CONTAMINATING THE WILD?

Gene Flow from Experimental Field Trials of Genetically Engineered Crops to Related Wild Plants

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EXECUTIVE SUMMARY

Genetically engineered (GE) crops present different risks than other agricultural technologies, such as chemical pesticides and fertilizers, in part because genes can be perpetuated indefinitely by the reproduction of the plant. One way the persistence of engineered genes, or "transgenes," can occur is by spreading into sexually compatible wild relatives of crops.[i] This process, known as "gene flow," can perpetuate whatever harm may be caused by transgenes, because once they escape into wild relatives, some will become a permanent part of the environment.[ii] This is because wild relatives, unlike most of their related crops, can survive in the environment without human intervention. For this reason, transgenes in wild relatives may harm parts of the environment, such as natural areas, that would not be directly harmed if the same genes were restricted to crop plants. Transgenes could harm the environment by increasing the weediness of wild relatives; or by harming plants, animals, or environmental processes. Many wild relatives are also serious crop weeds, and therefore gene flow may also harm agriculture. The possibility of irrevocable impact on the environment should serve as a warning to prevent gene flow, or to ensure that harm will be minimal if gene flow occurs.

To better understand the risks from gene flow to wild relatives, this report evaluates the regulation in the U.S. of GE field trials, which are outdoor plantings of experimental GE crops. All genetically engineered plants must acquire USDA approval to be grown outdoors in field trials. This report evaluates whether current USDA regulation is adequate to prevent gene flow from field trials, or environmental harm if gene flow occurs.

Gene flow from GE field trials is of particular concern because these trials contain experimental genes that have undergone little or no risk assessment by USDA or other regulatory agencies. In most cases, GE crops grown in field trials are not formally assessed for safety by USDA until the crop is slated for commercialization, which usually occurs after many field trials have already been completed. The types of experimental genes tested in field trials far

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[i] Sexually compatible wild relatives of crops will be referred to in the report simply as "wild relatives."

[ii] The term "gene flow" is somewhat ambiguously defined, but is used here to mean the process resulting in the long-term presence of a gene in a crop wild relative, or crop-wild hybrid, after transfer from the crop. Pollination is considered a step in the gene flow process.

outnumber the few types of transgenes found so far in commercialized GE crops, so these genes present risks that have not been carefully evaluated. And because most transgenic crops are never commercialized, many genes in field trials never undergo more than a cursory safety assessment.

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Concern about gene flow from GE field trials to wild plants recently acquired new urgency with the discovery that an herbicide resistance gene had spread via pollination into wild creeping bentgrass plants in Oregon. Transgenic seed was found on creeping bentgrass plants and another related species up to 13 miles beyond field trial boundaries—far beyond the 900 foot isolation distance accepted by USDA.[1] This breach of confinement is not unique; another occurred during a previous creeping bentgrass trial. As these data indicate, USDA's existing standards for transgene confinement, which typically have been based on the less-critical needs of conventional plant breeding, are unlikely to prevent gene flow. The likelihood of gene flow is further magnified by the large number of field trials for crops with wild relatives. For example, in addition to the aforementioned trials, 168 other bentgrass field trials have been approved, often for genes that may spread more easily in the wild than herbicide resistance. Whether pollination or gene flow from other trials has already occurred is unknown, because tests to detect gene flow to wild relatives are rarely conducted.

This report concludes that it is virtually inevitable that gene flow from GE crop field trials to wild weedy relatives will occur unless additional steps are taken to prevent it.

FINDINGS

1) Numerous field trials have been conducted, and continue to take place, for genetically engineered crops that have wild relatives, often in states where at least one wild relative is found.

Since 1987, when USDA began regulating GE crop field trials, over 2694 trials for crops with wild relatives have been approved for the 20 crops examined in this report.[iii] This is about a quarter of all approved field trials. There were 294 such field trials approved in 2004 alone. 1710 GE field trials—16% of the total of all field trials—have been approved for states where wild relatives are reported to grow, increasing the likelihood of close proximity that facilitates gene flow. Furthermore, wild relatives are often common weeds of crops, and therefore are even more likely to be found close to GE field trials.

2) Some field trials are large, covering hundreds or thousands of acres, substantially increasing the likelihood of gene flow.

Although it may be assumed that field trials are very small because they are often used for research, many are quite large. Large field trials produce more pollen, increasing the probability of more gene flow over greater distances. At least 290 trials of 50 or more acres have been approved, including the recent creeping bentgrass trial in Oregon, which was granted for 600 acres. There have been 17 large trials of creeping bentgrass, with an average size of 214 acres, and 32 large field trials of canola with an average size of 563 acres. The largest field trial, for cotton, was 34,350 acres.

3) USDA guidance on confinement will not eliminate gene flow to wild relatives.

The USDA guidance on GE confinement is based on standards developed to maintain the purity of conventional crop varieties. Those standards are not intended to completely prevent gene flow. The result, as demonstrated for herbicide resistant GE creeping bentgrass, is that pollination and possible gene flow can occur even when USDA confinement standards are followed. Some crops, like rice and wheat, have less gene flow over shorter distances than bentgrass, but also have correspondingly reduced confinement recommendations. Still other major crops like alfalfa,

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[iii] Data on field trials are through March 31, 2005.

canola, sunflower, and many forest trees may pollinate relatives over substantial distances. Furthermore, USDA confinement guidance for many GE crops is vague, leaving interpretation to the field trial proprietor. those crops. In general, APHIS seems willing to accept confinement methods that are unlikely to ensure the absence of gene flow. Whether more stringent methods are used is apparently left largely to the GE crop owner.

Pollination and possible gene flow can occur even when USDA confinement standards are followed.

4) USDA rarely conducts Environmental Assessments (EAs) prior to GE field trials.

Environmental assessments (EAs) are the most thorough risk assessments so far conducted by USDA for field trials. They have been prepared for a small minority of trials, and they describe both USDA environmental risk evaluations and accepted confinement methods. Of the 2694 field trials reviewed for this report, only about 130, or 5%, had an EA, and these EAs addressed even fewer different genes. In 1995 a categorical exclusion exempted most trials from EAs, and since then only 7 EAs have been prepared for the crops examined in this report. Except for the recent EA evaluating transgenic tall fescue and ryegrass, the most recent EA was conducted in 1997.[iv]

5) Confinement measures accepted in USDA Environmental Assessments (EAs) are often inadequate to prevent gene flow.

EAs provide the most detail available on the confinement measures used for specific field trials. However, confinement measures for GE field trials described in EAs vary widely, and many are likely to allow some gene flow to wild relatives. Some EAs allowed use of isolation distance alone for confinement, usually based on conventional seed purity standards. For example, canola isolation distances of 1320 ft or less were accepted by USDA, although recent data show some gene flow at three kilometers. Some permits proposed several confinement measures (usually two) and others used a single measure. At least 45% of the 11 EAs reviewed could have allowed gene flow to occur if wild relatives were present, based on published pollination distances for

6) Risks assessments in EAs, as well as requirements for reporting harm observed in field trials without EAs, may not identify environmental impacts that could occur due to gene flow.

The risk assessments conducted in EAs are usually restricted to conjecture based on a known function of the gene, or based on limited observations, rather than formal risk assessment. Formal risk assessments should include testing for harm to non-target organisms, use of protocols specifically designed for collecting environmental impact data from the field trials, and determining whether the gene is likely to spread in the wild relative. When EAs are not prepared, permits and notifications require reporting of any observed harm. However, environmental impacts may go undiscovered because there is no requirement for field trials to be properly designed to detect possible harm. Instead, most field trials are intended to evaluate the efficacy of the introduced trait and agronomic properties of the crop.

7) Available data, modeling, and population genetics theory, all suggest that introducing a gene at low frequencies can lead to permanent escape and spread.

For example, the National Academy of Sciences wrote in a recent report that: "Generally, if an allele confers a fitness advantage—once introduced into a population—it is expected to increase in frequency, *even if it is introduced only once*" [emphasis added].[2] Since determining the contribution of transgenes to the fitness of wild relatives is not a regular part of USDA risk assessment for field trials, the possibility of permanent escape of transgenes, should pollination occur, is largely unknown. Although gene flow

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[iv] Prior to 1995, exemption from an EA was decided on a case-by-case basis. This number does not include a few of the recent field trials for pharmaceutical-producing GE crops.

from individual field trials may occur infrequently, the large number of field trials substantially increases this possibility.

8) Many field trials contain transgenes that could enhance the fitness of wild relatives under particular environmental conditions.

Genes for stress tolerance or pest resistance may make wild relatives more fit by increasing their survival under conditions such as drought or insect attack that could otherwise limit the spread of these plants. For the nine crops with wild relatives that have the most field trials, about 600 trials, or 28%, contained genes that confer stress or pest resistance. Another 1257 field trials, or 59%, contained genes for herbicide resistance that could have enhanced survival if the herbicide was applied. This may be the case when the wild relative is a weed of the conventional crop, as with jointed goatgrass, which infests wheat.

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RECOMMENDATIONS

The following recommendations are made to prevent gene flow from field trials, or harm if gene flow occurs:

1) The USDA should immediately evaluate and strengthen confinement requirements for crops that have wild relatives in the U.S.

Field trials for GE crops with wild relatives should not be conducted unless gene flow can be prevented, or minimal risk ensured, with considerably more confidence than under current requirements. Where environmental harm is possible or unknown, strict confinement measures could include geographic isolation, or several simultaneous means to prevent gene flow. Where confinement data or methods are uncertain, a cautious approach should be taken.

2) The current "categorical exclusion" from the requirement to perform environmental assessments should be rescinded, or drastically revised, for crops with wild relatives.

Adequate risk assessments are rarely conducted under the current categorical exclusion for EAs. Further, the categorical exclusion regulation fails to account for the cumulative impacts of large numbers of field trials in a given area, as is required under the National Environmental Protection Act. Therefore the categorical exclusion should be rescinded for crops with wild relatives, or rewritten to include only field trials that contain the same test parameters as previous trials. Too little is known about the risks from experimental transgenes to support a categorical exclusion, especially considering the injunction of many scientific bodies to evaluate the safety of GE crops on a case-by-case basis.

3) USDA should develop detailed risk assessment guidance.

Even when risk assessments are performed, they are often inadequate due to the lack of USDA guidance on appropriate safety tests. It is therefore imperative that USDA develop risk-assessment guidance, with the assistance of independent scientists, based on a transparent public process. More guidance is also needed to determine cumulative impacts due to multiple field trials in a given area.

4) Careful risk assessments should be carried out in contained facilities or under stringent, redundant confinement prior to growing in an environment where gene flow to wild relatives is possible.

Initial safety tests should be performed under confinement that ensures that no gene flow can occur if there is uncertainty about the risk of the transgene.[v] Crops where risk assessment should be required include those that increase biotic or abiotic stress tolerance,

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[v] Stringent confinement is here defined as confinement that ensures, to the extent possible, that gene flow cannot occur.

where fitness of the wild relative may be enhanced by the transgene. Other GE crops should undergo risk assessment unless there is strong evidence, beyond speculation, that they pose minimal harm. Risk assessment should include testing for harm to relevant non-target and beneficial organisms and the ability of the gene to spread in wild relatives. If the risk assessment shows a propensity for the transgenic crop to cause harm, the crop should be safely contained.

5) Large field trials, where gene flow is more likely, and where it is often more difficult to provide reliable confinement measures, should not be allowed without first conducting a thorough risk assessment.

Large field trials have an increased likelihood of gene flow over longer distances because they produce much more pollen than small trials and because some confinement measures, such as those that require manual intervention, may be impractical. These trials should not be allowed without a thorough evaluation of risk.

6) USDA should survey the proximity of wild relatives to existing GE field trials, and should sample wild relatives to determine if gene flow has occurred.

Determining the proximity of wild relatives to field trials is not required by USDA unless they are within the prescribed isolation distance (and data on this proximity, if reported to APHIS, has generally not been made public). To remain in the dark about such important information severely limits our ability to determine the risks from gene flow. These data should be made public if they exist, or acquired if they do not, and if any gene flow has occurred, wild relatives containing transgenes should be eradicated if possible. Because the reagents or data (such as DNA sequences of transgenes) needed to carry out these tests may not be available to USDA or the public, trials should be made contingent on supplying such materials.

7) As recommended in a recent NAS report on biological confinement, USDA should conduct research on improving confinement methods and to study gene flow from crop plants.

Currently, such research is not well coordinated or funded. Although it should be a priority of genetic engineering research, it should not come at the expense of research into sustainable methods of agriculture. This research should not include genetic use restriction technologies (GURTs), because they are controversial in restricting farmers from saving seed, as well as potentially reducing gene flow.

To remain in the dark about the proximity of wild relatives to field trials severely limits our ability to determine the risks from gene flow.

8) Data about field trials should be available to the public.

Data on confinement methods, risk assessments, and the locations of field trials are not made public except in the rare cases where an EA is prepared. Also, the identities of many genes in field trials are not made public. Without this basic information, the public cannot have confidence that adequate safety is being ensured. This is especially true in light of recent incidents of contamination, and research showing gene flow from crops to wild relatives. The argument that these data are confidential business information is largely unjustifiable. For example, risk assessment data are routinely made public by EPA for some of the same genes that are reviewed by USDA. As a first step, county locations of all field trials should be disclosed.

WHAT DOES THE FUTURE HOLD FOR GENE FLOW?

The science of genetic engineering is still new, and new types and combinations of transgenes are being developed that may have consequences for gene flow risk. It is therefore important to consider some possible directions in transgenic crop research, and the implications for gene flow. Some recent developments, such as chloroplast transformation, may reduce rates of gene flow. Other developments suggest that environmental risk from field trials may increase unless improved confinement and risk assessment is required by USDA. For example, experimentation with genetically engineered forest trees is progressing, and both gene flow and resulting environmental harm may be substantial in some cases because of the importance of trees to natural ecosystems. In general, confinement will be more difficult for trees than for other GE crops because of longer pollen and seed dispersal distances for many tree species.

Under current regulations, new types of experimental GE crops could be field tested, and possibly transferred to a wild relative prior to any risk assessment from FDA, USDA, or EPA.

New synthetic genes not found in nature are also being explored, as well as more complex metabolic changes in crops. The risks from some of these genes may be higher than for many genes used in the past. For example, an experimental hybrid gene fusing the non-toxic cell binding portion of the ricin gene with the toxin portion of an insecticidal Bt gene has been engineered into rice and corn. It is reported to kill more species of insects than the unmodified Bt protein, and hence may be commercially attractive, but may also have greater potential to harm non-target organisms.

GE crops developed for new markets, such as altered food or feed quality, or that contain genes for industrial products, may present additional challenges. These genes often intentionally modify crop biochemical pathways, unlike currently commercialized GE crops. Such crops may contain more unintended changes than previous GE crops, making their impact on gene flow more difficult to predict.

Under current regulations, however, new types of experimental GE crops could be field tested, and possibly transferred to a wild relative prior to any risk assessment from FDA, USDA, or EPA.

There is also increased interest in crops containing stress-tolerance genes, such as for drought tolerance. There may be a higher likelihood that such genes will confer a competitive advantage to a wild relative in some environments, compared to many current genes. Increasing numbers of field trials containing stress-tolerance genes may therefore increase the likelihood of gene flow and environmental harm.

The number of crops combining several transgenes, often referred to as “stacking,” is also increasing. Stacking may magnify environmental harm. Several GE crops already exist that combine several insect resistance or herbicide and insect resistance genes. Stacking, and the development of pest resistance genes that control several pests at once, will become increasingly attractive because they address more crop problems than single transgenes found in most current GE crops. Most crops are attacked by several insect and disease pests not controllable by a single transgene, and therefore crops with multiple pest-control genes may have larger markets.

In conclusion, once a gene is widely distributed in a wild relative, experience with weedy or introduced species tells us that it will be very difficult, costly, and often impossible to eradicate. The presence of escaped transgenes will often not be obvious in wild relatives. Transgenes typically will not change an easily observed attribute like flower color or leaf shape. Without genetic testing, transgene escape will often not be detected until the gene is widely dispersed. It is important to understand, therefore, that the alternative to ensuring containment and conducting improved risk assessments will likely be the permanent escape of experimental transgenes, with unknown consequences, into the environment.

INTRODUCTION

Transgenes escaping from GE crop field trials into wild relatives, many of which are serious weeds, may cause environmental harm or add to the cost of weed control. Gene flow into wild relatives is important because those relatives, unlike most crops, are capable of growing and spreading in the environment without the help of farmers. Because gene flow is not usually monitored, by the time it is discovered eradication will often be impractical.

Concern about the spread of genetically engineered transgenes from GE crops has been highlighted by events over the past several years. In 2000, the gene from StarLink corn, which was not approved as safe for human consumption, contaminated food corn, resulting in hundreds of millions of dollars of damage to the food supply and other costs. In 2002, corn producing pharmaceutical proteins contaminated soybeans, resulting in the destruction of 500,000 bushels.[3] More recently, a report by the Union of Concerned Scientists has shown that much of the seed supply of major crops such as corn, soybeans, and canola has been contaminated by transgenes from commercialized GE crops.[4] These and most other previous examples of contamination have involved GE crops and their conventional counterparts, but recent revelations about contamination from GE bentgrass is the first case of widely recognized pollination of wild plants from a GE field trial.[5]

Although it has not yet been determined whether transgenic offspring from the bentgrass contamination have become established in the environment, this research reveals that experimental genes may have been escaping from field trials. This is especially disconcerting because the bentgrass field trial was only one of several thousand for GE crops with sexually compatible wild relatives in the U.S.

This report examines whether USDA regulation of outdoor field trials prevents gene flow to wild relatives. The data set consists of field trials for the 20 crops with sexually compatible wild relatives and at least 10 field trials. Reviewed are the number of these field trials, the number of larger field trials, the location by state, and the subset of field trials that are more likely to lead to transgene escape.

Also examined are confinement requirements and risk assessment for GE field trials. Both the confinement guidance provided by USDA and environmental assessments (EAs) of GE crops with wild relatives, conducted by USDA's Animal and Plant Health Inspection Service (APHIS), are reviewed. Although they are prepared for only a small minority of field trials, EAs are the only readily available source of information on transgene risk assessments and specific confinement methods accepted by USDA/APHIS.

Research on transgenic creeping bentgrass reveals that experimental genes may have been escaping from field trials. This is especially disconcerting because the bentgrass field trial was only one of several thousand for GE crops with sexually compatible wild relatives in the U.S.

Finally, this report concludes that because USDA confinement requirements could allow pollination of wild crop relatives, and because of the large number of field trials, permanent transgene flow is likely to occur over time.

Gene flow by pollination, rather than by the spread of seed or vegetative parts of the GE plant, is evaluated in this report. For species like creeping bentgrass or forest trees that can survive in the wild, these latter types of gene flow may sometimes be even more important than the pollen flow discussed here. However, there are few data available on the escape from fields of seed or vegetative material of transgenic crops. Gene flow by seed is therefore discussed only briefly and in general terms.

Field trials are of particular interest for several reasons. First, field trials are numerous, with over 10,900 approved since 1987 in the U.S.[6] Many of these have been, and continue to be, conducted with crops that have sexually compatible wild relatives in the U.S. And many of those wild relatives are known to exchange genes with crops. For example, Ellstrand found at least some evidence of gene flow between 11 of the 20 most important U.S. crops, such as wheat and grapes, and their wild relatives in the U.S.[7] Many other important crops, such as carrots and canola, also have wild relatives in the U.S. Therefore, field trials provide many opportunities for gene flow to occur.

Additionally, the National Academy of Sciences (NAS) concluded in 2004 that current methods cannot ensure that gene flow will be prevented, that reliable methods of biological confinement are not generally available, and that the careful application of several gene confinement methods at once may be needed in some cases.[8] The NAS report specifically addressed commercialized GE crops, but in principle the inadequacy of confinement methods applies to field trials as well.

The risks to the environment if gene flow occurs are largely unknown in many cases. This is because there is no requirement for environmental or human health risk assessments by USDA or the Food and Drug Administration (FDA), respectively, prior to field trials. Instead of requiring formal risk assessments, USDA typically assumes that confinement procedures will be successful, and that environmental risks are therefore minimal.[vi] As a result, field trials for genetically engineered crops are often conducted for several years before either an environmental risk assessment by the USDA or Environmental Protection Agency (EPA), or a human health risk assessment by either the FDA or the EPA. Formal risk assessments are usually only conducted for GE crops that are approaching commercialization, and many transgenic crops are never commercialized. So for many experimental transgenes used in field trials, formal risk assessments are never carried out.

[vi] USDA's conclusions in environmental assessments, that there will be minimal environmental impact are based largely on confinement of the transgene(s). For example: "It is unlikely that any trait from the transgenic wheat will be transmitted to any other wheat or sexually compatible relative. Hence, there will be no significant impact of the transgenic wheat plants in this field trial," (USDA Permit number 94-054-05, p. 1), and "Any such unexpected effects [on non-target flora and fauna] would be minimal by virtue of being confined to the area within the field plot," (94-024-01, p. 5).

As seen with previous incidents of gene flow, however, confinement requirements are sometimes inadequate and often imperfect. And once a transgene escapes, even at very low frequency, it may spread in the wild relative, depending on the characteristics of the gene, plant and environment. This ability of the transgene to increase in frequency is of critical importance because it subverts USDA's assumption of limited environmental exposure and harm.

Wild relatives are found growing in many states; therefore transgenes that find their way into those species may eventually spread over large areas. However, the ability of a particular gene to increase and spread if it escapes is typically not considered in granting field trial permits or notifications. Indeed, in the absence of rigorous environmental assessments, it is unlikely that the potential for increase in the wild plants will be known for most genes.

> *Proceeding blindly, without more data on the occurrence of gene flow from field trials, and without better requirements for confinement and safety testing prior to field trials, is taking unnecessary risks with the wild.*

The potential harms of transgene spread in wild relatives are numerous. They range from increased weediness or invasiveness, to negative impacts on environmental functions, to harm to wildlife or beneficial insects that normally use the plants as a source of food. [9, 10, 11]

Gene flow to wild relatives may have already occurred, in addition to the potential gene flow from GE bentgrass. Unfortunately, we do not know if pollination and gene flow into wild relatives has happened because testing for gene flow from field trials has rarely been conducted. This "don't look, don't tell" policy undermines assurances about the absence of gene flow from field trials, and the safety of the experimental genes they contain. Such statements can only be described as whistling in the dark. Proceeding blindly, without more data on the occurrence of gene flow from field trials, and without better requirements for confinement and safety testing prior to field trials, is taking unnecessary risks with the wild.

BACKGROUND

Field trials for GE crops have been regulated by the USDA Animal Plant Health Inspection Service (APHIS) since 1987. Researchers and companies can obtain APHIS approval for a GE crop field trial in one of two ways: they can either submit a "notification" or apply for a "permit." Notifications only require the completion of a short form. Permits require more detailed descriptions, including the genes and confinement methods, and give APHIS a longer period to come to a regulatory decision. APHIS regulates most field trials by applying performance standards rather than requiring specific confinement methods or safety tests. In this approach, APHIS sets performance requirements and allows the applicant to decide how to meet these requirements.

Initially, all field trials had to be approved under permits, but beginning in 1993 for six major crops, and in 1997 for most other crops, notifications were accepted.[12] The vast majority of trials are now conduced under notifications, which comprise 90% of all approvals, and 97% since 2000.[13]

National Environmental Protection Act (NEPA) compliance is required for USDA approval of field trials of genetically engineered crops, which are regulated under the Plant Protection Act. Environmental assessments (EAs) are prepared for a small percentage of field trials under NEPA to evaluate both the transgene confinement methods and environmental risks. They are usually the most thorough risk assessments prepared for field trials by APHIS. NEPA, however, allows for exclusion from requirements for EAs or environmental impact statements in some cases, including categorical exclusions for entire classes of subjects. Most field trials before 1995 were excluded from EAs on an individual basis. In 1995 USDA enacted a categorical exclusion regulation (7 C.F.R. § 372.5(c)(3)(ii)) for allegedly "confined" outdoor field trials of genetically engineered crops, which excluded almost all trials from the requirement of EAs as a group. Risk assessments were deemed unnecessary largely because trials that were performed outdoors were considered to be "confined" when procedures were used to limit gene flow.[14] Although relatively few EAs were prepared prior to 1995, far fewer EAs have been prepared since the categorical exclusion.

Because the vast majority of field trials are now conducted under notification, it is especially important to consider notification confinement requirements. The field trial applicant signs a form certifying compliance with the confinement performance standards in 7 CFR 340.3. Those performance standards, in turn, do not spell out clear methods to prevent gene flow for most crops, but point to guidelines developed for seed purity standards for breeders of conventional crops by the Association of Official Seed Certifying Agencies (AOSCA). For example, section 5 of, 7 CFR 340.3(c), "persistence in the environment," describes recommendations for confinement:

USDA performance standards do not discuss how close to the field trials the applicant needs to "survey" for wild relatives, leaving this to the interpretation of the applicant.

> It was also noted that methods that have been shown to give genetic isolation equal to certified seed standards are also acceptable [to minimize persistent offspring outside the field trial]. However, for plants with sexually compatible wild or weedy relatives, more stringent requirements may be necessary for the applicant to certify that no progeny will be produced that can persist in the environment. It may be necessary to survey the area surrounding the test site for the presence of these plants or select a test site where no sexually compatible species are known to exist.[15]

Although the language in this paragraph suggests that gene flow to crop wild relatives is impermissible, this report shows that in practice, gene flow may occur from field trials to wild relatives. For example, a review of EAs shows that the actual confinement methods accepted for field trials have often not been adequate to prevent gene flow.

Conventional certified seed purity standards typically allow 0.1% to 2.0% contamination with other conventional varieties, and foundation seed standards allow about 0.02% to 0.1% contamination.[16] The language in the citation above specifically refers to certified seed purity standards. However the isolation distances in Table 1, listed by USDA as a resource for meeting performance standards, appear to provide isolation distances for "foundation" seed, which allows less contamination than certified seed.[17]

The performance standards also do not discuss how close to the field trials the applicant needs to "survey" for wild relatives, leaving this to the interpretation of the applicant. For example, given the context provided by the AOSCA standards, should wild relatives be removed within a radius determined by the AOSCA certified or foundation seed purity standards, or from a greater distance?

The performance standards also provide a subsection with further guidance for sunflower, strawberry, and poplar species, noting that the standard…

> …provides some examples of points *to consider* for containment of these plant species which have already been field tested and for which sexually compatible wild or weedy relatives occur in the United States.[18] [emphasis added]

The discussion for these three species provides additional guidance, such as the use of caging or flower removal in sunflowers to prevent pollinator access to flowers. But no similar additional guidance is provided for other crop species, for which the above guidance to "survey the area surrounding the test site" for wild relatives is all that is discussed. However, recent studies, considered in the "Findings and Discussion" section below, point to the general inadequacy of isolation distances to prevent gene flow.

In addition to the limited confinement guidance that addresses pollination, USDA typically requires that the applicant destroy the crop after the field trial is complete, check for and destroy volunteer plants (self seeded progeny of the crop) in the test plot, and take precautions against seed or vegetative propagule dispersal, such as during transportation. The activities addressed by performance standards for aspects of confinement other than pollen flow may also lead to gene escape, but are beyond the scope of this report.

No risk assessment data are required prior to the first field trial, so if some dramatic problem is observed in that initial field trial, there may have already been an opportunity for gene flow to occur.

In addition to confinement requirements, it is also important to understand the risk assessment requirements for permits and notifications. As noted above, permits are only rarely subjected to EAs, and EAs are not prepared for notifications. For field trials performed under a permit without a formal EA, or for notifications, the applicant is required to submit field test reports to APHIS after the field trial is completed that include:

> …methods of observation, resulting data, and analysis regarding all deleterious effects on plants, nontarget organisms, or the environment.[19]

Although such data may reveal some potential environmental harm, informal observations are likely to miss many potential environmental impacts. This is because the main purpose of most field trials is testing the efficacy of the transgene and agronomic traits, rather than risk assessment. No risk assessment data are required prior to the first field trial, so if some dramatic problem is observed in that initial field trial, there may have already been an opportunity for gene flow to occur.

In the minority of examples where an EA is prepared, testing for potential harm to wildlife, crops, or people is usually not required. Instead, prior knowledge about the transgene is heavily relied upon to evaluate risk. There may be some sense of the relative safety of a transgene prior to formal risk assessment based on the source of the gene and its function, or the field trial applicant may have performed some risk assessment at his or her own discretion. But without requirements for a formal risk assessment, there is often inadequate data on the safety of the transgene.

Finally, permits and notifications may be monitored for compliance, but there is no requirement to test nearby wild relatives, if they occur, for gene flow. There appear to be extremely few instances where wild relatives have been tested for gene flow.

RESEARCH METHODS USED IN THIS REPORT

Data on GE field trials are compiled for USDA by Virginia Tech University.[20] In this report, the Virginia Tech database was used to collect information for the 20 crops that have wild relatives in the U.S., and that have been subject to 10 or more field trials. Each field trial permit or notification may consist of several outdoor sites, often in several different states, so the number of field sites is considerably greater than the number of approved notifications or permits. The database was also used to determine total acreage for approved field trials for each crop, locations, and EAs. Field trials were considered from the first trials in 1987 through March 31, 2005, unless otherwise noted.

Only approvals (for permits) or acknowledgments (for notifications) were counted, rather than actual planting data. Approved or acknowledged trials are not always planted, and when they are, they can comprise fewer acres than allowed. However, data on actual planting are not readily available.

The list of crops with compatible wild relatives covered in this report is based largely on Ellstrand.[21] Other crops were included if they have feral or wild populations in the U.S. For most of the crops in this report, there is strong evidence of hybridization with wild relatives in the U.S. For a few cases, hybridization has only been documented outside the U.S., although the wild relative also occurs in the U.S. A few examples where hybridization evidence came from outside the U.S. are included because it is likely that hybridization between these same species can occur in the U.S. as well. This is consistent with U.S. regulatory policy. For example, Ellstrand lists cotton as a crop where there is strong data for hybridization from outside of the U.S. Nevertheless, the U.S. EPA restricted the cultivation of commercialized Bt cotton, preventing its cultivation in those areas of the U.S. where wild relatives exist.[vii]

Isolation distance between a field trial and wild relative has a substantial impact on gene flow. If the tri-

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[vii] USDA does not restrict the location of field trials for GE cotton (or other GE crops) based on the presence of wild relatives, except to the extent that performance standards are followed.

als are geographically isolated from wild relatives, there is a much lower likelihood of gene flow. Therefore, the general proximity of the wild relative to the GE crop has been determined for this report. Proximity was only determined at the state level, because USDA does not typically disclose more precise locations for field trials. The primary data for the distribution of wild relatives was determined from the U.S. Plant Database.[22] This database only approximates the distribution of wild relatives, and does not claim to comprehensively document the geographic extent of occurrence. Although county distribution is available for some plants in some states, and county locations are available in EAs, county-level coverage in the Plant database is too sporadic to be relied upon.[viii] Wild relatives may occur in additional areas not noted in the database, but may also occasionally be absent from states where they are said to occur, or to be rare in those states. Some listed states may be only of historical importance for the wild relative. For example, several northerly states are listed as containing wild or feral cotton, although it is unlikely that these would be more than small and ephemeral populations. Similarly, wild red rice is listed as occurring in California, although it is believed to have been eradicated from that state.

Some genes confer multiple phenotypes, one of which may be associated with possible fitness effects.

For these reasons, the Plant Database information was revised for several crops where the author was aware of contrary information. In particular: California and several other states are not included as containing red rice; states or territories containing wild cotton are limited to Hawai`i, Florida, Puerto Rico, and the Virgin Islands;[ix] and sugar beet wild relatives are only considered for California.

The number of field trials of 50 or more acres was determined for each of the crops reviewed in this report. Although a somewhat arbitrary size, 50 acres is usually large enough to distinguish between trials of crops being seriously considered for commercialization, and smaller trials at the research stage of development. However, it is also common for a number of test sites to be covered under a single permit or notification, where the listed acreage is the cumulative total for all of the sites. Therefore, test plot sizes may be smaller at individual locations than the total acreage of the field trial.

Field trials containing genes that may confer a fitness advantage are considered for the nine crops with the largest number of field trials. These genes are highlighted because traits that confer a fitness advantage are more likely to spread in wild relatives after low levels of initial gene flow that may occur from field trials. Fitness is roughly defined as the ability of the plant to reproduce compared with other members of the species. Fitness depends not only upon the gene, but also on how it is expressed, the plant species, and the environments in which the plant is found. However, genes for some traits are generally considered to have a higher *a priori* probability of conferring a fitness advantage in some environments. In particular, genes that code for traits that confer tolerance to biotic or abiotic stresses are generally considered to be more likely to confer increased fitness than, for example, genes conferring altered nutritional content.

Also, some genes confer multiple phenotypes, one of which may be associated with possible fitness effects. For example, resveratrol is a secondary metabolite found in several crops, especially grape, which has been associated with health benefits. In addition, however, resveratrol has anti-fungal properties, which may confer fitness benefits to the plant.[23] Although the field trial database does not include resveratrol under the fungal resistance phenotype, it

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[viii] The disclaimer for county distribution maps, where they exist, is a follows: "Our county data are based primarily on the literature, herbarium specimens, and confirmed observations. Not all populations have been documented, however, and significant gaps in the distribution shown above may not be real."

[ix] These areas largely agree with the areas restricted from commercial GE cotton cultivation by EPA out of concern for gene flow.

is included here as a disease resistance gene. Other genes are included here if they are known to confer a phenotype that is potentially fitness-enhancing.

It is not possible to determine from the disclosed information whether field trials containing several genes represent stacked traits, with several genes in each plant,[x] or whether the genes have each been transformed into separate plants. Therefore, genes from a few of the field trials that contain several genes for different fitness categories were counted separately in each of the appropriate categories.

The number of EAs for field trials of crops with wild relatives was determined. EAs are the most thorough risk assessments performed by USDA for field trials, and therefore the majority of trials, which had no EA, probably had less thorough risk assessments.

Actual confinement methods for specific field trials are readily available only when an EA was prepared. Therefore, to better understand actual confinement methods accepted by USDA, several of the most recent EAs for field trials from states with a wild relative were examined. Both wind and insect pollinated, and self- and cross-pollinating crops were chosen. Because EAs are generally prepared for trials considered to represent the greatest risk, they are likely to contain the most stringent confinement methods and most thorough risk assessments of field trials.

> ***Because EAs are generally conducted for trials considered to represent the greatest risk, they are likely to contain the most stringent confinement methods and most thorough risk assessments of field trials.***

Even where EAs were not performed, field trials for crops containing pesticidal transgenes such as the Bt Cry proteins (but not GE herbicide resistant crops), known as plant incorporated protectants (PIPs), may be regulated by EPA when field trials exceed 10 acres. EPA requires some preliminary risk assessment for those Experimental Use Permits (EUP) before allowing outdoor planting. However, in practice there have been relatively few EUPs, mostly for Bt crops that have since been commercialized.

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[x] Most transgenic plants contain more than one gene, often a selectable marker gene (such as for the antibiotic kanamycin), as well as the gene of interest. Reference to multiple genes is here intended to mean more than one gene of interest, such as for pest resistance or drought tolerance, rather than associated genes such as markers.

FINDINGS AND DISCUSSION

QUANTITY OF FIELD TRIALS AND PROXIMITY TO WILD RELATIVES

Table 1 shows the number and total acreage for field trials for 20 crops with wild relatives in the U.S. There have been 2694 approved field trials for these crops, beginning in 1987.[xi] In 2004 there were 294 trials, indicating that trials for crops with wild relatives are continuing at a pace similar to the recent past. The total area covered by the trials examined in this report was over 200,000 acres. Although most of the trials were small, often less than a few acres, the large number of such field trials increases the risk of gene flow. In addition, a substantial number of trials covered hundreds or thousands of acres. However, there was no evidence in USDA documents to suggest that the number or cumulative acreage of field trials were considered during approval.

Several crops with relatives that are major weeds have had numerous field trials, including creeping bentgrass, with 170 trials, and wheat, with 368 trials. Creeping bentgrass has about a dozen wild relatives in the U.S., some native and some introduced, which occupy a variety of habitats.[24,25]

The crops found in Table 1 are diverse in their biological characteristics. These crops include annuals such as rice and wheat, biennials such as sugar beets, carrots and lettuce, herbaceous perennials such as creeping bentgrass and tall fescue, and long-lived native forest trees such as eastern cottonwood and sweetgum. Some of the crops, such as canola and sunflower, are pollinated primarily by insects, and others, such as pines and creeping bentgrass, are pollinated by wind. Primarily self-pollinating species like rice, and highly outcrossing species like creeping bentgrass, are both included. Many crops, for example wheat, are incapable of surviving for long outside of the agricultural environment. But some crops, such as forest trees and perennial grasses, can thrive in the wild. Several crops, such as wheat and lettuce, have wild relatives that are serious non-native agricultural weeds—jointed goatgrass (*Aegilops cylindrica*) and prickly lettuce (*Lactuca serriola*),

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[xi] Numbers may vary slightly over time, because the database adds pending field trials to the approved category by application date rather than approval date. We determined the numbers used in this report for field trials with application dates through March 31, 2005, and that were approved by early July 2005.

Table 1: Field Trials of Genetically Engineered Crops with Compatible Wild Relatives in the U.S.[1]

CROP	WILD RELATIVES(S)	# FIELD TRIALS[2]	ACREAGE[3]
Alfalfa	Feral alfalfa, *Medicago sativa* ssp. *sativa*, Feral sickle medic, *M. sativa* ssp. *falcata*[4]	312	21,739
Apple	*Malus* ssp. of section Chloromeles and *M. fusca*	36	102
Beet	*Beta vulgaris* ssp. *macrocarpa*	192	2,695
Carrot	Wild carrot, *Daucus carota*	13	6
Cotton	Creole cotton *Gossypium barbadense*, Upland cotton *G. hirsutum*, Hawai'i cotton *G. tomentosum*	728	143,825
Creeping bentgrass	Wild creeping bentgrass, *Agrostis stolonifera*, approximately 8-10 other *Agrostis* species, and approximately 3 *Polypogon* species	170	4,476
Eastern cottonwood	Eastern Cottonwood, *Populus deltoides*	11	28
Grape	Numerous Vitis species: *V. aestivalis, V. californica, V. girdiana, V. labrusca, V. novae-angliae, V. riparia, V. vinifera, V. vulpina*	36	194
Lettuce	Prickly lettuce, *Lactuca serriola*	79	176
Pines	Loblolly Pine *Pinus taeda,* and Loblolly X Pitch *P. rigida*	57	57
Poplar	Black cottonwood, *Populus trichocarpa*[5]	99	134
Rapeseed (canola)	Wild mustards, *Brassica rapa, B. napus, B. campestris, B. juncea*	210	19,234
Rice	Annual red rice, *Oryza sativa*	195	5,005
Squash	Wild squash, *Cucurbita pepo* var. *texana*	60	118
St. Augustine grass	St. Augustine grass, *Stenotaphrum secundatum*	17	193
Strawberry	Wild strawberry, *Fragaria virginiana*	40	13
Sunflower	Wild sunflower, *Helianthus annuus*	32	202
Sweetgum	Sweetgum, *Liquidambar styraciflua*	24	25
Tall fescue	Tall fescue, *Festuca (Lolium) arundinacea*	15	4
Wheat	Jointed goatgrass, *Aegilops cylindrica*	368	2,683
Total		2,694	200,909

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[1] This list is not intended to be comprehensive, and is based primarily on Elstrand N., 2003, and only contains crops with 10 or more field trials

[2] Data through March 31, 2005, field trials approved or acknowledged

[3] Acreage is an underestimate, because some field trials do not disclose acreage data

[4] Transgenic Crops: An Introduction and Resource Guide, available at http://www.colostate.edu/programs/lifesciences/TransgenicCrops/croptoweed.html This resource applies to information about cranberry, lettuce, oats, rapeseed, rice, sorghum, squash, strawberry, sunflower, wheat, and beet, as well.

[5] Slavov, G.T., "Gene flow in forest trees: From empirical estimates to transgenic risk assessment," from the Gene Flow Workshop, March 5, 2002. Available at http://www.biosci.ohio-state.edu/~asnowlab/Proceedings.pdf

Table 2: Field Trials of Genetically Engineered Crops in States with Compatible Wild Relatives in the U.S.

CROP	STATES AND TERRITORIES WITH WILD RELATIVES[1]	FIELD TRIALS IN STATE(S) WITH WILD RELATIVES[2]	
		Number	% of Total Crop
Alfalfa	All but VI	312	100
Apple	AL, AK, AR, CA, DE, FL, GA, IL, IN, IA, KS, KY, LA, MD, MI, MN, MS, MO, NE, NJ, NY, NC, OH, OK, OR, PA, SC, SD, TN, TX, VA, WA, WV, WI, WY	36	100
Beet	CA	27	14
Carrot	All but HI, AK, VI	13	100
Cotton	FL, HI, PR, VI	146	20
Creeping bentgrass	All but PR and VI	170	100
Eastern cottonwood	All but AK, CA, HI, ID, NV, OR, PR, VI, WA	11	100
Grape	All but AK, HI	36	100
Lettuce	All but AK, PR, and VI	78	99
Pines[3]	AL, AR, CT, DE, FL, GA, IL, IN, KY, LA, MA, MD, ME, MO, MS, NC, NH, NJ, NY, OH, OK, PA, RI, SC, TN, TX, VA, VT, WV	56	100
Poplar	AK, CA, ID, MT, NV, ND, OR, UT, WA, WY	44	44
Rapeseed (canola)	All states and territories	210	100
Rice	AR, LA, MS, MO, PR, TX, VI	107	55
Squash	LA, NM, TX	6	10
St. Augustine grass	AL, CA, FL, GA, HI, LA, MS, MO, NC, OK, PR, SC, TN, TX, VI, VA	17	100
Strawberry	All but HI, PR, VI	40	100
Sunflower	All but VI	32	100
Sweetgum	AL, AR, CT, DE, FL, GA, IL, IN, KY, LA, MD, MA, MS, MO, NJ, NY, NC, OH, OK, PA, RI, SC, TN, TX, VA, WV	24	100
Tall fescue	All except IN, MS, NH, NJ, ND, PR, RI, VI	13	87
Wheat	AZ, AR, CA, CO, ID, IL, IN, IA, KS, KY, LA, MI, MO, MT, NE, NV, NM, NY, NC, ND, OH, OK, OR, PA, SD, TN, TX, UT, VA, WA, WV, WY	332	90
Total		1,710	64

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[1] State distributions for wild relatives were found on USDA's PLANTS database (http://plants.usda.gov/). According to the website, "The state and county maps show the distribution of native and naturalized populations only, not planted or horticultural populations."

[2] Field trials were counted if at least one site for the trial was in a state with at least one wild relative of the crop. Since many trials have multiple sites, and many crops have multiple wild relatives, this is an underestimation of the number of times test crops had the opportunity to cross with wild relatives.

[3] Until recently APHIS did not distinguish between pine species. States here are a combination of locations for the two pine species, loblolly and pitch x loblolly. Field trial locations were assigned for the appropriate species.

respectively. Others have native relatives that can be agricultural weeds, such as wild sunflower (*Helianthus annuus).* Or the wild relative may be important to native ecosystems, as with forest trees like sweetgum (*Liquidambar styraciflua*) and loblolly pine (*Pinus taeda*). The variety of crops with wild relatives demonstrates the complexity of gene flow and its potential to impact several natural and agricultural ecosystems.

Several crops with relatives that are major weeds have had numerous field trials, including creeping bentgrass, with 170 trials, and wheat, with 368 trials.

The distance between a crop and its wild relative is of critical importance for assessing the likelihood of gene flow. In general, the closer the field trial to the wild relative, the greater the chance for gene flow. Table 2 shows the number of field trials with at least one site in states where one or more sexually compatible wild relatives occur. Although more precise measures of the proximity of wild relatives to field trials would be desirable, most publicly accessible data disclose only the states where field trials are conducted, rather than specific locations. Very few field trials, only those with EAs, disclose the counties where field trials take place, and none provide specific locations. The nearest wild relatives to field trials are not disclosed, and are not generally determined. Therefore, important data needed to assess the risk of gene flow from field trials are not available. Statewide data are not precise enough to demonstrate a high probability of gene flow, but are a preliminary indication that trials may be close to wild relatives. No systematic study of the occurrence of wild relatives in or near field trials could be found, although such occurrences may occasionally be noted incidentally.[26]

1710 field trails, or 64% of the total for the crops examined in this report, were approved for states with wild relatives. This is roughly 16% of all field trials.[xii] All creeping bentgrass trials have been conducted in states where their wild relatives are reported to grow, as have all trials of several other crops. Ninety percent of wheat field trials have been conducted in states with jointed goatgrass. Only sugar beet, cotton, poplar (black cottonwood) and squash trials have been conducted mostly in states that do not contain a wild relative.

State proximity data are important for focusing on field trials where gene flow is more likely to occur. Higher probability of gene flow is due both to closer proximity facilitating higher gene flow rates, but also by deemphasizing crops with rare wild relatives. For example, although cotton and sugar beet comprise a disproportionately large share of all field trials (Table 1), the substantial majority of those trails are not counted in Table 2. This is because wild relatives of those crops are greatly restricted geographically, so that most field trials occur in states without their wild relatives. As noted above, the beet wild relative, *Beta macrocarpa,* is found only in California, although the crop is also grown in a number of other states. Furthermore, sugar beet seed is not produced in California, reducing gene flow potential. The wild relatives of cotton are limited to the extreme southern U.S., some Caribbean islands, and Hawa`i.

Unlike sugar beet or cotton wild relatives, canola wild relatives, *Brassica rapa* (field mustard) and *B. juncea* are reported to be found in every state. Finally, some other wild relatives have restricted ranges, but their distribution is widespread where the crop is typically grown. For example, weedy annual red rice (*Oryza sativa*) is generally found only in Southern U.S. rice growing regions that produce most of the rice in the U.S. However, except for California, it is relatively widely distributed in those areas, and therefore may be found near field trials, which are also restricted to southern states due to the climatic requirements of the crop.

Although proximity at the State scale is not close enough to support gene flow by pollination, other

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[xii] There have been 10,859 field trials issued as of March 31, 2005.

considerations suggest that much closer proximity, that could allow gene flow, may be common. First, because many wild relatives are adapted to habitats similar to the crop, they tend to become established in areas where the crop is grown commercially. This is often true even for wild relatives that are not native to the U.S. For example, jointed goatgrass (*Aegilops cylindrica*) is a compatible wild relative of wheat from the eastern hemisphere which was introduced into the U.S. where it has become a major weed of winter wheat.[27] Morrison et al. note that jointed goatgrass infests over 7.4 million acres of winter wheat cropland in the U.S.[28] Second, many trials are conducted in areas where the crops are grown commercially, and therefore, where their wild relatives may be found nearby. This is because it is important to test the GE crop in these areas to ensure that it is well adapted to commercial production conditions. Field trials in other regions that have different climate and pest conditions may be acceptable for some field trials, but ultimately many trials will need to be carried out where the crop is usually grown.

Because many wild relatives are agricultural weeds, gene flow frequencies may be enhanced in many instances compared to natural habitats. This is because seedlings resulting from pollination of the wild relative typically must compete with established vegetation to survive in undisturbed natural habitats. Disturbed environments like agricultural fields usually reduce the barrier to establishment, and therefore the probability that a transgenic hybrid could survive may be enhanced.[xiii]

The dearth of information about the proximity of wild relatives to field trials is a substantial gap in the data needed to evaluate gene flow, and should be remedied. The quickest way to improve this situation is to publicly identify the counties where field trials take place in all of USDA's online databases, instead of only identifying the state. This will not be sufficient to accurately determine gene flow potential due to lack of precise locations of wild relatives in relation to the field trials, but it would improve information on proximity compared to currently available data. Disclosing county locations would be a very feasible step toward connecting wild relative and trial locations without revealing any confidential information.

Disclosing county locations would be a very feasible step toward connecting wild relative and trial locations without revealing any confidential information.

Several of the crops can survive outside an agricultural environment, and so do not depend on nearby wild relatives for gene flow to occur. These crops are capable of escaping as seed as well as through pollination. In fact, because seed development does not depend on low-frequency pollination events with wild relatives, because the progeny will not suffer from possible reduced hybrid fitness, and because seed from some species can be easily dispersed, the risk of escape by seed may be higher for these crops than gene flow from pollination.

Crops in this category include the forest trees (pines, cottonwoods, sweetgum, poplar) and grasses (creeping bentgrass, tall fescue, and St. Augustine grass), which account for 369, or about 14%, of all field trials covered in this report. Black cottonwood in particular was grown in states with the wild species in only 44 of 99 field trials. But most of the 99 trials may have led to escape, in the absence of adequate confinement, had they set seed, even without nearby wild trees.

Area totals for field trials (Table 1) of specific crops range from as little as 4 acres for tall fescue to 143,000 acres for cotton. Several crops had substantial acreage including alfalfa with 21,000 acres, canola with 19,000 acres, rice with 5,000 acres, creeping bentgrass with 4,400 acres, and wheat with almost 2,700 acres.

[xiii] Competition with the weed seed bank will still occur, and in agricultural fields weed control measures may counter the advantage from disturbance to some extent.

Table 3: Large Field Trials

CROP	NO. FIELD TRIALS 50 ACRES OR LARGER	AVERAGE SIZE* (ACRES)	LARGEST FIELD TRIAL* (ACRES)
Alfalfa	41	465	1,520
Beet	12	118	320
Cotton	165	849	34,350
Creeping bentgrass	17	214	600
Rapeseed (canola)	32	563	4,000
Rice	13	327	1,259
Sunflower	2	65	70
Wheat	8	84	200
TOTAL	290	--	--

* Size is for approved acreage. Actual planted size may be less

NUMBER OF LARGE FIELD TRIALS

An important influence on gene flow is the size of the field trial (pollen source), as well as the size of the recipient wild-relative population. Greater numbers of crop plants produce relatively more pollen to fertilize wild plants.[29,30] Although field trials are often used for research purposes, and are often therefore small, they also may be much larger, such as when grown to increase the amount of seed prior to commercialization (and before approval for deregulation).

Table 3 shows the number of field trials for crops with wild relatives that are 50 acres or larger. A total of 290 such field trials were found for 8 crops. The average size of these field trials, by crop, range from 65 acres for sunflower to about 850 acres for cotton. The largest was a cotton field trial of 34,350 acres (where single field trials can consist of multiple sites).

Fewer confinement methods are available for large field trials, which may increase the potential for gene flow. Some confinement measures, such as placing bags over flowers to prevent pollen release, may not be feasible for large field trials containing millions of plants. Field trials used to produce enough seed to grow a commercial crop are typically much larger than typical research trials. The size of large seed-increase trials will depend, among other things, on the needs of the crop, with larger-acreage crops such as cotton or wheat requiring more acreage to supply adequate seed.

The greater risk of gene flow from large field trials suggests that more careful risk assessment is needed to ensure that they are safe. Environmental assessments are the most thorough means used by USDA to assess field trial risk, so these larger trials were examined to determine how many had been subjected to EAs. Of the 290 large field trials, only one was the subject of an EA.

It is possible that EAs had been prepared for earlier, smaller trials containing the same crop/gene combination as the large trials in Table 3. However, the many genes that are claimed to be confidential business information (CBI) in those smaller trials make it difficult to determine if this was the case (data not shown). For example, genes were claimed to be CBI in at least 153 alfalfa field trials, 119 beet trials, 623 cotton trials, 100 creeping bentgrass trials, and 146 wheat trials.[xiv]

ENVIRONMENTAL RISK ASSESSMENTS

Overall, relatively few EAs have been prepared for field trials of crops with wild relatives. Table 4 lists the number of EAs for the crops examined in this report. Of the 2694 field trials, only 130 EAs were prepared, or less than 5% of the total. And these EAs were for even fewer different genes. That is, the same gene, such as for kanamycin resistance (neomycin phosphotransferase II), was the subject of many EAs in several crops, while many other genes had no EAs.

Despite 170 approved creeping bentgrass trials, only three EAs were prepared, or less than 2% of the total. These EAs examined only three different genes, for the blue color-marker beta-glucuronidase, for resistance to glufosinate herbicide, and for the *pin-2* insect resistance gene (reviewed in the EA section in Appendix A). EAs were prepared for only 3 of 368 wheat trials, or less than 1%.

Similarly, for other crops, only a small percentage of the field trials have had EAs. A number of crops in Table 4, such as grape and strawberry, have had no EA. Therefore, it is clear that many genes have been used in field trials without any formal risk assessment.

Because so many genes are claimed as CBI, an accurate count of genes subjected to EAs cannot be made.[xv] Nonetheless, very few of the field trials with genes claimed as CBI were evaluated by an EA. For example, none of the 153 CBI genes of alfalfa or 100 CBI genes of creeping bentgrass were subjected to EAs.

It is clear that many genes have been used in field trials without any formal risk assessment.

The dearth of EAs has been exacerbated by the categorical exclusion decision by APHIS, which concluded that EAs are generally unnecessary for field trials. Beginning in 1996, after the categorical exclusion rule, only 7 EAs have been prepared for the crops of this report.

USDA justified adopting the categorical exclusion rule because it concluded that there was minimal risk from field trials based on previous EAs, and because field trials were said to be confined.[31] However, the justification for using the risk assessments from previous EAs as a basis for the categorical exclusion is dubious. For example, in an extensive review of USDA regulation of GE crops in 2002, the National Academy of Sciences found that the environmental reviews performed by USDA lacked needed rigor.[32] Therefore it is doubtful that the EAs that served as the basis for the categorical exclusion were rigorous enough to justify the rule.

One of the case studies reviewed most carefully by the NAS was GE virus-resistant summer squash, a crop with a U.S. wild weedy relative, the Texas gourd (*Cucurbita pepo* var. *texana*). The NAS report found that the USDA risk assessment did not adequately evaluate whether virus resistance genes might spread through the wild population and increase weediness. The NAS cited several examples of shortcomings of the USDA evaluation, such as insufficiently evaluating the impact of the viruses on wild populations of squash.

The NAS report also criticized USDA for evaluating risk based on lack of evidence rather than on data that demonstrated that a risk did not exist. In other words, USDA often claimed that there was no evidence of risk when there were no data evaluating the risk. In the absence of tests designed to assess the

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[xiv] Genes may be claimed as CBI in earlier field trials, but disclosed later, so the disclosure of genes in larger field trials does not necessarily mean that these genes were not earlier considered to be CBI.

[xv] The number of non-CBI genes is approximate, because gene nomenclature is not standardized in the database, and there may be several different genes with the same designation, or the same gene with different designations. For example, there are several entries for "glutenin" from wheat, and although there are several different wheat glutenin genes, these different genes are not distinguished. In addition, similar genes from different donor organisms were counted separately. The number of genes is also an underestimate due to the large number claimed as CBI.

Table 4: Approved Field Trials[1] and Environmental Assessments

CROP	FIELD TRIALS APPROVED	ENVIRONMENTAL ASSESSMENTS
Alfalfa	312	14
Apple	36	3
Beet[2]	192	8
Carrot	13	2
Cotton	728	39
Creeping bentgrass	170	3
Eastern cottonwood	11	0
Grape	36	0
Lettuce	79	5
Pines[3]	57	0
Poplar	99	3
Rapeseed (canola)	210	21
Rice	195	5
Squash	60	19
St. Augustine grass	17	0
Strawberry	40	0
Sunflower	32	4
Sweetgum	24	1
Tall fescue	15	0
Wheat	368	3
Total	2,694	130

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[1] Includes both permits issued and notifications acknowledged

[2] The sum of listing for beet and sugar beet. All, or nearly all, are likely to be sugar beet.

[3] The database recently listed separate pine species, and these consist almost entirely of (*Pinus taeda*) or loblolly x pitch pine (*P. rigida*). Both are native U.S. species.

risk, such statements are meaningless. Such "lack of evidence" statements, often implicit, were found in virtually all of the EAs evaluated in this report (see Appendix A), and were often the primary arguments used by USDA to reach a finding of low risk.

Beginning in 1996, after the categorical exclusion rule, only 7 EAs have been prepared for the crops of this report.

The squash EA critiqued by the NAS was for the deregulation of the crop, rather than for a field trial. As is evident by comparison to the specific EAs examined in this report, the EAs for field trials are less rigorous and rely on fewer risk data than the example analyzed by the NAS. For example, none of the EAs reviewed in this report attempt to determine the risk that the transgene would spread in the wild relative if gene flow occurred. And, as almost all EAs were prepared prior to 1997, they do not include the substantial amount of newer data on gene flow.

The decision by USDA to allow the categorical exclusion for field trials was based on EAs prepared prior to the NAS report, and therefore did not take this evaluation into account. In addition, as noted above, the majority of EAs have evaluated only a few different genes, so the number of EAs substantially over-represents the number of risk issues examined.

Finally, there has been considerable scientific consensus that the risks from GE crops should be evaluated on a case-by-case basis.[33] This judgment is based on recognition of the unique risk profiles of each GE crop based on the gene, its expression, the organism, and the environment. This perspective argues against a categorical exclusion approach, which is based on the argument that a limited number of risk assessments can predict the risk of new GE organisms. Accordingly, the categorical exclusion for field trials should be reevaluated in light of the current understanding. The lack of adequate EAs greatly limits our understanding of risk should gene flow occur.

TRANSGENES AND THE FITNESS OF WILD RELATIVES

The fitness of hybrids that result from pollination between a crop and a wild relative is critical to gene flow. Transgenes that increase the fitness of the hybrid or wild relative are likely to spread widely once the gene reaches a sufficient number of plants to become established.

Although it is often difficult to predict the fitness effect of a transgene without experimental data, there is general consensus that some genes commonly used in GE crops are more likely to increase the fitness of wild relatives than others. For example, the NAS noted that genes for pest resistance or stress tolerance, such as to drought or cold, are more likely to increase fitness than some other genes.[34] Several studies indicate that diseases,[35,36] or insects[37] reduce weed populations or plant invasiveness in some cases. Since many transgenes are intended to reduce disease or insect damage, these genes may increase the fitness of wild relatives by improving survival when the pest is present.

Table 5 lists the number of field trials for genes with a reasonable potential for increasing the fitness of wild relatives. The nine crops with the highest number of field trials are included. The genes in these trials confer pest resistance, abiotic stress tolerance, herbicide resistance, or enhanced nutrient utilization. For the 2175 field trials approved for these crops, 1900, or 87%, are for genes associated with increased fitness. The largest category is for herbicide resistance, with 1286 field trials, or 59% of the total.

Herbicide resistance, in the absence of treatment with the herbicide, is not likely to increase fitness. But because many wild relatives are crop weeds, they may be exposed to very strong selection by the herbicide after they acquire the transgene. For example, glyphosate is used to control weeds in wheat, includ-

Table 5: Field Trials with Genes Associated with Possible Increased Fitness[1]

CROP	TOTAL[2]	DISEASE RESISTANCE[3]	INSECT RESISTANCE[3]	ABIOTIC STRESS TOLERANCE[4]	HERBICIDE RESISTANCE[5]	OTHER[6]
Alfalfa	312	14	1	0	274	3
Canola	210	4	20	2	71	5
Cotton	728	6	332	19	361	0
Creeping bentgrass	170	33	2	24	105	0
Rice	195	21	9	3	111	0
Sugar Beet	192	16	0	0	176	0
Wheat	368	90	0	7	192	3
Totals	2,175	184	364	55	1,290	11

1) A small number of trials contained multiple genes in more than one category, and were counted in each appropriate category

2) Totals through March 31, 2005. Totals are for all approved or acknowledged field trials (including genes less likely to have a positive effect on fitness), not only those included in the other categories in this table.

3) In a few instances, field trials were included for genes that were not identified by the applicant or USDA under these categories, but where the genes are known to have properties that fit the category. These included resveratrol, lactoferrin, lysozyme, and aprotinin.

4) Stress tolerance includes traits such as drought, salt, and aluminum tolerance.

5) Herbicide tolerance is generally not believed to confer a fitness advantage in the absence of the herbicide. However, many of the wild relatives are agricultural weeds and are likely to be exposed to the herbicide in some situations.

6) The only trait included was for nitrogen metabolism.

ing jointed goatgrass, and is especially useful to control volunteer wheat, and in wheat-fallow cultivation in drier regions of the western U.S.[38, 39] Jointed goatgrass that acquires glyphosate resistance from a field trial may be favored by selection if it grows in a wheat field treated with glyphosate.

Many genes reduce fitness in the absence of selection for the trait they confer, and such genes would not spread in a crop wild relative unless selection for it occurs. For example, in the absence of insects, some insect-resistance genes may actually reduce the fitness of a plant. This reduced fitness may eventually eliminate the gene. But glyphosate resistance genes do not appear to substantially reduce fitness, even in the absence of glyphosate. In general, herbicide resistance and herbicide resistance genes often do not seem to confer substantial fitness costs in the absence of herbicide use.[40,41] If the gene is fitness-neutral, or only slightly detrimental, it may persist for at least several generations at levels determined by the rate of gene flow.[42] This may allow wheat-goatgrass hybrids, for example, to persist at low levels until eventually exposed to selection by glyphosate applications.

The wild relative may also be a weed in an approved GE herbicide-resistant crop, where it could be selected after gene flow from a field trial. Alternatively, an herbicide may be used to control the wild relative itself, as is the case with glyphosate used to control creeping bentgrass in undeveloped habitats.

Excluding herbicide resistance, the remaining 28% of "fitness genes" comprise 614 field trials. Even removing the large number of Bt genes used in cotton field trials (some of which, for beetle control, have not completed regulatory review) leaves 245 trials, or 11% of the field trials from Table 5. These genes code mainly for insect resistance, disease resistance, and abiotic stress tolerance such as drought or salt tolerance. For example, the 170 field trials for creeping bentgrass include 59 for fungal disease resistance, insect resistance, or tolerance to abiotic stresses. Three trials for fungal resistance were 20 or 30 acres, in one or more states (presumably 10 or 20 acres per state).

The 170 field trials for creeping bentgrass include 59 for fungal disease resistance, insect resistance, or tolerance to abiotic stresses.

Ninety of the 368 approved wheat field trials, or about 24%, were for disease resistance. About 52% of wheat field trials were for herbicide resistance, and about 2% were for abiotic stress tolerance.

Genes for traits such as nutritional enhancement, ripening, male sterility, yield enhancement, oil composition and others were not included in Table 5. It is possible that some of these genes could confer fitness advantages in some environments, but this is less likely than for stress-resistance genes, and mechanisms for this to occur are often not obvious. For example, yield enhancement could increase fecundity, and thereby survival, but may also reduce survival due to the increased metabolic cost devoted to seed production. On the other hand, recent work with GE canola suggests that some types of altered seed oil content may increase fitness by increasing seed dormancy or survival.[43, 44, 45, 46]

It is important to understand that the fitness conferred by a gene is highly context-dependent. For example, Swiss chard hybridized with sugar beets containing a transgenic virus resistance gene had increased fitness only in environments where high levels of the virus were present. A Bt gene increased the fitness of canola only where susceptible insects were present.[47] A trait such as drought or salt tolerance may confer advantage only where water stress or saline soils are present.[48] Although the fitness effects of these genes may be anticipated, others may not. For example, the fitness increase due to seed lipid composition in canola, discussed in the previous paragraph, may not have been easily predictable.

Some crop traits, such as the non-shattering seed heads of maize, are likely to be deleterious to a wild relative in any environment. These considerations have led to the suggestion that transgenes that contribute to crop domestication are unlikely to be maintained, or cause environmental harm, in wild relatives because they would decrease fitness.[xvi, 49] Beyond a few examples, however, these genes have not been well defined. It is also not clear that all proposed domestication traits would reduce the fitness of wild crop relatives in all environments. For example, seed lipid composition has been mentioned as one of these traits,[50] but as noted above, some changes in seed lipid chemistry may provide a fitness advantage in some environments.[51]

There is evidence that crop genes have previously contributed to weed fitness. Johnsongrass (*Sorghum halapense*), considered one of the world's worst weeds, is believed to be a hybrid of crop sorghum and a wild species, *S. propinquum*.[52] This suggests that at least some crop genes contributed to the increased weediness of johnsongrass. Furthermore, the northward extension of johnsongrass into Canada was associated with several traits that are found in sorghum, presumably due to hybridization between the weed and the crop.[53] These traits are generally contrary to properties associated with the weediness of johnsongrass in the warmer parts of its range. This raises the question of whether crop genes involved in the northern extension of johnsongrass would be considered to be "domestication" genes.

[xvi] Domestication can be defined as adaptation to the demands of cultivation, and may include traits such as reduced dormancy and large seeds that do not disperse when mature.

Gene flow from crops to wild relatives is also implicated in the evolution of aggressiveness of several other important weeds.[54] As these examples illustrate, the fitness conferred by a gene often cannot be determined in the absence of experiments that consider the environmental context, and such experiments are not typically conducted before or during field trials.

> ***The fitness conferred by a gene often cannot be determined in the absence of experiments that consider the environmental context, and such experiments are not typically conducted before or during field trials.***

Rate of hybridization and fitness of hybrids are also important in determining rates of gene flow. Some of the crops considered in this report apparently hybridize readily with some of their wild relatives, for example, creeping bentgrass[55] and grape.[56] In both species, hybrids are reported to often be vigorous and fertile. Creeping bentgrass hybridizes with several related species producing fertile offspring, and is also perennial and able to survive and reproduce vegetatively.[57] Some hybrids between cultivated rice and red rice in the southern U.S. have also been reported to have increased vigor,[58] and others are nearly as fit as the parents.[59,60]

For other crops, first (and sometimes subsequent) generation hybrids often have low fertility. These fitness-reducing effects may overwhelm a competitive advantage conferred by the transgene alone. However, fertility may increase with subsequent backcrossing into the wild relative, as has been observed with wheat and jointed goatgrass.[61, 62, 63]

Even if the initial hybrid is less fit and has reduced fertility, it may persist long enough to backcross with the wild relative over several generations, leaving the transgene and relatively few other crop genes in the wild relative. This process is known as introgression. Introgression has been documented for hybrids between wild plant species, where similar issues of low fertility and fitness often occur.[64] Initial hybrids of radish (*Raphanus sativus*) and the important weed called jointed charlock (*Raphanus raphanistrum*) were less fit than the wild relative. Fitness improved with introgression of radish genes into the weed, demonstrating that crop genes do not necessarily reduce fitness of the wild relative, despite somewhat lower fitness of hybrids.[65]

For crops examined in this report, there is substantial evidence of persistence of crop genes, either in hybrids or in the wild relative through introgression.[66] These data, although developed mostly with conventional non-GE crops, provide support for possible survival and spread of agricultural transgenes in wild plants.

Only a few experiments have been conducted to determine the fitness of transgenes in wild relatives. In one of these, carried out under environmental conditions, wild sunflowers containing the *cry1A* Bt gene produced significantly more progeny than their non-transgenic counterparts at one of two field locations, a preliminary indication of increased fitness and possible increased weediness.[67] Wild sunflower containing a transgene for protection against an important fungal pathogen did not provide increased fitness under the conditions of another experiment (although the sunflowers were artificially infected in a way that does not represent typical natural infection processes, and which may have influenced the results).[68, xvii]

Testing for transgene effects on the fitness of a wild relative is important where the prevention of gene flow cannot be ensured. However, as noted above, USDA does not require testing for fitness effects of transgenes for field trials. Testing for possible fitness advantage should be conducted in a range of environments in which the commercial crops are grown.

[xvii] Stem inoculation at flowering, the approach in this study, does not replicate the more common modes of infection; infection of seedlings from fungus in the soil, or infection of the flower by spores. The former mechanism gives the fungus more time to damage the plant at earlier stages of growth, and the latter impacts seeds production.

> *The lack of careful evaluation by USDA of transgene fitness, combined with inadequate confinement, means that the risks of gene flow from field trials are largely unknown.*

Absence of increased fitness in some environments may merely reflect the absence of selection for the genetically engineered trait in that locality or time rather than a general lack of fitness advantage. As noted above, the National Academy of Sciences criticized the USDA for conducting an inadequate fitness assessment for deregulation of GE virus resistant squash.[69] This was in part because of USDA's inadequate consideration of the prevalence and importance of the virus in controlling the wild relative at different locations. As with herbicide resistance, even if a trait is fitness-neutral or only slightly deleterious in the absence of selection, it may persist long enough to be exposed to conditions that would select for its spread.

Increased fitness is not always synonymous with increased weediness. For example, although lepidopteran insect-resistant canola survived better than non-resistant canola in the presence of susceptible insects, this trait did not seem to increase competitiveness with native vegetation (however, this experiment was conducted with a crop that is not generally competitive outside the agricultural environment).[70] So even if fitness is enhanced, the population of the wild relative may not necessarily increase.[xviii]

On the other hand, increased competitiveness with other species is not always necessary to cause environmental harm. That is because a fitness-enhancing gene would still be expected to spread through the wild relative, even if the population of the wild relative is not thereby increased. In that case, if the gene product harms non-target species, such as beneficial insects, environmental harm may occur even without increased weediness.

Several factors that are independent of the transgene may affect rates of gene flow. Crop genes that are close to the transgene insertion site will be transferred with the transgene to most of the progeny, so fitness effects of those crop genes may affect transgene retention in the wild relative. Because transgene insertion into plant chromosomes occurs randomly, different crop genes will flank the transgene in each transformed plant. Other factors, such as chromosomal variation in the ability to recombine (transfer the transgene from the crop genome to a wild-relative chromosome) can also affect rates of introgression.[71]

For low rates of initial gene flow, as would occur from field trials, random, or stochastic, events are likely to play an important role in the survival of the transgene. These events will often eliminate rare hybrids.

It is important to understand, however, that a transgene may survive after only a single or a few transfers if it confers a fitness advantage to the wild relative, as noted by the National Academy of Sciences.[72] For example, "permanent" gene flow was observed between cultivated and wild sunflowers several years after the single growing season of the crop in California.[73] Because it is unusual for a crop to be grown in a particular location only once, and because gene flow from crops to wild relatives has only rarely been tested, we do not know whether this kind of rapid gene flow is unusual. The fact that such rapid gene flow was found raises the question of how commonly such gene flow occurs.

In summary, many transgenes may be neutral or fitness-enhancing in wild relatives in various environments, and therefore may persist or spread after initial gene flow occurs. The lack of careful evaluation by USDA of transgene fitness, combined with inadequate confinement, means that the risks of gene flow from field trials are largely unknown.

[xviii] However, this paper argued that the low dormancy of canola probably makes it unable to persist in undisturbed areas, although later work (Simard M-J et al., 2003, Weed Technol. 16:433-439) demonstrated that canola seed can acquire secondary dormancy, even on undisturbed soil.

OTHER FACTORS THAT DETERMINE GENE FLOW: CROP AND WILD-RELATIVE BIOLOGY

Several factors in addition to proximity, field size, and fitness determine whether a transgene will become a “permanent” part of the wild population. Many aspects of crop and wild-relative biology influence gene flow rates. Outcrossing crops are generally much more likely to pollinate wild relatives than self-fertilizing crops. However, even crops that are considered to be self-fertilizing outcross to some extent. Crops like rice and wheat are largely self-fertile, and usually outcross at low levels over relatively short distances. On the other hand, plants like creeping bentgrass are largely outcrossing, and therefore are expected to pollinate wild relatives at higher frequencies. All of these crops are wind pollinated, with pollination distances dependant on weather conditions such as wind speed and turbulence, duration of pollen viability, weight or size of pollen grains, and overlap of anthesis (flowering period) between the crop and wild relative.

> *The “flat” distributions of pollen at longer distances mean that pollination may occur at distances well beyond the conventional crop isolation distances recommended by USDA.*

These biological parameters can in turn be influenced by environmental conditions. For example, duration of pollen viability can be influenced by air temperature, humidity, and cloud cover.[74, 75] Unusual conditions, such as severe weather, may extend the range of pollination. Many pollination studies also have used small (e.g. less than 1 acre) pollen sources that may underestimate pollination distances for commercialized crops or larger field trials. Since most published experiments on gene flow explore only a portion of the range of these parameters, their results are usually only an approximation of possible gene flow distances.

Crops like canola, alfalfa, and sunflower are primarily insect pollinated, and effective pollination distances are therefore determined by the types of pollinators, their numbers, their foraging behavior, and their range.

Another factor that may influence initial rates of gene flow is whether the crop and the wild relative share matching (homologous, or genetically similar) chromosomes. Several crops in this report are allopolyploid, meaning that that they have multiple sets of chromosomes that originated from several species of plants. Often only one of the chromosome sets is “shared” with those of the wild relative. For example, wheat and jointed goatgrass share the “D” chromosomes, but each species also has other chromosome sets, designated by different letters, not shared by the other species. Insertion of a transgene on one of the wheat “D” chromosomes may provide relatively higher initial gene flow rates than insertion in the other wheat chromosomes not shared with jointed goatgrass. Several other crops with wild relatives in the U.S., such as canola, are also allopolyploids that share some, but not all, chromosomes with wild relatives.

One important and often overlooked factor in determining gene flow is the distribution of pollen over distance, which fits a “leptokurtic” distribution. Although pollination frequencies decrease fairly rapidly from the pollen-source, further from the source the distribution plateaus, and low pollination frequencies continue over long distances. Leptokurtic distributions are widely observed, and seem to be the rule.[76, 77, 78, 79]

These “flat” distributions of pollen at longer distances mean that pollination may occur at distances well beyond the conventional crop isolation distances recommended by USDA. Pollination levels may remain fairly constant for considerable distances beyond these isolation distances. Current isolation distances do not account for total gene flow at longer distances, which is the sum of gene flow frequencies from the limit of the isolation distance to the distance where negligible gene flow occurs (often not determined in gene flow studies).

Some aspects of crop biology may reduce gene flow. For example, some crops that are not grown for seed consumption, such as carrots, beets, and lettuce, are biennial, and these crops typically do not flower and produce pollen and seed until their second year of growth. First-year field trials for those crops are therefore less likely to produce pollen that can fertilize wild relatives. However, biennial crops may produce a small percentage of early "bolters," or plants that flower during the first year. If those plants are not eliminated prior to flowering, they may pollinate nearby wild relatives, although their lower numbers reduce this possibility (bolting, however may be affected by temperature, so higher numbers of bolters may occur unexpectedly in some years). There is evidence of gene flow from sugar beets grown in California to the wild relative *Beta macrocarpa*, despite the lack of beet seed production there, which may have resulted from bolters or abandoned (unharvested) fields.[80]

Long-lived perennial crops such as grapes, orchard trees or forest trees may not flower during a field trial, limiting the possibility of gene flow. As of 2000, confinement requirements in individual tree field trials prohibited flowering, and USDA reportedly did not allow flowering of any genetically engineered trees.[81] However, the author is aware of no current policies that prohibit flowering of forest tree field trials. If unpublished, internal, policy prohibitions against flowering exist, they can be easily revoked, unlike regulations or statues.

Flowering may not be needed during most tree field trials when the transgene is not intended to affect the flower, fruit or seed. Where the GE trait involves vegetative properties, such as the amount of lignin in the trunks of trees, flowering may not be needed. On the other hand, some GE traits are intended to affect the flowers or seed of trees. For example, GE apple trees resistant to the important disease called fire blight would need to flower at some point because the pathogen of interest, *Erwinia amylovora*, typically infects twigs and branches by gaining entrance through flower nectaries.[82] A similar situation would apply to traits intended to protect the fruit or seed from insects or diseases.

Even if the GE trait is unrelated to the reproductive structures of the plant, if the fruit or flower is an important part of the crop, field trials will eventually be needed to test their performance in the field. For example, orchard and nut trees will eventually need to be grown to maturity to test yield of the fruits or nuts, and to test for susceptibility of flowers and fruits to diseases and insects. This is because pleiotropic (unintended) effects of GE may alter any aspect of the plant in unpredictable ways. In addition, if the crop reproduces by seed, late-stage field trials will be used to increase the amount of seed that will be needed commercially. This consideration also applies to biennial crops. And even some early stage trials may be needed to produce seed for additional trials. Therefore, development of GE trees will inevitably lead to flowering in field trials.

EVALUATION OF ENVIRONMENTAL ASSESSMENTS OF CROPS WITH SEXUALLY COMPATIBLE WILD RELATIVES

Environmental assessments (EAs) provide the most readily available examples of specific confinement methods for field trials accepted by USDA. Since most field trials are not subjected to EAs, and EAs are the only avenue by which confinement methods are made public, there is no confinement information available for the vast majority of trials. In addition, because EAs are prepared only for field trials considered to present the greatest risks, the confinement methods in EAs probably exceed those of many notifications.[xix] EAs are also important for illustrating actual confinement methods because detailed guidance on confinement is not provided by USDA, and therefore actual methods of confinement are difficult to derive only from USDA guidance. Eleven of the

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[xix] For example, USDA may require supplemental confinement requirements, as with the recent EA for tall fescue and Italian ryegrass, which exceed the proposed confinement methods of the applicant.

most recent environmental assessments for genetically engineered crops with wild relatives were reviewed to evaluate their confinement methods (see Appendix A for detailed reviews of these EAs).

In addition to providing confinement methods, EAs evaluate environmental risks from field trials. EAs provide the most rigorous risk assessments available for field trials,[xx] and therefore the large majority of field trials, conducted without an EA, have been subjected to considerably less risk assessment, unless undertaken at the initiative of the field trial applicant. Confinement and risk should be considered together because negligible risk to the environment may reduce concern if the gene escapes, while higher risk indicates the need for stringent confinement to prevent gene flow. Because of the possible environmental consequences if gene flow occurs, the author reviewed environmental risk evaluations conducted in the EAs.

The categorical exclusion decision in 1995 has greatly reduced the number of EAs prepared. The few exceptions consist mainly of crops producing pharmaceutical or industrial compounds, which now have more stringent confinement requirements than other GE crops, or for genetically engineered microorganisms. The EAs evaluated for the report date from 1997 or earlier, with the exception of the recent EA for tall fescue and Italian ryegrass (fescue/ryegrass). The fescue/ryegrass EA may be atypical because of USDAs concern about gene flow from turf grasses caused by the escape of transgenic creeping bentgrass pollen. This EA is useful, however, because it may approach high levels of concern about gene flow by USDA, and thereby represent some of the more stringent confinement requirements set by APHIS.

Of the 11 EAs reviewed in Appendix A, at least 5 could have allowed pollination and gene flow when using the described confinement methods if the wild relative grew in the vicinity, based on published studies of pollination distances for these crops.

The confinement practices described in the older EAs also appear to be relevant to current practices, because they are largely consistent with the current confinement performance standards found in 7 CFR 340.3, and with the fescue/ryegrass EA. In addition, the recent field trials showing gene flow in creeping bentgrass support the contention that isolation distances used for conventional crop varieties are generally acceptable to USDA for confinement of transgenes for crops with wild relatives. Therefore, it is likely that the confinement methods in the EAs examined in this report are similar to, or exceed, currently accepted methods for most field trials conducted under notification.

Even when field trial confinement measures evaluated in an EA failed, USDA did not adequately strengthen its requirements for subsequent field trials. Data from creeping bentgrass trials in 1998 and 1999 found hybridization well beyond the 900 ft isolation distance that was subsequently accepted by USDA for a later field trial. These data were published in 2001, in part with the specific intention of informing USDA, as noted in the paper:

> "These data will be available to USDA-APHIS for use in creating a risk assessment model for commercial transgenic seed production."[83]

Although that sentence is directed specifically at commercial cultivation, the data clearly also have implications for preventing gene flow from field trials. Furthermore, that trial was very small, containing only 286 plants, and would have produced a limited amount of pollen compared to larger field trials. Despite the publication of these data in 2001, and repeated written requests by the Oregon

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[xx] Environmental Impact Statements (EIS) are more demanding that EAs, and may be prepared for field trials, but this has never occurred. Recently, the first EIS has been undertaken for a proposed creeping bentgrass deregulation.

Although a small sample, the EAs reviewed in this report confirm that USDA accepted confinement methods for several different crops that would not ensure the absence of gene flow.

Department of Agriculture based on them, USDA approved a 600 acre (400 acres planted) creeping bentgrass field trial for 2002 with a 900 ft. isolation distance.[xxi, 84, 85] This latter field trial resulted in hybridization with wild creeping bentgrass miles beyond the trial boundaries, reported in the paper by Watrud et al. Although USDA is now subjecting creeping bentgrass to its first environmental impact statement for a GE crop, this did not occur until after the field trials and the publication of the Watrud et al. paper in 2004. In addition, despite the unresolved issues that led to the EIS, APHIS continues to approve field trials of creeping bentgrass under permits, including one for increased shade tolerance, one allowing 45 acres, and one with locations in 20 different states.[xxii] The confinement conditions for these trials have not been disclosed to the public. EISs have not been scheduled for other crops with wild relatives.

More recently, USDA prepared an EA for a field trial application for tall fescue and Italian ryegrass submitted late in 2005. The EA notes that the conditions of the proposed field trial suggest that it could be "categorically excluded…under 7 CFR 372," but that "the recent scientific study in creeping bentgrass demonstrating pollen gene flow over large distances," leads APHIS to prepare this EA.[86]

The fescue/ryegrass field trial consists of three groups of 360 GE plants containing either the hygromycin resistance gene, the beta-glucuronidase marker gene, or an antisense gene that reduces expression of the pollen allergen gene, *lol p1*. An isolation distance of 400 m, or 1312 ft., was accepted by USDA, with additional requirements to limit seed escape, and a cleared border of 10 feet. This isolation distance is incrementally greater than the 900 ft. isolation distance for turf grasses of the AOSCA-based performance standards. The proposed isolation distance was based largely on a single study of transgenic tall fescue by the crop developer that found no pollination beyond 150 m, and secondarily on a study of perennial ryegrass that found pollination at the longest distance measured, 144 m.[87, 88] The latter study, however, showed a leptokurtic pollen distribution, unmentioned in the EA, that suggests low-level pollen flow may occur at distances considerably farther than measured in the study. The EA also suggested that because of its larger size, the 400 acre creeping bentgrass field trial may not accurately represent gene flow distances for the smaller fescue/ ryegrass trial. However, the EA failed to mention a creeping bentgrass field trial of similar size to the proposed fescue/ryegrass trial that documented pollination at 1400 ft., and estimated pollination at least as far as 1300 m.[89]

Although USDA believed that "…tall fescue pollen should be effectively contained" in the field trial, it acknowledged that "The bentgrass studies raise some uncertainty with regard to the confinement of field releases of flowering transgenic grasses."[90] Additional data and arguments were presented that the transgenes would not cause environmental harm or increase the fitness of wild relatives, should gene flow occur. USDA did not consider the possibility that the genes may be fitness-neutral, and therefore persist in wild relatives for a considerable period of time.

For the other EAs, confinement measures varied widely. Some, as with creeping bentgrass, are likely to allow gene flow to occur. Some permits proposed using isolation distance alone, usually based on conventional certified or foundation seed purity standards, which are known to allow low levels of gene flow. Others used a combination of isolation distance and trap crops, prevention of flowering, flower cov-

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[xxi] Forty other creeping bentgrass field trials were also approved in 2002.

[xxii] Data as of Jan. 12, 2005; http://www.isb.vt.edu/cfdocs/fieldtests3.cfm

ers to prevent pollen movement, or screen cages around plants to prevent access by pollinators. Some of these latter measures are likely to prevent gene flow from pollen, providing human error does not occur. Some permits employed several measures (usually two) and others used a single measure.

Because the risks of the transgene in the crop may be very different from the gene in a wild relative, observation of the GE crop may be of limited value for predicting risks from gene flow.

Of the 11 EAs reviewed in Appendix A, at least 5 (1 creeping bentgrass, 2 canola, and 2 wheat) could have allowed pollination and gene flow when using the described confinement methods if the wild relative grew in the vicinity, based on published studies on pollination distances for these crops. For example, confinement for a canola field trial consisted of a 15 ft trap crop of canola, surrounded by 200 m of perennial grasses (unspecified species). The trap crop was to be destroyed at the end of the season, and the 200 m zone checked for wild relatives. Several studies have demonstrated that trap crops often have limited efficacy, and that cross pollination from canola can occur from at least 400 m for small field trials and several kilometers for large fields.[91, 92, 93] Four canola wild relatives are found in the county where this field trial was conducted according to the USDA Plants database. Similarly, several wheat EAs apparently would have allowed jointed goatgrass to within 20 feet of those field trials, although pollination between wheat plants at over 40 m has been shown.[94]

In addition to the five EAs with inadequate confinement, methods for the fescue/ryegrass and an alfalfa field trial may allow gene flow if wild relatives are in the vicinity beyond the isolation distances. The EA for the fescue/ryegrass trial claims that the fields around the trial site contain Bermudagrass or are unsuitable for fescue or ryegrass, but there is no description of the size of these fields and no indication that they were actually surveyed for wild tall fescue or ryegrass. Both species (as well as the closely related perennial ryegrass) are known to grow widely in parts of Oklahoma, and there are water sources in the area of the field trial that may provide suitable habitat.[95, 96, 97, 98, 99] Confinement measures for the alfalfa field trial were ambiguously described. But if taken at face value in suggesting that a small amount of flowering would occur, could also have allowed pollination and gene flow.

The performance standards note that for crops with wild relatives, steps in addition to conventional breeding isolation distances may be needed to ensure that no gene flow occurs:

> It may be necessary to survey the area surrounding the test site for the presence of these plants [wild relatives] or select a test site where no sexually compatible species are known to exist.[100]

The recommendations made in this statement, however, were either not generally followed or interpreted to apply only to the area within standard isolation distances. For example, only one EA, for canola, noted that it was "believed" that no wild relatives were nearby. The fescue/ryegrass EA suggested that the habitat near the field trials was unsuitable for wild relatives, but as noted above, did not indicate that the area was surveyed. The most recent creeping bentgrass EA acknowledged, by contrast, that wild relatives may be in the area. This EA also noted that the area around the border of the farm was checked for weeds, but the extent of that area was not disclosed. One alfalfa EA observed no weedy relatives adjacent to the test plot, but did not disclose the extent of the adjacent area surveyed.

Four other EAs noted that areas of various sizes within or up to the standard isolation distances would be checked for wild relatives. These statements suggest that a prevalent interpretation of the recommendation to "survey the area around the test site" for the presence of wild relatives pertained to the area within conventional crop isolation distances, rather than areas extending beyond these distances.

Although a small sample, these EAs confirm that USDA accepted confinement methods for several different crops that would not ensure the absence of gene flow. Whether more stringent methods are used is apparently left largely to the permit holder or the notifier.

Many field trials with experimental genes have been conducted with inadequate confinement and where the environmental risks from gene flow are largely unknown.

Environmental risk evaluations in the EAs relied heavily on the predicted hazard based on the known properties of the gene in the source organism, as well as limited environmental exposure assumed to result from confinement provisions. For example, two pest resistance genes were used in the canola field trial discussed above, the Bt *cry1Ac* and potato *pin-2* genes. No risk assessment was conducted for these genes. Instead, APHIS concluded that they present no plant pest risk, based only on their origin in non-pest organisms. This argument amounts to the type of "lack of evidence" risk assessment that was criticized by the NAS (see "Environmental Risk Assessments" section above). In some EAs, informal reports from the crop proprietor are sometimes cited as observing no adverse impact (usually determined from earlier field trials), but with no detail concerning how these observations were made, and whether the field trials were designed to test for adverse effects.

Because the risks of the transgene in the crop may be very different from the gene in a wild relative, observation of the GE crop may be of limited value for predicting risks from gene flow. For example, a gene to control a disease in wheat or grapes would be functioning as expected if the disease was prevented. But control of this or other diseases in the wild relative may allow it to become a more troublesome weed, making it more competitive by reducing damage from the pathogen. The same limitation is true for virtually any transgene. In addition, in the absence of methods and procedures specifically designed to test environmental impact, observation of transgenic crops are unlikely to be a sensitive means for detecting harm. More subtle or sporadic effects, although they could be very important if the gene escaped to a wild relative, may go unnoticed. Even important crop pathogens, insects, or stresses (such as drought) may be absent from the site for several years while field trials are conducted.

There is no requirement to conduct field trials in a manner designed to detect environmental impacts, and no requirement for direct toxicity tests on important groups of non-target organisms. Even the best designed studies done in the laboratory or at the small scale of field trials may miss significant environmental impacts, and these limitations are magnified by lack of adequate experimental design requirements for field trials.[101, 102] USDA should therefore require testing to determine the likelihood of transgene persistence in wild relatives.

In summary, APHIS has accepted confinement methods that cannot ensure the absence of gene flow, and field trials have been conducted prior to any risk assessments, or with only informal risk assessments that would be of limited value for determining risk from the transgene in a wild relative. Therefore, many field trials with experimental genes have been conducted with inadequate confinement and where the environmental risks from gene flow are largely unknown.

SUMMARY AND CONCLUSIONS

Recent field trials of genetically engineered creeping bentgrass have led to pollination of wild relatives when USDA-accepted confinement measures were followed. Although species like creeping bentgrass have a particularly high propensity for cross pollination, USDA confinement requirements are unlikely to prevent gene flow, even for crops that are less prone to cross-pollinate. Data demonstrating pollination beyond USDA accepted isolation distances for several crops supports the possibility of gene flow to a variety of different wild relatives. Similarly, introgression of conventional genes into several wild relatives of crops with different propensities to outcross supports the possibility of gene flow from GE field trials to wild relatives.

Since 1987, almost 2700 field trials have been approved for GE crops with wild relatives in the U.S. This is about a quarter of all field trials. Proximity between the crop and wild relative facilitates gene flow, and the majority of these trials, about 65%, have been in states that are reported to contain one or more wild relatives of the crops. Large field trials also increase gene flow, and many field trials have been much larger than a typical research plot, consisting of hundreds or thousands of acres. In addition, many field trials have utilized genes that may enhance the fitness of wild relatives, also increasing the possibility of gene flow.

EAs were reviewed to determine whether confinement methods accepted by USDA can prevent gene flow. Almost half, and probably more, of those examined could allow gene flow. The confinement methods in these EAs, consistent with current USDA confinement recommendations, are based primarily on isolation distances for non-GE crop varieties that are known to tolerate some gene flow.

The vast majority of GE field trials are now performed under a notification process that involves considerably less oversight and risk assessment than EAs. A recent report by the USDA Inspector General (IG) revealed that written notification protocols are not typically reviewed by USDA biotechnology staff prior to planting the trial.[103] Therefore, it is unlikely that any substantive review of confinement methods is performed prior to planting. This is consistent with the “performance standard” approach used by USDA, whereby the GE crop developer asserts that adequate confinement and other standards will be maintained. However, because guidance for meeting performance standards is based largely on conventional crop isolation distances that do not ensure complete containment, it is likely that many field trials use methods that could allow gene flow. Although USDA/APHIS agreed with the IG to correct many of the other oversight problems identified in the IG report, the agency declined to begin reviewing methods used in field trials performed under notifications.

Unfortunately, gene flow from GE field trials has not been measured in more than a handful of cases, so data are not available on the frequency of gene flow. There are also virtually no data on the proximity of wild relatives to specific field trial sites. However, the available data suggest that it is possible that pollination or gene flow has occurred (in addition to the noted creeping bentgrass trials), based on: 1) the large number of trials that have been conducted for GE crops with sexually compatible wild relatives, 2) the general proximity of many of these field trials to wild relatives, 3) documented gene flow from conventional crops to those wild relatives, 4) USDA confinement requirements that often do not ensure the absence of gene flow, and 5) the possible fitness-enhancing effects of many of the genes in these trials, and hence the increased likelihood of spread in the wild relative.

Because of the multitude of factors that determine whether permanent gene flow occurs for any specific trial, it is impossible to predict precisely whether gene flow has already occurred, or when it will happen in the future. But over time, permanent gene flow is virtually inevitable unless confinement requirements for these crops are strengthened considerably. Because wild relatives that acquire transgenes will usually have the same appearance as their non-GE counterparts, and because USDA does not check for gene escape, gene flow is likely to be irrevocable by the time it is discovered.

The possibility that gene flow will harm our nation's forests, and the organisms that depend upon them, will increase unless more stringent confinement requirements and better risk assessments are implemented.

The prospect of gene flow occurring from field trials is especially troubling because most of these genes have not undergone meaningful risk assessment at the time the field trials occur. Examination of EAs shows that only cursory risk assessments, at best, are typically performed. Therefore genes that may be more harmful than those already approved for commercial use by U.S. regulatory agencies may be transferred permanently to wild plants.

Looking ahead, the prospects are even more daunting. The genetic engineering of trees and horticultural crops is still in the early stages of development, with transgenic papaya the only currently commercialized GE tree. The ability of many trees to breed with their wild counterparts in the U.S. is especially pronounced. This is because, unlike most food and feed crops, forest-product trees are not domesticated and are therefore more similar to, and more capable of mating with, wild counterparts. Many forest trees may also disperse pollen and seeds over long distances.[104, 105] In addition, although most crops do not form "natural" ecosystems (although they may still be important for biodiversity in some respects), many trees are critical components of natural ecosystems that many native species depend upon. The potential harm to native species, if harmful transgenes escape into native trees, is therefore in some ways magnified compared to most transgenic crops.

Although GE trees in current field trials reportedly were not allowed to flower as of 2000,[106] thereby greatly limiting the likelihood of gene flow, pressure to allow flowering will mount with the need to produce seed and to examine the properties of flowering and fruiting, and as GE trees approach commercialization or permanent release. The author found no evidence that current policy prohibits the flowering of trees in field trials. When flowering is allowed, the possibility that gene flow will harm our nation's forests, and the organisms that depend upon them, will increase unless more stringent confinement requirements and better risk assessments are implemented.

As genetic engineering increases in sophistication, more complex and novel transgenes will be developed with even less predictable consequences, or that may have greater propensity to increase the fitness of wild crop relatives and to cause harm. Multiple genes may be "stacked" in a single plant, as we are now seeing with several Bt and herbicide resistant crops. Where each gene may provide only a small increase in fitness, stacking of several of these genes may make a transgenic wild relative substantially more fit in more environments. As better-regulated promoters are found to express these genes, fitness costs in the absence of selective pressure may be reduced compared with current transgenes that are expressed constitutively (constantly) at high levels.

Stacking of transgenes is likely to increase substantially because such gene combinations may simultaneously address several problems that are important to farmers, and provide higher revenue for seed companies. Crops are typically attacked by several pests, so there is substantial motivation to stack several genes that confer resistance, as well as other agronomic traits. Control of multiple pests expands the potential markets for the GE crop, and may be more competitive with traditional methods such as chemical pest control that have a broader spectrum of activity than some genes. For example, the *cry1A* Bt genes control only a few Lepidopteran (moth) pests, but not other Lepidoptera such as army worms, nor beetle pests like corn rootworm. Other Bt genes do control these other pests, and may be combined with a *cry1* gene in one plant. Stacked *cry* genes have been approved in cotton, as well as stacked Bt and herbicide resistance in cotton and corn. Corn containing Bt genes to control European corn borer (Lepidoptera) and rootworm (Coleoptera) are awaiting approval. Similar combinations will be desired in crops with wild relatives in the US. Several genes for insect resistance together may have a higher likelihood for harming non-target insects and other animals compared to a single gene, as well as providing higher fitness. Combinations of insect resistance, disease resistance, and abiotoic stress tolerance may also be produced.

Several genes for insect resistance placed together may have a higher likelihood for harming non-target insects and other animals compared to a single gene, as well as providing higher fitness.

Modified or synthetic genes that are not found in nature may also be developed, with unpredictable risks. For example, an experimental (non-commercial) hybrid gene produced from the binding domain (non-toxic part) of the castor bean toxin, ricin, and the toxin domain of a Bt gene, has been engineered into corn and rice.[107] This fusion broadens the range of insects controlled by the hybrid gene compared to the normal Bt gene, probably because the ricin portion of the protein binds to the digestive track of more insect species. This broadened target insect range may be attractive to farmers and genetic engineering seed companies, but may also increase the fitness of a wild relative, such as weedy red rice, and may harm beneficial insects or animals that feed on these plants. The ricin binding domain also binds to the human and mammalian gut,[108] and it is unknown whether such binding would allow the Bt toxin portion of the fusion protein to harm humans or other higher animals.

In another experimental example, a human cytochrome P450 gene was used to make rice resistant to several herbicides.[109] It was found that the gene conferred resistance to herbicides in nine different activity classes (typically called "modes of action"). The authors suggest that this broad-spectrum resistance could be very useful, but the gene could also be transferred to weedy red rice, making all of these herbicides useless to control this important weed. Although rare examples of simultaneous resistance to a few herbicides have occurred, resistance to nine different classes of herbicide at once is probably unprecedented. Under current policy, both the ricin/Bt and P450 genes could be tested in field trials, where they may escape into wild relatives prior to any risk assessment for either human or environmental safety.

The desire to develop GE crops for new markets, such as altered food or feed quality, or that contain genes for industrial products, may present additional challenges. These crops often contain genes that intentionally modify biochemical pathways in the crop, unlike currently commercialized GE crops. Such genetic modification may produce more unintended changes in the plant than previous transgenes. For example, several experimental transgenic potatoes containing different sugar metabolism transgenes had numerous alterations in metabolite expression, with some compounds not previously observed.[110] Unlike the changes that can result from the tissue culture process used to make GE crops (called somaclonal variation), unintended effects due to the transgene or transgenic protein cannot be eliminated by further conventional breeding. The possibility of greater unintended changes than in current crops makes their impact on gene flow more difficult to predict.

Under current policy, both the ricin/Bt and P450 genes could be tested in field trials, where they may escape into wild relatives prior to any risk assessment for either human or environmental safety.

For all of these reasons, it is imperative that USDA substantially increase the stringency of its confinement requirements for genetically engineered crops with sexually compatible wild relatives, and develop and require thorough risk assessments for these crops, conducted before gene flow can occur. It is past time for USDA to stop playing genetic roulette with the wild.

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APPENDIX A: REVIEWS OF APHIS ENVIRONMENTAL ASSESSMENTS

INTRODUCTION

As discussed in the main text of this report, APHIS regulation of field trials has often been inadequate to prevent gene flow from experimental GE crops, potentially allowing gene flow of untested traits into wild plants. This appendix serves to evaluate the effectiveness of eleven EAs, which provide specific examples of confinement measures accepted by APHIS and the agency's reasoning on risk assessment.

CREEPING BENTGRASS

EAs for two previous bentgrass field trials warrant examination (the recent 400 acre field trial that was responsible for the noted gene flow that was studied by Watrud et al., reference 1 in the main body of the report, was not the subject of an EA).

An environmental assessment was conducted for a small field trial, approved in 1997 for Oregon for creeping bentgrass containing both a gene for resistance to glufosinate herbicide and the *pin-2* gene from potato, which codes for a proteinase inhibitor that helps protect potatoes from pests (permit number 97-087-02r).

The *pin-2* gene has also been expressed from its own promoter in another grass, rice, where it conferred resistance to a major insect pest.[i] Other work indicates that *pin-2* genes may also confer plant pathogen resistance, so the possible selective advantage of this gene may be higher in some cases than for pest-resistance genes that protect against fewer pests.[ii] Because *pin-2* is a pest-control gene, it falls into a category that may enhance the fitness of wild bentgrass species (see discussion in the "Transgenes and the Fitness of Wild Relatives" section, and Table 5), thereby facilitating gene flow. Pin-2 protein may also harm beneficial or other nontarget insects.

The primary focus of this EA was on preventing contamination of nearby commercial turfgrass fields, because the trial was conducted at a turfgrass farm. The EA acknowledged that wild sexually compatible relatives may have been in the area, but accepted the typical conventional foundation seed purity standards, and a 6 ft wide border of cereal rye as a pollen barrier, as adequate separation. Possible contamination of wild relatives was surveyed in the "downwind" direction, where a wetland began about 600 ft from the test plot. The survey of wild relatives was to be conducted between 500 ft and 900 ft downwind from the field trial. There was no discussion about the variability of wind direction, based on local meteorology, for the period when the GE grass would be flowering. The EA also mentioned that the border of the farm was checked for weeds, but no details were provided about the distance of the border from the field trial site, or how extensively the area at the farm perimeter was surveyed. APHIS acknowledged that it had no data addressing the effective pollination distances for creeping bentgrass. Furthermore, seed from the plot could be used for three years to grow a continuously mowed putting green. No additional measures were mandated to prevent gene flow from the putting green.

The reliance on standard isolation methods used in conventional crop breeding is found on page 6 of the EA, which states that: "*Standard breeding practices and monitoring will be used* to prevent mixing and unwanted introduction of transgenic material into neighboring creeping bentgrass plants *or other sexually compatible plants* via pollen or seeds." [emphasis added]

It is clear that at least for the "downwind" direction, only areas within the conventional seed purity distance were required to be surveyed. Pollination of creeping bentgrass was detected up to about 1400 ft (using sentinel creeping bentgrass plants and the turf crop) and predicted at about 0.02% at 1300 m.

No data were presented on the possible risks of gene flow for *pin-2*. The EA only refers to a personal communication to USDA that the protease inhibitor "had not been shown to affect the phenotype" of creeping bentgrass. The data or tests on which this statement was based have not been disclosed. Phrases such as "affects the phenotype" often refer to determinations of "substantial equivalence" that primarily examine agronomic and morphological traits, rather than possible harm to the environment. Such tests may include data on susceptibility of the GE crop to diseases and insects, but seldom examine harm to non-target organisms or the effects on the environment of the gene in wild relatives. Also, personal communications are not typically used to provide substantial data, and the fact that no details were supplied suggests that supporting data for this communication may have been minimal or non-existent. Even such basic data as expression levels of the Pin-2 protein were not provided, making it impossible to know if levels were high enough to cause harm. Typically, however, the purpose of using such a transgene is to control pests, and therefore there is a presumption that it was expressed, possibly at levels high enough to harm non-target organisms.

Another EA was prepared for a creeping bentgrass field trial for New Jersey in 1994 (permit number 94-076-01). The trial was for creeping bentgrass containing glufosinate herbicide resistance and the marker gene for beta-glucuronidase (GUS) production. Confinement of the transgenes was to be accomplished by moving all test plants into a certified, contained greenhouse prior to flowering. Containment could have been achieved, providing that this greenhouse included means of preventing pollen from escaping and there was no human error (e.g. missing early-flowering plants).

Risk assessment was based on the known properties of the genes, and that exposure of the environment would have been limited by the confinement practices.

TALL FESCUE AND ITALIAN RYEGRASS

USDA/APHIS has recently prepared an EA for a field trial for both GE tall fescue (*Festuca (Lolium) arundinaceae*) and Italian or annual ryegrass (*Lolium multiflorum*) for Love County, Oklahoma (permit numbers 05-278-01r and 05-278-02r). Both grasses are wind pollinated and highly outcrossing, and both are considered to be serious agricultural weeds, invasive in some habitats.

This EA is probably atypical of requirements for confinement because USDA considers the gene flow risks presented by turf grasses to exceed those found in other types of GE crops.[1] Because USDA considers turf grasses to present greater confinement challenges than most other crops, it is likely that it represents some of the highest levels of stringency for confinement that USDA is likely to require.

The proposed fescue/ryegrass field trial consists of three groups of 360 GE plants containing either the hygromycin resistance gene, the beta-glucuronidase marker gene, or an antisense gene that reduces expression of the pollen allergen gene, *lol p1*. An isolation distance of 400 m (1312 ft) was accepted by USDA, with additional requirements to limit gene flow by seed escape, and with a cleared border of 10 feet to allow survey for vegetative escape.

The isolation distance accepted for this field trial is incrementally greater than the 900 ft isolation distance for the turf grasses of the AOSCA-based performance standards. The proposed isolation distance was based largely on a single study of transgenic tall fescue by the crop developer that found no pollination beyond 150 m, and secondarily on a study of perennial ryegrass that found pollination at the longest distance measured, 144 m.[2, iii, iv]

Although these studies provide useful preliminary data, they are insufficient to determine adequate isolation from wild relatives. The tall fescue study contained only one year of data where the number of pollen donor plants was comparable to the number of plants in the proposed field trial (225 in the published study, and 360 in the proposed trial). An earlier plot contained only 49 donor plants, and had considerably less pollen flow at shorter distances than the 225 plant plot the following year.

A significant limitation of the experiment was its relative insensitivity for detecting low, but biologically meaningful, levels of pollination. This limited the ability to draw general conclusions about gene flow at the longer distances from this experiment. For example, a total of 1405 seedlings from recipient plants from the longest distance, 200 m, were analyzed for hybridization. At the gene flow rates minimally acceptable for conventional foundation seed, about 0.02% - 0.1%, only about 0.3 to 1.5 out of 1405 hybrid seedlings would be expected at 200 m. Furthermore, only four of the eight directions around the donor plants contained the majority of pollen flow. In the other four directions, no pollen flow was detected at 150 m. If only seedlings from the 200 m distance from the four principle directions where hybridization was detected are considered, only 0.125 or 0.626 seedlings would be expected, on average, based on 0.02% or 0.1% hybridization frequencies, respectively (based on 626 seedlings from those four directions). Even higher levels of gene flow at 200 m could easily have been missed in this experiment.

USDA also noted that gene flow was not detected at up to 2294 m in this study. However, this experiment was even less sensitive than the experiment previously described. The recipient plants were placed only in the upwind direction from the field trial, where less gene flow is expected than downwind, based on the data from this and other studies. The closest recipient plants in this experiment were located about 890 m from the donor plants, leaving an untested gap of about 690 m between the 200 m limit of the primary experiment and this longer-distance experiment.

In the other study cited in the EA, for gene flow in perennial ryegrass, a leptokurtic pollen distribution was observed. Although unacknowledged by the EA, the leptokurtic distribultion suggests that low-level pollen flow may occur at distances considerably farther than the 144 m measured in the study.

The EA also suggested that, because of its larger size, the 400 acre creeping bentgrass field trial in Oregon

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[1] The EA notes that the conditions of the proposed field trial suggest that it could be "categorically excluded…under 7 CFR 372," but that "the recent scientific study in creeping bentgrass demonstrating pollen gene flow over large distances," leads APHIS to prepare this EA.

[2] About 1% pollination was found in the downwind direction at 150 m. USDA mistakenly indicated pollination at 200 m, which was instead the maximum distance tested in one of the experiments.

may not accurately represent gene flow distances for the smaller fescue/ryegrass trial. However, the EA failed to mention a creeping bentgrass field trial, of a size similar to the proposed fescue/ryegrass trial, which produced pollination at 1400 ft and estimated pollination to at least 1300 m.[v] Although this latter creeping bentgrass study was performed with a different turfgrass species than the current trial, it should not be dismissed until adequate studies with tall fescue are available, or other data show that pollination distances are likely to differ substantially between the two species. Research has been conducted on tall fescue and creeping bentgrass pollen viability, and shows similar survival curves, suggesting that pollen survival of both species may be similar.[vi, vii] Therefore, the impact of this parameter on gene flow may be similar between the two grass species.

The EA also claims that the fields around the trial site contain Bermudagrass or are otherwise unsuitable for fescue or ryegrass, because the area is too dry to support these cool-season grasses. There is no description of the size of these fields, however, and no indication that they were actually surveyed for wild tall fescue or ryegrass. Both species (as well as the closely related and sexually compatible perennial ryegrass) are known to be widespread in parts of Oklahoma, and there are water sources in the immediate area that would provide for a suitably irrigated habitat.[viii, ix, x, xi, xii]

Although USDA believed the data suggested that "…tall fescue pollen should be effectively contained," it also acknowledged that "the bentgrass studies raise some uncertainty with regard to the confinement of field releases of flowering transgenic grasses."[xiii] Therefore, additional data and arguments were presented that the transgenes would not cause environmental harm or increase the fitness of wild relatives, should gene flow occur. Reference was made to the safety of the beta-glucuronidase and hygromycin genes in previous USDA risk assessments, as well as other arguments beyond the scope of this report. The assessment of the *lol p1* antisense gene is based on lack of expected harm from the loss of a major allergen. However, the function of Lol p1 in the plant, which may provide preliminary risk information, was not discussed.

There is no explicit assessment of unintended or pleiotropic effects which could potentially be harmful. Sections on weediness and susceptibility to pests implicitly address this issue. However, although USDA cites a paper by the crop developer based on previous field trials as having addressed these issues, only two sentences, and no data, were devoted to the analysis of harmful and beneficial insects and diseases in the cited work. That paper is predominantly devoted to assessing agronomic properties of the transgenic grasses, and there is no indication that an adequate assessment of insects and diseases was performed.[xiv] Although several vegetative and seed production properties were assessed, several other properties that are often relevant to fitness, such as seed dormancy and survival, were not evaluated. Overall, the risk assessment in the cited paper was minimal, and seems to support a concern of this report that limited attention has been devoted in field trials to examining possible environmental impacts.

Finally, although the genes in this field trial do not present any obvious environmental impacts or increased fitness, USDA did not consider the possibility that the genes may be fitness-neutral, and therefore persist for considerable periods in wild relatives.

CANOLA

Canola (*Brassica napus*) is a moderately outcrossing crop grown primarily for seed oil content, but the seed meal may also be used as livestock feed. It has several introduced and widely distributed sexually compatible weedy relatives, and *Brassica rapa* and *B. juncea* in particular can readily form fertile hybrids with canola.[xv] It may also form fertile hybrids at very low rates with the serious weeds jointed charlock (*Raphanus raphanistrum*) and charlock mustard, (*Sinapis arvensis*).[xvi, xvii] Canola is primarily insect pollinated, and has typical characteristics of insect pollinated flowers.[xviii]

A field trial application from 1994 (permit number 94-326-01) contained two insect resistance genes, *cry1Ac* and potato *pin-2* (see Creeping Bentgrass section above for discussion of *pin-2*). Although both genes may increase the fitness of canola wild relatives, APHIS concluded that they present no plant pest risk. This is based only on their origin in non-pest organisms, and without any risk assessment data. Both genes were controlled by the CaMV 35S promoter, and therefore high levels of expression in most plant tissues would be expected. The trial also contained a kanamycin resistance gene. The field trial proposal consisted of 2000 plants in an area of 39 x 49 m in DuPage County, Illinois. The USDA Plants database lists all four of the canola wild relatives mentioned at the beginning of this section as growing in DuPage County.

Containment for this field trial consisted of a 15 ft trap crop of canola, surrounded by 200 m of perennial grasses (unspecified species). The trap crop was to be destroyed at the end of the season, and the 200 m zone checked for wild relatives. APHIS acknowledges that foundation seed purity requirements are for 660 m.

Current APHIS guidance provides for an isolation distance of 1320 ft, or a 30 ft trap crop (so this trial apparently used a combination of these methods). However, recent studies demonstrate that GE canola can pollinate non-GE canola at distances of at least 3 km at low frequencies.[xix] Furthermore, some pollinated canola plants in that study were embedded in recipient canola fields, in effect surrounded by a "trap crop," but still experienced pollination. Direct study of the efficiency of a trap crop for bee-pollinated cucumbers demonstrated that a comparable trap crop reduced, but did not eliminate, pollination of recipient cucumbers 50 m away.[xx] That study also noted the importance of the ratio of the test plot to the trap crop, and contained only 9-25 cucumber pollen-source plants. Some pollen flow was found when the ratio was 25:1, trap crop area to pollen donor (test crop) area. APHIS guidance does not consider the relative size of the test crop and the trap crop.

Furthermore, the perennial grass buffer zone would be unlikely to act as an additional pollen trap crop because canola is primarily insect pollinated, while most perennial grasses are primarily wind pollinated. Therefore pollinating insects leaving the test canola plants would largely pass over the grass. In addition, it would be necessary for the grasses to flower while the canola was flowering to act as a pollinator trap even if insect pollinated species of grass were used. No discussion of these issues was included in the EA.

In summary, the containment methods approved for this field trial cannot ensure the absence of gene flow. Furthermore, it is possible that either the *cry1Ac* or *pin-2* genes could provide a fitness advantage to a pollinated wild relative, increasing the possibility of gene flow as well as environmental harm.

Another field trial was approved for Georgia (permit number 94-200-01) for the Bt gene *cry1Ac* for Lepidopteran (moth larvae) insect resistance. The proposed trial area was 10,000 square meters, containing 60 one or two square meter plots. The field trial was approved for three years, where the second and third years would involve self-seeding of canola from the previous years. Experimental evidence suggests that canola may survive as self-seeding feral plants for four or more years.[xxi, xxii] During the first two years, the plots were to be tented and pest insects added. Tenting was apparently for the purpose of retaining the added pest insects, but was also recognized as a means to prevent gene flow. However, neither pest insects nor tenting were mentioned for the third year. The purpose of the experiment was to test survival of the GE compared to non-GE canola.

A 15 ft perimeter of conventional canola trap crop was to be planted around the test plants. Other confinement information was contradictory, because in two places the EA mentions surveying and destroying wild relatives within 500 ft of the plots, while in another the applicant was said to survey and destroy wild relatives within 500 m. Canola volunteer plants found within the 500 ft zone (and beyond the field trial plots) were required to be destroyed. The applicant was said to "believe" that there were no wild relatives of canola in the "vicinity," but there was no requirement to check beyond 500 m, and no indication of the basis for the belief that wild relatives did not grow close by. If Bt canola survived and flowered in the proposed third year of the experiment, it is possible that gene flow could have occurred.

The risk assessment for this field trial was based on confinement and the known properties of the gene. Part of this assessment includes the statement that: "No factor unique to this field test has been identified that would have an effect on any nontarget vertebrate or invertebrate species." (EA, p. 5) Clearly, the Bt gene is intended to harm certain invertebrates (some Lepidopteran insects). So, in addition to the intended target insects, some nontarget insects may be harmed. The meaning of this assertion that the field trial would not harm invertebrate species is therefore unclear.

A subsequent publication appears to have resulted from this field trial, based on a comparison of the descriptions, revealing that tenting was used over all test plots, and the experiment was apparently carried out for only two years.[xxiii] Therefore, in practice, gene flow was unlikely, although at the beginning of the experiment, when the permit was granted, this was not clear, and the lack of survival of transgenic canola or discontinuation of the research after two years was not discussed when the permit was issued.

A field trial application for 1996 (permit number 96-215-01r) was submitted for canola containing a pat gene for glufosinate herbicide resistance, and CBI pharmaceutical and industrial genes, for Imperial County California. Proposed confinement consisted of caging flowering transgenic canola to prevent access by pollinating insects, and monitoring the surrounding 200 m for any sexually compatible *Brassica* species, which are characterized as important and serious weeds. *A. sinapsis* and *R. raphanistrum* are not considered to be sexually compatible in this EA. Because canola is primarily insect pollinated, these measures may prevent pollination of wild Brassicas, although the contribution of wind pollination in canola has not been completely resolved. However, if *A. sinapsis* or *R. raphanistrum* are found close to the caged canola, wind pollination may occur, although hybrid fertility with these species is extremely low.

Risk assessment was based on confinement and that the gene product is not expected to cause harm, based on the published properties of the proteins. Because two of the genes are claimed as CBI, they cannot be evaluated here for potential environmental impact. However, several pharmaceutical and industrial genes are known to control plant diseases or insects. For example, the industrial protein avidin has insecticidal properties, and the pharmaceutical proteins lactoferrin and lysozyme each have antimicrobial properties and have been used in other field trials for that purpose.

Another EA (permit number 94-168-01) was performed for GE canola containing 10 added genes from multiple plant species to modify oil content of the seeds and confer kanamycin resistance (it is not disclosed how many genes were contained in a single transformant). The EA indicates that the field trial is for a maximum of 2 acres, although the field trial database records 50 acres.

The described confinement methods are not entirely clear. For example, the section on "containment" (page 5) stated that an isolation distance of 1320 feet "will be maintained between the field test and any *cultivated* <u>Brassica napus</u>, <u>B. juncea</u>, and <u>B. rapa</u>" [Emphasis added], while the conclusion section noted that "an isolation distance of 1320 feet will be maintained between the field test and any compatible weed species…" In addition, a 15 ft non-transgenic trap crop was to surround the test plot, and a zone within 200 m would be surveyed for wild relatives. Although compatible with conventional seed purity standards, these isolation methods would not ensure the absence of gene flow.

The EA made conclusionary statements, not supported by data, that the genes involved in this field trial were not expected to cause harm. Although changes in oil content are not obviously associated with competitiveness, there are several ways that these changes could conceivably alter fitness. For example, the oil content of seed has the potential to affect seed survival, dormancy, or seedling vigor, which may increase or decrease the fitness of a wild relative. Several studies suggest that certain types of altered canola seed oil content enhances fitness by improving seed survival and germination, although this has not been examined in wild relatives.[xxiv, xxv] Interaction between the new enzymes and existing biochemical pathways may also cause unintended effects that could affect fitness.

ALFALFA

Alfalfa is an important perennial forage crop that probably has no wild relatives in the U.S. capable of forming hybrids. However, both alfalfa (*Medicago sativa* ssp *sativa)* and the subspecies, sickle medic (*M. sativa* ssp *falcata*) can become feral, and are widespread naturalized weeds in the U.S. that can cross with cultivated alfalfa.[xxvi] Several sources have listed these feral plants as weeds in some environments, displacing native vegetation in some cases.[xxvii, xxviii, xxix] Alfalfa is primarily pollinated by honey bees and several native bee species.

An EA was conducted in 1994 for a small two-year alfalfa field trial in several states, with the plot at each location measuring about 50 ft by 70 ft (APHIS number 94-166-01). All of the states where the field trials were to be conducted are reported to have feral alfalfa, and many states in the northern half of the US contain sickle medic. The GE alfalfa plants contained two genes: the Bt *cry6A* gene to control the alfalfa weevil (a beetle), and the *pat* gene for glufosinate herbicide resistance.

Containment of pollen was to be accomplished by mowing the plots prior to flowering (commercial alfalfa is typically mowed several times per year for forage production purposes, but often not until some flowering occurs). After the field trials, the plots were to be destroyed and monitored for volunteers for one year. Part of the plot in two locations was to be covered by a mesh screen to allow introduction of weevils.

Risk assessment was based on the known activity of the transgenic protein and confinement to the field trial location. APHIS asserts without supporting data that no harm is expected from the field trial.

Barring human error, gene flow to feral relatives would have been unlikely providing that no flowering was allowed.

Another EA was produced in 1994 for a proposed four year field trial in five states for alfalfa containing a coat protein gene for resistance to alfalfa mosaic virus (AMV) and a kanamycin resistance gene (APHIS number 94-027-01). Gene flow was to be controlled by regular mowing, and no cultivated alfalfa was said to exist within 900 ft (the accepted isolation distance for foundation seed). However, for this field trial, mowing was said to "reduce" rather than eliminate flowering. Elsewhere (page 6) the EA noted that the GE alfalfa would be mowed and would thereby "virtually eliminate flowering," and in the conclusion it is noted that the plants will be "mowed regularly to *reduce* flower development…" [Emphasis added] Common practice for alfalfa production is to mow when up to 5%-10% of the plants are flowering. The EA also remarked that "no weedy species related to alfalfa have been observed in the areas adjacent to the field test plot." But there was no discussion about the distance from the plot that was checked, or how thoroughly the area was surveyed.

Based on the statements in the EA, it can be concluded that a limited amount of flowering was accepted in this four year field trial. Depending on the species, bees are capable of transporting pollen up to several miles. Therefore, this field trial would likely not ensure the absence of gene flow.

The EA also determined that there was no risk to the environment, apparently based on the limited exposure from the restricted size of the field trial. However, alfalfa mosaic virus is widespread, infecting many plant species.[xxx] It was therefore possible that acquisition of AMV resistance by feral alfalfa may have increased the fitness of the feral plants, bolstering their weediness, and facilitating gene escape and spread.

WHEAT

Wheat is wind pollinated and primarily self-fertilizing, but may outcross at low frequencies. Wheat has a non-native sexually compatible wild relative called jointed goatgrass (*Aegilops cylindrica*), which is a serious noxious weed of winter wheat in many areas, and is reported in most states. Some cohorts of jointed goatgrass have also recently been found infesting spring wheat.[xxxi] Fertile hybrids between wheat and jointed goatgrass have been produced artificially and in the field.[xxxii, xxxiii, xxxiv]

An EA was prepared (APHIS number 94-024-01) for a 1994 field trial on about one acre (not more than 500,000 plants) of GE wheat (*Triticum aestivum*) in Montana. The wheat transgene was for herbicide resistance (the specific gene was not revealed because it was claimed as confidential business information), and also contained transgenes for kanamycin, anthocyanin, and the marker GUS.

Confinement was to be provided by a 20 ft isolation distance from non-GE wheat to prevent mechanical mixing. APHIS maintained that gene flow was unlikely because wheat is normally self-pollinating, and pollen remains viable for 15-30 min. However, low levels of outcrossing of up to several percent at short distances are well known in wheat, and can vary by variety and environmental conditions.[xxxv] Lower levels of outcrossing have been measured at over 40 m.[xxxvi] USDA describes an acceptable isolation distance for foundation seed as 33 ft for non-hybrid production, and 660 ft for hybrid production (Table 5, 7 CFR part 201.76), both of which exceed the 20 ft distance accepted for this field trial.[3]

Although APHIS acknowledged that wheat may hybridize with jointed goatgrass, it stated that if hybridization occurred, it would be controlled by "…acceptable practices for wheat using labeled herbicides and or hand weeding," and "site monitoring and management practices…should provide the necessary degree of both biological and physical containment." However, it is not clear how transgenic hybrid jointed goatgrass would be identified, since testing for transgenes in wild relatives is not usually conducted. There is no explicit indication of the distance from the field test that might be checked for transgenic hybrid wheat-jointed goatgrass.

The EA asserted that environmental harm would not occur due to the limited area, duration, and containment of the field trial. No data addressing possible harm were presented or mentioned.

A second EA was performed in 1994 to assess a permit application for GE wheat containing a *pat* gene for resistance to glufosinate herbicide and the GUS marker gene. The field trial was proposed for Illinois and North Dakota for 0.4 acres (permit number 94-054-05).

The confinement requirements were the same as for the above field trial, although this field trial was from a different applicant. Therefore, this field trail may also have allowed some gene flow to occur.

APHIS expected the risk from the field trial to be insignificant based on confinement and the known properties of the gene.

In summary, evidence for wheat field trials indicates that confinement measures may often have been inadequate to prevent gene flow if jointed goatgrass was present.

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[3] The much longer isolation distance for hybrid wheat is due to the lack of pollen production by emasculated wheat parents used for hybrid production. Because these emasculated wheat plants cannot self-pollinate, they are more susceptible to outcrossing.

CONCLUSION

Several of the EAs reviewed in this report have repeatedly proven to be inadequate for ensuring the absence of gene flow to wild relatives. These EAs often allowed confinement measures that could have permitted low level pollination that may eventually lead to gene escape. This is especially true for genes that increase the fitness of wild relatives, where even low levels of gene flow may be amplified by selection. Several of the genes in these EAs were for pest resistance, and therefore may have contributed to increased fitness.

Risk assessments were based on expected properties of the gene, defined by APHIS, without the benefit of testing for harm, or the potential for increasing fitness in wild relatives.

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