Planted Acreage	Pounds of Soybean Seed Required	Acres of Soybean Seed Production Required	Acres of Foundation Seed Production
50	3,000	1	0.0
100	6,000	3	0.1
500	30,000	13	0.3
1,000	60,000	25	0.6
2,000	120,000	50	1.3
3,000	180,000	75	1.9
5,000	300,000	125	3.1
10,000	600,000	250	6.3
75,000,000	4,500,000,000	1,875,000	46,875.0

The calculations in this table assume an average planting rate of 150,000 seeds per acre, 2,500 seeds per pound, and a soybean yield of 40 bushels per acre. A bushel of soybeans weighs 60 pounds.

The main concerns for contamination during seed packaging are spilling and mixing, particularly if seed containing experimental transgenes is packaged in the same facilities with food/feed-grade seed. And there is always the risk of the planter not being properly cleaned before and after each use, especially if it is used for planting different types of corn.

The main problem during controlled pollinations, which are made either by hand or wind depending on the breeding procedure, is preventing pollen from moving to where it is not intended or wanted. Generally speaking, there are many more plants shedding pollen than are needed for use in backcrossing. The seed resulting from controlled pollinations in breeding nurseries is usually collected by hand and any seed left on the plants is harvested with machines.

Harvesting involves several points of vulnerability. There are always plants that produce grain that is not harvested for seed. The disposal of both this grain and the plants that produce the grain, as well as the cleanup of machines that glean the fields, poses special challenges. Seed may be spilled in the field during the harvest or during transportation to the shelling/threshing facility.

The shelling/threshing process generates a lot of loose seed, as does the associated handling of the seed and spillage. Seed from different lots, for example, can be easily mixed or improperly labeled after shelling by inexperienced personnel. If the shelling/threshing machines are not properly cleaned, they too may become a source of contamination.

One of the more serious problems during shelling is that there is frequently more seed than breeders need. This excess seed must be properly discarded so that it does not become mixed with other seed or grow the following year and produce pollen and more seeds. Volunteer plants emerging in the field the following year are always a problem in breeding nurseries, where the frequency of dropped ears and partially shelled ears is high because of the variability among plant types.

Field testing of new varieties poses many of the same vulnerabilities as described for variety development. The main difference is that field tests are more frequently conducted on land rented or leased from farmers. The field test may be located some distance from the breeding nursery, making daily or even weekly monitoring of the test site difficult. Otherwise, the vulnerabilities of field testing are almost identical to those encountered during the variety development phase.

Breeder Seed Production. Breeder seed is produced in breeding nurseries similar to those used for variety development. Therefore, the points of vulnerability are similar to those described above for backcrossing. The amount of breeder seed produced varies considerably depending on the circumstances, but a typical range would be 5 to 50 pounds per variety. Breeder seed is produced by making hand (controlled) pollinations.

Although the sources of contamination of breeder seed are the same as for backcrossing (Table 4-3, p. 66), the probability of detecting the contamination and its consequences are much different in breeder seed production using good practices (e.g., iterative self-pollination and observation). During the observation phase, mixing and outcrossing is nearly always detected before it can cause problems. Such a breeder seed production system is less vulnerable to contamination than backcrossing and other research operations.

Foundation Seed Production. Foundation seed production is the first large-scale seed increase that occurs in the seed production process. The amount of foundation (parent) seed of a corn inbred line produced will depend on the anticipated sales of the hybrid for which that line is a parent. For example, if the hybrid is expected to be grown on about 10,000 acres, then about

0.4 acre of foundation production will be needed. In practice, the acreage may be larger if a multiple-year supply of seed is desired, if the inbred line is used as the parent of more than one hybrid, or if there are facilities in which to store the seed.

The points of vulnerability for foundation seed production are similar to those described for breeding and field testing; the primary difference is scale and frequency. Foundation seed increases in corn are almost always done in isolation and pollination occurs via wind movement. Most companies use the standards listed in Table 4-1 (p. 56), which do not guarantee virtually zero contamination, and may need to produce the seed of 10 to 50 inbreds depending on the size of their product line, sales, and storage capacity. There is no easy way to contain corn pollen, and plants must produce pollen in order to produce seed.

Because of the need to produce foundation seed for a large number of inbred lines, the steps following pollen movement become even more important. Harvesting, transporting, drying, shelling, conditioning, and storing would all be problematic if the same equipment and facilities were used to process seed. If pharma corn were processed in the same manner and the same facility as commodity corn, the vulnerability to contamination due to seed mixing during these steps would be very high. The use of separate equipment and facilities is feasible for pharma corn.

Commercial Seed Production. Hybrid seed production poses some of the same vulnerabilities to contamination of the food system as foundation seed production. The most obvious source of potential contamination is pollen movement from the production field to a farmer's field or a field producing another hybrid. Physical mixing associated with harvesting equipment is another source. Hybrid seed production also has the same contamination risks as on-farm production (Chapter 5) and risks similar to those for shipping and storage (Chapter 6).

Seed processing has several points of contamination, including the mixing of seed lots, imperfect cleaning of equipment in the processing plant, and mislabeling of bags. Although seed production plants are designed to maintain the purity of individual hybrids, virtually zero contamination is not necessary under normal circumstances and is never achieved. Separate equipment and facilities for pharma corn are feasible and could prevent cross-contamination with seed of varieties used for food.

Soybean

Pharma soybean has far less risk of contaminating the commodity crop via windborne pollen than corn. In other ways, however, soybean can pose greater risks than corn. For example, soybean is visited by insects that facilitate pollination (Palmer et al. 2001). Bees have the potential to travel long distances before depositing pollen, though the success of such long-distance pollination depends primarily on pollen longevity.

Otherwise, soybean has similar vulnerabilities to corn when it comes to contamination risks, including issues related to transformation, backcrossing, foundation and commercial seed production, and seed processing. Because a larger volume of seed is required to plant an acre of soybean than an acre of corn, there may be a greater risk of seed mixing with soybean than with corn.

ACHIEVING VIRTUALLY ZERO CONTAMINATION

As discussed, commodity corn and soybean share many of the same points of vulnerability. The major points are pollen movement, physical mixing of seed, and seed left in the field to become volunteers the following year.

Pollen Movement

The fact that soybean is predominantly self-pollinated has led many to believe that contamination from outcrossing in soybean is not a concern,[33] whereas the wind-pollinated nature of corn suggests that contamination from pollen movement cannot be eliminated. Surprisingly, some fundamental biological characteristics of corn make pollen control easier than in soybean production, particularly with regard to achieving the stringent goal of virtually zero contamination.

Soybean produces between 3,740 and 7,600 pollen grains per flower (Palmer et al. 2001). If the average soybean plant produces 100 flowers, then that plant produces between 374,000 and 760,000 pollen grains. Since soybean is typically planted at the rate of about 150,000 plants per acre, the number of pollen grains produced per acre is around 75 billion. This is less than that produced by corn, but obviously a large amount nevertheless.

Rates of outcrossing vary from less than one percent to more than 25 percent in soybean depending on the variety, environment, and availability of pollinators (Palmer et al. 2001). There is no published information on long-distance pollen movement in soybean, but there are many anecdotal accounts suggesting that soybean pollen can be carried long distances by insects under the right environmental conditions.[34] Because little is known about insect-mediated long-distance pollen movement in soybean, spatial barriers may not be effective. Temporal barriers are ineffective because soybean tends to flower over long periods of time.

Mechanical and biological barriers to pollen movement are also unavailable in soybean, which is very difficult to emasculate manually and could never, therefore, be emasculated on a large scale. Biological sterility systems such as CMS only recently became available for soybean, and producing seed on male-sterile soybean plants is difficult (Palmer et al. 2001). For these reasons, the production of pharma crop soybean seed in areas where commodity soybean is grown cannot be recommended until more is known about insect-mediated pollen movement.

More tools are available to control pollen movement in corn than soybean. Though spatial and temporal barriers are not completely effective, there are good mechanical and biological barriers available. For example, corn can easily be emasculated on a large scale either manually or with special machines; the key is to start before the beginning of pollen shed. Excellent CMS systems, which have been used for commercial seed production, are also available for corn. In theory, combining these four barriers—spatial, temporal, mechanical, and biological—could effectively achieve virtually zero contamination from pollen movement in corn. This assumes, of course, no human errors and a 100 percent effective CMS system.

The ability to use all four barriers effectively to achieve virtually zero contamination from pollen movement depends on the size of the fields, the amount of time devoted to monitoring fields for potential problems, and the isolation distances. It is difficult to quantify the probability of a contamination event. There are, for example, no published data on the frequency of failure for emasculation or CMS—this is an area that needs additional research on both the plant side and the modeling and simulation side. Strong qualitative statements are also difficult to make because they

33 See, for example, the document on soybean available at the U.S. Department of Agriculture Animal and Plant Health Inspection Service (USDA APHIS) website (*http://www.aphis.usda.gov/brs/soybean.html*), accessed September 19, 2004.

34 Personal communication with R.G. Palmer, U.S. Department of Agriculture Agricultural Research Service, 2003.

require too many assumptions about processes under human control and thus depend on measurement of human error rates.

Another possible approach to achieve virtually zero contamination is for farmers to plant inbred lines rather than hybrids. The male-sterile inbred could then be pollinated with normal corn, and commercial production could proceed in much the same way it does for hybrids. The main disadvantage of this method is that larger seed production acres of the pharma inbred would probably be required, since inbreds yield substantially less than hybrids.

Growing inbred lines eliminates the hybrid seed production step but requires much larger increases of the inbred line carrying the transgene of interest. Since there is no easy way to produce this quantity of inbred seed other than by allowing it to open-pollinate in isolation, inbred seed production would require greater isolation distances than CMS production and may need to be conducted in areas where the commercial crop or a seed production crop is not grown. CMS could still be used, but it would require the creation of the appropriate sterile and maintainer lines as described earlier.

The type of hybrid corn seed produced for pharma crops is also important in preventing pollen movement. Assuming that the pharma transgenes are dominant, the hybrids to be grown by farmers might need to be 100 percent male-sterile. This can be accomplished in one of two ways. A male-sterile hybrid that is hemizygous[35] for the transgene could be produced as outlined in Figure 4-5 (p. 72, Option 1). The female inbred of the hybrid would be homozygous for the transgene and reside in sterile cytoplasm without nuclear restorers. The male inbred of the hybrid would be produced with a normal inbred as the male that lacks both the transgene and restorer alleles. The resulting hybrid would be male-sterile and hemizygous for the transgene.

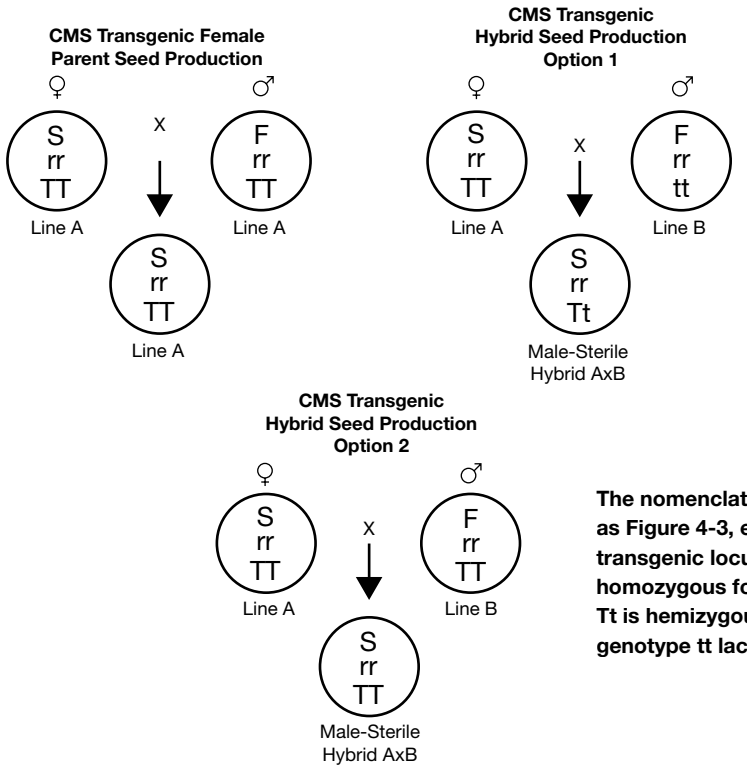
The farmer would plant the field in the same manner as a seed field with the male being a normal hybrid (Figure 4-4, p. 64). After pollination, the male rows in the field could be destroyed so they are not inadvertently harvested. The advantage of this system is that pollen would not be shed by transgenic plants. In case the CMS system failed or partially failed, the plants would also be emasculated as the tassels emerge.

Option 1 has an advantage during hybrid seed production as well, in that the only pollen shed in the field is from a non-transgenic inbred. The disadvantage of this option is that only 50 percent of the kernels on the ear will produce the pharma crop trait, a result that would presumably lower product yield at the extraction plant.

The second hybrid production option is to produce a CMS hybrid that is homozygous for the transgene. Seed is produced by using the same female inbred used in Option 1. The male inbred of the hybrid differs from Option 1 in that it is also homozygous for the transgene; otherwise, it has fertile cytoplasm and carries non-restorer alleles. The hybrid produced by Option 2 will be CMS and homozygous for the transgene. The farmer would plant the field as with Option 1. The primary advantage of producing hybrids with Option 2 is that 100 percent of the kernels harvested will carry the transgene; the disadvantage is that the transgene has to be backcrossed into two inbred lines instead of one, but this can occur simultaneously.

35 Hemizygous means each plant cell carries only one copy of the transgene.

Figure 4-5 **Using CMS to Create Mail-Sterile Transgenic Commercial Hybrids**



The nomenclature in the figure is the same as Figure 4-3, except the genotype at the transgenic locus is shown. The genotype TT is homozygous for the transgene, the genotype Tt is hemizygous for the transgene, and the genotype tt lacks the transgene.

Physical Mixing

Preventing contamination of the food chain with pharma crops is primarily a function of keeping pharma crop seeds separate from commodity seeds during all phases of seed handling outlined in Table 4-3 (p. 66). Accomplishing this would require rigorous standard operating procedures (SOPs) to track the flow and quantity of seed and the use of dedicated machinery and infrastructure. The use of SOPs would ensure that employees were aware of the requirements, allow accidents or mistakes to be traced, and enable the process to be stopped or changed to prevent mistakes.

Volunteer Plants and Crop Residue

Volunteer plants are often a significant problem with regard to pharma crop contamination of the food system. If, however, dedicated seed production facilities and locations are used and no commodity crops are grown in that location, volunteer plants, even if they occur, would not have opportunities to contaminate commodity crops. Crop residues are also an issue in that they can be moved from field to field by equipment and wind.

CONCLUSIONS AND RECOMMENDATIONS

Establishing effective isolation distances between pharma and other crops is difficult at this point. One strategy would be to determine distances as some function of the furthest distance that pollen of a given species has been found to travel. This would require conducting high-quality experiments to measure long-distance pollen movement. For example, pollen movement in corn can

be detected at 0.5 mile from the source when there is no temporal isolation (Halsey et al. 2002).

Another good option would be to grow the pharma crops in irrigated desert regions of the world where corn or soybean is not currently grown. This would reduce the likelihood of contamination by pollen movement, but the likelihood of contamination from seed mixing will depend on compliance with standard operating procedures, especially in the dedicated use and cleaning of harvest equipment and the use of sealed transport containers.

Because all seed essentially looks the same, one measure that should be adopted is the use of colored seed for all pharma crops at all stages of seed production. For example, almost all seed corn sold in the United States is treated with fungicides, and a colorant is added during the treatment process so that fungicide-treated seed can be identified and kept out of the food chain. A unique color could be assigned to pharma crop seed and required as a coating to be applied as soon as is practical following harvest. The color would identify this grain and help prevent pharma crop seeds from entering the food chain.

The use of natural color genes in corn and soybean has been suggested as another way of uniquely identifying pharma grain. The primary advantage of this system is that the color system would be genetic and always present. Depending on the genes used, this system could also help identify contamination events via pollen movement. The disadvantage of this system is its inherent complexity during breeding, backcrossing, and hybrid development. In addition, this system could impinge on those who sell naturally colored commodity crop seed as a specialty product (blue corn for example).

The only way to guarantee virtually zero contamination of the food supply by pharma crops is to maintain dedicated machines, facilities, and processes. Even though pollen movement in a crop such as corn can essentially be eliminated during seed production, seed can be moved long distances and can easily become mixed with commodity crop seed during harvest and transportation. For this reason, the author recommends that all pharma crop breeding and seed production activities be conducted in areas of the world where commodity crops and seed production crops of the same species are not grown.

Acknowledgments

The writing of this chapter required many informal conversations with scientists in both the public and private sector. I am grateful to all who contributed, but listing them by name would mean that I would inevitably leave someone out. I am especially grateful to Paul Christensen, who reviewed the entire chapter and offered numerous changes, some of which I used.

—Kendall Lamkey

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A Growing Concern

Protecting the Food Supply in an Era of Pharmaceutical and Industrial Crops

Technical Report
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Introduction, Conclusions, and
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December 2004