Conference Report

Workshop on Safeguards for Planned Introduction of Transgenic Corn and Wheat

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Preface

New varieties of crop plants produced with modern biological techniques are moving through the field testing stage toward the large scale plantings typical of performance trials and commercialization. The U.S. Department of Agriculture has worked closely with the developers of these new crop varieties, numerous other interested parties, and the public to ensure the continued safety of American agriculture.

Some have wondered if certain phenomena that may have'a relationship to safety might be more or less likely to occur, or have different consequences, when considered in the context of large scale plantings as opposed to small scale field trials. APHIS has convened a number of workshops to consider these and related issues with respect to specific crops. A meeting at Cornell University in Ithaca, New York (October, 1990), considered transgenic oilseed crucifers. Additional workshops have been or will be held on potatoes, cotton, rice, and other crops. The results of these meetings will assist USDA in the evolution of regulatory review procedures and requirements. This report conveys the results of a workshop which considered these issues for wheat and corn.

The conclusions of these workshops have been generally enlightening. The safety issues have been clarified, and the nature of relevant concerns has been sharpened and narrowed. The Department of Agriculture thanks the participants in this workshop for their expert contributions.

Terry L. Medley, Director
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Acknowledgements

The workshop on which this report is based would not have been successful without the contributions and cooperation of many individuals. The staff of the Keystone Center -- especially Ms. Denise Siebert, Dr. Martha Tableman, Ms. Abbey Dilley, Dr. Mike Lesnick, and Mr. John Ehrmann -- were instrumental in establishing the agenda, making logistical arrangements, and ensuring their smooth execution. Professor Mike Clegg performed above and beyond the call of duty as a superb scientific chairman, keeping the discussions on track and focussed, and ensuring that all voices were heard.

The participants in the workshop (listed in Appendix II) brought the knowledge and experience required for a critical consideration of the questions at hand, and provided the judgement and wisdom essential to make the information useful to regulators. This is often a difficult bridge for scientists to cross. The participants worked hard at it. We are enormously grateful for their efforts.

The staff of the Biotechnology, Biologics & Environmental Protection Division of the Animal & Plant Health Inspection Service of the U.S. Department of Agriculture conceived of the workshop and composed the questions listed in Appendix I as a basis for discussions. Particular mention is due a committee convened and chaired by Dr. Sivramiah Shantharam, for which Dr. James Lackey served as scribe. Salient contributions to the development of the questions were made by Dr. James Lackey, Dr. Sivramiah Shantharam, Dr. Quentin Kubicek, and Dr. Val Giddings. Additional input to the questions, the plans for the workshop, and comments on the various editions of this document came from them as well as from Dr. James White, Dr. Sally McCammon, Dr. John Payne, Dr. Michael Schechtman, Dr. Cathy Joyce, Mr. Chuck Kastner, and Ms. Nancy Sweeney.

Safeguards for Planned Introductions

of Transgenic Corn and Wheat

In the last decade, considerable research advances have been made in the genetic engineering of significant food crops such as wheat and corn (maize), The aims of this genetic manipulation include introducing resistance to insects and plant pathogens, disease resistance, improved protein or fiber quality, and other characteristics.

As research efforts have begun to achieve these aims in important food crops, concerns have been raised about the possibility of changing the basic genetic structure of wild plant species, creating crops that become weeds, or other possible environmental impacts. Further, could such changes in genetic structure exacerbate the weedy properties of some already existing weeds, or result in the replacement of indigenous species or locally generated cultivars with homogenous varieties? If scientists modify gene pools, will the world lose the opportunity to develop natural variants of crops that might better meet human needs? Could genetic diversity be inadvertently reduced?

As of December, 1990, about 150 permits have been issued in the United States for small-scale field tests of transgenic (genetically engineered) crops[1], ranging from half an acre to about 10 acres. A number of applicants are poised to move on to large-scale, multiple-site field testing and commercial sale of transgenic crops. Therefore, the U.S. Department of Agriculture (USDA) is faced with developing a regulatory framework for large scale plantings of transgenic crops to ensure that due care is given to safety considerations.

To consider this issue. the Keystone Center (Keystone, Colorado) convened a workshop in December 1990, at the request of the Biotechnology, Biologics, and Environmental Protection (BBEP) division of USDA's Animal and Plant Health Inspection Service (APHIS). The *48* people attending the meeting included a broad range of individuals involved with or interested in these issues: university researchers in plant genetics and pathology, ecology and evolutionary biology, agronomy and range science, entomology, zoology, and rural sociology; regulatory officials from the Federal and State levels; plant breeders and lawyers; researchers and managers of the leading companies in the field; and representatives of environmental groups.

Workshop participants considered three general questions:

1) What is the likelihood that genetic material of transgenic crops could transfer from experimental or large-scale plantings to adjacent crops or to wild or weedy relatives?

2) What are the potential consequences of gene transfer to other crops and/or to wild-or weedy relatives?

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[1]As of 21 April, 1992, the tally stands at 234 permits issued, with an additional 80 pending for the 1992 growing season.

3) What potent-a1 safeguards should be considered against any undesirable gene transfer?

After considerable examination of these questions in plenary sessions and in special work groups, the Chairman, Dr. Michael Clegg, summarized the discussions and outcome on each issue. This report follows the format of the workshop itself, drawing on the Chairman's summary statements, the presentations of panelists, and the general discussions during the meeting. The report attempts to capture the range of issues discussed. Explicit consensus was not an objective of the workshop. However, this report does indicate those areas where general agreement was reached. The report was primarily produced by an editor from workshop transcripts and notes. Participants were given the opportunity to review a draft of the summary for. comment, but did not review the final text. Therefore, this report is a meeting summary, not a consensus document.

Potential for Gene Transfer

Because of the differences in the reproductive biology of corn (maize) and wheat, the meeting was divided into two sessions.

Each session discussed the following general questions:

1) What is the likelihood that pollen could transfer genetic material from plantings to adjacent crop plants or to wild or weedy relatives?

2) If genetic material (pollen) was transferred beyond the deliberate plantings, under what conditions could it survive and be introduced and maintained in crop plants, or in wild or weedy relatives?

3) What is the likelihood that escaped genetic material would be incorporated into gene banks or other germplasm stocks?

4) Are there events of low frequency, but potentially high consequence (for example, floods or hurricanes), that could increase the potential for gene transfer?

Corn

Dr. Peter Day reported that the panel that considered the potential for corn pollen transfer from transgenic crops to wild or weedy relatives included several people with practical, commercial experience in producing hybrid corn for seed. The panel was well aware of the importance of protecting hybrid crops from pollination by other corn plants. Years of experience producing corn with specific traits have led to a wealth of scientific information on the viability of corn pollen. the distance corn pollen will travel in the wind, and the probability of unintentional transfer through field workers collecting pollen on their clothes, and other mechanical means. However, it was noted that much of this information has not been analyzed specifically for the purpose of addressing questions associated with transfer of traits from genetically engineered plants. Additional information would be needed to answer all the questions that arose concerning gene transfer in corn.

When corn tassels release pollen, the surrounding vegetation can be enveloped in a cloud or fog of pollen grains. To date there has not been a documented example in the United States where heavy exposure to pollen has led to introgression (the addition of genes through hybridization and back crossing from one cultivar to another, or to a wild or weedy relative) that has resulted in a problematic viable plant. Thus, extensive experience with temporal, physical, and biological barriers to unintentional gene transfer in the United States led the group to conclude there is very minor cause for concern on this issue outside the centers of origin of the crop (i.e., Mexico and Guatemala). However, as some participants point out, while the risk from first generation hybrids may be very small to negligible, introgression of novel genes into teosinte populations can be of great concern.

Hybridization and introgression could occur in Mexico or Guatemala if transgenic corn were to cross with native teosinte (a group of annual and perennial grasses that are the nearest wild relatives of corn). Although the frequency of gene transfer is low, the recent research by John Dobley showed, using biochemical techniques, that introgression from corn to some teosinte has occurred in the past (Bioscience 40(1990): 443-448). The rate of introgression is severely limited by the low fitness of first-generation hybrids; their seed does not disseminate well because they have some of the traits of cultivated corn, and they are of no use to farmers because they do not produce corn for human or animal consumption. Thus, corn and teosinte have maintained separate identities for hundreds of years in Mesoamerica, in spite of occasional introgression.

Dr. Ronald Meeusen argued that there was nothing to suggest that possible transfer of genetically engineered traits from corn to teosinte should be of greater concern than that of naturally occurring traits, given that conventionally bred corn has been cultivated in regions with teosinte for centuries without adverse consequences. But Dr. Robert Colwell pointed out that, given the documented potential for introgression, the possibility cannot be completely discounted that entirely new traits in transgenic corn (such as cultivars with new types of pest or disease resistance not previously feasible) might increase the fitness of teosinte, and thus lead to undesirable effects. The immediate effect might be the displacement of natural populations by introgressed strains of teosinte. The practical implications of such a development, however, were judged uncertain, as teosinte germplasm has so far not been useful in corn (maize) breeding programs.

Wheat

The panel on wheat, chaired by Dr. Calvin Qualset, noted that *two* types of wheat are cultivated: hexaploid, used for making bread, and tetraploid, such as durum and spaghetti-type-wheats. The wild and ancestral species of these cultivated wheats occur primarily in Mesopotamia, Anatolia, Ethiopia, or other areas to which the species have been carried.

Hexaploid and tetraploid wheats do cross with each other, though at low rates estimated only occasionally to reach as high as 3 or 4 percent. Thus, the potential for the hybridization of cultivated wheat with weedy diploid wild relatives exists, though it is extremely low -- lower even than the frequency of corn with and teosinte. Further, such a hybrid wheat would be cytogenetically dysfunctional, and therefore sterile.

The panel also noted that tetraploid cultivated wheats could cross with wild polyploid relatives; however, the probability of gene transfer in this case was considered very low. Nevertheless, some workshop participants expressed concern about gene transfer in wheat's centers of origin.

<u>Summary</u>

In summary, the potential for gene transfer from transgenic corn and wheat in the United States should pose no serious environmental concerns for government regulators: However, consideration should be given to the possibility of such transfer and its potential associated risks when dealing with, or advising, regulators in these crops' centers of origin, including areas throughout the Fertile Crescent, Anatolia, Mesoamerica, and Ethiopia.

Environmental Conseauences of Gene Transfer

A panel of 11 speakers considered the potential consequences of genetic transfer from transgenic crops. The following questions served to focus the workshop's discussion:

1) Applications for an APHIS permit have involved gene insertions for the following characteristics: insect and virus resistance, herbicide tolerance, alteration of nutritional components, and antibiotic resistance. How likely are these classes of inserted genes to continue in the future? Are there additional categories of gene insertion that will emerge?

2) In light of the above, what environmental issues should be addressed to assess the realistic potential of adverse consequences for different classes of gene insertions?

3) For each environmental issue, what are the likely and the most extreme possible effects on the environment from the introduction of genetic material? Formulation of these issues should consider any effects on gene pools, nutritional value, weediness, agronomic characters, and the potential for any secondary or unintended effects.

4) What is the order of likely environmental effects, ranked from the level of highest concern to the level of least concern?

5) What is the possibilfty of modified crop plants becoming weedy and resulting in an agricultural problem?

6) What is the possibility of a transgenic crop becoming a wild (feral) plant?

7) What is the possibility of a gene escaping to wild and weedy relatives and resulting in a more invasive weed?

8) What is the possibility of a gene escaping to a wild relative and conferring greater fitness to that plant?

9) How should plantings be monitored to detect any of the above scenarios?

The panel discussed two prerequisites for genetic transfer to have any negative environmental consequences. First, there must be the sexual transfer of a gene from a transformed population into a cultivated crop of the same species, or into a wild or weedy relative. The second prerequisite is that the transferred gene would have to spread, or in other words, be favored by selection pressure.

The introduced trait should be the first consideration when looking at any possible consequences. A good deal of the discussion centered on herbicide tolerance genes and on the potential for transformation with DNA encoding the delta-endotoxin gene from Bacillus thuringiensis, but it was noted that many other traits may be the targets of genetic engineering in the future.

Several other questions must be answered to assess both the likelihood of gene transfer (see previous panel discussion) and the potential consequences: Is the trait in question dominant or recessive? Has it been engineered to be expressed in specific tissue? Are multiple genetic changes to be introduced in the engineered species, as part of a broad spectrum approach to insect or pathogen resistance?

To assess the ability of the trait to spread, the most important consideration is the impact on fitness of the new trait, both in the crop being altered and in any relatives in its natural environment. In other words, does it confer an improved ability to survive and produce offspring? Experiments to test for such fitness consequences in cases where a negative impact might be expected were highly recommended.

Two possible environmental consequences of the escape of a transformed gene were discussed. Of the two, more concern was expressed about the potential loss of biodiversity as wild genetic resources are altered. Biodiversity could be affected if increased fitness of a wild relative of a crop plant led to the disruption of wildland plant communities -- either through direct competition with other wild plant species, or through indirect effects on other plants by increasing the densities of pests whose host ranges, for example, might include both teosinte and other plants.

The second point discussed was the possible creation of new weeds or the strengthening of existing ones through the introduction of genes that might increase a weeds' adaptability. Possible examples include those encoding herbicide tolerance, insect resistance, disease or drought resistance.

To help regulators consider the relative importance of these various issues in general and on a case-by-case basis, it was suggested that a decision tree be used to establish criteria about the level of acceptable risk and to set aside the trivial consequences of transgenic crop gene transfer. Some of the obvious elements of the decision tree included the nature of the trait to be altered, scientists' familiarity with the gene being modified, any information about fitness effects, and knowledge about the crop's wild relatives.

An additional criterion proposed by Dr. Day for assessing risk is that whatever is done through genetic engineering should not pose an .additional' threat to the environment over and above what farmers, consumers, rural residents and scientists accept under current agricultural practices in wheat 'and corn. Some participants noted a heightened concern shared by several groups, and more specifically articulated by the environmental and conservation biology communities, about changes in the gene pool. This concern meant that researchers might be held to a higher standard of proof in the future than had been applied previously.

The public affected by the decision-making process on this issue is larger, it was noted, than those groups represented at the workshop. Dr. Joseph Molnar talked about the societal framework in which genetic engineering is being done. He pointed out that the workshop was discussing food crops, and that the general public is sensitive about anything that appears to affect safety, whether it is in fact a risk or not. Any assessments of potential consequences must be done in a way that assures the larger public that their best interests have been considered.

potential Safeguards Against Gent Transfer

Three work groups considered possible safeguards to the transfer of genes in small- and large-scale field tests and in commercial settings.

Physical Safeguards

The group on physical safeguards was asked to consider:

1) What would be an acceptable isolation distance to minimize gene movement?

2) Would fallow ground or border rows of non-transgenic plants around the trangenic plants serve as an adequate safeguard?

3) Could detasseling, emasculation, or bagging the inflorescence be a practical means to prevent gene transfer?

4) Are there any walls, nets, traps, or other physical structures or plot layouts that could be used to minimize gene transfer? How practical would it be to use them in the field?

5) What are acceptable termination protocols for field trials of transgenic plants?

For small-scale testing of both corn and wheat, the isolation distances set for seed certification programs were considered adequate to minimize gene movement. For corn this is usually 660 feet, while for wheat a shorter distance is sufficient. In either crop, care should be taken to clear fields of rogue volunteer plants in subsequent growing seasons.

Regarding large-scale tests and commercialization, the group maintained that significant safety issues should be resolved for both crops before getting to the point where gene flow cannot be controlled--i.e., any such concerns should be resolved during small-scale testing.

Temporal Safeguards

The group on temporal safeguards considered two issues:

1) Could planting dates, irrigation, fertilization, or any other agronomic practices be used to alter flowering dates so as to prevent pollen-mediated gene flow out of the plantings?

2) Is there any method to alter flowering time during the day so as to prevent pollen-mediated gene flow out of the plantings?

The second safeguard, changing the time of day for planting, was not considered relevant.

Regarding seasonal considerations, changing the planting date would not help for winter wheat, but may help for spring wheat, depending on the latitude of the area. For both these crops, this method would not be appropriate for varietal testing. In wheat's centers of origin, wild relatives bloom at such different times that temporal separations might not be useful unless test fields were planted in the dry season and irrigated, at great expense.

For corn, temporal safeguards could be used in principle, in combination with other safeguards. In the centers of origin, since corn usually flowers earlier than teosinte, this method of minimizing gene transfer could be used.

In all these situations, the group noted that changing the time of planting could defeat the purpose of the test, as the crop might not then be tested under normal growing conditions.

em ca Sa e ds

The group on biological and chemical safeguards dealt with four basic questions:

1) Could genetic sterility factors be used to prevent pollen-mediated gene transfer?

2) Could chemicals be used to induce sterility?

3) Are there any genetic factors that could be introduced into the transgenic plants that would render progeny of a fertilization via gene transfer incapable of self-perpetuation?

4) Could "suicide" genes or other constructs be used to prevent undesired genetic transfer?

Chemical methods of minimizing gene transfer did not seem appropriate, the group reported. They considered several biological safeguards, such as inducing male sterility, but these were called a "plant breeders's nightmare" by Dr. Qualset. In trying to mitigate a possible risk, scientists would be introducing a new trait for male sterility which could create other problems. Currently no biological or chemical safeguards appear worth pursuing.

Summary

From the reports of the sub groups to the meeting as a whole, it is clear that physical or spatial safeguards -- e.g., specified isolation distances similar to those used in seed certification programs, or the use of walls, nets, or other physical structures -- should be relied upon to minimize gene flow only in small-scale tests.

For transgenic corn and wheat, any safeguards to minimize gene flow should be applied during small-scale testing, when control is still a possibility. The ability to control results could in fact be used as a definition of small-scale, rather than relying primarily upon the size of the area in the test. In other words, experiments at numerous sites could be considered small-scale in that any potential for pollen movement could be controlled. After that, no clear and practical distinction regarding safety considerations could be drawn between large-scale testing and commercialization of a product. It was noted, however, that corn and wheat in the United States have presented little concern. Issues other than those related to gene flow, such as non target impacts, were not the focus of discussion of this workshop.

Research Priorities

The meeting ended with a general discussion of possible research priorities, although no specific research findings were identified as essential prerequisites to the large-scale testing of transgenic corn and wheat.

While the meeting had been mandated to consider corn and wheat, Dr. Colwell noted that the discussion should not be taken to mean that these were the crops of greatest concern regarding possible genetic transfer. Furthermore, the meeting's conclusions on the efficacy of physical safeguards should not be taken to imply that similar precautions would necessarily suffice for other crops, according to Dr. Robin Manasse.

The lack of answers to many basic questions was noted throughout the discussions. Topics incompletely understood include the survival of pollen in nature and the frequency with which genes are transferred from domestic crops to their wild relatives as a consequence of traditional plant breeding. Dr. James Hamrick suggested that research could be done on the ability of a variety of genes to introgress, since the lack of introgression of a neutral marker may not provide useful information about the introgressive ability of, for example, disease resistance genes. Studies on the effects of new traits when they are introduced into crop plants could provide a sound basis for predicting the probability of gene movement into populations of wild or weedy relatives. Review articles on these or other topics raised at the meeting could make useful contributions to knowledge in this area. Dr. Qualset queried whether a broad study of herbicides and herbicide tolerance would be valuable, as it relates not just to transgenic wheat and corn but to many crops.

One problem may be the difficulty of funding studies'and publishing "negative" results, or definitively proving that something does not happen: few researchers or companies want to invest resources in a demonstration of the impossibility of a conjectural phenomenon. There are few ecological data requirements that would encourage such research. It was noted that the USDA granting program is setting aside funds for risk assessment studies related to transgenic plants, and that perhaps such funds could be used to investigate some of these questions.

Workshop Summary

* The potential for gene transfer from transgenic wheat and corn in the United States should be a matter of minor concern to government regulators because there are no wild relatives in the United States with which they interbreed.

* Two possible'environmental consequences of the use of transgenic wheat and corn in their centers of origin are a reduction of biodiversity among wild relatives and the creation or exacerbation of related weeds.

* When identified, all relevant environmental or health concerns must be resolved before moving the crops from a small-scale testing stage, where pollen flow can be controlled, to large-scale testing or commercialization.

APPENDIX I: DISCUSSION QUESTIONS

INTRODUCTION:

The Workshop will be concerned with two genetically engineered crops: corn and wheat. For each crop, workshop participants are expected to examine three major issues.

- potential for gene transfer;
- environmental consequences of gene transfer; and
- safeguards to prevent or minimize gene transfer.

The participants are welcome to use the following list of questions, and additional questions as necessary. Responses to questions would be most helpful if organized from general to more specific in identifying potential negative or undesirable genetic material transfer and subsequent means of mitigating any problems. These issues also could be addressed from the local to national and international context.

I. Potential for eene transfer:

I-1. What is the likelihood that pollen could transfer genetic material out of plantings to adjacent crop plants or wild or weedy relatives?

1-2. If genetic material (pollen) were transferred beyond the deliberate plantings, under what conditions would it survive and be maintained in crop plants, or wild or weedy relatives?

1-3. What is the likelihood that any escaped genetic material would be incorporated into gene banks or other germplasm stocks?

1-4. Are there events of low frequency but potentially high consequence (e.g., floods or hurricane) that could increase the potential for gene transfer?

II. Environmental Conseauences of Gene Transfer:

2-1. Past permit applications have involved gene insertions for the following characters: insect and virus resistance, herbicide resistance, alteration of nutritional components, and antibiotic resistance. How likely are these classes of inserted genes to continue in the future? Are there additional categories of gene insertion that will emerge?

2-2. What environmental issues should be addressed to assess realistic potential adverse consequences for different classes of gene insertion in light of 2-1 above?

2-3. For each environmental issue, what are the likely (if any) and the most extreme possible, effects to the environment from introduction of genetic material? Formulation of issues should consider any effects to gene pools, nutritional value, weediness, agronomic characters, and the potential for any secondary or unintended effects.

2-4. What is the rank order of likely environmental effects, arrayed from the level of highest concern to the level of least concern?

2-5. What is the possibility of modified crop plants becoming weedy and resulting in an agricultural problem?

2-6. What is the possibility of the transgenic crop becoming a wild plant?

2-7. What is the possibility of a gene escaping to wild and weeds relatives and resulting in more invasive weeds?

2-a. What is the possibility of a gene escaping to a wild relative and causing fitness of the plant, either wild relative or crop, to change?

2-9. How should monitoring of plantings be structured and conducted for identifying the above scenarios?

III. Safeguards

Physical .safeguards

3-1. What would be an acceptable isolation distance to minimize gene movement?

3-2. Would fallow ground or border rows of non-transgenic plants around the transgenic plants serve as an adequate safeguard?

3-3. Could detasseling, emasculation, or bagging the inflorescence be a practical means to prevent gene transfer?

3-4. Are there any walls, nets, traps, or other physical structures or plot layout designs that could be used to minimize gene transfer? How feasible would it be to use them in field practice?

3-5. What are acceptable termination protocols for field-trials of transgenic plants?

Temporal safeguards

3-6. Can planting dates, irrigation, fertilizing, or any other agronomic practices, be used to alter flowering dates so as to prevent pollen-mediated gene flow out of the plantings?

3-7. Is there any method to alter flowering time during the day so as to prevent pollen-mediated gene flow out of the plantings?

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Biological/Chemical Safeguards

3-8. Can genetic sterility factors be used to prevent pollen-mediated gene transfer?

3-9. Can chemicals be used to induce sterility?

3-10. Are there any genetic factors that could be introduced into the transgenic plants that would render progeny of a fertilization via gene transfer incapable of self-perpetuation?

3-11. Can "suicide" genes or other 'constructs be used to prevent undesired genetic transfer?

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