PROCEEDINGS OF A WORKSHOP ON:

ECOLOGICAL EFFECTS OF PEST RESISTANCE GENES IN MANAGED ECOSYSTEMS

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FOREWORD

The idea for a highly focused, multi-disciplinary risk assessment workshop emerged from conversations with scientists, regulatory officials and members of public interest groups. Discussions about the environmental release of transgenic crops, either for field tests or for commercial use, seemed always to touch on what constitutes the basis for decisions, and how those decisions could be strengthened.

In 1989, the National Research Council published *Field Testing Genetically Modified Organisms; Framework for Decisions*, the so-called Green Book. Ten years later, it's worth taking a look at one of the issues surrounding the use of genetically engineered crops—the impact of introducing pest resistance into crops, and the potential for related species to benefit by acquiring the trait. Combining our collective experience with conventional crops and what we know about engineered varieties brought into focus what we know now, and helped identify the gaps in our knowledge. From this came recommendations for experimental approaches that would generate the needed data.

Most participants found the multi-disciplinary science-based approach used in this workshop to be surprisingly effective in bridging gaps between participants from different disciplines, and in stimulating new ideas for research. This format could well serve as a model for similar evaluations of other risk issues associated with the commercial use of transgenic crops in the US and other countries. It is our hope that the reports in this volume will serve to support decision making at all levels and will stimulate greater interest in and funding for risk assessment research.

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INTRODUCTION

Genetically engineered crops have become a visible part of the US agricultural landscape. The first transgenic varieties in or near commercial production have been modified for a range of characteristics conferring improved agronomic performance, herbicide tolerance, pest and disease resistance, handling and storage properties, as well as other traits. However the use of biotechnology to address constraints in agricultural production brings with it questions regarding the potential of genetically modified organisms (GMOs) to cause unacceptable impacts on the environment.

Among the ecological issues associated with transgenic crops is the possibility that some newly introduced traits, such as pest or pathogen resistance, could confer added fitness to the crop. As a result, the crop may gain weedy characteristics if its ability to survive and spread outside of cultivation is enhanced. A second issue arises if such crops are grown in the vicinity of compatible wild or weedy related species; transfer of the trait by natural hybridization may produce hybrid progeny that are more aggressive or more difficult to control. These issues are no longer hypothetical, as at least seven groups of crops being engineered for pest resistance are known to have sexually compatible wild or weedy relatives in the US.

Pest resistance has been a primary objective of farmers and breeders throughout the history of agriculture. Genes identified in wild germplasm or recovered as spontaneous or induced mutations have been incorporated into cultivated varieties of many major crop species. This process is now being supplemented by the techniques of genetic engineering, and dozens of crop species are being engineered for improved pest resistance. Pre-release risk assessment of these crops addresses the question: Does releasing such crops pose any special risk of creating or exacerbating a weed problem?

Assessing the potential for transgenic pest resistant crops to become problem weeds, or to enhance the weediness of nearby sexually compatible relatives, is a complex task. Information is required from many disciplines – weed science, agronomy, population biology and genetics, entomology, plant breeding, ecology, plant pathology, molecular biology, and more. Scientific evidence in support of informed risk assessment and decision making thus lies in the collective knowledge of experts from these fields.

The workshop on *Ecological Effects of Pest Resistance Genes in Managed Ecosystems* was organized to promote multidisciplinary discussions that would lead to a synthesis of what we already know, and what we don't know, regarding the environmental impact of pest resistant crops. In so doing, the workshop provided an opportunity to reexamine a key issue related to the responsible development and use of agricultural biotechnology products.

APPROACH

The workshop focused on seven groups of crop species that have weedy relatives in North America: berries, certain grains and grasses, poplar, sunflower, squash, and *Brassica* species. A 13-member steering committee drawn from academic, private sector, and government institutions defined the objectives, identified plenary speakers, and nominated participants known to have expertise and interest in the subject.

Formal workshop objectives were to:

1. review existing evidence that the introduction of pest resistance into a crop species has affected the establishment,

persistence, and spread of the crop or sexually compatible species; and

2. identify, and recommend research strategies to address, gaps in information concerning the effects of pest resistance genes on the establishment, persistence, and spread of a cultivated crop or sexually compatible weedy species.

The focus throughout was to support and promote sound decision making by those who set research priorities, plan breeding programs, make regulatory decisions, or address public concerns regarding the use of genetically engineered crops.

In their invited talks, plenary speakers gave overviews and insights into crop breeding, weeds, pest resistance, ecology, and regulatory concepts. This material provided the background and context for the discussions that followed. Participants were invited on the basis of their expertise and with an eye towards achieving a balance of disciplines and institutional affiliations. They were organized into small multidisciplinary working groups, each centered around one of the seven target crop groups.

Group leaders, in consultation with experts, collected background information on their crop group and sent it in advance to participants. Topics included:

- major pests and diseases of the crop;
- pest resistance traits introduced by breeding or by genetic engineering;
- weed complexes associated with the crop;
- type of crop management system and degree of crop domestication; and
- weed management approaches for the crop and its sexually compatible relatives.

At the workshop, the groups were asked to assess ***what is known*** using the following guidance questions as a framework for their discussions:

- What is the evidence that introduction of a pest resistance trait could increase the ability of the crop to become established, persist, or spread?
- What is the evidence that pests have a significant effect on populations of plant species that are sexually compatible with the crop? Are any such pests common to both the crop and the sexually compatible species?
- If the crop is made resistant to such pests, what are the potential consequences of the pest resistance trait moving from the crop to the sexually compatible relatives? How likely is introgression of the resistance trait?

The groups were then asked to identify ***what is needed*** for sound decision making by considering:

- What specific information is not currently available but would be important in providing a stronger scientific basis for evaluating the effect of pest resistance genes on the establishment, persistence, and spread of the crop or its sexually compatible relatives?
- What are the available sources and/or experimental approaches that would provide such needed information?
- What characteristics of the crop affect our ability to extrapolate from small-scale field tests to large-scale use in terms of evaluating its establishment, persistence, and spread?

The following summaries highlight the main conclusions and recommendations emerging from the group discussions. The full reports, which integrate the working groups' collective knowledge and insight, should be a valuable resource for persons involved in making decisions on the appropriate development and use of pest resistant varieties of these crops.

BRIEF SUMMARIES OF GROUP REPORTS

Berries

Strawberry (*Fragaria* spp.), blackberry and raspberry (*Rubus* spp.), and blueberry (*Vaccinium* spp.) are small berry crops with potential to hybridize with feral populations of weedy relatives. Strawberries are known to escape from cultivation and to cross with wild

relatives, but generally lack aggressive weedy characteristics. Although cultivated strawberries are subject to attack by a wide variety of diseases, little evidence of disease has been noted on leaves or fruit of wild populations, supporting the assumption that environment is a greater limiting factor than pest pressure on strawberry establishment. Nevertheless, important information needed to verify this hypothesis is missing. Data is needed on the ecology of wild strawberry populations, the level of hybridization between crop and wild relatives, and the impact of pests on wild populations.

Most of the pest resistance traits incorporated into blackberry and raspberry have been derived from weedy relatives. As a result, there is currently little concern about escape of pest resistance genes to wild relatives. Furthermore, there is no evidence that resistance traits bred into raspberries over the past 60 years have increased the weediness of the crop. In contrast, the working group felt that engineered herbicide tolerance in *Rubus* would be unwise because it was likely to confer a selective advantage on weedy relatives in agricultural settings and eliminate important weed control options. Important information for the risk assessment of pest resistance genes should include surveys of pest incidence on weedy species.

Introgression between cultivated and wild blueberries has been documented, but the group did not think the impact of pest resistance genes on either the crop or relatives was a matter of serious concern. Neither cultivated nor wild blueberries have characteristics associated with aggressive weeds, and the transfer of a single pest resistance trait was not seen as likely to alter this.

Brassica crops

Discussion focused on the common *Brassica* species that are currently most subject to modification by genetic engineering, the oilseed crops *B. napus* and to a lesser extent, *B. rapa* and *B. juncea*. All are capable of cross hybridizing among themselves and with other related species. Many commercial cultivars already contain resistance to common fungal pathogens; because the resistance was derived from wild relatives, it was considered unlikely that movement of such genes back to weedy relatives would have a significant impact on fitness of the weeds. However, the situation may be different for other genes encoding traits such as insect resistance and herbicide tolerance (or most problematic, a combination of both), which could confer a substantial fitness advantage on a recipient plant.

Available information indicates that cultivated transgenic *Brassica* will hybridize with a number of weedy species and that introgression of transgenes is probable. Ecological studies show that in many environments insects are the principal factor limiting plant population growth, suggesting that acquired pest resistance genes could increase the fitness and hence the population range of weedy *Brassica* species. However, too little information is available to definitively state that this risk would outweigh the benefits of having crops with enhanced pest resistance.

The working group identified seven areas of research that would contribute to our knowledge of pest resistance gene impact on *Brassica* species:

1. The creation of a database of sexually compatible species and varieties.
2. The development of a geographic information system of pest influence. This would combine species ranges with environmental information required to predict the impact of pests on a given host.
3. Long-term studies on weed populations to examine changes in pest resistance gene frequencies and the effect of such changes on pest populations.
4. Pest exclusion studies to measure the influence of pest pressure on plant reproductive rates.
5. Hybridization and introgression experiments using resistance-conferring transgenes to measure the performance and persistence of transgenes in the environment.
6. Observational studies of basic reproductive biology of lesser-studied related species.
7. Modeling projects to synthesize available knowledge and direct future research.

Cereal Grains

Sorghum (*Sorghum bicolor*), rice (*Oryza sativa*), and wheat (*Triticum aestivum*) have close weedy relatives capable of hybridizing with the respective cultivated crops, although the ease of introgression depends on the specific crop-weed complex. Pest resistant varieties of these crops are currently being bred using traditional and (except for sorghum) genetic engineering techniques. The group could find no evidence that pest resistance traits introduced into these crops or their weedy relatives would affect their ability to establish, persist, or spread. Because these crops (and associated weeds) are already subject to integrated weed management programs, there was little concern about exacerbation of a weed problem within the managed agroecosystem. However, insufficient data exists to make the same conclusion about less managed ecosystems.

The working group concluded that pest resistance genes derived from the same gene pool (i.e., characterized and predictable genes from conventional breeding or genomics programs) are of low risk. More information is needed to assess the risk of introducing genes derived from diverse sources. Recommended research topics on the environmental effects of novel genes introduced into crop species include:

1. Inventories of pest infestations of the related weedy species.
2. Presence of pest resistance traits in the weed population.
3. Impact of pests on weed population dynamics in the absence of resistance.
4. Where impact is significant, quantitation of pest infestation.
5. Studies of crop-weed hybrids if fitness or population dynamics is affected.

When an engineered pest resistant crop deemed to present low risk based on small scale studies, is released commercially, the first five years following release provide a unique opportunity for risk assessment on a larger scale. It was recommended that funding and research efforts be targeted to this time period.

Cucurbits

The diversity in origin and genetic composition of wild and weedy cucurbit crops makes generalizations about crop-weed complexes difficult. Cucurbits grown in the US have both Old World and New World origins and are generally interfertile with wild native or introduced cucurbit species in the US. Weedy relatives include dudaim (*Cucumis melo* subsp. *melo*) and varieties of *Cucurbita pepo*. Evidence indicates that wild *C. pepo* has experienced hybridization and introgression with cultivated relatives, and perhaps that some weedy species have evolved from escaped ornamental gourd varieties.

It is possible that introduced pest resistance traits in *Cucurbita* spp. could enhance weediness, but this would depend on whether the trait conferred a selective advantage on the recipient plant. An introduced gene for virus resistance has been demonstrated to flow from squash to wild relatives and confer virus resistance to the wild plants. Although viruses have been reported on wild *C. pepo*, the impact of viruses on such wild populations has not been investigated.

The group concluded that our ability to evaluate the risk of pest resistance genes enhancing a weed problem would benefit from a greater understanding of the biology of wild cucurbit species. This would include weediness characteristics (i.e., degree of aggressiveness), genetic similarity of crop and weed, geographical distribution, ecological requirements, sympatry (degree of genetic interaction among crop and related weed), reproductive biology, pests of wild species, and pest resistance in wild species (i.e., type, frequency, stability). Because of the release of transgenic virus resistant summer squash, efforts should focus on wild *C. pepo* as well as other weedy *Citrullus* and *Cucumis* species.

Grasses

The turfgrasses creeping bentgrass (*Agrostis palustris*) and Kentucky bluegrass (*Poa pratensis*) were considered by the group because of recent efforts to genetically engineer these crops. Turfgrasses are highly domesticated species that are subject to intense management. Although they are capable of hybridizing with

wild relatives, they are relatively slow growing, small, and quickly out-competed by most plants. These traits, combined with the fact that mowing normally prevents these plants from setting seed, suggests that crop to weed gene flow and introgression would be rare.

Various disease and insect resistance traits have been bred into turfgrasses, and these have contributed to an expanded geographic range of cultivation. However, the working group was unaware of any evidence that introduction of a pest resistance trait had resulted in turfgrasses or their sexually compatible relatives overcoming any control exerted by those pests.

Despite the low weediness potential of turfgrasses, the group identified several gaps in our knowledge that could be filled by research on:

1. The life history and invasiveness of the various turfgrass species.
2. The geographic range of related species, as well as their cross-compatibility with crop species.
3. The diversity of pests and pathogens that attack the sexually compatible relatives.
4. The factors (including pests and pathogens) that limit populations of sexually compatible relatives.
5. The rate of increase of populations of sexually compatible relatives, and the factors that control them.
6. A greater understanding of the characteristics of weedy grasses in general.

It was proposed that this information could be obtained through several avenues, including existing literature, from which information could be compiled into a useful database; introduction experiments, in which transgenic plants could be monitored after controlled introduction into populations of sexually compatible wild relatives; simulation experiments, which simulate greater reproductive fitness by artificially increasing seed output in target plant populations; and experimental crosses, which directly characterize the weediness potential of hybrid progeny of transgenic crops and weedy relatives.

Poplar

Poplar (*Populus* spp. including cottonwoods, aspens, and related hybrids) differ considerably in their biology from other crop groups considered in the workshop. They become reproductively active between the ages of five to fifteen years, have long life spans, and exhibit a high capacity for vegetative regeneration. Dispersal by sexual reproduction can be extensive whether pollen or seed is considered, however, seeds rapidly lose viability and thus do not persist in the seed bank. Many species have stringent habitat requirements (e.g., for moisture), most environments harbor large wild populations compared to poplar plantations, and seedlings are not competitive in closed stands of trees or herbs. This creates a condition of "genetic inertia" in which significant changes in population genetics due to transgenes may take dozens to hundreds of years to occur. Nevertheless, poplars are very closely related to their wild relatives, and gene flow and introgression have been documented.

Development of poplar with fungal resistance is proceeding primarily by conventional breeding, but genetic engineering of insect resistance (and herbicide tolerance) is well under way. The ability of poplars to disperse pollen and seeds over great distances suggests that transgenes from plantation trees will escape, but the genetic inertia of these trees makes it difficult to predict when, or even if, transgenes might significantly impact wild populations. Despite the large areas and long time required to study this issue, the working group concluded that the risks of releasing transgenic poplar do not outweigh the benefits, but that releases should be coordinated with monitoring programs to follow the impact of these genes on the environment.

Seven areas were identified where research would be useful to inform both scientific and regulatory decisions on pest resistance genes in poplar. Ranked from highest to lowest priority, these are:

1. Isolation of additional kinds of insect and disease resistance genes.
2. Development of reliable containment methods to prevent seed and pollen

movement of transgenes (e.g., engineered tree sterility).
3. Information to support management of pest resistance (e.g., insect dispersal, refugia design).
4. Poplar reproductive biology, seed, and pollen dispersal, and fertility of crop-wild hybrids.
5. Ecology of natural pest resistance mechanisms in relation to species interactions and ecosystem function in the wild.
6. Evaluation of the economic and legal impacts of transgene spread.
7. Analysis of contributions that transgenic poplars could make to economic and environmental sustainability.

Sunflower

Cultivated sunflower (*Helianthus annuus*) overlaps in range with its weedy, wild progenitor (also *H. annuus*) and the two are fully capable of hybridizing. Pollen from cultivated sunflower may be spread to adjacent wild populations through the movement of insects, and thus crop genes may introgress and persist in populations of wild sunflower.

Disease resistance in cultivated sunflower has been obtained through both conventional and transgenic approaches. Insect predation on cultivated sunflower is considered to reduce yield both by directly consuming seed heads or by spreading disease agents, thus engineering resistance to insects (i.e., Bt toxin) is a high priority for transgenic commercial hybrids. No studies have examined whether gene flow from cultivated to wild sunflower has had an impact on the wild population. Since the pest resistance traits bred into crop cultivars to date have largely been derived from wild germplasm, it is not likely that these traits would add anything new to the wild populations.

The working group developed a series of questions that should be addressed for each new type of transgene that confers resistance to insects or disease. They provide a framework for identifying important research areas and aid in making decisions about the release of transgenic sunflower.

1. Is the transgene inherited as a stable, Mendelian trait when it is crossed into wild plants? Experiments to address this question should examine genetic behavior of the new trait and its expression under various environmental conditions.
2. Do insects or diseases targeted by the transgene occur in populations of wild sunflower, and if so, how common are they? A recommended approach is to conduct detailed surveys to examine the influence of targeted pests on wild sunflower populations.
3. When the transgene has introgressed into wild plants, will these plants exhibit greater survival or fecundity than their nontransgenic counterparts? Suitable experiments would examine the impact of pest resistance genes either through simulation or controlled introgression of the gene into wild relatives.
4. If the transgene leads to greater survival or fecundity, will this cause wild populations to become more troublesome weeds? A combination of field experiments and modeling to predict potential impacts could provide important insights.

OVERALL CONCLUSIONS

Despite the diversity of participants' backgrounds and the range of crops discussed, there were several points of general consensus. The common baseline was a recognition that conventional agricultural activity entails certain environmental and ecological risks. Given that, the group concluded that the genetically engineered pest resistance traits currently being field tested or commercially released present no fundamental differences from similar traits bred into crops using traditional techniques. It should be noted that some participants disagreed, however, and contended that transgenes will have more profound effects on crop phenotype than traditional genes, and thus potentially greater impact on weed species.

The second point of consensus was the view that cases in which crops are engineered with multiple pest resistance or other fitness traits present more complex ecological questions. Such

"gene stacking" to confer resistance against a broad spectrum of pests may give recipient plants a greater selective advantage and lead to ecological consequences that are less predictable than the single-gene pest resistance traits which constitute much of our experience to date. Participants agreed that the general consensus on the nature of risks posed by current transgenic crops could not be extended to the next generation of crops engineered with multiple pest resistance traits.

Organization of the working groups around crop types proved to be a very effective approach for synthesizing what is known and what needs to be known about ecological effects of introduced pest resistance genes. Although all groups were given an identical set of guidance questions, each group struggled with a unique crop-weed situation and set of issues. The biology and ecology of the crop and its weedy relatives dictated which issues were most relevant, and was perhaps the most important factor in determining the groups' level of concern over the risk of transgene escape. The weediness of the crop and its wild relative, the probability of crop/weed hybridization and transgene introgression, the life spans of the crop and weed, and the persistence of each in the seed bank all influenced the groups' thinking. Other important parameters that varied by crop group were the susceptibility of crops and weeds to pathogens and the role such pathogens play in limiting populations.

Discussions during the workshop were based on information compiled by group leaders prior to the meeting and the knowledge and experience participants brought to the table. Within every working group, members agreed that much information is lacking about the ecology of crop-weed complexes, in particular the level to which pests limit weed populations. This shortcoming hampered their ability to accurately predict the consequences of novel pest resistance traits. More importantly, it resulted in specific recommendations for more research on basic plant biology and ecology, as well as applied risk assessment. Although most commercially important crops have related weedy species somewhere in the world, not all of these crops are expected to be engineered for pest resistance in the near future. It is therefore a feasible task to generate essential biological and ecological information on the more widespread outcrossing crop species, which would increase our ability to make educated determinations of risk posed by release of genetically engineered varieties.

PLENARY PAPERS

THE CONCEPT OF FAMILIARITY AND PEST RESISTANT PLANTS[1]

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INTRODUCTION

Meetings such as this workshop provide an all too rare opportunity for scientists from different disciplines to share their perspectives on a topic of common interest. In this case we examine the use of pest resistant plants in managed ecosystems. USDA-APHIS has a clear interest in this subject because it is involved in regulating transgenic plants, many of which have been engineered with some sort of pest resistance, within its broad authority to protect plants under the Federal Plant Pest Act and the Plant Quarantine Act. Since 1992, when APHIS received its first request to determine non-regulated status for a transgenic crop, the agency has approved 43 petitions for non-regulated status; 16 of those are for crops with engineered pest resistance. The agency authorizes controlled field testing of transgenic plants in which test plants are isolated from other plants that might be affected. APHIS grants nonregulated status once it determines that the transgenic plant does not present a plant pest risk. In the regulations, the concept of plant pest risk is associated with direct or indirect injury or damage to plants or plant products.

How does APHIS decide if a transgenic plant poses a plant pest risk? As part of its assessment, the agency asks two questions: 1) What is known about the properties of the plant and the environment into which it will be introduced? and 2) What are the probable effects of the plant on the environment?

THE CONCEPT OF FAMILIARITY

Familiarity has consistently been a prominent criterion for evaluating the risks associated with transgenic organisms. The concept of familiarity was presented 10 years ago in the document entitled "Field Testing Genetically Modified Organisms: Framework for Decisions," which was produced by a panel of experts selected by the National Research Council and published by the National Academy of Sciences (NAS). That 1989 NAS report considered how to evaluate the relative safety of testing transgenic plants in the field. The panel summarized some critical observations and principles that were relevant for field testing. APHIS has used these conclusions in the process of assessing transgenic plants, on a case-by-case basis.

APHIS assesses risk by considering what is known about the following factors: the biology of the crop, the introduced trait, the receiving environment, and the interaction between these. The biology of the crop includes, for example, the mating system, mode of pollination, and compatibility with wild relatives. Aspects of the introduced trait to consider include the source of the resistance and how it was introduced. In the case of pest resistance, consideration of the introduced trait also includes the pests to which resistance is conferred. Examples of points to consider about the receiving environment are the presence of sexually compatible wild relatives, pest populations, and the cultivation practices for that crop. Knowledge of and experience with any and all of these factors provide familiarity, which plays an important role in assessments. This concept of familiarity allows the decision-makers to draw upon past experience with introduction

[1] Paper presented at the "Workshop on Ecological Effects of Pest Resistance Genes in Managed Ecosystems," in Bethesda, MD, January 31 – February 3, 1999. Sponsored by Information Systems for Biotechnology.

of plants into the environment, and to compare genetically engineered plants to their non-engineered counterparts.

GENETICALLY ENGINEERED VERSUS CLASSICALLY BRED CROPS

One conclusion in the NAS report is that crops modified by genetic engineering should pose risks that are no different from those of crops modified by classical genetic methods (including bridging crosses, wide crosses, mutagenesis, etc.) for *similar* traits and grown in *similar* environments. Similar traits means traits that produce similar phenotypes in an engineered or a traditionally bred crop, for example: resistance to similar insects in an engineered or a traditionally bred crop; and resistance to similar viruses in an engineered or a traditionally bred crop. A similar environment means an environment similar to one where the plant has always been grown. Generally, plants engineered for pest resistance will be grown in the same places that their non-engineered counterparts have always been grown. One important point of this first conclusion is that it is more important to evaluate the phenotype produced, rather than the process/techniques that were used to produce it. In the context of this workshop, this is a very important point, because what needs to be addressed and focused on are the effects of any pest resistance genes, traditionally bred or engineered, in managed ecosystems.

A second important conclusion made by the panel is that plants modified by classical breeding techniques have a history of safe use. This is not to say that traditional practices pose zero risk, but that the level of risk has been acceptable and manageable. Familiarity does not necessarily mean safe, but that enough is known about the plant to determine the level of safety.

These points are generally agreed upon by scientists who have been concerned with the issue, as in the frequently cited paper by Tiedje *et al*. (1989). In that comprehensive overview of engineered organisms the authors state that "transgenic organisms should be evaluated and regulated according to their biological properties (phenotypes), rather than according to the genetic techniques used to produce them . . ." and "Long term experience derived from traditional breeding provides useful information for the evaluation of genetic alterations similar to those that might have been produced by traditional means, and such alterations are likely to pose few ecological problems."

In many cases, plants developed through genetic engineering and traditional breeding are similar. Consider how a new variety is developed. Traits are initially introduced through genetic engineering or through traditional techniques involving crossing a standard or elite variety with a particular relative that has a desirable trait, such as disease resistance. After a promising new variety has been identified, whether in a greenhouse or a laboratory, it is typically tested in the field for several seasons to see how it performs in a variety of agricultural settings. Once in the field, it may also be backcrossed a number of times to restore the desired genetic background. Regardless of how the trait was initially introduced, the subsequent development follows a well established and formal process.

The information gathered in these steps is extensive. A great number of characteristics are considered in detail during the process of developing a new variety because the developer is keenly interested in being certain that the new variety behaves just like other successful varieties of the crop in as many agronomically significant ways as possible. As part of a petition seeking nonregulated status, APHIS requires applicants to report any differences that are observed between the transgenic lines and the parental organism during this variety development process. So aside from the desired phenotypic change, engineered plants are usually similar to their non-engineered parents, and that allows the agency to assess them based on previous experience with the biology of the crop and its environment and what is known about the introduced trait.

FAMILIAR TRAITS

What kind of traits are we familiar with? Familiarity varies from case to case. Consider one example. Table 1 shows all of the pests in melon for which traditional sources of resistance have been identified and can be used by breeders

(Pitrat 1994). This is part of what forms our basis for familiarity with pest resistance in melon. The only transgenic melons that have been approved by APHIS for field testing have similar non-transgenic phenotypes, which are shown in italics in Table 1. Generally, many traits for pest resistance available from traditional breeding can be used as a base for our familiarity with genetically engineered traits.

Consider, as another example, the transgenic pest resistant plants that APHIS has deregulated or that are pending deregulation. For some of these plants, there are comparable pest resistant cultivars obtained by traditional breeding (Table 2). Resistance genes found in traditional breeding sources are not the same as those introduced by genetic engineering, but they confer similar phenotypes. In other deregulated pest resistant crops the resistance traits are found in the gene pool, but are not necessarily found in commercial lines. Although there is less experience with these traits that are not found in commercial lines, there is some familiarity with these traits based on reports where these traits have been found in relatives of these crops.

Table 1. Pests for which traditional sources of pest resistance/tolerance exist in *Cucumis melo* (melon).
(Those with comparable field-tested transgenic resistance are shown in italics.)

Aphid	Downy mildew	Pickle worm
Anthracnose	*Erwinia tracheiphila*	Powdery mildew
Cucumber scab	Fruit fly	PRSV
CMV	*Fusarium oxysporum*	*Pseudomona lachrymans*
Colletotrichum lagenarium	Gummy stem blight	Root knot nematode
Corynespora melonis	Hypocotyl rot	Spider mites
Corynespora cassiicola	Leaf blight	SqMV
Cucumber beetle	Leaf miner	WMV2
CGMMV	MNSV	ZYMV
Diabrotica		

Table 2. Deregulated transgenic pest resistant phenotypes and genetic resources available for traditional breeding.

Transgenic Plant	Conventional Source of Similar Phenotype	Reference
Lepidopteran resistant corn	Resistant commercial hybrids available	Barry and Darrah 1997
PLRV resistant potato	Resistant cultivars available	Swiezynski 1994
PVY resistant potato	Resistant cultivars available	Khurana and Garg 1998
Coleopteran resistant potato	15 resistant accessions in the genus *Solanum* L., subgenus Potato, section petota	GRIN 1994
ZYMV, WMV2 resistant squash	Resistant cultivar available	Sold by Harris Moran
CMV resistant squash	Resistant cultivar available	Quemada, pers.comm.
Lepidopteran resistant cotton	Gossypol, Factor X in *Gossypium* ssp.	Dilday and Shaver 1976; Perceval, pers. comm.
PRSV resistant papaya	Tolerance genes identified	Gonsalves, pers. comm.
Lepidopteran resistant tomato	Resistance in *Lycopersicon* ssp., particularly *L. hirsutum*	Stevens and Rick 1986

POTENTIAL FOR PEST RESISTANCE GENES TO ENHANCE WEED PROBLEMS

One of the main concerns with the ecological effects of transgenic plants is that the engineered genes will escape to their wild or weedy relatives and enhance the recipients' weediness in agriculture ecosystems or their invasiveness in natural communities. Is this a probable effect in the case of pest resistance traits?

There are many well-studied examples of hybridization and introgression between domesticated plants and their wild relatives. Many of these involve hybridizations that have been implicated in weed evolution. One of the best examples is Johnsongrass (*Sorghum halepense*), one of the world's most noxious weeds, which arose from the hybridization of cultivated sorghum (*Sorghum bicolor*) and the wild *Sorghum propinquum*. Some of the ecologically important traits thought to have been acquired from the crop include earlier flowering, greater seed production, larger individual seed weight, and earlier emergence (NAS 1989), traits that are often associated with weediness. But there is no evidence that any pest resistance genes from cultivated sorghum have enhanced the weediness of Johnsongrass. In fact, APHIS is not aware of any evidence that weeds have benefited from the acquisition of crop pest resistance genes. Clearly, genes, including pest resistance genes, flow from crops to their sexually compatible wild relatives. The lack of evidence for beneficial effects on weeds may be due either to a lack of effect, or because not enough time and effort have been spent looking for effects.

One thing to consider is that in order for pest resistance to have a noticeable effect in natural populations, the pest itself should have a significant effect on the natural populations. All of the deregulated pest resistant crops have compatible wild relatives somewhere in the world. There is no evidence, however, to indicate that the pests these deregulated crops are engineered to resist have an ecologically significant role in limiting populations of the wild relatives. Is that because no one has looked? Obviously, there are examples of plant pests that do have significant effects on natural populations of plants. The devastating effects of gypsy moths on forest trees are a striking example of this. Other examples are chestnut blight and Dutch elm disease, both caused by fungal plant pathogens that were introduced into North America during the past century. Clearly, resistance to these pests could have had a significant effect. However, these examples may not reflect the same sort of potential interactions exhibited by some pest-crop-wild relative complexes. In the examples above, the pests are all introduced or exotic species and the hosts are long-lived species that have not co-evolved with them. In contrast, most crop species have co-evolved with their pests, including repeated, often annual, selections mediated by humans.

EVALUATING THE RISK

How should a regulatory agency assess whether genes for resistance to crop pests, traditionally bred or engineered, will confer an advantage on a wild relative that may cross with the crop? This issue needs to be considered on a crop-by-crop and a trait-by-trait basis. To improve the effectiveness of using the concept of familiarity in assessing ecological consequences of pest resistance, some important questions need to be addressed for individual crops.

- Are there examples of traditionally bred or naturally occurring crop pest resistance genes that confer or enhance weediness? APHIS does not know any examples of a pest resistance gene that has enhanced weediness, but this needs to be addressed in individual crops, and for individual pests or types of pests.
- Are there examples of pests that limit natural populations of wild relatives of crops where the acquisition of resistance would clearly make a difference? For crops in which pest resistance is being engineered (i.e., against *Rhizoctonia* in the grasses, fungal diseases in strawberries, viruses in the cucurbits, etc.) are there examples where the pests do have a significant effect on the natural populations? Hopefully we will be able to identify other questions over the course of this workshop.

CONCLUSIONS

Familiarity can always be increased as a result of a trial or experiment, and the increased familiarity can then form a basis for future assessments. The Biotechnology Risk Assessment Research Grants Program, administered by the Cooperative State Research, Education, and Extension Service (CSREES) and the Agricultural Research Service (ARS) of the USDA, supports research that will assist Federal regulatory agencies in making science-based decisions about introducing genetically modified organisms into the environment. Proposals should be designed to identify risks, quantify the likelihood of these risks, and quantify their probable effects. Ideally, these grants support projects designed to bring together scientists from many relevant disciplines. Plant breeders, plant pathologists, entomologists, biochemists, molecular biologists, and ecologists should pool their expertise to investigate questions that will increase familiarity with specific issues related to risk assessment.

Returning to the concept of familiarity, two documents referenced in this presentation, Tiedje *et al*. (1989) and the NAS (1989) report, were published ten years ago and were written from a broad perspective on genetic engineering. Reasoning from such broad premises for all organisms and their potential uses can sometimes yield statements that are too general and not always useful. In order to generate useful discussion, it is necessary to identify and focus on specific issues that are components of risk. This workshop on the ecological effects of pest resistance genes in managed ecosystems presents an opportunity to do just that. APHIS recognizes the importance of observational information from individuals who are the true experts on the biology of a particular crop or its pests and does not hesitate to request additional information from those experts when questions arise. APHIS strives to keep its reviews science-based, and it cannot be emphasized strongly enough how important it is to focus on identified risks supported by facts. Speculation without facts may be valuable, but it is not risk assessment.

The objectives of this workshop are 1) to review existing evidence that the introduction of pest resistance into a crop species has affected the establishment, persistence, and spread of the crop or of species related to the crop; and 2) to identify gaps in the information concerning the ecological effects of pest resistance genes, and recommend strategies to address those. These objectives call us to improve upon that with which we are already familiar regarding pest resistance in crop species.

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WEED MANAGEMENT: IMPLICATIONS OF HERBICIDE RESISTANT CROPS[1,2]

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ABSTRACT

Crops made resistant to herbicides by biotechnology are being widely adopted in North America and entering other parts of the world. Those containing transgenes that impart resistance to post-emergence, non-selective herbicides such as glyphosate and glufosinate will have the major impact. These products allow the farmer to more effectively use reduced- or no-tillage cultural practices, eliminate use of some of the more environmentally suspect herbicides, and use fewer herbicides to manage nearly the entire spectrum of weed species. In some cases, non-selective herbicides used with herbicide resistant crops reduce plant pathogen problems because of the chemicals' toxicity to certain microbes. There is concern among weed scientists that over-reliance on fewer weed management strategies will result in evolution of resistance to the more useful herbicides and/or population shifts to naturally resistant weed species. Although environmentalists are concerned with the potential impacts of gene flow from transgenic crops to wild relatives, herbicide resistance transgenes confer no fitness advantage outside of fields treated with the herbicide. Thus it is unlikely that they would affect plant populations in natural areas. The next decade should clarify the eventual impact of these powerful new tools on weed science and weed management.

INTRODUCTION

Weed science became an organized discipline with the introduction of synthetic herbicides in the 1940s. The discipline grew with and focused on an expanding array of new herbicides with increasing efficacy and utility in crop production. The success of this paradigm has generally satisfied farmers and those that control public funding of weed science research. Compared to other pest management disciplines, considerably less effort has been expended on alternative methods of weed management. The proportion of pesticides used in the US that are herbicides continues to grow and is now close to 75% of the crop protection pesticide market (see Figure 1).

The herbicide market for major crops has been mature for several decades. Discovery of weed control compounds better and more economical than what is already available is very difficult. Furthermore, the cost of regulatory approval has increased significantly. Nevertheless, introduction of new herbicides for major crops continues unabated because of the profit potential of a successful new product. In most of the world, however, there is a strong sentiment to reduce synthetic pesticide use.

Biotechnology is now providing an alternative to the discovery process for new herbicides. Crops are being genetically modified to be resistant to existing herbicides, thus widening the potential market and usefulness of these established products. In some cases, resistance has been achieved by simple selection in cell or tissue culture. The most successful approach has been to introduce resistance genes by genetic engineering. Opposition to transgenic crops is variable, with some of the strongest opposition in certain European countries (Burghardt 1998). The impact of this new technology on the pesticide industry, weed science, and weed management may be profound. This paper attempts to predict some of these impacts.

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[2] Modified from: Duke SO. 1998. Herbicide resistant crops—their influence on weed science. *Journal of Weed Science and Technology* (Zasso-Kenkyu, Japan) 43:94-100.

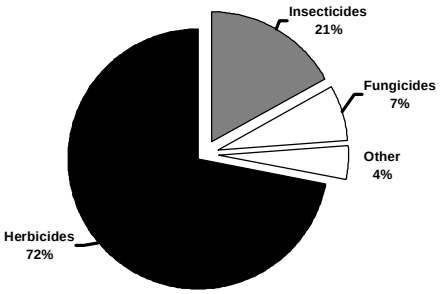

Figure 1. The chart shows crop protection pesticide sales in US in 1997 (Anonymous 1998b).

DRIVING FORCES

Significant external forces will influence weed science and weed management and thus how biotechnology will be utilized for weed management. In Europe and North America, there are rapid and profound changes in the pesticide industry. Companies that historically relied on new and better pesticides for future profit are investing heavily in plant biotechnology, presumably with the intention of making a significant portion of future profits from transgenic crops.

Population pressure on land resources will increase dramatically in the near future unless agricultural productivity (yield per unit area) grows concomitantly with population. New technology will be needed to increase crop productivity in a sustainable fashion, without converting more natural areas to cropland.

Within weed science, there are more specific influences that will affect how herbicide resistant crops (HRCs) are used. These include the movement toward integrated pest management, which until recently has largely ignored weed management. In the US, there is a strong and steady adoption of reduced- and no-tillage agriculture, resulting in greater reliance on post-emergence herbicides for weed management. The occurrence of weeds with evolved herbicide resistance is growing rapidly. This problem has not yet reached the severity of insecticide resistance, but in isolated cases the impact has been severe. Precision agriculture is being readily adopted and is expected to reduce herbicide use. Expert decision-making computer programs have the potential to more accurately determine the most appropriate timing, application rate, and pesticide to apply for maximum economic return. Considering the many external and internal forces and changes that are affecting weed science, predicting the impact of HRCs on weed science carries a significant level of uncertainty.

THE IMPACTS OF HERBICIDE RESISTANT CROPS

Over the past few years, several HRCs, both transgenic and non-transgenic, have become available in North America (see Table 1); others will soon be introduced. Of these, glyphosate- and glufosinate-resistant crops appear to have the greatest potential for wide adoption. These two herbicides are non-selective, so the farmer may be able to substitute one herbicide for several. Furthermore, they are foliar-applied herbicides that lend themselves well to no- or reduced-tillage agriculture. Finally, they offer manufacturers the significant advantage of linking their own chemical product to the resistant crop, because there are no analogues of either glyphosate or glufosinate that could be used with these crops. The economic advantage for the manufacturer could be lost when the patents on these herbicides expire. At that point, manufacturers could shut out competitors by engineering the HRC with an inducible promoter and formulating the herbicide with a compound that will induce the expression of resistance gene(s) in the HRC.

The herbicide industry appears to be rapidly transforming from a chemistry-based to a biotechnology-oriented industry. The larger pesticide producers of the US and Europe have invested heavily in plant biotechnology and the seed industry. Each year since the first experimental releases in 1987, HRCs have accounted for nearly one-third of field tests conducted under USDA authority. Imparting resistance to a successful herbicide in a new crop can be an economical method of expanding the market for a product for which the company has already gained approval, recognition, and manufacturing expertise.

Table 1. Herbicide resistant crops now available in North America.

Herbicide	Crop	Year Available
Bromoxynil	cotton	1995
Cyclohexanediones*	maize	1996
Glufosinate	canola	1997
	corn	1997
Glyphosate	soybean	1996
	canola	1996
	cotton	1997
	corn	1999
Imidazolinones*	maize	1993
	canola	1997
Sulfonylureas*	soybean	1994
Triazines*	canola	1984

*not transgenic

Whether production of crops resistant to broad herbicide classes (*e.g.*, protoporphyrinogen oxidase inhibitors) will be a viable strategy for the agrochemical industry is unclear because of potential problems in linking the crop to only one herbicide from a class in which there are many commercially available analogues. Furthermore, most currently used herbicides are selective and do not have the advantages conferred by a broad target spectrum such as glyphosate and glufosinate. An increasingly attractive herbicide discovery strategy is to find broad-spectrum phytotoxins with few effective analogues and to co-develop them with crops made resistant by biotechnology.

HRCs offer several advantages to the farmer. In most cases, the farmer can design simpler weed management strategies based on fewer herbicides. Glyphosate and glufosinate are ideal herbicides for no-tillage agriculture, allowing the farmer to spray at or near planting and then as needed during crop development. In many cases, HRCs will lower the cost of weed control. As with any new technology, the economic benefits are greatest for those who use it first. The overall environmental impact of managing weeds in HRCs is generally lower than that of using selective herbicides combined with tillage. HRCs can be especially useful for eradication of parasitic weeds (Joel *et al.* 1995). Finally, with certain non-selective herbicides, the herbicide may also have activity against plant pathogens. For example, glufosinate inhibits the infection of glufosinate-resistant creeping bentgrass with several plant pathogens (Liu *et al.* 1998). More research needs to be done on the secondary effects of pesticides in order to fully determine their roles in integrated pest management (Altman 1993).

Although transgenic herbicide resistant varieties of most major crops will be available in the near future, comparable minor crops will lag behind. Companies are slow to develop and introduce minor HRCs for the same reason they are reluctant to register their pesticides for small markets—a poor economic return, considering the investment and risk. At this time there is no strong sentiment for public funding for the creation of minor crop HRCs.

A few potential problems exist with HRCs. Overreliance on a single weed management technology gives existing weeds more opportunity to evolve resistance to that control mechanism. Alternatively, overuse of one management strategy may allow other weed species to become adapted in the ecological vacuum created by effective control of the weed species now present. Resistance will probably be slower to evolve to glyphosate and glufosinate than to many other herbicides (Bradshaw *et al.* 1997; Devine *et al.* 1993). Nevertheless, glyphosate resistance has already appeared in more than one population of ryegrass in Australia (Powles *et al.* 1998; Pratley *et al.* 1996). Most weed scientists agree that with these herbicides, population shifts to naturally resistant weed species will be a bigger problem than evolution of resistance (Owen 1997). Where crop rotation is practiced, HRCs can become weeds in a crop rotation system if the second crop is an HRC engineered to be resistant to the same herbicide to which the original crop was resistant.

Introgression of crop genes and transgenes into weeds is possible with some crops. For example, rice can interbreed with red rice (Langevin *et al.* 1990), a feral form that is a serious weed problem in some rice-growing areas of the world.

A herbicide resistance transgene alone confers no fitness advantage in areas where the herbicide is not sprayed. Thus, if it is transferred from the crop to a related weed species, the biggest concern is for the farmer who must cope with the herbicide resistant weed. An herbicide resistance transgene in a crop can greatly increase the chance of survival of interspecies crosses by eliminating competition of other herbicide susceptible weeds (Keeler *et al*. 1996). If the crop also contains transgenes conferring other survival-enhancing traits, such as resistance to insects and/or pathogens, the resulting cross and further backcrosses with the weedy parental species might confer enhanced fitness outside the agricultural setting, resulting in ecological disruption.

There is perhaps more potential for unexpected pleiotropic effects with transgenes than non-transgenes because these genes have not evolved to function in coordination with the rest of the genome. Furthermore, positional effects in the genome, independent of pleiotropic effects, can be problematic. Lastly, inconsistent expression of the transgene in time or in the proper tissues is a potential problem. Some transgenic, herbicide resistant varieties have not been evaluated by public sector scientists to the extent that traditional varieties have been tested, leaving unresolved questions about yield and quality (e.g., Anonymous 1998a).

Despite these potential problems, in most cases HRCs have largely been welcomed enthusiastically by North American farmers. In fact, the success of HRCs will probably delay the intensive search for non-herbicide-based weed management technology. However, the utility of the most successful HRCs will eventually decrease, resulting in the need for alternative herbicides or weed control methods. There is some concern that the increasing consolidation of biotechnology and agrochemical industries may reduce competition in finding new commercial weed management solutions, perhaps increasing the importance of public sector research in this area.

Current trends indicate that within a few years almost all acreage of the major crops grown in North America, except perhaps wheat, will be herbicide resistant. This level of acceptance by farmers strongly indicates that this technology has improved the economics and efficiency of weed management. Weed science research will be strongly impacted.

CONCLUSIONS AND SPECULATIONS

Several unpredictable factors can affect how and to what extent HRCs are used and the resulting impact of their use. These factors include international regulation of transgenic crops, unforeseen new technologies, ability of the pesticide/biotechnology industry to protect and recoup their investments, and the speed with which weeds evolve, adapting in response to new technologies.

Clearly, in most major crops, HRCs are (or soon will be) strongly impacting weed management choices. In many crops their use will decrease the cost of effective weed management in the short to medium term. Their use will speed the adoption of reduced- and no-tillage agriculture, greatly reducing the environmental damage of farming by reducing soil erosion by both wind and water, and by reducing use of herbicides more likely to be found in surface and ground water. Herbicide resistance and new weed species problems that arise as a result of this technology will be dealt with by traditional methods, such as rotating herbicides, mixing herbicides, and rotating crops. Overreliance on HRCs could prematurely reduce their usefulness. However, they offer the farmer a powerful new tool that, if used wisely, can be incorporated into an integrated pest management strategy that can be used for many years to more economically and effectively manage weeds.

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ESCAPE OF PEST RESISTANCE TRANSGENES TO AGRICULTURAL WEEDS: RELEVANT FACETS OF WEED ECOLOGY[1]

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INTRODUCTION

In this paper, I will sketch some features of weed ecology and evolutionary biology that, in my view, are relevant to assessing the prospects of pest resistance transgene escape into populations of agricultural weeds. I will focus on weeds of field crop agroecosystems, rather than addressing the broader category of invasive plants in general.

This discussion is organized around a model for transgene escape that distinguishes three phases leading to the establishment of widely distributed populations of weeds carrying a transgene. In this scheme, the first event is hybridization between a weed and a transgenic crop. Second, a process of introgression and adaptation occurs in which evolutionary mechanisms improve maladaptive features of the early-generation products of hybridization, resulting in a weed bearing a pest resistance transgene and having a reasonably high level of adaptation to certain agroecosystems. Finally, a process of dispersal distributes this 'neo-weed' over the landscape accompanied by local adaptation to variable conditions encountered when dispersal is over a sufficiently broad area.

I will survey aspects of weed ecology and evolutionary biology that appear important to the operation of each of these three phases. Frequently, I will be in the uneasy position of suggesting plausible implications of suspected features of weed ecology. Unfortunately, in many instances neither these features nor their implications have received more than fragmentary documentation. There are enormous gaps in our knowledge of weed ecology. Many aspects that would likely be widely agreed-upon by weed scientists simply have not been described by published observations and experiments. This paucity of data reflects the prevailing focus of weed science in recent decades on herbicidal weed control to the neglect of ecological inquiries and, especially, of theoretical frameworks.

HYBRIDIZATION

Clearly, hybridization between transgenic or conventional crops and sexually compatible relatives (Snow and Palma 1997) occurs in many crops and has produced new forms of weed behavior in resulting populations (Barrett 1983). Recent work has documented such hybridization in detail and makes clear that transgenes can be expected to escape even across large spatial barriers and significant barriers of genetic incompatibility (Snow and Palma 1997). Transgene escape by hybridization appears inevitable in some systems. However, in other cases it is unclear whether hybridization is a rate-limiting phase in the escape of transgenes. My premise is that hybridization may indeed be rate-limiting in some circumstances, for example when hybridization is occurring across a substantial incompatibility barrier. Aspects of weed ecology that may affect hybridization rates in these situations include weed breeding systems and effects of spatial and temporal distributions of weeds at several scales.

The most common breeding system among weeds of field crop agroecosystems is a mixed mating system in which both self-fertilization and cross-fertilization occur, although other reproductive systems are also known (Barrett

[1] Paper presented at the "Workshop on Ecological Effects of Pest Resistance Genes in Managed Ecosystems," in Bethesda, MD, January 31 – February 3, 1999. Sponsored by Information Systems for Biotechnology.

1992). Therefore, the most prevalent weed breeding system permits hybridization, but such crosses must occur in the face of a substantial rate of self-fertilization.

Breeding systems and other aspects of genetic systems and reproductive ecology that affect hybridization rates are known to vary within and among weed populations. For example, jimsonweed (*Datura stramonium*) populations in North Carolina have flowers that open to pollinators and show approximately 10% outcrossing rates (Motten and Antonovics 1992). In contrast, certain populations in Indiana are exclusively self-pollinating, with flowers that do not open to pollinators (Weller and Jordan, unpubl. data). In some cases, this variation may reflect adaptation of the breeding system after range expansion (Barrett 1992); pollinator behavior may also vary geographically as well. These aspects of reproduction, therefore, should not be regarded as fixed characteristics within weed species (Barrett 1992).

The spatial distribution of weeds may strongly affect weed-crop hybridization. First, many weeds have a highly patchy distribution within fields, and recent work suggests that patches in some species have some degree of temporal stability (Walter 1996). Patches may result from edaphic factors or from persistent effects of high seed production. Within fields, patchy weed distribution may mitigate against weed-crop hybridization if weeds occur in patches of sufficient density that the proportion of weed individuals on the periphery of patches is small, thus limiting the population rate of hybridization. More homogenous and sparser spatial distributions may favor considerably higher rates of outcrossing, because isolated individuals may experience much higher local abundance of crop pollen or because of changes in pollinator movement as a function of local density. Alternatively, weed density may have the opposite effect on hybridization rates when the crop serves as female parent. In this case, high-density patches may promote hybridization by virtue of attaining high local densities of weed pollen, and homogenous weed density may reduce hybridization.

The distribution of weeds in the broader landscape around field crop agroecosystems also has potential importance in modulating rates of weed-crop hybridization. If conditions permit weed establishment in non-cropped areas in this landscape, then many small isolated populations may exist (Wilkinson *et al*. 1995). Weed-crop hybridization may occur at higher rates in these populations than in field populations for a variety of possible reasons. For example, due to the plasticity of reproduction in many weed species, flowering can occur over a broad time period during the growth season for a given species. Commercial-scale fields of both wind and insect pollinated crops have been shown to disperse pollen for more than 1 km beyond field boundaries (Wilkinson *et al.* 1995). Therefore, crop pollen can be expected to reach non-field weed populations in agricultural landscapes within this distance. Thus, when weed populations are considered on a landscape scale, extensive plasticity of flowering time and availability of crop pollen across the landscape may markedly extend the range of opportunities for hybridization in many weed-crop systems.

Finally, weed abundance is highly variable. In certain years, weather factors can lead to weed control failures over extensive regions, producing very high weed densities in some fields. Weed density also varies on a regional basis due to interactions between weed biology and regionally variable weed management practices and other cropping system factors. Both forms of variation may result in substantial increases in the absolute number of hybridization events. Increased rates of hybridization may result in cases in which the rate is affected by density-dependent variation in pollinator behavior or spatial distribution. For example, increased local abundance of a species may allow it to colonize marginal habitats in agricultural landscapes that are not occupied at lower densities, perhaps increasing probability of hybridization. Thus hybridization rates may fluctuate considerably over years and over portions of a weed species range.

INTROGRESSION AND ADAPTATION

The evolutionary process that ensues after hybridization (Adam and Köhler 1996) is likely

to be affected by a number of features of weed ecology in contemporary field crop agroecosystems. First, the nature of these systems appears to impose on weeds only a few strong population-regulating factors (Barrett 1992), compared to most annual and short-lived perennial plant populations that inhabit other sorts of ecosystems. This may favor introgression of transgenes even if hybrids and initial backcross generations have many modestly maladaptive features compared to weeds not bearing transgenes. Secondly, weed populations often appear small and perhaps transient, so that evolution of introgressants is likely to be governed by the joint effects of selection, migration, and random genetic change. Finally, seed ecology is a primary determinant of weed fitness; thus, effects of transgenes or other crop genes on seed ecology are likely to exert strong selective effects on these genes.

The few-but-strong selective agents seemingly result from the biological simplification that appears typical of contemporary high-input field crop agroecosystems. Apparently, although data are lacking, weed populations in these ecosystems are often limited by only a few management practices or natural enemies. The implication is that introgression of genes that improve weed adaptation to these predominant selective agents can dramatically increase the average fitness of a weed population. Moreover, tradeoffs among adaptations to different limiting factors (e.g., competitors vs. herbivores) resulting from introgression of a single gene may also be minimal. A prime example is the evolution of herbicide resistance in weeds. The advent of herbicide resistance often dramatically increases average fitness and growth rate of weed populations. Moreover, herbicide resistant mutations can have high absolute fitness despite major functional impairments that result from pleiotropic effects of resistance mutations. This example illustrates how selection can favor mutants that overcome key limiting factors despite performance tradeoffs. Another line of evidence for this notion comes from the multiple examples of increased distribution and abundance of weeds resulting from acquisition of a crop trait via hybridization (Barrett 1983). Finally, the many cases of major increases in distribution and abundance of certain weeds following modest changes in cropping systems (e.g., herbicide or fertility regimes) provide additional evidence that many weed populations are regulated by a few powerful factors (Froud-Williams 1998).

If accurate, this conjecture suggests that post-hybridization adaptation of weeds bearing escaped transgenes is greatly facilitated by the biological uniformity of current field crop ecosystems. Weeds may require relatively little evolutionary 'refinement,' such as breaking of linkages to disadvantageous crop traits, in order to acquire adaptation to large areas (Adam and Kohler 1996). One suggested criterion for assessing spread of transgenes into weed populations is that the fitness of the weed-crop hybrid bearing a transgene should be greater than the fitness of non-hybrid weeds. This criterion may more easily be met in contemporary field crop ecosystems than in most other ecosystems. Thus, transgene escape may be a rapid process compared to what it would be if cropping systems were, in effect, less forgiving of maladaptive features. Even quite poorly fit hybrids and early backcrosses may persist in agroecosystems at sufficient densities to allow opportunities for introgression and adaptive refinement, provided that these forms have a key adaptation that facilitates their persistence. These considerations may apply most strongly to escape of transgenes affecting tolerance to abiotic factors (e.g., herbicides or drought). However, it is possible that over extensive areas weed populations are limited by a single biotic factor to which adaptation would confer major increases in fitness. For field crop weeds, virtually nothing is known about this possibility.

A distinctly different mechanism by which escaped transgenes can affect weed adaptation is via increased fitness in weed populations in non-cropped parts of agricultural landscapes. Weed populations in areas such as field margins or roadsides may be subject to a different range of selective pressures than weeds in cropped fields. For example, seed predation rates may be markedly higher in field-margin habitats, while selective factors affecting fitness in cultivated fields may be absent. Therefore, these non-cropland populations may offer refugia from certain selective factors such as seed germination

behavior during early generations after hybridization. Also, introgression of transgenes into non-field populations may allow adaptation to unrecognized biotic population-regulating factors, such as herbivores, pathogens, and seed predators that are not active in field populations. This mechanism is speculative, since the role of non-field populations in the dynamics and evolution of weed populations in agricultural landscapes is currently unknown. Recent simulations (Blumenthal and Jordan, unpubl.) suggest that populations of perennial weeds along field margins can sometimes be important to maintaining field populations.

Another weed ecology feature likely to affect adaptation of crop-weed hybrids is the frequency of episodes of low effective population size due to small census sizes and high levels of selfing (Barrett 1992), particularly in the process of colonization. Low population sizes cause random changes in genetic composition through genetic drift and founder effects. These mechanisms can act on the genetic novelty produced by hybridization, producing a range of genetically differentiated small populations from a genetically diverse early-backcross weed population.

The implication here is that adaptation in weed populations containing escaped transgenes is likely to be affected by both selection and random genetic change. When both factors are present, evolutionary events can occur that would not occur when selection is the dominant evolutionary mechanism. Specifically, the adaptive effects of combinations of transgenes, other crop genes, and weed genes can be much more thoroughly "explored" by the joint action of random genetic change and selection than by selection acting alone (Wade and Goodnight 1998). This mechanism can be particularly forceful when weed populations experience high levels of extinction and recolonization, thus forming ecological and genetic metapopulations. Although it is not yet empirically clear whether agricultural weeds have a metapopulation structure, the occurrence of such structure, in combination with small population size and varied selection pressures, creates favorable conditions for the plausible operation of the shifting balance process. However, the action of this process may itself be unpredictable due to geographical variation in population structure in some weed species due to breeding system, local adaptation after colonization, time since colonization, and hybridization with related taxa (Barrett 1992).

A final dimension of weed ecology relevant to adaptation after hybridization is seed ecology. Ability to maintain persistent seed or propagule populations in soil, along with efficient dispersal and ability to rapidly and efficiently use available resources for reproduction, are the apparent hallmarks of successful weeds of field crop agroecosystems (Ghersa and Roush 1993). Many of the most intractable weed species are so because of the ecology of their seeds. Simulations of weed population dynamics show that seed demography (e.g., seed survival and germination rates) strongly affects weed population growth rates (Colbach and Debaeke 1998). Weed seeds vary substantially among species in longevity. Many soil management factors affect seedbank demography by preventing germination, evoking fatal germination, or by otherwise increasing seed mortality rates. These factors include use of cover crops, conservation tillage, and residue burning; they may have direct effects on seeds, or indirect effects via effects on seed predators and pathogens. Thus, the germination/dormancy behavior of weed seeds is a critical determinant of their survival rates in a given cropping system. The importance of seed ecology to weediness suggests that if transgenes affect seed ecology, these effects are likely to be a primary determinant of their fitness (Landbo and Jorgensen 1997). Similarly, maladaptive effects on seed ecology may be a major mechanism by which non-transgenes from crops hinder adaptation after hybridization.

There are several other aspects of weed seed ecology that appear relevant to the adaptation stage. First is the well-known effect of dormancy whereby weed genotypes, produced by plants growing under past environmental circumstances, can again be selected for despite intervening periods of unsuitable conditions. Thus, seed populations augment the genetic variability of weed populations. Also, seed populations, as a form of temporal dispersal,

allow weed genotypes to be tested over a wider range of conditions than would otherwise be possible. This effect may significantly increase the opportunity for a weed carrying an escaped transgene to encounter conditions to which it is adapted.

DISPERSAL

Effective spatial dispersal of seeds is considered a primary attribute of a successful weed (Ghersa and Roush 1993), and the dispersal ecology of weeds is expected to affect the fate of escaped transgenes in a number of ways. On a field scale, simulation modeling indicates that high rates of weed seed dispersal generally greatly increase weed populations (Perry and Gonzalez-Andujar 1993).

For most weeds of field crops, dispersal is determined by the interaction of weed attributes and human activities, such as contaminated crop seeds (Ghersa and Roush 1993), equipment such as combine harvesters, irrigation water, livestock, and trucking of grain. Management actions in agroecosystems can affect weed dispersal, perhaps regulating weed populations in some cases. When human activities serve as principal weed seed dispersal vectors, dispersal distances are difficult to characterize. They are strongly affected by the particular dispersal vector and geographically variable due to variations in cropping system factors. As a result, the extremes of the dispersal distance distribution are poorly known in most cases.

On a broader scale, many cases of rapid, sub-continental scale dispersal of weed species are known. Weed species have been observed to disperse and become abundant over large regions of the western US (Mack 1986). Due to cropping system changes that promote its abundance, jointed goatgrass (*Aegilops cylindrica*), a sexually compatible weed of wheat, substantially expanded its range in Utah during a period of eight years. Expansion over hundreds of kilometers of roadsides in less than a decade has been observed in herbicide resistant weeds. These observations suggest that roadside and other non-field weed populations may be important to weed range expansion, again suggesting the importance of weed ecology across agricultural landscapes in the escape and dispersal of transgenes.

Finally, because of the apparent biological simplification of current field crop agroecosystems, a weed may gain markedly higher fitness across a large spatial domain from an escaped transgene. In theory, the resulting spatial homogeneity of favorable habitat (Tomiuk and Loeschcke 1993) and the absence of a need for local adaptation of colonizing populations promote rapid range expansion by colonizing organisms. Therefore, the ecology of weed dispersal and population regulation in current agroecosystems and agricultural landscapes appear to permit large and rapid range expansions of adapted weeds.

Weed attributes affecting dispersal (e.g., seed size, shape, resemblance to crop seed, etc.) should be regarded as adaptive traits that are probably subject to strong selection. As for seed ecology, any effects of transgenes on dispersal ecology are likely to be primary determinants of the fitness effects of those transgenes, and effects of crop genes on dispersal ecology may cause major fitness costs in hybrids. Weed dispersal may also have an evolutionary role, as mentioned above. Dispersal of small founding populations can trigger adaptive processes in these small populations that would not occur in larger populations. Also, in the shifting balance process, dispersal has a critical role in distributing evolutionary products of events in small populations across the landscape and in triggering change in other populations.

CONCLUSIONS

There are several summary points to emphasize. First, the ecology of weeds in contemporary cropping systems may facilitate transgene escape by permitting survival of weed-crop hybrids that are maladapted, relative to “wild-type” weeds, in a variety of fitness components. This most likely occurs when the hybrids and subsequent backcross progeny carry a transgene of sufficient adaptive value. Second, seed and dispersal ecology are major determinants of weed fitness and population growth rate, although this is not widely appreciated as such among non-weed scientists. Effects of crop-derived transgenes and

all other crop genes on these traits will strongly affect the adaptation of weed-crop hybrids and backcross progeny. Third, most major weed species show extensive spatial and temporal variation in reproductive, seed, and dispersal ecology on several scales. This variation has both genetic and environmental causes and may strongly affect processes involved in all phases of transgene escape. Finally, a landscape perspective may be important for proper assessment of prospects for transgene escape. Populations of agricultural weeds are distributed across agricultural landscapes, including many populations that occur outside of cropped fields. Particularities of the ecology of these populations may affect all three phases of transgene escape.

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MECHANISMS OF PEST RESISTANCE IN PLANTS[1]

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INTRODUCTION

In their long association with pests and pathogens, plants evolved an impressive array of defensive tools. At the same time, pests and pathogens developed mechanisms to compromise plant resistance mechanisms in what must have been an evolutionary game of ping-pong. Natural pest resistance mechanisms occurring in higher plants can be classified into preformed resistance mechanisms and inducible resistance mechanisms. Agricultural pest control throughout this century has attempted to harness these mechanisms wherever possible. Natural resistance has several obvious advantages over the use of chemical pesticides or other methods for pest control. These include nominal genetic permanency, negligible cost once cultivars are developed, and quite high efficacy. The major downside of natural pest resistance is the reality that selection pressure is placed on pest populations to develop means of overcoming the resistance, thus practically limiting the time of effectiveness.

PREFORMED RESISTANCE MECHANISMS

Resistance mechanisms of this type are usually broken down into preformed structural, morphologic, and chemical factors. In entomology, it has long been known that innate morphological and anatomical features such as leaf and flower color, presence of trichomes, and even the texture of cuticle may cause certain insects to avoid a plant, thus constituting resistance mechanisms. Anatomical features may also deter or discourage insect feeding. These include the degree of secondary wall thickening, stelar structure, and other aspects of basic plant structure. They all fall under the category of preformed resistance mechanisms.

Plant pathogens include viruses, fungi, bacteria, and nematodes, all of which must gain entry into the plant and contact living plant cells in some way for success. Accordingly, structural and morphological barriers could be expected to provide resistance against many potential invaders. Recognized examples include features as sophisticated as stomatal guard cell anatomy, for instance the height of lips of the guard cells. As shown in work by Harvey Hoch and colleagues (Hoch *et al*. 1987) at Cornell University, certain fungal rust pathogens initially colonize the surface of leaves and have exquisite sensing mechanisms that measure the height of stomatal guard cell lips encountered on susceptible plants. When the fungus hyphae encounter a lip of the proper height, they are programmed to undergo a developmental program resulting in the formation of invasive structures that enter the stomate and begin colonization of the leaf interior. It has been noted that if one could alter the height of guard cell lips, this rather benign change should provide resistance against the rust fungus.

Plants typically contain significant amounts of preformed chemicals produced via secondary metabolism. These include phenolics of varying structural sophistication, terpenoids, and steroids. The concentrations of these compounds in particular tissues may be very high. Some preformed compounds are directly toxic, while others exist as conjugates such as glycosides that are not directly toxic but become toxic following disruption of the conjugate. For instance, plant glycosides are often hydrolyzed following insect damage or pathogen ingress that releases vacuolar glycosidases. The aglycones thus produced may be quite toxic to the invader as well as neighboring plant cells. Since the toxic response is local, however, only a small portion of the plant is affected.

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On the other hand, some plant preformed compounds are toxic as glycosides, but lose toxicity when deglycosylated. Elegant work done with fungal plant pathogens has proven the role of several such compounds as bona fide resistance factors. In one example, the preformed saponin glycoside, avenacin, was shown to inhibit the growth of a root pathogenic fungus, and oat plants producing the compound exhibited resistance to the pathogen. A related fungus strain, however, was observed to produce a glycosidase that removed the sugar residue from avenacin, effectively detoxifying it. This strain was not inhibited by avenacin and oat plants were susceptible to it. Anne Osborne and colleagues (Bowyer *et al*. 1995) at the John Innes Institute in England cloned the gene for the fungal glycosidase from the detoxifying strain and showed that mutation of this gene rendered the fungus sensitive to growth inhibition by avenacin. More importantly, oat plants were now resistant to the mutant strain, strongly arguing that avenacin is a resistance factor unless a pathogen can deal with it.

INDUCIBLE RESISTANCE MECHANISMS

Inducible resistance mechanisms are active, energy-requiring systems typified by specific recognition of an invader that ultimately leads to the production of proteins or metabolites that are antagonistic to the invader. These resistance mechanisms have been most studied in regard to plant pathogens, but the same or similar mechanisms clearly function against insect pests. Such active resistance mechanisms are usually referred to collectively as the hypersensitive response (HR).

RECOGNITION ASPECTS IN THE HR

Invocation of the HR requires that the plant recognize or key on at least one molecule produced by the invading pest. These factors have come to be called elicitors and may be peptides or proteins, fatty acid derivatives, sterols, or other low molecular weight chemicals produced by a pest or pathogen. Elicitors themselves, in the absence of the living pests, initiate the active plant defense response.

Plants have been known since early in this century to contain particular genes, called disease resistance genes, that confer resistance to some but not all biotypes or strains of a pest or pathogen. These genes have been widely used in practical agriculture, and have allowed farmers to avoid using countless tons of chemical pesticides. There are, unfortunately, cases where certain plants do not have an identified resistance gene against an important pest, and pesticides still have to be used. There is also the problem of pests mutating to virulent forms that are no longer recognized by the disease resistance gene, effectively rendering it useless. Strains of pathogens that initiate plant defenses harbor genes called avirulence genes. These genes direct the production of specific elicitors, which when purified, have the rather remarkable property of initiating the HR only in plant cultivars containing the cognate or matching disease resistance gene. Pest strains that have escaped resistance conferred by a certain plant resistance gene have either eliminated production of an elicitor by losing the corresponding avirulence gene or (if the elicitor is a protein) have modified its structure such that the resistant plant no longer detects it.

In the last few years, many different plant disease resistance genes have been cloned and sequenced. Almost all of them fall into the leucine-rich repeat (LRR) class of proteins, typified by imperfect repeats of blocks of amino acids, usually with about 24 residues per repeat element. The LRR resistance gene proteins may also have nucleotide binding sites, leucine zipper domains, or kinase domains suggestive of signal transduction functions. In a few cases, disease resistance genes have been transferred to foreign plants by transformation and generally shown to be functional. Although no commercial plant cultivars have yet been developed, it is suspected that transfer of disease resistance genes by transformation will become a commonly used method to develop new pest-resistant plants.

A few LRR plant disease resistance genes have been shown to exhibit dual specificities—that is, the plant harboring them either recognizes two different pests or two different elicitors. Especially exciting was the recent finding by Valerie Williamson and colleagues at the Univ.

of California, Davis (Rossi *et al.*1998) that the cloned *Mi* resistance gene in tomato against the root knot nematode also recognizes a species of aphid. It is not known whether the nematode and aphid produce the same elicitor, as is likely, but the finding is of considerable importance and has practical implications that should stimulate the search for additional disease resistance genes that target insects. While several examples of insect-targeting resistance genes are recognized, they are relatively rare compared to resistance genes known against fungi, bacteria, nematodes, and viruses.

INDUCED RESISTANCE RESPONSES

When resistant plants recognize cognate or matching elicitors, intracellular signal transduction pathways are activated that ultimately result in the derepression of a battery of genes called defense response genes. These latter genes encode toxic proteins such as chitinases, glucanases, lysozyme-active proteins, or cell wall strengthening proteins such as hydroxyproline rich glycoproteins. Response proteins may also be enzymes in biosynthetic pathways for lignification of cell walls or the production of phytoalexins, low molecular weight toxic chemicals that antagonize the invader.

Our knowledge of signal transduction in the HR is incomplete, but several interesting genes have been identified from mutagenesis screens or biochemical studies. These genes include protein kinases and phosphatases, calmodulin genes, and others of unknown biochemical function that ultimately activate transcriptional activators of defense response genes.

FUTURE APPLICATIONS

The *Arabidopsis* genome sequencing project should be completed by the end of 1999. These results will add significantly to the repertoire of genes available for producing transgenic plants. Indeed, understanding the functions of unknown genes identified by the sequencing project will be greatly aided by routinely transforming them into the same or heterologous plants and screening the resulting transgenics for various traits, including pest and pathogen resistance. We can accordingly expect a revolution in approaches to improvement of plants by the construction of transgenics.

There are several strategies that are being evaluated for harnessing what is known about active pest and disease resistance to crop improvement. There have been attempts by conventional plant breeding to introduce genes that alter the morphologic or chemical composition of plants such that they become unattractive to pests and pathogens. I suspect that much more of this kind of work will occur now that it is possible to routinely produce transgenic plants and, in principle, introduce genes for new biochemical pathways.

Several investigators have transformed pathogen avirulence genes responsible for production of elicitors into plants that carry the cognate disease resistance gene. There are some clever approaches underway in this arena, generally involving wound or defense response gene promoters used to regulate expression of the avirulence genes, such that they will only be expressed (and the HR elicited) following pathogen challenge. Various viral genes, such as coat or replicase genes, have also shown promise for producing resistance when transformed into plants.

Several HR signal transduction genes have been experimentally over-expressed in transgenic plants and some of them lead to enhanced pest and disease resistance. Accordingly, these genes are being studied for possible use in future disease resistant plant cultivars.

The LRR domains of disease resistance gene products have been shown to account for the specificity of these proteins to recognize only one pest elicitor. Thought has consequently been given to designing synthetic resistance genes with the LRR domains targeted to a certain elicitor of a pest or pathogen. Although this approach has not yet progressed beyond the experimental stage, it is clearly an area that will be heavily investigated in the future to generate new and unique disease resistance genes, hopefully some of them targeted to currently refractory pests and pathogens.

ECOLOGICAL CONCERNS

There is naturally the concern that heterologous natural disease resistance genes, engineered resistance genes, or synthetic resistance genes could be passed to weed populations and accordingly present hazards. Several factors make me think such dangers are minimal. First, natural disease resistance genes have been used throughout this century for pest and pathogen control and I know of no case where horizontal transfer of these genes has led to new weed problems. Intrinsically, there is no reason to think that engineered or synthetic resistance genes should behave any differently. Secondly, the big tactical advantage of creating transgenic plants is that genes of interest can usually be introduced into elite cultivars directly and relatively rapidly. Unlike classical plant breeding, this process drastically reduces the requirement for backcrosses and testing before a new cultivar can be released. The result of this 'time-line shortening' will be the ability to rapidly change the resistance genes present in crop plants, thus confounding pests and pathogens and their efforts to evolve and overcome resistance. This sleight of hand will also minimize the dangers of horizontal gene transfer. Newly inserted genes can be removed rapidly by simply substituting transgenics with new resistance genes for the old cultivars. As such, exposure time of any one gene can, in theory, be minimized, and pathogens accordingly will have less time to overcome it.

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CONSEQUENCES OF CLASSICAL PLANT BREEDING FOR PEST RESISTANCE[1]

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TEN MILLENNIA OF PLANT BREEDING FOR PEST RESISTANCE

Resistance Breeding Before Mendel

Wild relatives of crop plants such as beans, wheat, and maize are not uniformly resistant to insect and disease pests. This can be demonstrated in simple fashion—when selections of these wild populations are set out in plant-rows, some of them are highly susceptible, others are resistant, and some are intermediate in resistance to the common pests of the region. The first plant breeders, those women and men who domesticated crops such as beans, maize, and wheat, could save only those genotypes that had some level of resistance, i.e., those individual plants that did not succumb to pest depredation. In effect, therefore, they selected for pest resistance and thus changed the population structure of their crop species in favor of resistance genes. This change made it possible to grow the crops in monoculture, which was convenient for food production and harvest. It was also convenient for multiplication of disease and insect pests that might not be affected by the limited sample of resistance genes.

Plant breeding thus set the stage for sequential cycles of pest resistance and pest susceptibility of crop plants. We have no direct record of the consequences of this ancient ecological meddling, but myth and historical accounts tell of disastrous disease epidemics and insect outbreaks, so one can assume that from time to time large plantings of crops that were uniformly susceptible to a new kind of insect or disease fostered increases of that pest to epidemic proportions. Resistance genes were essential for crop domestication and monoculture but they did not guarantee perfect safety.

We have no record at all and little or no speculation about how the newly domesticated crops might have affected their wild relatives, which no doubt were growing in close proximity to the domesticates.

Resistance Breeding After Mendel

Genetics-based plant breeding, launched in the early years of the 20th century, produced new crop varieties with improved resistance to major disease and insect pests. Usually such resistance was developed as a second phase—a rescue operation—after new varieties, selected primarily for high yield, were discovered to be susceptible to a particular insect or disease. Breeders found early on that they could identify single genes (usually dominant) that conferred essentially complete resistance to the pest in question. Varieties containing such excellent resistance were developed and released for large-scale farmer use. But breeders then discovered, all too often, that the "perfect" resistance lost its effectiveness after a few seasons. They soon learned, with the aid of entomologists and plant pathologists, that insect and disease pests are highly diverse genetically, and that almost without fail a rare pest genotype will turn up (or perhaps be created *de novo* by natural mutation) that is not affected by the newly-deployed resistance gene. The new pest genotype multiplies and the crop variety's resistance "breaks down."

As years went by, breeders found that some kinds of resistance did not fail, and that such resistance often was less than complete; the plants suffered some damage but gave satisfactory performance overall. This longer lasting resistance was dubbed "durable" resistance. Further, the breeders discovered that durable resistance usually (but not always) was

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governed by several genes rather than by one major gene. The multifactorial kind of resistance has been called "horizontal resistance." The major-gene resistance has been called "vertical resistance."

The good news, then, was that breeders could identify and breed for durable resistance. The bad news was that the breeding was more difficult because several genes had to be transferred at one time, thus requiring larger populations for selection, as well as multiplying the usual problems with "linkage drag" (undesirable genes that are tightly linked to the desired ones). To this day, breeders use both kinds of resistance in varying proportions, according to the crop and where it is grown.

At first, breeders found and used resistance genes from the adapted, local landrace populations that also were the initial gene pool as a source of resistance genes for their new varieties. As years went by, these gene pools began to dry up and breeders looked further afield, turning to exotic (unadapted) landraces, and even to wild relatives of their crop. Sometimes they made extraordinary efforts to hybridize the domestic crop with a very distant wild relative—making a cross that could not succeed under natural conditions. Embryo rescue and even x-ray treatments were used to make "unnatural" crosses and derive breeding progeny from them. The breeders fooled around with Mother Nature; they moved genes farther than natural processes would allow.

But the breeders as a whole preferred to not breed from exotic varieties or distant and often wild relatives. They used exotic material only when there was no other choice. This preference was due not only to the difficulty of wide hybridization, but also to the fact that exotic germplasm exacerbates the problem of undesirable linkages. Few or none of the foreign genes—except the desired resistance genes—were suitable for the needs of high yielding, locally adapted varieties. But often the breeders had no choice; either they got the needed resistance genes from a distant relative, or they got nothing at all.

At about this time, breeders realized that it would be important to conserve remnant seed of landraces from all around the world, but especially from the centers of diversity of their crop. As farming worldwide grew more commercial, farmers turned more and more to professionally bred varieties that were better suited to commercial production, and in so doing they abandoned their landraces. If remnant seed of those landraces was not collected and saved in special storage facilities, the genetic base for crop breeding in the future would be drastically narrowed. Seed "banks" were needed. Through the efforts (especially in the 1960s and 1970s) of a few far-sighted plant breeders, seed banks were established in several countries and in international research centers.

So at the end of the 20th century, plant breeding for pest resistance had laid out the genetic framework of vertical and horizontal resistance, and identified important sources of new resistance genes, i.e., plant germplasm from anywhere in the world. Sources were limited, however, to the crop species itself or its relatives, either wild or cultivated. All of the introduced genes therefore came from plants.

Plant breeders selected not only for tolerance or resistance to disease and insect pests, they also selected for tolerance to abiotic stresses such as heat and drought, cool temperatures, or nutrient imbalance. Much of this selection was involuntary; in selecting varieties with top performance over many seasons and many locations the breeders necessarily selected varieties with tolerance to the prevailing abiotic stresses of the diverse seasons and localities. In selecting for tolerance to environmental stresses, breeders necessarily changed the genetic makeup of the crop species, altering it still further from that of the original wild species, which had been restricted to certain environmental niches. Witness teosinte (the probable parent of maize), restricted to certain habitats in Mexico as compared to maize that now is grown in nearly every country of the world except Iceland.

Global distribution of crop plants often means that they are grown with no proximity to wild relatives that might intercross with them. Teosinte is not found in Germany or China, nor

for that matter in the US Corn Belt. In other cases, however, wild species with hybridization potential coexist with their cultivated crop relatives, often as weeds. Canola, sunflower, and grain sorghum are examples of crops with hybridization potential with either a related species (canola with wild mustards) or with a weedy form of the same species (sorghum with shattercane, cultivated sunflower with wild sunflower).

FOUR QUESTIONS ABOUT PEST RESISTANCE TRAITS

The above discussion shows that plant breeders have changed the genetic composition of crop species to a large degree as they selected for pest resistance and also for resistance to environmental stresses. Such changes are in addition to the major phenotypic changes (e.g., non-shattering, uniform and fast germination) that were a consequence of domestication. What have been the consequences of such alterations, either on the crop species and its near relatives or on the ecosystems in which those species are grown? Twenty experienced plant breeders addressed this question as they responded to four queries I sent to them. My questions were:

1. Have the resistance traits been stable over time?
2. Have they led to undesirable consequences with respect to weediness of the crop or its relatives?
3. What have been the major sources of pest resistance genes as used in classical breeding (e.g., same species, related species, mutation)?
4. Are there relevant differences between the resistance genes currently being engineered into plants and those that have been transferred by conventional breeding?

In the following sections I summarize the responses from the breeders, and add commentary of my own.

Have Resistance Traits Been Stable Over Time?

The breeders say that as a general rule, resistance traits governed by major dominant genes have not been stable over time, whereas those governed by several genes have been more durable. But there are exceptions to both statements. One cannot say categorically that single gene resistance will always be undependable, or that multiple factor resistance will always be durable.

It is important to remember that the phrase "stability of resistance" refers to whether or not a previously resistant variety is overcome by a particular species of disease or insect. It does not infer that individual resistance genes lose their power to hold individual pest biotypes in check. The resistance genes are stable, but new (or previously undetected) pest biotypes appear, with types of virulence that are not curbed by the now-outdated resistance genes. The variety succumbs to the disease or insect pest once again, albeit to a new race of the pest, and breeders say that the variety's resistance was unstable.

Has Introduction of Conventional Resistance Genes Led to Undesirable Consequences with Respect to Weediness of the Crop or Its Relatives?

The breeders know of no undesirable consequences (such as enhanced competitive ability in a related weed species following the unintended transfer of resistance genes from crop to weed) from any introduction of resistance genes into crop plants through classical breeding. Some of the introduced genes have come from very distant relatives, but all have been derived from plants. Chances of introgression from crop species to wild relatives vary by crop. Ease of hybridization and the genetic complexity of transformation from wild to domesticated plant type (or vice versa) are major determinants for the rate and amount of introgression that might be expected. In the US, sunflower and sorghum are highly cross-compatible with related weeds and would be the most likely crops to exhibit undesired movement of pest resistance genes from crop to weed. Breeders, however, have not yet observed this kind of introgression.

What Are the Major Sources of Resistance Genes in Classical Breeding?

The breeders say that resistance genes from within the crop species are preferred when they can be found, because of ease of breeding with

them, but they will go far afield if they have to. The practice varies with the crop; e.g., tomato breeders commonly use genes from wild relatives whereas sorghum breeders do not. The amount of genetic diversity within the crop species and its ease of breeding with alien species are major determinants of breeders' actions.

Are There Important Differences Between Classical and Engineered Resistance Genes?

The breeders say that engineered resistance genes now in use appear to have different modes of action than traditional resistance genes, but they point out that we know very little about structure and mode of operation of the traditional genes and so have little basis for sweeping judgments about difference. Further, we have few specifics about how a radically different genetic background might affect expression of a transgene.

Genes for herbicide resistance (the archetype example of potentially dangerous genetic transformation) are not necessarily imparted by means of genetic transformation. Such genes are found within crop species or their relatives, or have been created by means of mutation. These genes, bred into a specific crop variety, theoretically could move from the crop to cross-compatible weed species and impart unwanted herbicide resistance to the weeds. But in order to cause a new problem, resistance genes would have to introgress into weeds that had not contributed the resistance genes in the first place. This example shows how difficult it can be to decide whether or not a given resistance gene in a crop plant will increase competitiveness in weeds or make crop plants into weeds. Presence or absence of genetic engineering is not the major determining factor.

The breeders look to a future generation of engineered plant genes that will provide greater diversity and utility than genes presently available in any one crop. Genes from related taxa, from very distant taxa, or from within the crop species may be altered to provide improved resistance, but they will be plant genes rather than genes from extremely different organisms. It may be difficult to identify the point at which such new genes should be called "unnatural."

CONCLUSIONS

Until recently, plant breeders did not worry about how their breeding affected weeds, or whether their crops could become weeds. Weeds were looked on as potential sources of genes for pest resistance if they could hybridize with crop species, but almost no one thought about whether or not the population genetics of weeds could be altered by introgression from crop species. A very few students of crop evolution studied the weeds that may have been ancestors of cultivated plants. Plant taxonomists and ecologists usually ignored weeds because they weren't considered as parts of natural ecosystems.

Genetic engineering has changed all of that. If genes from far afield can be added to crop plants, giving them marvelous gains in pest resistance, tolerance of environmental stress, or enhanced seed production, one can imagine that those transgenes could enhance the power of weeds in the same ways.

The analogy may not be as simple as it sounds, however. Two concepts must be clarified and data need to be assembled before one can make firm predictions.

Do crop plants as a class have the same requirements for survival and luxuriance as weeds as a class?

- To consider this question one must lay out the ways in which crop plants and weeds are similar and ways in which they differ.
- Perhaps even before that, one must decide whether it is possible to make a definitive description of crop plants as a class, and another one for weeds as a class.

What is the functional role of resistance genes in weeds as compared to their role in crop plants?

- Will a gene that greatly enhances survival chances for a crop plant perform the same service for a weed? (Crops grow in crowded monocultures; weeds usually grow in dispersed "polycultures.")
- Will the presence or absence of genetic diversity within a crop or weed population, or among crop or weed species in a site, affect the utility of a given resistance gene? (Crop varieties usually are genetically uniform, weed populations are not.)
- Should one distinguish between dangers of imparting genes for resistance to natural restraints, such as disease or insect attack, and resistance to man-made restraints, such as herbicides?
- Do we have any reason to believe that selection for new (or previously undetected) kinds of herbicide resistance in weed species operates on different principles than selection for new (or previously undetected) kinds of virulence in disease or insect species?

The breeders, in answering my four questions, were considering these two main points and the subsequent questions that they raise. My sense is that they did not want to classify resistance genes into only two categories—natural or engineered. Further, the breeders said we know so little about the molecular nature of resistance genes that we cannot yet categorize them in any meaningful way. I think they do not believe that mode of transfer or kingdom of origin is a meaningful classification. But I did not get any hints as to what would characterize a meaningful classification.

Despite their reluctance to sort genes into "engineered-bad" and "natural-good," the breeders acknowledged that whenever we fool around with Mother Nature we get surprises, some of them bad. Therefore we need to look with caution at any novel breeding technology, predicting possible consequences as well as we can, with the modicum of data we may have in hand.

We need to know more about the effects of genetic background on gene action. Location within a genome seems important, and the entire genetic background seems important. We have little or no understanding of these interactions.

We need to know more about the consequences of hybridization of crop species with related weeds and the potential for introgression in both directions. Jointed goatgrass hybridizes with common wheat and viable backcross offspring can be produced. Have resistance genes from wheat moved into jointed goatgrass and changed its survival potential? A similar question can be asked for sorghum and shattercane, sunflower and wild sunflower, canola and mustards, or maize and teosinte.

So we must ask ourselves, do we have data to answer either of these key questions—effect of genetic background, or consequences of hybridization—or at the least do we have enough data to let us speculate from a firmer foundation than we have at present?

In my opinion, we have fragments of data for some crops and/or their weed relatives, but rarely do we have enough for firm predictions about gene introgression or about gene action in the genome or the population.

What are the consequences of adding new pest resistance genes to a wild species, either a weed or otherwise? How plentiful and how powerful must the genes be to change the genetic balance of the wild species, make it a stronger weed, transform a non-weed into a weed, or, conversely, reduce the weed's viability as a competing population?

How about the "function" of related weeds as a reservoir of new biotypes of pest species, disease, or insects? Are the weeds more dangerous to crop plants when they lack resistance and so are a constant source of pest infection and infestation? Or are they more threatening when they contain many of the same resistance genes as carried in the crop species and therefore encourage the multiplication of new pest biotypes (biotypes that are not bothered by the weeds' resistance genes)?

The recommendation arising from these questions seems obvious. Whenever a worrisome outcome seems likely but data are too sparse for firm conclusions, scientists need to work hard to fill the void. They need to plan the right experiments, gather the needed data, and publicize the results in both public and specialist media. And the public needs to provide the funds—the tax dollars—to support this work, since most of it will need to be done by scientists in public institutions.

A final consideration: sometimes the odds of a bad outcome from not doing a particular action may be much higher than the odds of a bad outcome from performing that action. Sometimes it may be better to take action with uncertain outcome than to stand still. Life always works on probabilities.

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INSECT LIMITATION OF WEEDY PLANTS AND ITS ECOLOGICAL IMPLICATIONS[1]

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INTRODUCTION

The ecological consequences of releasing a genetically modified organism into a novel environment will depend upon its establishment, dispersal, and interactions with other organisms. Establishment and dispersal in the population dynamics of weedy plants are discussed by Jordan (these proceedings). My task is to discuss the known effects of plant-feeding insects on populations of native weedy plants, which provide the best current predictive basis for assessing the potential ecological consequences of the movement of resistance genes from genetically modified crops into their native weedy relatives.

The focus of our research over the last 20 years has been on understanding and predicting the quantitative outcome of plant-feeding by insects on the density, distribution, and lifetime reproductive success of native weedy plants. In the context of this workshop, these data can be used to address: (1) what is the evidence that insect herbivores can limit plant population density, restrict plant distribution, or reduce lifetime reproduction of native weedy plants? (2) under what circumstances is such limitation strongest or most likely? and, (3) when might increased resistance to insects alter the weed status of presently innocuous weedy plants?

Experimental evidence that feeding by insect herbivores can influence the growth, reproduction, and population density of native herbaceous plants has accumulated over the last 25 years (see reviews: Crawley 1983, 1997; Parker 1985; Hendrix 1988; Weis and Berenbaum 1989; Louda 1989, 1995). From these studies it is clear that herbivorous insects, either singly or in combination, often significantly limit both the success of individual plants and the densities of populations of native weedy plants. Increased insect resistance in such cases would result in a reduction in control exerted by insects on plants and would lead to a prediction of increased weediness of the native plant species.

To illustrate the influence of insects on plants and to explore the circumstances under which insect herbivores have been shown to be crucial in limiting plant density, I would like to review the highlights of two of our research projects. The first concerns a native crucifer that is related to canola and wild radish (Brassicaceae). The second project concerns a group of native thistles that are related to sunflower (Asteraceae). In addition, I will review some of the implications of these data for anticipating potential ecological responses to altered insect pest resistance introduced into related weedy native plants. In the absence of direct tests on the role of insects in the dynamics of crop-related weedy native species, these studies can be used as the best models presently available to assess the role of insects and insect resistance in the dynamics of native weed populations.

Before considering the studies in detail, three main points from this work emerge as relevant to the question at hand:

1. Foliage-feeding insects on the crucifer and inflorescence-feeding insects on the thistles altered the growth, reproduction, recruitment, and density of both types of plants in some environments. These results suggest that increasing plant resistance to insects has the potential to increase individual plant performance and

[1] Paper presented at the "Workshop on Ecological Effects of Pest Resistance Genes in Managed Ecosystems," in Bethesda, MD, January 31 – February 3, 1999. Sponsored by Information Systems for Biotechnology.

population density for such species in some portions of the environment.

2. The severity of limitation by insects in these studies depended on the physical and biological environment in which the interaction occurred. Thus, our results suggest that prediction of the quantitative effect of altered plant resistance to insects requires knowing something about the environments under which the native plants grow, or would grow as performance increased.

3. Changes in insect herbivore load associated with the introduction of resistance to one guild of insects can alter total herbivore load and augment the impact of other native insects on plant performance and density. Thus, prediction of the quantitative effects of altered resistance to herbivores such as leaf beetles, in response to a coleopteran specific Bt, will require knowing how other groups of plant-feeding insects respond to the change in plant growth, phenology, and reproductive success.

CRUCIFERS (BRASSICACEAE)

Crucifers, such as canola (*Brassica napus*) and cabbage (*Brassica oleraccea*), are among the crops targeted to receive transgenes conferring increased insect resistance. Our research on bittercress (*Cardamine cordifolia* A. Gray), a potentially weedy perennial crucifer found in the Rocky Mountains, provides a model for the effects of insects on crucifer dynamics. Closely related species are circumboreal, occurring in our northeastern deciduous forests and plains grasslands as well as in the montane environment. Bittercress density is highest in moist, moderately shaded areas. The aim of our studies with this species was to determine experimentally the role of herbivorous insects, especially a particularly damaging chrysomelid leaf beetle (*Phaedon* sp. nr. *oviformis*: Louda 1984), in plant growth, density, and distribution. In addition, we evaluated the role of plant resistance factors, such as the mustard oil defenses, in mediating insect influence. The results of our studies over a decade suggest several points relevant to this workshop.

First, foliage-feeding insects limited the growth and reproduction of bittercress under field conditions (Louda 1984). To determine the overall effect of the foliage-feeding insects, an insecticide check test was used to reduce insect load compared to controls. Since the chrysomelid leaf beetle was the predominant herbivore at the study site, the results are relevant to discussions of the ecological effects of coleopteran specific Bt resistance genes. In addition, this experimental technique could be used with native weedy relatives of crop plants to simulate reduced insect herbivore load associated with increases in plant resistance. Such an experiment could eliminate concern about demographic effects of introgression of the resistance gene, if the insecticide check showed that insects had no significant effect on plant reproduction and recruitment. Alternately, if the insecticide check led to increased plant performance or density, as was the case here, further tests would be merited to assess the relative contribution of a specific insect guild, for example beetles vs. moths. The simplicity of the experimental design, and its ability to eliminate concern if done properly, argue in favor of requiring such an experiment prior to any release of an insect resistance gene into a crop with native weedy relatives.

Second, insect limitation of plant performance and density was much greater in the exposed drier habitat than in an adjacent moister environment (Louda and Rodman 1996). The two experimental protocols used in the study could both be used to quantitatively evaluate the effect of environmental variation on the outcome of insect feeding for the density of weedy native relatives of modified crop plants. Clonal material was transplanted from naturally-occurring plants into plots in an exposed sunny habitat and in a nearby moderately shaded habitat; half in each habitat were protected with insecticide. When plants in the exposed site were protected from insect herbivory, their performance was comparable to plants in the preferred shaded habitat. Also we removed the shade cover over half the shaded plots and quantified both foliage loss to herbivores and change in plant density over three years. Herbivory increased and density decreased dramatically over time when exposure was increased (Louda and Rodman 1996). Over the same three-year period, cumulative levels of

insect herbivory in the exposed sunny habitat limited density of plants there, demonstrating that insects restricted the occurrence of this crucifer to the shaded habitat. The implication of this result is that increased resistance to insects in this crucifer, and likely in similar species, would increase plant density within the current preferred habitat and expand the habitat range over which the native weedy relative could become abundant.

Third, in the exposed habitat where insects limited plant density, the variation in the level of impact was determined by the interaction of three factors: lower defensive compound concentrations (glucosinolates, the mustard oil precursors), higher insect abundance, and higher plant stress (Louda and Rodman 1983, 1996). We tested the role of plant physiological status directly in several experiments that manipulated plant water status (see Louda and Collinge 1992, and references therein). In every case, insect feeding damage was greater on plants that exhibited moderate leaf water deficits and higher nitrate-nitrogen concentrations (a symptom of plant water deficit). Clearly, insect impact on plant density, and consequently the potential for increased weediness in response to altered insect resistance, can be severe and can vary among growing environments. These results suggest that realistic, multi-factor models will be needed to predict plant density responses to increases in insect resistance.

THISTLES (ASTERACEAE)

Thistles are members of the Asteraceae family, which also includes cultivated sunflowers, a crop targeted for improved pest resistance. Our work on native thistles, which are characteristic native species in the prairie grasslands of the upper Great Plains, provides data on the role of insects in limiting weedy plants in this family.

The genus *Cirsium* is circumboreal, and plants occur in disturbances in a wide range of habitats in North America and Eurasia. Several species, such as bull or spear thistle (*C. vulgare*) and Canada thistle (*C. arvense* L.), are considered agronomically important weeds in some places. The aim of our studies of these species has been to determine the degree to which insects limit plant performance and contribute to limiting the weed potential of these species under normal conditions. In addition, these studies have fortuitously provided quantitative information on the ecological effects of increased insect herbivore load caused by the host range expansion of an insect deliberately released for the biological control of exotic thistles species (Louda *et al*. 1997; Louda 1998, 1999). The results of our studies over the last 15 years suggest three points that are relevant here.

First, insect herbivores significantly reduce growth and reproduction of thistles under indigenous conditions. Leaf-feeding insects restrict individual growth of both weedy thistles, such as tall thistle (*C. altissimum*) (e.g., Guretzky and Louda 1997) and a federally-listed threatened species, Pitcher's thistle (*C. pitcheri*) (Bevill *et al*. 1999). Inflorescence-feeding insects significantly reduced seed production and seedling establishment in every case we have studied experimentally to date (see references above). For example, using the insecticide check method, we found that inflorescence-feeding insects significantly lowered lifetime maternal fitness and limited population density of Platte thistle (*C. canescens* Nutt.) in the field (Louda *et al*. 1990, Louda and Potvin 1995). The implication of these results is that increased pest resistance that leads to reduced insect herbivore load in native weeds such as these would lead to significant increases in plant density and weediness.

Second, the role of insects in plant density limitation was more obvious, consistent, and important in the more disturbed open habitat than in the nearby grassland (Louda and Potvin 1995). After experimental reduction in insect damage to developing flowers and seeds, seedling recruitment and subsequent plant densities were higher in the disturbed sand prairie (stabilizing blowouts) than in the more heavily vegetated grass-dominated areas. Insects had a significant effect in both competitive environments, but the release from insect suppression was greater where the environment was disturbed. So, the prediction of the magnitude of insect suppression of thistles, in relation to other potentially limiting factors, is related to microenvironment, which is similar to findings described above for crucifers.

The implications of these results are that the quantitative response to increased pest resistance in a population that is being limited by insects will depend on the environment in which reproduction and recruitment are taking place. This is not reassuring for fugitive plant species, such as sunflower, which are well adapted to the disturbances that are a characteristic feature of managed agricultural ecosystems and their adjacent vegetation.

Third, even well-intentioned, planned releases of novel species can have unexpected ecological side effects in complex biological systems (Louda *et al*. 1997), and current protocols for risk assessment fall short of providing a definitive, unambiguous determination of ecological risk (Simberloff and Stiling 1996; Arnett and Louda 1999, in review). Release of a weevil (*Rhinocyllus conicus*) for the biological control of exotic thistles has led to its widespread feeding on native thistles, including species in several national parks and nature reserves (see Louda *et al*. 1997). For native Platte thistle, the effects of this alteration of herbivore load on fitness and population density are quantifiable based on our previous studies. The outcome may model the quantitative effects of increased insect resistance in native species subsequent to the development of insect resistant crops.

The population growth of *Rhinocyllus* on Platte thistle has been exponential since its first discovery in 1993 (Louda 1998), and the weevil population now significantly reduces seed production of this seed-limited species (Louda *et al*. 1997). Native insects alone, which were shown to limit recruitment and density, already reduce seed production by 65% (Louda and Potvin 1995). The weevil, superimposed on the damage by native insects, led to a 94% reduction in seed by 1996 (Louda *et al*. 1997).

The implications of these studies for releases of genetically-modified organisms are two-fold. First, increased resistance to insects by native weedy relatives of crop species could lead to increases in seed production, recruitment, and weediness. Second, it is clear that accurate prediction of ecological risk associated with releases is still in its infancy. We are only now learning what needs to be measured to predict insect feeding and impact on plant performance and dynamics. Nevertheless, the data from native thistles provides testable predictions for native weedy relatives of crop species. Based on what we know now, the plant types most likely to have insect herbivory as a significant determinant in their densities in disturbed areas are larger annuals and short-lived perennial native weeds with fugitive life histories like sunflowers and thistles (see Louda 1989).

CONCLUSIONS

The inferences of these studies for the three main questions posed at the beginning of this presentation are as follows: (1) There is evidence that insect herbivores can limit plant population densities and restrict distributions of native weedy plants. Since many of the native plant species related to crops targeted for improved pest resistance genes are weedy, quantification of the role of insects in limiting their densities are needed in order to have reliable risk assessments. The quantification process should start with insecticide exclusion studies. Manipulation of specific groups of insect herbivores is then merited if the insecticide tests show that seed, seedling, and older plant densities are affected by insect feeding. (2) The importance of insect limitation of weed density varied with environmental conditions. Insects played a significant role in limiting plant populations in open, disturbed, and potentially stressful growing conditions. Since disturbance is characteristic of agricultural fields and their margins, conditions are favorable to facilitate increases in weediness if insect resistance becomes incorporated into the native weedy relatives of genetically modified crops. (3) The studies to date suggest that increased resistance to insects could alter the weed status of presently innocuous weedy plants in disturbances in agricultural and native plant communities when those plant populations are limited by their insect enemies. Further research in this area is merited, and specific studies of native weedy relatives of crop plants are needed.

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PATHOGENS AND PLANT POPULATION DYNAMICS: THE EFFECTS OF RESISTANCE GENES ON NUMBERS AND DISTRIBUTION[1]

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INTRODUCTION

The scenario that has stimulated this workshop is a scary one: resistance genes developed for protecting our crops escape into natural populations of weeds and crop relatives. These populations then become resistant to the pests and pathogens that hold them in check. Resistant populations explode in abundance and invade back into our crop fields, or displace and disturb the ecological balance of our natural communities.

Circumstantial evidence that this scenario might be a cogent one comes from natural experiments that result from the inadvertent introductions of weeds from different continents. Success of such introductions is often attributed to the absence of native pathogens and pests that previously kept the plants in check in their original habitats. The success of biological control agents subsequently introduced to control these alien weeds is then cited as supporting evidence for the importance of pests and pathogens in regulating the natural populations. Conversely, introduced pests can devastate native populations, and this is further cited as evidence for their controlling influence.

However, there are many reasons why the natural experiments provided by introductions and biological control may not be good models for assessing the risks of the escape of resistance genes. First, we only focus on extreme cases and "horror stories"—the many introductions and biological control agents that fail are never at the forefront of our minds. Biological control itself is often preceded by intensive screening and deliberate selection for agents that will have a large effect. Should we then be surprised that they are sometimes effective? Second, there is the assumption that losing a whole suite and community of ecological interactants is equivalent to acquiring a resistance gene. Resistance genes are very diverse in their effects and may carry substantial fitness costs. Third, introductions and biological-control activities disrupt population genetic structure, and this itself may have a large impact on the outcomes of host-pathogen interactions. For example, it has been shown that biological control is more effective when the weed is inbred or largely clonal, than when it is outcrossing (Burdon and Marshall 1981). Understanding the relationship between disease and mating systems remains one of the most important issues in evolutionary biology.

How then do we get an alternative, perhaps more balanced view? The approach I want to take is to examine how resistance to pests might be expected to impact the abundance of their host populations, focusing on plant pathogens in particular. We can get considerable insight by examining how pathogens impact natural populations of plants, and then "imagining" what the consequences would be if those plants became resistant to the pathogens.

This paper describes some simple models of host-pathogen dynamics to establish some principles and generalities. Very similar and no more complex models are then used in a real-world context to better understand the impact that pathogens have on abundance and distribution of plants in nature. Understanding these impacts for species capable of acquiring resistance genes from crops will provide a more scientific basis for attempting to "reverse predict"

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the consequences of eliminating pathogens through the introduction of resistance.

EFFECTS OF PATHOGEN RESISTANCE GENES ON PLANT ABUNDANCE: EXISTING EVIDENCE

Learning from Theory

Although comprehensive reviews on the subject have appeared only recently, there is abundant evidence that plant diseases affect components of plant fitness in nature (Burdon 1987; Alexander 1988). However, the effect that a pathogen (or any pest) has on an individual is a poor guide to predicting the effects that the pathogen will have on a population. Far fewer studies have investigated the effect of pathogens on the abundance of species in natural populations or on the structure of whole communities.

Basic models

We will focus on models of so-called "micro-parasitic" infections (e.g., fungi, bacteria, and viruses). These models consider the host population to consist of healthy and diseased individuals and disregard the degree to which specific individuals are diseased or infected. This is in contrast to the so-called "macro-parasitic" models, where the number of pathogens or parasites per individual is considered to be an important variable.

In a diseased population, the rate at which healthy individuals become diseased is determined not just by their physiological resistance but also by the likelihood that they receive infectious stages of the pathogen from other diseased individuals. This is a function of the number and proximity of other diseased individuals in the population and on the transmission mode. In the most basic models, "resistance" is often represented by a disease transmission parameter (often denoted by the symbol β). A low value of β implies that the host is resistant, while a high value indicates it is susceptible. Healthy individuals produce new individuals (by birth or seed production) at a rate b and they die at a rate d. Disease either lowers reproductive output or increases mortality of the hosts. The magnitude of this effect is often represented by the symbol α. This parameter represents the "aggressiveness" of the pathogen (the term "virulence" is often used instead in animal and human contexts). The inverse of α represents the "tolerance" of the plant to the disease. Many such models also include the rate of recovery of diseased hosts, but to avoid parameter overload, I will ignore this for the moment. Full account of such models and their analysis is given in Anderson and May (1981).

We will use the convention that X represents the number of healthy hosts, Y the number of diseased hosts, and N the total population size (= X + Y). If disease transmission to a healthy host increases linearly with the number of diseased individuals in the population, then β N is the rate at which healthy individuals become diseased. Assuming further that the disease only affects the mortality rate of the hosts, we can generate a simple dynamical model that describes the rate of change over time in numbers of healthy and diseased individuals.

$$dX/dt = (b - d)\,X + b\,Y - \beta\,X\,Y$$

$$dY/dt = \beta\,X\,Y - (d + \alpha)\,Y$$

The sum of these gives an equation for the rate of change of total population size:

$$dN/dt = (b\text{-}d)\,N - \alpha\,Y$$

If the host is susceptible enough for the disease to spread (β large), a population represented by this model reaches an equilibrium population size (which we will call N*). In this model, N* is actually given by a somewhat involved expression ($N^* = \alpha\,(\alpha + d) / \beta\,(\alpha - b + d)$), which tells us immediately that the effect of a disease on population size is not a simple function of host resistance and disease aggressiveness.

With regard to resistance, the outcome meets our qualitative expectations. More resistance (smaller value of β) leads to a larger population size, but the effects are very non-linear. With regard to aggressiveness affecting mortality, translation of individual effect to population effect fails completely. Indeed, pathogens that are of intermediate aggressiveness have the largest effects on reducing population. By killing their

own hosts, very lethal pathogens have much less of an impact on the equilibrium population size. Resistance to very aggressive pathogens may therefore have negligible consequences for the population! This result is different for pathogens that reduce the reproductive output of their hosts without affecting their survival; in this case increasing sterility results in increasingly reduced population size, albeit in a non-linear manner.

What is the effect of the spread of a gene for complete resistance on population size? Obviously, when the disease is eliminated (β is set to zero), the population increases exponentially to infinity. Here then, in algebra, is our "scary scenario."

Effects of population regulation

We know that very few populations grow exponentially for any length of time. In most ecological settings, the birth and death rates of a host will be influenced by factors such as limited resources, predators, or alternative parasites. In other words, the population size of the host itself will be subject to “density-dependent” regulation in addition to the pathogen of interest.

When there is density-dependent regulation, the effects of a newly acquired resistance gene in increasing the population size are much less than if there is unconstrained growth of a healthy population. The population cannot "explode" because it is limited by other factors. A critical parameter now becomes the intensity of this regulation. All other things being equal, when the effects of density-dependent population regulation are more severe, the impact of a particular pathogen on equilibrium population size will be less. Therefore, an escaped resistance gene is likely to have much more effect on populations that have weak density-dependent regulation. The effect of a gene that causes broad-scale or multiple-pest resistance would be to eliminate a number of other pests, perhaps causing a concomitant loss of a suite of ecological interactions important in regulating the host population. Thus a broad scale resistance gene would have the effect of decreasing the severity of density dependence.

Effects of transmission mode

The disease transmission mode will change the model construction, as well as our predictions about the effects of introduced resistance genes. Such a transmission process may be applicable to a number of diseases important in agriculture such as fire-blight of pears and the pollen borne virus diseases.

Here, the rate at which healthy individuals become diseased is more likely to be a function of the fraction of individuals in the population that are diseased rather than a function of the absolute number that are diseased (Antonovics *et al.* 1995). In the case of such "frequency-dependent" disease transmission, the increase in population size due to resistance becomes independent of the level of density dependent regulation. Therefore, the proportional effect of introducing a resistance gene will be the same regardless of the intensity of density-dependent regulation, and knowledge of only four parameters (b, d, α, and β) is necessary to predict the magnitude of such an effect.

Genetic models

Genetic models also make the point that simplistic, seemingly "intuitive" approaches are likely to fail. Consider the matter of resistance costs. It may seem that if a resistance gene had a large cost, then it could still escape into the natural environment and spread if the benefits to the host were sufficient. It is likely that some broad scale resistance genes might be costly to the plant, e.g., constitutively induced systemic acquired resistance that activates a whole suite of pathogenesis related proteins. For example, resistance of *Silene alba* to an anther-smut disease can have costs approaching 30% (Alexander 1989; Biere and Antonovics 1996).

Analysis of a simple genetic model of resistance costs, in which resistance variation in the host was controlled by a single locus with two alleles and there was no genetic variation in the pathogen, showed that an allele that gave a very high resistance could spread even if it resulted in a large decrease in reproductive output (Antonovics and Thrall 1994). Despite an initial increase, however, the allele did not spread completely but remained in a polymorphic equilibrium with the alternative more susceptible

allele which did not lower reproductive output. Moreover, the disease could persist in such populations even if it could not persist in a population that was completely fixed for the resistance allele. The advantage of the resistance allele depended on its frequency in the population—once it reached a high frequency, disease frequency decreased and the advantage of the allele declined.

Large costs therefore do not necessarily prevent the spread of resistance alleles into diseased populations. However, if such genes do invade, they may have much less effect than would be predicted from a model where the population was monomorphic for those alleles. Monomorphism is an implicit assumption in models that only consider changes in numbers, such as those described at the beginning of this section.

Effects on a regional scale

The models developed above can be scaled up to a regional metapopulation (a collection of populations) level, with very little loss of generality. Individual populations within the larger metapopulation have a dynamic that is determined by their colonization and extinction dynamics. A region is thus conceptualized as consisting of habitat patches that may be occupied or empty. Empty patches are created by extinction of the local population and become colonized by dispersal from occupied patches.

When plant pathogens cannot exist independently of the host (as we have assumed throughout), three kinds of patches are possible: unoccupied (= "empty"), occupied by the host alone (= "healthy"), and occupied by the host and its disease (= "diseased"). Such a scenario results in a model that closely resembles the dynamics of a pathogen in a single population with density-dependent regulation. The empty patches can be seen as equivalent to a limiting resource, the occupied patches represent individual healthy hosts, and the diseased patches represent individual hosts that are diseased. A model of the form presented above is therefore usable (in a heuristic sense) to gain understanding of host-pathogen interactions at a metapopulation level.

By drawing certain parallels, it can be seen that the spread of a resistance gene may both decrease the population extinction rate and increase the reproductive (= seed) output of individual patches. However, the consequences for the regional abundance of a host now depend on the population level effects of the pathogen. The hierarchical structuring of this simple model shows that the effects of a pathogen on the individual are now subsumed two levels below its effect on abundance at a regional effect. Therefore, the magnitude of regional effects is only predictable by a study of metapopulation dynamics.

The extension of metapopulation models to include the details of host-pathogen interactions and their genetics has barely begun (Frank 1993; Thrall and Burdon 1997). This is an exciting area in which more research is needed if we are to gain anything more than an anecdotal understanding of how diseases impact plant populations of weeds, weeds harboring resistance genes from crops, or endangered species.

Disease and range extension

Limits to distribution along local ecological gradients occur when immigration and recruitment rates no longer exceed the local death rates (Watkinson 1985; Antonovics and Via 1988). At a metapopulation level, geographical limits occur as suitable patches become farther and farther apart, or as emigration from those patches or their persistence decreases (Carter and Prince 1988). The question of how disease presence (or absence due to invasive resistance genes!) affects distribution of a plant along a habitat or geographical gradient has not yet been explored.

The precise outcomes are hard to predict, especially as there will be feedback between host and pathogen dynamics. The explicit study of how pathogens limit range distribution is an area that needs serious inquiry and promises rich dividends in the future. It is critical for understanding both the risks associated with release of genetically engineered organisms and the overall impact of pests and pathogens on species distributions.

Learning from Nature: Getting Data That Says More Than the Models

The models presented here have enormous practical utility. Such models capture the essential features of the life cycles of both the host and the pathogen. They are iterative and therefore they can be used as a starting point for estimation and prediction purposes.

In most ecological settings, one simply cannot control all the factors that ecologists are prone to study (e.g., light, nutrients, soil, water, or temperature). Predicting the effects of resistance genes on population size is therefore best done by direct study of the population dynamics of the organisms at risk, rather than by extensive studies of factors that may affect these populations.

For over a decade, we have been studying the population biology of *Silene alba* (= *S. latifolia*, or white campion) and its pathogen *Ustilago violacea* (= *Microbotryum violaceum*, or anther-smut disease). The disease, somewhat unusual in being pollinator transmitted, sterilizes the host rather than increases its mortality. As a model system, it incorporates approaches that might be useful for examining both the local and regional dynamics of any naturally occurring host-pathogen system. It illustrates how population data on disease incidence, gathered over a number of years, can provide critical information for predicting the potential effects of resistance escape.

Local predictions

A minimal model of the kind described above has very few parameters and these can often be estimated from field and experimental studies (Alexander *et al*. 1996). The assumptions inherent in the model can also be confirmed by experimental and field studies (e.g., that the disease transmission term is non-linear rather than linear, and that transmission is a function of frequency of disease in the population rather than density of disease; Antonovics and Alexander 1992).

Defining symbols as described earlier, we use the following model:

$$dX/dt = (b - d)\ X - \beta\ X\ Y/N$$

$$dY/dt = \beta\ X\ Y/N - d\ Y$$

The sum of these gives an equation for the rate of change of total population size:

$$dN/dt = bX - d\ N$$

The above model contains only three parameters, and if we can estimate these, we can estimate the increase in population size due to acquisition of resistance by the host. Data obtained by averaging the results from several field experiments gave values of $b = 2.0$, $d = 0.5$, and $\beta = 5.86$ (Antonovics *et al*. 1998). Using these data in the above model and adjusting the density dependence so that the size of a healthy population would be 100, results in 18.2 healthy and 17.1 diseased individuals in the equilibrium population of 35.3. The disease reduces the population to 35% of its normal equilibrium size. Introducing a resistance gene into a diseased population of *Silene alba* would increase the population size by around 200%.

The ability of "minimal" models to predict the dynamics of single populations was elegantly shown by Thrall and Jarosz (1994a,b) also using the *Silene-Ustilago* system. They used model parameterization from field experiments in one year to predict outcomes in the following year. Their experimental populations were started either with hosts that were susceptible or hosts that were relatively resistant and were replicated over a range of initial disease frequencies. They showed that the theoretically predicted dynamics closely matched the observed dynamics and that the long-term predictions for resistant and susceptible populations were quite different. They predicted population sizes of generally around 20-40 individuals (depending on model details) in disease susceptible populations, and population sizes of 80-100 individuals in resistant (and usually disease free) populations. This is direct evidence that resistance genes have a substantial effect on the size of *Silene alba* populations.

Regional predictions

Just as it is dangerous to extrapolate the effects of a disease on an individual into population level effects, so it is potentially very misleading to extrapolate from single populations to a

regional level. This is best illustrated by first doing an overly simplistic calculation of how a 200% increase in population size due to introduction of a disease resistance gene might translate into a regional effect. For example, in our study area only about 20% of the populations are diseased. Assuming this value, four-fifths of the populations (i.e., the healthy ones) would have a size of 100, while a fifth (i.e., the diseased ones) would have a size of 35.3. Eliminating the disease would increase only this latter fifth to a size of 100. The overall impact of the resistance gene at a regional scale (about 15%) is therefore almost negligible from a practical standpoint.

However, such a simple summation fails to take into account the overall metapopulation dynamics. We have used the *Silene-Ustilago* system to model disease effects at a regional level (Thrall and Antonovics 1995; Antonovics *et al*. 1998). The main effect of the disease at a metapopulation level is that the number of sites occupied by the plant in the presence of disease is much less than in the absence of disease (17.4% vs. 51.7%; average of 10 simulation runs). The spread of a gene for complete resistance to the fungus would therefore result in a 300% increase in numbers of host populations, and this is an order of magnitude greater than predicted by summing individual population effects. There are two main reasons for this effect. First, the increased seed output of resistant populations increases the rate at which new sites are colonized. Second, the extinction rates in the model are dependent on the size of the populations (based on empirical data), and therefore the increase in the population size as a result of disease resistance also decreases extinction risk.

Our results therefore confirm a general expectation that hosts and their pests and pathogens would regulate each other to low levels in natural populations. This has the important corollary that simply assessing disease incidence at one period of time or estimating the magnitude of disease effect on individuals is a poor predictor of the impact of these agents on populations. There is a real danger in recourse to casual natural history or feelings that "well, we don't see a lot of disease." More importantly, these studies show how it is possible to understand the consequences of disease for the abundance and distribution of any species. These methodologies and principles can be applied to understanding the impact of pests and pathogens of crop relatives that may acquire resistance genes. A challenge for the future is to do precisely this and to move the regulation and management process away from being based on general impressions derived from hearsay, anecdote, and familiarity.

FUTURE RESEARCH STRATEGIES: OBTAINING EVIDENCE

Studying the effects of pathogens on plant population dynamics is a very recent enterprise. A paper on the dynamics of the anther-smut disease (Alexander and Antonovics 1988), published only a decade ago, was perhaps the first study to deal exclusively with joint numerical dynamics of a plant host and its pathogen! Only within the last few years has there been a surge of interest in applying these approaches explicitly to plant populations in natural and agricultural contexts (Thrall *et al*. 1997; Gilligan and Kleczkowski 1997; Gilligan *et al*. 1997).

The example cited here is perhaps unique in being the only case in which we have any substantial understanding of how a pathogen influences plant abundance at either a local or regional level. It is therefore not surprising that our current ability to predict pathogen effects is so often limited to discussions focused around scary scenarios and circumstantial evidence.

Experimental studies of disease effects at a population level are urgently needed to assess the role of pathogens in population regulation at both local and regional scales. We simply don't know if plant species are strongly limited in their abundance by resources or if pests play a substantial role. If pests and pathogens have a minimal effect on these species, then we have little to fear. If, however, pathogens are an important regulatory force on those populations, then the escape of resistance genes could cascade into population and community effects that might parallel the drastic effects of introduced species.

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EXTRAPOLATING FROM FIELD EXPERIMENTS THAT REMOVE HERBIVORES TO POPULATION-LEVEL EFFECTS OF HERBIVORE RESISTANCE TRANSGENES[1]

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INTRODUCTION

In 1996, transgenic crops (cotton, corn, and potatoes) containing the gene from *Bacillus thuringiensis* (Bt) that encodes for an insecticidal toxin were first commercially produced in the United States. Because we can expect the commercialization of numerous crops with Bt insecticidal genes, environmentalists have cautioned regulators to explore the risks of this new technology. In response, industry has argued that this really is not a new technology, since plant breeders have a long history of artificially selecting for herbivore-resistant crop varieties using conventional methods. Both sides of this debate have merit—herbivore resistance is in fact a selected trait in many crops, but on the other hand these particular Bt genes are entirely novel to the plant species into which they are introduced. For instance, a Bt gene product that acted as an insecticide against cabbage worms (*Pieris rapae*) inserted into a *Brassica* crop would be expressed as an entirely novel trait—*Brassica*s that are immune to attack from cabbage worms have never been produced by artificial selection. Rather than splitting hairs about what is "novel," the more pertinent question is: what are possible effects of Bt resistance genes entering wild populations of plants? The assumption here is that the genes will escape into wild populations; even if gene flow is extremely infrequent, extensive commercialization makes such a scenario highly likely. To answer this question we examine quantitative field experiments that have manipulated the number of herbivores attacking plants in a manner that well simulates the action of a Bt gene. Second, we use the effects detected by these field experiments to simulate a "risk assessment" and compare how well various risk assessment strategies would fare in predicting long-term population trends. Finally, we discuss results from previous studies that add insight into the challenge of conducting a risk assessment for transgenic crops containing resistance genes.

SYNTHESIZING DATA FROM FIELD EXPERIMENTS THAT REDUCE HERBIVORE ATTACK RATES

To assess the potential risks associated with the escape of herbivore resistance genes into wild populations of plants, we reviewed the strength of herbivore effects documented in the recent ecological literature. Specifically, we compiled and reviewed a collection of studies that compare the reproductive performance of plants protected from herbivores versus plants exposed to herbivores. The increased success of plants in the absence of herbivores provides a hint of how the performance of a non-crop plant might be affected if it were to obtain herbivore resistance genes from a transgenic crop. We used a formal statistical approach called meta-analysis (Hedges and Olkin 1985) to quantify the overall effects that insect herbivores have on plant reproductive success. Meta-analysis involves standardizing the difference between treatments in each experiment and using these standardized differences as individual data points. We searched the contents of eight ecological journals (volumes from January 1983-June 1997 of *American Naturalist, Ecological Applications, Ecological Monographs, Ecology, Journal of Applied Ecology, Journal of Ecology, Oecologia,* and *Oikos*) and selected terrestrial field studies that measured plant reproductive responses to manipulated densities of insect and/or mollusc

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herbivores. We excluded agricultural and greenhouse studies because we were interested in assessing the effects of real herbivores on natural plants and maximizing the relevance of our findings to our scenario of a transgenic weed that has obtained herbivore resistance genes. To minimize the number of non-independent contrasts, we used only one measure of plant reproduction for each experiment, according to the following ranking (starting with most preferred): viable seeds per plant, total seeds per plant, fruits per plant, inflorescences or flowers per plant, seeds per inflorescence, fruits per inflorescence, and fruits per initiated bud.

We located 18 publications that satisfied our criteria (Table 1). Several of these papers reported data from multiple experiments, multiple plant species, or multiple sites, resulting in a total of 52 comparisons. This data set includes responses of 28 plant species in 19 genera (Table 1). To compare the strength of herbivore effects, we used the weighted standardized mean difference, Hedges' *d*, as the measure of effect size for each study. Hedges' *d* is calculated as the difference between the means of the experimental and control treatments divided by the pooled standard deviation, weighted by sample size (Hedges and Olkin 1985). This metric compares the difference *between* treatments to the difference *within* treatments: the numerator is the difference between the means of the two groups and the denominator is the presumably random variation within groups. Thus, using this metric we can determine whether the difference in reproductive performance between treatments is large enough that it probably did not occur by chance alone. We defined as controls the treatment that exposed plants to a natural abundance of herbivores. The sign of *d* was reversed for studies that augmented herbivores. Thus, a positive value of *d* indicates an increase in reproductive performance in the absence of herbivores. For example, a study might report that plants protected from herbivores produced 165 seeds on average, whereas those exposed to herbivores produced 150 seeds on average, with a standard deviation within each group of 25 seeds. For this study, the average difference between treatments is $d = (165 - 150) / 25 = 0.6$ standard deviations. Assuming that seed production is normally distributed, $d = 0.6$ means that the average protected plant produced more seeds than 73% of the exposed plants. By convention, $d = 0.2$ is considered a small effect, $d = 0.5$ a medium effect, and $d \geq 0.8$ a large effect (Cohen 1969). We calculated *d* for each comparison and then combined these values across comparisons. The statistical significance of *d* can be simply assessed by examining whether its 95% confidence interval overlaps with zero. We also compared the strength of herbivore effects between studies that excluded herbivores versus those that augmented herbivores using mixed model homogeneity analysis, roughly analogous to mixed model analysis of variance.

The meta-analysis of our collection of herbivore studies demonstrated that, on average, herbivory caused a statistically significant and large reduction in plant reproductive performance (Figure 1; overall effect size = 0.86; 95% CI= 0.55-1.17; n = 52). Note that $d = 0.86$ standard deviations means that the average protected plant (such as might occur with a Bt resistance gene) produced more reproductive structures than 81% of the unprotected plants. If we make the analogy between the herbivore treatments used in these experiments and two plant genotypes (herbivore resistant vs. susceptible), the strength of the observed effects could represent extremely large selection coefficients favoring resistance. Studies that augmented or enclosed herbivores onto plants demonstrated a slightly higher effect of herbivory than studies that excluded herbivores. However, the difference between these types of experiments is clearly not statistically significant, as can be assessed simply by noting the extensive overlap of the 95% confidence intervals (Figure 1). Another way to place the result of this meta-analysis for herbivore protection in context is to compare it to the *d* calculated for experiments in which competitors, as opposed to herbivores, are removed and plant response is measured. Whereas protection from herbivory yielded a *d* of 0.86, the effect of competition on plant biomass was significantly smaller ($d = 0.34$; 95% CI = 0.29-0.39; n = 74; Gurevitch *et al*. 1992).

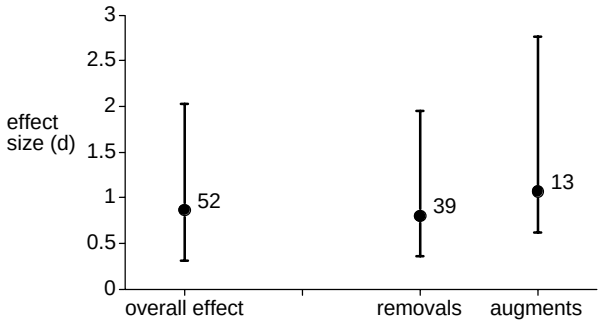

Figure 1. Effect of insect and mollusc herbivores on plant reproductive success. Error bars are 95% confidence intervals with number of comparisons within each group indicated. Positive values of *d* indicate an increase in reproductive performance when herbivores were excluded (the sign of the effect was reversed for augment studies).

One caveat from this analysis concerns its relevance to the insertion of a Bt gene. In particular, do the levels of herbivore protection used as treatments in the experiments listed in Table 1 reflect the likely levels of herbivore protection afforded by a Bt gene, which typically works against only a subset of herbivore species? Most of the reduced herbivore treatments in Table 1 targeted only a subset of herbivores (just as Bt does) and not all herbivore species. Of course, the treatments tended to focus on dominant herbivores, but this is exactly the case with Bt (one does not insert Bt genes into a group of plants to target minor herbivores). Indeed, 10 of 18 studies in Table 1 removed five or fewer species of herbivores and 8 removed only one herbivore species. Secondly, the main lesson from Figure 1 is that herbivory is clearly a potent demographic factor in natural populations of plants. Thus, the generality evident from field experiments is that any trait that protects a plant against a major herbivore will likely enhance that plant's rate of reproduction.

Table 1. References used for meta-analysis. These studies measured the effects of manipulated insect and/or mollusc densities on plant reproductive performance.

Citation	Plant spp
Bergelson 1990	*Senecio vulgaris*
Brown *et al.* 1987	*Vicia sativa* and *V. hirsuta*
Declerck-Floate and Price 1994	*Salix exigua*
Fay and Hartnett 1991	*Silphium integrifolium*
Greig 1993	*Piper arieianum, P. culebranum, P. phytoaccaefolium, P. sacti-felicis,* and *P. urostachyum*
Hanley *et al.* 1995	*Agrostis capillaris, Ranunculus acris, Senecio jacobae, Stellaria graminea,* and *Taraxucum officionale*
Islam and Crawley 1983	*Senecio jacobaea*
James *et al*. 1992	*Senecio jacobaea*
Jordano *et al*. 1990	*Astragalus lusitanicus*
Kelly 1982	*Euphrasia pseudokerneri* and *Linum catharticum*
Louda and Rodman 1996	*Cardamine cordifolia*
Louda 1983	*Happlopappus venetus*
Parker 1985	*Gutierrezia microcephala*
Peart 1989	*Anthoxanthum odoratum*
Pysek 1992	*Senecio ovatus*
Reader 1992	*Medicago Iupulina* and *Centaurea nigra*
Rosenthal and Welter 1995	*Zea diploperennis* and *Zea mays parviglumis*
Sacchi *et al*. 1988	*Salix lasiolepis*

SIMULATION OF HERBIVORE RESISTANCE AND PLANT POPULATION GROWTH

One reason environmentalists are concerned with plant resistance genes is the worry that these genes may exacerbate weed problems. It is important to note that "weed" is used here in a broad sense, not simply to include agronomic problems—indeed, exotic invasive plants are the major threat to maintaining native vegetation and biodiversity within the nature reserves of North America (US Congress, 1993). The question is whether a plant like a weedy *Brassica* might acquire a Bt gene and, because of reduced herbivore pressure, become more invasive in natural communities. Such an increase in invasiveness might actually be more likely in natural communities, which, unlike agricultural settings, harbor large populations of insect herbivores because they are not treated with insecticides. Clearly, any change that increases rates of population growth will cause a plant to become more invasive. However, our simulation was aimed at a slightly different question: given the variability in "effect size" uncovered by our meta-analysis, how likely are short-term field experiments to detect the "true" effect of an herbivore-resistance gene in a wild plant? This is a pertinent question because in the absence of a "similarity argument" (which cannot be applied to a novel trait like production of Bt toxin) direct experiments are a major tool for risk assessment.

First, we compared rates of population growth obtained by using effect sizes either from herbivore augmentation studies or from herbivore removal studies. Second, we asked how the number of sites used in a field trial affects our conclusions about the risks posed by herbivore resistant weeds. We were particularly interested in how these methodological differences affect the frequency with which we would *erroneously* conclude that gene escape would not be a significant problem. The model of plant population growth was built on a few simplifying assumptions. First, we assumed that herbivore susceptible (control group) plant populations are stable. We set mean production of seeds to 20 per individual with the standard deviation in seed production equal to 10. This rate of seed production combined with the probability of survival from seed to adult = 0.05 gave stable populations of herbivore susceptible plants. We assumed that plant populations grow exponentially and that population growth is seed limited, so an increase in seed production due to herbivore resistance translates directly into a higher rate of population growth.

For our first set of simulations we used the results from all herbivore removal studies as one pool of effect sizes and results from all herbivore augments as a second pool of effect sizes. Assuming equal variation in the control and herbivore resistant groups, the mean performance of herbivore resistant (HR) individuals in any given year is: $(\text{mean seeds per HR individual})_t = \text{sd} * (\text{effect size})_t + \text{mean seeds per control individual}$. We then drew the population size for the next year from a normal distribution with mean = (mean performance per HR individual * N_t), and standard deviation = $(\text{sd}^2 * N_t)^{0.5}$, where N_t is the number of individuals in the population at time t. We grew the population for 50 generations, calculated λ, the population growth rate for each run and found the average and standard deviation of λ across 100 replicate runs of the model. Populations of herbivore resistant weeds are predicted to grow quickly when effect sizes are drawn from removal studies and even more quickly when effect sizes are drawn from augment studies (Figure 2).

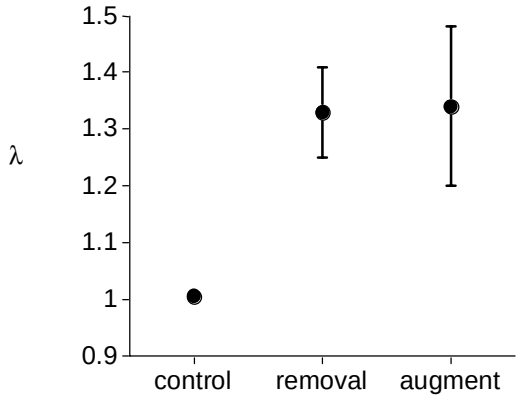

Figure 2. Population growth rate (λ) for herbivore susceptible plants (control group) versus herbivore resistant plants (removal and augment groups). Mean λ's are for 100 replicates. Error bars are one standard deviation.

We also used our simulations to examine how variation in herbivore impacts affects our ability to predict the risk associated with herbivore resistance genes. For this set of simulations we assembled lists of effect sizes reported from multiple sites but for a single plant species. From the set of papers used in the meta-analysis, we found three papers that reported data for more than two unique sites (Table 2). For each iteration, the model selects a subset of the effect sizes reported for any one plant species with 1, 2, ... n sites per subset where n equals the total number of effect sizes reported. Growth of the plant population is then projected using effect sizes randomly selected each year from the subset. We calculated λ for each iteration of the model and found the average and standard deviation for λ across 500 replicate runs. We also calculated an error rate for each set of simulations by recording how many times we would erroneously conclude that the herbivore resistant plant was not as significant a pest as indicated by the simulations using data from all possible sites. For example, we recorded how often λ was predicted to be less than 1.0, 1.05, and 1.1.

Measuring the impacts of herbivores at multiple sites gives a more consistent estimate of λ (Figure 3: standard deviation decreases as the number of sites increases), and the magnitude of herbivore effects is less likely to be underestimated as more sites are sampled (Figure 4: error rate is dramatically reduced as more sites are sampled). The results presented in Figures 3 and 4 are for simulations based only on the results of Jordano *et al*. (1990), but our findings were qualitatively consistent across sets of simulations tailored to the additional studies listed in Table 2. Clearly, the potential risks associated with herbivore resistance genes can only be accurately assessed when trials are performed at multiple sites that offer potentially different environments for plant growth as well as different background densities of herbivores.

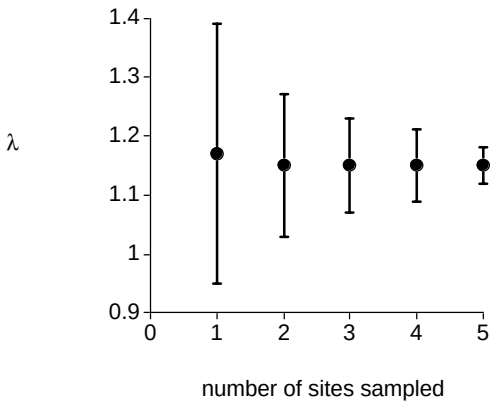

Figure 3. Risk posed by herbivore resistance genes. Scatter points are mean λ's for 500 replicate populations of herbivore resistant plants. Larger values of λ indicate increased potential weediness of resistant genotypes. The number of sites used to estimate λ was varied up to the total number of sites reported by Jordano *et al.* (1990). Error bars are one standard deviation.

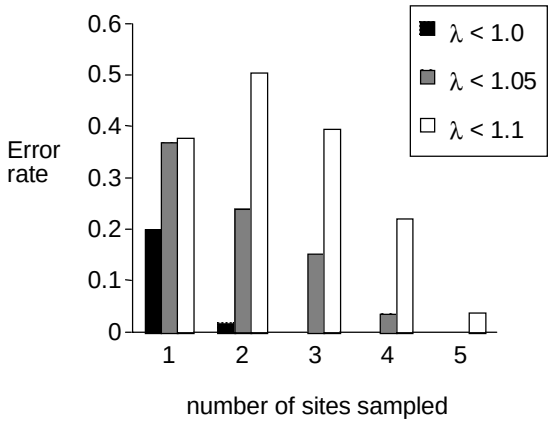

Figure 4. Rate of underestimation of the risk posed by herbivore resistant genes. When all five of the sites from Jordano *et al*. (1990) are used, the mean λ is 1.15 (Figure 3). Error rate is the proportion of the 500 replicates where the predicted λ was less than either 1.0, 1.05, or 1.1.

Table 2. References that tested herbivore effects at more than two unique sites.

Reference	Plant species	Herbivore	Comparison
Jordano *et al*. 1990	*Astragulus lusitanicus*	Lepidopteran seed predators	5 sites
Louda 1983	*Haplopappus venetus*	Mixed insect herbivores	3 zones
Brown *et al*. 1987	*Vicia hirsuta*	Folivorous and sucking insects	3 successional stages
Brown *et al*. 1987	*Vicia sativa*	Folivorous and sucking insects	3 successional stages

TIME LAGS, MONITORING, AND OTHER WORRIES ABOUT ANTI-HERBIVORE INSECTICIDES IN PLANTS

Two points emerge from the analyses above: (i) protecting a plant against herbivory, even against just one species of herbivore, is likely to markedly increase that plant's rate of reproduction, and (ii) field tests for assessing a plant's enhanced invasibility are prone to mistakenly assure safety unless they are repeated at multiple sites or under multiple conditions. Both of these points have been anticipated previously (e.g., Williamson 1993; Parker and Kareiva 1996; Kareiva *et al*. 1996) with the admonition that caution and tenacious monitoring are warranted for certain transgenic crops. Although it will be hard to exercise that caution given the current pressure to ease regulations on the basis of the safe record to date, caution should clearly be maintained. A survey of historical records for past invasions by weeds in the northwestern United States indicated that the median timelag between the first record of a weed and the onset of widespread infestation was on the order of 30-50 years (Marvier *et al*. 1999). Timelags between the introduction of ornamental woody plants and their escape into the wild in Germany are on the order of 150 years (Kowarik 1995)! Moreover, for most monitoring efforts, early detection of weed problems will remain unlikely and timelags are still expected on the order of decades (Marvier *et al*. 1999). Examples from the "exotic species" literature are often rejected in the biotechnology arena because it is pointed out that exotic species contain thousands of "novel genes" whereas a transgenic plant contains only a few novel genes. However, the point of learning lessons from exotic species is NOT to claim that such introductions are one and the same as those of transgenic plants, but to point out some inherent features of biological invasions. Those features include extensive timelags and the observation that most invasions require the chance concordance of a suite of favorable conditions before taking off. It is entirely reasonable to expect that invasions of transgenes will share these features.

WHAT DOES THIS MEAN FOR RISK ASSESSMENT?

Like most biological invasions, the majority of transgenic plants will not become pests, and most will have minimal impact. However, it would be arrogant to assume that transgenic plants represent a fail-safe technology. We have previously witnessed such brazen overconfidence. For example, in 1947, the President of the Entomological Society of America gave a presidential address commenting on the implication of pesticides for the control of pest insects. He said,

> *"The recent progress in the development of new insecticides has not been equaled in all history . . . at no previous time in history have the achievements of entomologists been of such universal value . . . . The entomologist has become a wizard in the eyes of the uninitiated—and indeed some of the achievements seem little short of magic. . . ."*
>
> Lyle, C 1947 Presidential Address entitled, "Achievements And Possibilities In Pest Eradication."

We are in danger of repeating this blind arrogance with transgenic plants. Fortunately, biotechnology truly has the potential to promote

sustainability in ways that chemical insecticides never could. Nonetheless, because engineered resistance genes, such as Bt endotoxin, are entirely novel to their recipient plants (indeed to the entire plant kingdom), they do entail some risk. In addition, we know from ecological field experiments that protection of plants against even small subsets of herbivore species generally causes dramatically enhanced seed production. The information that remains lacking is a broader understanding of what regulates weedy plant populations in nature. Thus, the way to enhance risk assessment of transgenic plants is to explore weed population dynamics and the role of biotic stresses (herbivores and pathogens) in governing those population dynamics. Until we have that understanding, the most sensible strategy is a triage approach that recognizes that most transgenes are likely to be safe, but that resistance genes introduced into crops with close, weedy relatives bear extra scrutiny. A few experiments under a narrow range of conditions should not be accepted as proof of safety; neither should comfort be drawn from any argument of "similarity"—production of Bt toxin is an entirely novel plant trait. Finally, we should not allow a prior record without ecological ill-effects to lull us into complacency—large time lags between introduction and the onset of weed spread are the norm, and we have not been looking very long or all that closely for problems involving transgenes. Herbivory is a potent demographic force in plant populations, and the implications of resistance for plant invasiveness cannot be easily extrapolated across sites or over long periods of time.

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WORKING GROUP REPORTS

REPORT OF THE BERRY WORKING GROUP[1]

(Strawberry, Raspberry/Blackberry, Blueberry)

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STRAWBERRY (*FRAGARIA*)

Strawberries are a relatively recently domesticated crop. The most commonly cultivated strawberry, *Fragaria* x *ananassa*, is a hybrid of the North American *F. virginiana* and the South American *F. chiloensis* (Maas 1998). These parental species are still grown in some areas and *F. virginiana* is the primary wild, sexually compatible relative to the cultivated strawberry. In addition to *F. virginiana*, *F. vesca* and its subspecies are also present in the United States. *Fragaria* x *ananassa* and *F. virginiana* readily cross. Introgression of pest resistance traits into the wild strawberry population is likely, as substantial amounts of crop-weed introgression has already occurred throughout the midwest, northeast, and southeast United States (Jim Hancock pers. comm.). Introgression has occurred to the extent that it is difficult to find "pure" populations of *Fragariae virginiana* in many areas. Strawberries suffer from several limiting diseases, insects, nematodes, and weed problems (Table 1).

In addition to those listed above, resistance to root-lesion nematode (Potter and Dale 1994), *Phytophthora cactorum*, *Sphaerotheca macularis*, and strawberry aphids (Shanks and Moore 1995) have been identified in cultivars of strawberry. Much of the pest resistance that has been incorporated into strawberry breeding programs has been derived from wild relatives. A substantial effort has been targeted specifically towards *Phytophthora fragariae* resistance (van de Weg *et al.* 1997), but in most breeding programs, elite types have been screened after selection for other horticulturally important traits. New efforts to breed for resistance to *P. fragariae* and *P. cactorum* are underway (Maas *et al*. 1993).

Strawberry is considered to be relatively amenable to transformation using *Agrobacterium* (Nehra *et al.* 1992), and the technique is being used to genetically engineer virus resistance. Strawberry plants have been transformed using the coat protein gene from strawberry mild yellow edge virus (Finstad and Martin 1995), and resulting plants are being evaluated. Other traits that are currently under investigation include glyphosate resistance, broad-spectrum fungal resistance through the use of the stilbene synthase gene and genes for systemic acquired resistance, and nematode resistance through the use of transgenes producing protease inhibitors (Morgan and Gutterson 1998).

Although sexually compatible relatives to strawberry are found in the United States, they are not considered to be a weed problem in strawberry fields. Cultivated strawberries are not capable of persisting outside the area of cultivation in the California production system, but have been found to escape in many areas of the midwestern and southern United States. Strawberries lack significant weedy characteristics and the addition of pest resistance would be unlikely to substantially increase the crop's ability to persist.

[1] Group Report from the "Workshop on Ecological Effects of Pest Resistance Genes in Managed Ecosystems," in Bethesda, MD, January 31 – February 3, 1999. Sponsored by Information Systems for Biotechnology.

Table 1. Strawberry pests and status of resistance in cultivated and wild species.[1]

Strawberry Pest	Status of Resistance in Cultivated Varieties	Resistance in Wild Relatives
Angular leaf spot *(Xanthomonas fragariae*[2)]	Some cultivars with resistance	*F. moschata* tolerant. *F. virginiana* and *F. vesca* moderate resistance (Maas 1998; Maas, Pooler, and Galletta 1995)
Leaf scorch (*Diplocarpon earlianum*[2])	No commercial resistance; Resistance in wild relatives and some non-horticultural varieties (Xue *et al*. 1996)	Not reported
Leaf spot (*Mycosphaerella fragariae*[2])	Commercial cultivars with resistance (Darrow 1962; Horn, Burnside, and Carver1972; Nemec 1971)	Not reported
Botrytis fruit rot (*Botrytis cinerea*[2])	[3]Engineered resistance; Resistant cultivars	Not reported
Anthracnose *(Colletotrichum* spp.[2])	Resistant cultivars through directed breeding (Olcott-Reid and Moore 1995)	Not reported
Red stele root rot (*Phytophthora fragariae* var. *fragariae*[2])	[3]Resistance genes identified to races 1 and 2; Resistant cultivars through directed breeding (Scott *et al*. 1976; 1984)	*F. virginiana* (Gooding 1973) *F. chiloensis* (Galletta *et al*.1994)
Verticillium wilt *(Verticillium* spp.[2)]	[3]Engineered resistance; Moderate resistance in some cultivars (Shaw *et al*. 1997)	*F. chiloensis* (Shaw *et al*. 1996)
Black root rot (*Pythium ultimum*, *Rhizoctonia fragariae*, and *Pratylenchus* spp.[2])	[3]Engineered resistance to *Pythium* spp.; Moderate, regional resistance in some cultivars (Wing *et al*. 1995)	Not reported
Strawberry mottle virus	[3]Coat protein mediated resistance	Not reported
Strawberry mild yellow edge virus	[3]Coat protein mediated resistance	Not reported
Pratylenchus, *Aphelenchoides*, *Xiphinema*, *Belonolaimus*, *Meloidogyne*[2]	[3]Protease inhibitor transgenes. Tolerance in some cultivars	*F. chiloensis* and *F. virginiana* (Potter and Dale 1994)
Spider mite (*Tetranychus urticae*[2])	Cultivar resistance (Easterbrook and Simpson 1998)	*F. chiloensis* and *F. virginiana* (Shanks and Moore 1995; Easterbrook and Simpson 1998)
Lygus bugs (*Lygus lineolaris*[2])	Cultivar resistance	*F. virginiana* and *F. chiloensis* (Maas 1998)
Bud weevil (*Anthomonomus signatus*)	No commercial resistance	Not reported
Flower thrips (*Franliniella* spp)	No commercial resistance	Not reported
Sap beetles (*Stelidota geminata*)	No commercial resistance	Not reported
Root weevils (*Otiorynchus* spp)	No commercial resistance	Not reported
Weeds	[3]Round-Up Ready	None

[1] Farr *et al*. 1989; additional information compiled from Maas 1998
[2] Pest occurs on wild relatives, resistance derived from or identified in wild populations
[3] Under consideration or in development

WHAT IS NEEDED?

The impact that pest populations have on the spread of wild, sexually compatible relatives is not clear. It is assumed that the environment limits the growth and spread of strawberries more than pest pressure. Little evidence of disease has been noted on the leaves and fruit of natural populations, but the root pathogens of wild strawberry have not been characterized at all; therefore broad-spectrum resistance to fungal plant pathogens may or may not provide an advantage. Information is also lacking on the competitive interactions within natural communities, and we do not know if engineered traits could make the strawberry a more effective competitor within its natural community. For example, even if the insertion of a pest resistance gene did not cause wild strawberry to be a significant weed problem in agriculture, are there endangered plants that might be replaced by a more aggressive strawberry? This information could minimize any concerns that the environmental community might voice about minimizing diversity in native plant populations. It would be helpful to determine if broad-spectrum resistance to fungal plant pathogens already occurs in native species.

Experiments are currently underway in which *Frageriae chiloensis* and *F. virginiana* growth is being compared in methyl bromide fumigated and non-fumigated soils. This will provide information concerning general pest resistance in the native species. Lists of endangered species and primary locations are maintained by various government agencies. These could provide information concerning the co-existence of wild strawberry relatives and endangered native species.

Strawberry producers have used pest resistance genes incorporated through conventional breeding for several decades. These resistant cultivars have been grown over large acreages for long periods of time, but increased weediness of strawberry has not been observed. There does not appear to be any evidence that there should be concern about the introduction of pest resistance genes in this crop, particularly those that are specific to a single pathogen.

RASPBERRY/BLACKBERRY (*RUBUS*)

Cultivated raspberries and blackberries are a diverse group. Most species have perennial root systems and biennial canes; however, some produce perennial canes, and others annual canes. Species producing edible raspberries that are used commercially include *R. idaeus* subsp. vulgatus, *R. idaeus* subsp. strigosus, *R. occidentalis*, and *R. glauca*. Commercial blackberries are most commonly in the subgenus *R. eubatus*. Hybrids between blackberries and raspberries are also commonly grown. Several *Rubus* species are found wild within the United States. Crosses within each subgenus are common and crosses between the subgenera are viable at higher ploidy levels. Diploid hybrids between *R.* subg. *Ideobatus* and *R.* subg. *Eubatus* are usually sterile.

Raspberries suffer from a variety of diseases, the most important of which are summarized in Table 2. Wild brambles are a problem weed in raspberry and blackberry production. Cultivated bramble primocanes are controlled in some areas using herbicide application. This practice accounts for a large portion of the weed management as well. In addition, weed control between rows is achieved through clean tilling, mulch, and herbicide application. Herbicides that are used to control *Rubus* spp. include imazapyr, sulfometuron-methyl, glyphosate, tebuthiuron, picloram, and hexazinone. Genes for resistance to pests, such as aphid and raspberry bushy dwarf virus resistance, were incorporated into raspberry cultivars and have been used since the 1940's. There is no evidence that these traits have caused an increase in the weediness of the species grown as crops. Red raspberry is not a weedy plant in areas where it has been grown commercially since the 1920's. There is also no evidence that these resistance traits have conferred any advantage to wild populations.

Most of the diseases that occur on cultivated *Rubus* spp. are likely to occur on the native wild relatives as well (Farr *et al*. 1989). Accordingly, most of the resistance genes that have been incorporated into commercially-grown species have been derived from wild relatives. Since the resistance genes introduced through breeding efforts have come from native species, there is a

high degree of familiarity with the traits that are being used in genetic engineering. However, there is the possibility that genes conferring broad-spectrum resistance could contribute to weediness of native species, but this risk is difficult to assess because little information exists concerning the impact that pathogen complexes have on wild relatives.

Introgression of pest resistance traits into wild *Rubus* populations is very likely and has occurred where commercial varieties are the same species as the native *Rubus*. In areas where different species exist together, introgression is also likely to occur, but at a slower rate. The consequences of pest resistance genes moving into the native species are considered to be of minimal risk in cases in which similar resistance phenotypes already occur in native species. Herbicide resistance would not be recommended.

WHAT IS NEEDED?

An extensive literature search on the occurrence of pathogens and pests on native species would contribute significantly to determining the impact of broad-spectrum resistance genes. For example, it would be important to determine if the Himalaya berry (*R. porcerus*) is sexually compatible with native and commercial *Rubus* spp.. While the majority of diseases found on cultivated red raspberry (*R. idaeus*) and blackcap (*R. occidentalis*) also occur on wild relatives, no information is available that would indicate whether or not these pests are limiting the spread of the wild species.

A survey of the authorities on *Rubus* spp. could be implemented to determine what "anecdotal" information exists about pest epidemics in native *Rubus*. When information is lacking, disease surveys could be conducted. Experiments could be conducted using a large number of genotypes of a single potentially weedy species. These plants could be used to screen for levels of resistance that might occur in native populations. Plants could be inoculated with a wide range of potential pathogens to determine if broad-spectrum resistance already exists. Due to the wide range of genetic variability within species and the distribution of native species, small-scale field trials to determine the extent of resistance in natural populations are less applicable than trials that test a wide range of genotypes.

Table 2. Raspberry and blackberry pests.[*]

Rubus Pest	Status of Resistance
Anthracnose (*Elsinoe veneta* [1])	Available through conventional breeding
Cane blight (*Leptosphaeria coniothyrium*[1])	Red raspberry-*R. pileatus* hybrids
Spur blight (*Didymella applanata*[1])	Conferred through "H gene." *Rubus* spp.
Gray mold and Fruit rot (*Botryotinia fuckeliana*[1]) (anamorph: *Botrytis cinerea*)	Engineered
Orange rust (*Arthuriomyces peckianus* and *Gymnoconia nitens* [1])	Blackberry and red raspberry
Phytophthora root rot (*Phytophthora* spp. [1])	Cultivars of red raspberry
Bluestem (*Verticillium* spp. [1])	None
Raspberry bushy dwarf virus	Engineered

[*] Ellis *et al*. 1991
[1] reported from wild relatives

BLUEBERRY (*VACCINIUM*)

Four species of blueberries are cultivated: highbush (*V. corymbosum*), lowbush (*V. myrtilloides* and *V. angustifolium*), and rabbiteye (*V. ashei*) (Caruso and Ramsdell 1995). Highbush blueberries are the most commonly cultivated of the group, with approximately 100 cultivars; the most common is Bluecrop. More than 20 cultivars of rabbiteye have been developed, and although cultivars of lowbush blueberry have been developed, these are rarely planted. Highbush and rabbiteye blueberries are planted in rows, which may be in raised beds. Low vigor canes are removed annually from highbush types everywhere, and bushes are regularly hedged in the southeast. Lowbush blueberries are allowed to grow in natural stands. These are managed with mowing or burning to rejuvenate stands.

Weed management is extremely important in blueberry production; the plants are not strong competitors with most weeds. Pre-plant weed control is of utmost importance. In established fields, mulching, cultivation, and herbicide application are used in an integrated approach to weed management.

Feral blueberries are not found in agricultural fields and would be unlikely to become weeds due to the introduction of pest resistance traits. Highbush blueberries are very closely related to wild relatives, and lowbush blueberries are undomesticated from a breeding standpoint. Pests occurring on blueberries in commercial areas occur on other native species (Table 3) (Farr *et al*. 1989). Blueberry viruses, such as shoestring and leaf mottle, have been documented in wild populations.

Introgression of pest resistance traits into wild *Vaccinium* is assumed to be due to their genetic similarity. Numerous hybrid swarms between cultivated and wild species exist in Michigan. However, it is highly unlikely that additional traits would lead to an increase in weed problems with this group.

In the case of all three of the berry groups discussed, it is unlikely that pest resistance genes that target a single pathogen or group of insects would cause significant increases in weed problems. There is concern, however, about broad-spectrum resistance genes. It was determined that a simple survey of authorities should be made to increase our knowledge base. A single question could be posed:

> "What diseases, nematodes, or insect pests have you observed on native species of *Rubus*, *Fragariae*, or *Vaccinium*? Based on information from your observations (not lists of diseases in the literature), please indicate the relative abundance or impact of these pests on the native species."

Table 3. Blueberry pests.

Blueberry Pest	Status of Resistance
Phytophthora root rot *(Phytophthora cinnamomi*[1])	Some (highbush and rabbiteye)
Botrytis blight (*Botrytis cinerea*[1])	None
Mummy berry (*Monilinia vaccinii-corymbosi*[1])	None
Stem blight (*Botryosphaeria dothidea* [1])	Limited (highbush)
Stem canker (*Botryosphaeria corticis*[1])	Limited (highbush)
Bacterial canker (*Pseudomonas syringae*)	Highbush only
Blueberry scorch carlavirus[1]	Highbush only
Blueberry shock ilarvirus	
Blueberry shoestring sobemovirus[1]	Highbush
Xiphenema americanum, Pratylenchus penetrans, and *Meloidogyne carolinensis* [1]	Cultivars available

[1] pests known to occur on other *Vaccinium* spp.

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REPORT OF THE *BRASSICA* CROPS WORKING GROUP[1]

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INTRODUCTION

The Brassicaceae family comprises about 3000 species, the majority of which are found in the Northern Hemisphere. Many common agricultural weeds, such as *Brassica nigra* (L.) Koch, *Brassica rapa* L., *Cardaria draba* (L.) Desv., *Raphanus raphanistrum* L., and *Sinapis arvensis* L., belong to this family. The most important crop species from this family are the oilseed Brassicas; *Brassica napus* L., *B rapa* L., and *B juncea* Coss., which are generally referred to as rapeseed, oilseed rape, or canola. Other widely cultivated species in this family are: *B. oleracea* L. (cabbage, kale, kohlrabi, Brussels sprouts, cauliflower, and broccoli), *B. chinensis* L. (syn. B. *napus* var. *chinensis*; Chinese cabbage), *Raphanus sativus* L. (radish), and *Armoracia rusticana* Gaertn. (horseradish). We agreed to limit our discussion to the rapeseed species because they are the focus of most of the gene transfer technology. Worldwide, rapeseed is grown on more than 20 million hectares; it is the third most important oil plant after palm oil and soybean. Major producers are China, India, Canada, the European Community, and Australia.

Reproductive Biology

Brassica napus can be self-pollinated or cross-pollinated. In cultivated fields, cross-pollination rates of about 20-30% have been reported (Rakow and Woods 1987). The frequency of cross-pollination is influenced by weather, availability of insect pollinators, and cultivar. *Brassica rapa* varieties are generally self-incompatible. Both species are primarily pollinated by honeybees (Williams *et al*. 1987), however wind pollination is possible over distances of up to 2.5 km (Timmons *et al*. 1995). *B. rapa* is a diploid with chromosome number of 2n=20, and *B. napus* is an allotetraploid with a chromosome number of 2n=38 (Hosaka *et al*. 1990).

Hybridization

Crosses between *B. napus* and other related species occur, but the rate of success varies depending on the species. The following spontaneous (without emasculation or manual pollination) hybridizations have been documented: *B. napus* × *B. rapa*, *B. juncea* × *B. napus*, *B. nigra* × *B. napus*, *B. napus* × *Hirschfeldia incana*, and *B. napus* × *Raphanus raphanistrum*. In all of the above hybridizations, F_2's and backcross progeny were produced (OECD 1997).

[1] Group Report from the "Workshop on Ecological Effects of Pest Resistance Genes in Managed Ecosystems," in Bethesda, MD, January 31 – February 3, 1999. Sponsored by Information Systems for Biotechnology.

Seed Dormancy

Seed dormancy in crop plants is generally undesirable because dormant seeds may germinate at inappropriate times, and because additional seed is required to compensate for the proportion that will not germinate. Breeders of *B. rapa* have not yet succeeded in removing this weedy trait completely. Seed dormancy is much less of a problem in *B. napus*, whose mature seed has virtually no primary dormancy (Lutman 1993; Schlink 1994). It is possible however, that *B. napus* seed can acquire secondary dormancy and remain viable in the soil for at least 5 years (Schlink 1994). Secondary dormancy can be induced in the absence of light when available moisture is insufficient for germination, conditions that may occur when seeds are incorporated into the soil after harvest (Pekrun *et al*. 1997; Pekrun *et al*. 1998).

In weedy relatives of rapeseed, seed dormancy is a very powerful survival mechanism. Dormant seeds may germinate over a period of several weeks during the growing season, which greatly improves the chance that at least some plants will be successful in replenishing the seedbank. The presence of flowering individuals during a large portion of the growing season increases the opportunity for cross-pollination with populations that produce only one cohort per season. Furthermore, seed dormancy may allow a plant population to survive one or several seasons of complete reproductive failure. Whether or not a particular hybrid will exhibit seed dormancy is rather unpredictable (Landbo and Jørgensen 1997).

Novel Resistance Traits

Within the last ten years, numerous novel traits have been genetically engineered into the *Brassica* genome, however, only a relatively small number have found their way into commercial varieties. Some of these traits were introduced to confer resistance to herbicides, insects, and disease organisms. The herbicide resistance traits were particularly successful because they provided farmers with new and much needed weed control options. Plants with engineered disease resistance genes have not yet been released commercially, but field trials are underway. The table below summarizes transgenic resistance traits in rapeseed varieties that are commercially available or currently being field-tested.

Table 1. Novel traits introduced into *B. napus* and/or *B. rapa* varieties.

Phenotype	Transgene(s)
Resistance to glufosinate-ammonium	Phosphinothricin acetyltransferase (PAT) gene from *Streptomyces hygroscopicus*
Resistance to glyphosate	Roundup-Ready™ gene
Resistance to imidazolinone (imazethapyr)	Acetohydroxyacid synthase (AHAS) gene from *Arabidopsis*
Resistance to chlorosulfuron	Acetohydroxyacid synthase (AHAS) gene from *Arabidopsis*
Resistance to Turnip Yellow Mosaic Virus	Noncoding regions of TYMV genomic RNA
Resistance to fungal infection	Chitinase
Insect resistance	Synthesis of *Bacillus thuringiensis* insecticidal crystal protein (Bt cryIAc)

EVIDENCE OF INTROGRESSION OF PEST RESISTANCE GENES

Pest resistance genes are bred into cultivated varieties to prevent yield loss. In agricultural systems, pest resistant varieties generally have a significant advantage over non-resistant varieties, and this advantage typically translates into a rapid spread of the resistant variety at the expense of the non-resistant varieties. Humans mediate the spread of pest resistant crop varieties. Whether introgression of a pest resistant gene into a feral population would increase the spread of a wild population is open to question. We, as a group, were not aware of any example where introgression of a pest resistance gene, or the consequences of such an event, had actually been documented.

There is, however, ample evidence that hybridization between closely related species occurs spontaneously (OECD 1997); introgression of resistance genes is therefore quite plausible. Available commercial varieties already have varying degrees of resistance to two of the most common fungal pathogens, *Leptosphaeria maculans* and *Albugo candida*. These traits, identified in related germplasm, were introduced into cultivars by conventional breeding. It is reasonable to assume that these resistance traits already exist within the genome of the closely related *Brassica* species complex, so their reintroduction via gene flow from cultivated transgenic varieties is not likely to have much influence on the fitness of feral populations.

The situation may be different with respect to many of the engineered resistance traits likely to be available over the next few years. Such new traits, imparted for example by a set of stacked genes that confer broad insect and fungus resistance, may protect plants from a wide range of pests. It is conceivable that this situation could trigger an increase in the size and range of the population in question.

There is little doubt that genes from transgenic rapeseed have the potential to escape into related varieties and species. Chèvre *et al.* (1997) documented the introgression of glufosinate ammonium resistance from B. *napus* to *Raphanus raphanistrum* under experimental conditions. Spontaneous production of crop-weed hybrid seeds under field conditions was reported from Denmark and the Netherlands (Jørgensen *et al.* 1996; De Vries *et al.* 1992). Consequently, the escape of transgenes is certainly a cause for concern.

Data from a field experiment with *Brassica napus* containing a *Bacillus thuringiensis* cry1Ac transgene suggest that this pest resistance gene is not likely to have a significant impact on weediness (Stewart *et al.* 1997). The Bt gene conferred increased fitness under moderate selection pressure by *Plutella xylostella*, however, this did not translate into increased competitiveness, nor did the transgenic plants exhibit greater weediness (Stewart *et al.* 1997).

IDENTIFICATION OF KNOWLEDGE GAPS

During the plenary session of this workshop, it was argued that scientists have numerous questions they would *like* to have answered, but what *really* needs to be known is a much more restricted set of information. We partially disagree with this statement because essential knowledge can only be identified once we have a sufficient general understanding of the system under consideration.

Our current knowledge tells us that pest resistant transgenic rapeseed varieties are likely to hybridize with a number of weedy species (OECD 1997) and that there is a good chance that transgenic traits will eventually introgress into populations of weedy species (Mikkelsen *et al.* 1996; Metz *et al.* 1997). We know from ecological work that herbivory by insects and other organisms is the principal factor in many environments that limits the abundance of plant species (Louda and Potvin 1995). Hence, there is a definite possibility that the newly acquired pest resistance will result in greater fitness of weedy *Brassica* species, which could be expected to become more abundant. Such an event could threaten biodiversity by displacing other plant species as well as their associated fauna. From a strictly agronomic standpoint, the increased abundance of a weed is also likely to be undesirable.

It may therefore be argued that transgenic pest resistant rapeseed varieties should not be released because potential negative effects due to the spread of pest resistance genes are unacceptable. However, before making such a decision we should also consider potential positive effects of this technology, including the health, environmental, and economic benefits due to a reduction in pesticide use. In order to weigh the positive effects against the negative, a more detailed analysis is required.

Current knowledge of the mechanisms that determine population dynamics of a weedy species is at best sketchy, even for the most extensively studied weeds. We have little quantitative information on the influence of insects, pathogens, and other organisms on the relative abundance of weeds. Data from

ecological research, as well as from biocontrol studies, show that reductions in population growth caused by insects and/or pathogens are often dependent on environmental factors (Louda and Rodman 1996). A geographic information system that matches the environment with pest and host species would provide valuable information about the potential impact of increased pest resistance. Introgression of a pest resistance gene has the potential to increase plant fitness only in areas where the pest is present and where environmental conditions are favorable to the pest.

We also need to know how frequently introgression would occur, how quickly such populations would spread, and the fate of the gene within the population. The frequency of hybridization events and subsequent introgression may depend on the time of flowering, presence of pollination vectors, and degree of sexual compatibility. Spread at the landscape level will largely depend on seed dispersal mechanisms. Seeds that rely on wind, water, animals, or humans for dispersal may travel long distances and spread their genes much faster than seeds that are only locally dispersed. Introgression and dispersal can easily be simulated, but actual data is needed to parameterize the model and to test underlying hypotheses.

Information on the frequency of hybridization events and on the rate at which pest resistance genes may spread could be obtained with the use of marker genes. Furthermore, such genes could help reduce the risk associated with gene escape by facilitating identification of hybrid individuals. The green fluorescent protein (GFP) gene is a commonly used transgenic marker that could easily be inserted along with a pest resistance gene (Stewart 1996).

A complete record of wild species that have the potential to hybridize with the rapeseed crops is needed. We should also be concerned with compatible species outside the US because transgenic varieties will eventually cross national, and therefore regulatory, boundaries. Furthermore, we need some measure of the likelihood of specific hybridization events and the level of fertility of subsequent generations.

Questions also remain on how transgenes are expressed when moved from one species to another. Current evidence shows that transgenes are expressed similarly in hybrids and parent plants (Chèvre *et al*. 1997; Stewart *et al*. *in press*). It is, however, conceivable that situations could exist in which a pest resistance gene may not function as expected when transferred to a different host. Unexpected behavior must be taken into consideration in order to evaluate the risks properly.

Filling these knowledge gaps should allow us to build simulation models that can quantify the impact of introgression of pest resistance traits on fitness and abundance of sexually compatible species. Based on this information it would be possible to make a more accurate risk assessment that would consider costs as well as benefits of a proposed technology.

RESEARCH NEEDS

Because of the above knowledge gaps, we recommend support for the following kinds of research projects:

Creation of a Database of Sexually Compatible Species and Varieties.

A database should be created to provide users with an exhaustive list of sexually compatible species, information on their geographic distribution, time of flowering by geographic region, details about hybridization success, and an exhaustive list of pests. This project would require the collaboration of numerous institutions and individuals from a wide range of disciplines. The database should be made accessible to the public, preferably over the internet, and would need to be updated as new information becomes available. Much of the information could be obtained from existing sources, but funds should be made available to conduct hybridization studies.

Development of Geographic Information System of Pest Influence.

This geographic database would combine distribution maps of compatible species with distribution maps of pest species and a range of layers for environmental variables. The objective would be to produce a map that indicates the

expected impact of a pest organism on a given host. The stronger the impact, the greater would be the effect of a corresponding pest resistance gene.

Creation of Long-Term Studies.

Long-term studies are needed to monitor weed populations for changes in gene frequencies and to determine the influence of such changes on pest populations. Far too few of these studies exist, especially in North America. The data would be very useful in validating simulation models of population genetics.

Exclusion Studies to Measure the Influence of Pest Pressure on Reproductive Rates.

Well-designed exclusion studies can explain the influence of specific pest populations on the fitness of plant populations. Such studies should be conducted over a range of environments, and critical environmental variables measured. Levels of pest pressure can be varied by adjusting pesticide application rates, as would occur with partial resistance. These kinds of data are required to develop and parameterize models that can simulate the influence of pest pressure on reproductive rates.

Hybridization and Introgression Experiments.

Hybridization and introgression experiments should be conducted with the most relevant compatible species and include follow-up studies to measure the persistence and performance of the transgene in the environment. In addition to using single pest resistance genes, these experiments may also be conducted with stacked genes that include herbicide resistance.

Observational Studies of Basic Reproductive Biology.

Observational studies describing the reproductive biology of the lesser known related species are needed. Such studies should provide information about the time of flowering, degree of outcrossing, principal pollination vectors, reproductive mechanisms that isolate the species, seed dispersal, and seed survival.

Modeling Projects.

Simulation models are needed to synthesize available knowledge and to direct further research. Risk assessment will have to be conducted on simulated outcomes. The quality of the risk assessment can be expected to be in proportion to the quality of the simulation model used.

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REPORT OF THE CUCURBIT WORKING GROUP[1]

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CUCURBIT BACKGROUND INFORMATION

Cucumber (*Cucumis sativus* L.) originated in India, melon (*C. melo* L.) and watermelon (*Citrullus lanatus*) in Africa, and squash, pumpkin, and gourd (*Cucurbita* spp.) in the Americas. Thus, cucumber, melon, and watermelon (including citron) are relatively recent introductions to the New World. Most domesticated species of *Cucurbita* were introduced from Mexico, Central America, and South America with the migration of native Americans centuries earlier. Wax gourd (*Benincasa hispida* (*Thunb.*) Logr.) is from Southeast Asia. Bottle gourd (*Lagenaria siceraria* (Molina) Stand.) is of African origin. South Asia is the probable center of origin for cultivated species of *Luffa*. Bitter melon (*Momordica charantia* L.) is a tropical Old World species. Chayote (*Sechium edule* (Jacq.) Swartz) is a New World species from southern Mexico and Central America.

Of the New World taxa, only *Cucurbita pepo* occurs as a significant weed problem in North America. *Cucurbita pepo* is a morphologically and ecologically diverse species composed of genetically distinct groups of cultivars and free-living populations (i.e., self-sustaining, including both wild and weedy populations). All of these diverse elements are completely interfertile and are classified as shown in Table 1.

Hybridization among *Cucurbita* species is also possible, with various of the 15 or so able to hybridize with some difficulty. Diversity in *C. pepo* is rooted in the ancient widespread distribution of free-living populations. Today, these populations range from northeastern Mexico and Texas, east to Alabama and north through the Mississippi Valley to Illinois. They occupy a diversity of environments and ecological niches—from upland, seasonally dry thornscrub habitat in northeastern Mexico, to primarily riverbanks and moist thickets in Texas, to a variety of riparian and other disturbed lowland habitats (e.g., agricultural fields, railroad tracks, highway embankments, etc.) throughout the Mississippi Valley. Different morphological and physiological adaptations have evolved in these areas, including early fruit abscission from the peduncle in response to riverine dispersal in Texas, as well as relatively quick seed germination in response to a shorter growing season in the more northerly populations (Decker-Walters *et al*. 1993).

Wild native taxa in the US and Mexico are listed in Table 2. In addition, many Old World cucurbits have been reported as feral species in the US and Mexico (Table 3), particularly in the coastal plain from Florida to Texas and into northern Mexico. The feral variety of *Citrullus lanatus*, which originated in Africa, is cross compatible with watermelon and occurs in the

[1] Group Report from the "Workshop on Ecological Effects of Pest Resistance Genes in Managed Ecosystems," in Bethesda, MD, January 31 – February 3, 1999. Sponsored by Information Systems for Biotechnology.

US. The remaining cucurbits in Table 3 are found sporadically to rarely in disturbed areas and are not major agricultural weeds. The same is true for the occasional escapes of melon and watermelon that have been documented in some North American floras (e.g., Steyermark 1963).

Production Patterns and Cropping Systems in the US

Cucurbits are grown in several commercial cropping systems and are popular garden crops. Worldwide, there may be more squashes grown in home gardens than are grown commercially for sale in local or distant markets. Although consumption figures are not readily available, production estimates are available for the US and many other countries (FAO 1992).

Cucurbit production in parts of the desert southwest US, e.g., Imperial Valley, California, is done on a large scale in areas of intensive agricultural production of a broad array of warm and cool season vegetables and agronomic crops. Weed control in the immediate vicinity of these production fields is generally very good, but control along river and canal banks is generally not carried out. In some of these areas, it is possible to find one or more cucurbits, usually a *Cucurbita* sp., grown on a small scale.

In the rest of the US, cucurbits are grown on a smaller scale and are not usually part of an intensive vegetable and/or agronomic crop production area. They are spatially and temporally dispersed. Early season production begins in Florida and moves northward to New York and New England in the East, and Michigan and Wisconsin in the mid-west. Weed control in these systems may be more difficult due to increased rainfall and the resultant native plant populations that may often be found growing immediately adjacent to cucurbit fields.

Pests of Cucurbits

Cucurbits are afflicted with a broad array of insect, pathogen, and nematode pests. With the exception of powdery mildew, which is one of few diseases that may be found in most production areas across the US, each production area requires a different complement of pest resistances. New pests (insect, fungal, viral, and bacterial) continue to be identified. Recently described pests include sweetpotato whitefly and silverleaf whitefly, zucchini yellow mosaic virus, lettuce infectious yellows virus, cucurbit aphid-borne yellowing virus, cucurbit yellow stunting disorder virus, squash leaf curl virus (= watermelon curly mottle = melon leaf curl), bacterial blotch of watermelon and melon, vine decline of melon (causal agent yet to be identified), and *Monosporascus cannonballus*.

Resistance breeding is the most active area of cucurbit germplasm, breeding, and genetics research in the US and worldwide. Most programs use traditional genetic and plant breeding procedures. Mapping (phenotypic, isozyme, molecular) of cucumber and melon has begun and progressed, but the maps are not yet saturated and few linked markers have been identified (Pitrat 1998). There has been little progress in the development of genetic maps of watermelon and *Cucurbita* spp.

Most pest resistance genes have been found in US or exotic cultivars or in landraces and cross-compatible relatives from centers of origin or diversity. Unsuccessful attempts have been made to produce fertile F_1 progeny from crosses of *Cucumis metuliferus* with *Cucumis melo* and *Cucumis sativus* in order to transfer several pest resistance traits from this distant relative to melon and cucumber. However, *Cucurbita okeechobeensis* ssp. *martinezii* was successfully used in crosses with *Cucurbita maxima* and *Cucurbita pepo* to transfer powdery mildew resistance to these two species (Contin 1978). Through its Asgrow Seed division, Seminis Vegetable Seeds has introduced transgenic resistance to two potyviruses (ZYMV and WMV) and one cucumovirus (CMV) in summer squash (*Cucurbita pepo*).

Many sources of pest resistance have been identified in cucurbits, although relatively few have been deployed in commercial cultivars (see McCreight 1998). Few of the identified resistance genes in the other cucurbits have been deployed or stacked in commercially available cultivars.

Table 1. Major cultivated cucurbit species in the US and worldwide.

Scientific name	2n	Common name(s); utilization
Cucumis sativus	14	Cucumber; fresh, cooked, processed
Cucumis melo	24	Cantaloupe, honeydew, exotic; fresh, cooked, juice, confections
Citrullus lanatus	22	Watermelon (seeded, seedless); fresh, candied, processed, juice
Cucurbita pepo[z]	40	
ssp. *pepo*		Cultivated pumpkins, marrows, a few ornamental gourds (e.g., orange and warted gourds)
ssp. *ovifera* var. *ovifera*		Cultivated crookneck, scallop, and acorn squashes, most ornamental gourd cultivars
ssp. *ovifera* var. *texana*		Free-living populations in Texas
ssp. *ovifera* var. *ozarkana*		Free-living populations in the central Mississippi Valley and the Ozark Plateau
ssp. *fraterna*		Free-living populations in northeastern Mexico
Cucurbita maxima	40	Pumpkin and winter squash; cooked, processed
Cucurbita moschata	40	Pumpkin and winter squash; cooked, processed
Cucurbita argyrosperma	40	Pumpkin, winter squash, and cushaw; cooked, processed

[z]classification of subspecies from Decker-Walters *et al*. 1993

Table 2. Free-living taxa of *Cucurbita* native to the US or Mexico. All have 2n=40.

Scientific name	Distribution
Cucurbita argyrosperma ssp. *Sororia*	Mexico
Cucurbita argyrosperma ssp. *argyrosperma* var. *palmeri*	Mexico
Cucurbita digitata ssp. *Cordata*	Baja California
Cucurbita digitata ssp. *cylindrica*	Baja California
Cucurbita digitata ssp. *digitata*	Southwestern US, Mexico
Cucurbita digitata ssp. *palmata*	Southwestern US, Baja California
Cucurbita foetidissima	Western US to the Mississippi Valley, Mexico
Cucurbita galeotti	Mexico
Cucurbita kellyana	Mexico
Cucurbita lundelliana	Mexico
Cucurbita okeechobeensis ssp. *okeechobeensis*	Florida
Cucurbita okeechobeensis ssp. *martinezii*	Mexico
Cucurbita pedatifolia	Mexico
Cucurbita pepo ssp. *fraterna*	Mexico
Cucurbita pepo ssp. *ovifera* var. *ozarkana*	Mississippi Valley
Cucurbita pepo ssp. *ovifera* var. *texana*	Texas
Cucurbita radicans	Mexico
Cucurbita scabridifolia	Mexico

Table 3. Feral Old World cucurbit taxa in the US and Mexico (listed by descending importance).

Scientific name	2n	Common name(s)	Distribution
Citrullus lanatus var. *citroides*	22	Citron, Colorado preserving melon, egusi	Florida to Texas, California, Mexico
Cucumis melo subsp. *melo* Group Dudaim	24	Smell melon, Texas smell melon, Queen Anne's pocket melon, chito melon	Florida to Texas, California, Mexico
Cucumis anguria var. *anguria*	24	Bur gherkin, West Indian gherkin	Florida to Texas, Mexico
Cucumis dipsaceus	24	Teasel gourd, hedgehog gourd	Florida, Texas, Hawaii, Mexico
Momordica charantia var. *charantia* (sometimes mistakenly given as *M. balsamina* in New World floras)	22	Bitter melon, balsam pear (sometimes mistakenly referred to as balsam apple)	Florida to Texas, Mexico, possibly as far north as Pennsylvania in eastern US
Lagenaria siceraria	22	Bottle gourd	Florida to Texas, Missouri, Illinois, North Carolina, Pennsylvania
Luffa cylindrica	26	Sponge gourd	Florida to Texas, North Carolina, Mexico
Coccinia grandis	24	Ivy gourd, scarlet gourd	Florida, Texas
Bryonia spp.	20	Bryony	Northwestern US

Weed Complexes

Cucurbits are affected by typical warm season weed species, which are controlled by conventional weed management practices. These include a limited group of nonselective herbicides and standard cultivation (discing, harrowing, hand hoeing, and weeding). Genetic resistance to herbicides has not been identified as a priority for cucurbit production.

There are no known feral species of cucumber in the US or Mexico. Citron, which is cross compatible with watermelon, may be a weed in cucurbit production fields (Robinson and Decker-Walters 1997). In Florida, citron is a weed in citrus groves. Dudaim is one of several cross-compatible groups of cultivated melons (*Cucumis melo* subsp. *melo*). It was reportedly feral in parts of Texas (Correll and Johnston 1970) and Florida (Wunderlin 1982). Dudaim was a noxious weed in melon production fields and other crops in the Imperial Valley, California beginning in the mid to late 1960's (K. Mayberry, pers. comm.). It was declared to have been eradicated from the Imperial Valley as well as from the entire state of California in December, 1998 (C. Valenzuela, pers comm).

A number of wild relatives of the squashes (*Cucurbita* spp.) occur in parts of the US (Table 2). Of these, only free-living populations of *C. pepo* occur in agricultural settings. In Arkansas, Louisiana, and Mississippi, *C. pepo* ssp. *ovifera* var. *ozarkana* is an aggressive weed in soybean and cotton fields (Boyette *et al*. 1984; Oliver *et al*. 1983). Whereas in wild habitats (i.e., those not directly influenced by human activity) individual plants or small groups of plants are widely dispersed along floodplain corridors, in weedy habitats (i.e., those created by human activities), populations are often very dense and cover large areas in agricultural fields.

Wild-habitat populations from northeastern Mexico, Texas, and many parts of the Mississippi Valley have been accepted as indigenous (e.g., Smith *et al*. 1992) with long histories of occupation in their general areas. However, morphological and isozymic evidence confirms that some of these populations have

experienced hybridization and introgression with cultivated material planted nearby (Kirkpatrick and Wilson 1988; Decker-Walters *et al*. 1993; Smith *et al*. 1992). Furthermore, this evidence suggests that some weedy populations in Illinois (Decker and Wilson 1987; Wilson 1990), Kentucky (Cowan and Smith 1993; Decker-Walters *et al*. 1993), and possibly elsewhere (Asch and Asch 1992) may have evolved purely as ornamental gourd escapes, which may or may not have experienced subsequent introgression with other nearby cultivated, weedy, or wild material of *C. pepo*. In short, the origins, histories, and genetic compositions of wild and weedy populations of this species are diverse. Consequently, it is difficult to make general conclusions about free-living populations based on observations or research conducted on a limited sampling of these populations.

Because of their similar usage as small, hard-shelled autumn decorations, ornamental gourds are typically thought of as a distinct grouping within *C. pepo*. Isozymic evidence has clearly shown this not to be true, with cultivars having originated in ssp. *pepo,* ssp. *ovifera,* and possibly ssp. *fraterna* (Decker-Walters *et al*. 1993). What many of these cultivars do share in common, though, are characteristics often ascribed to free-living populations, e.g., tough pericarps and bitter flesh, which serve to ward off predation in the wild. Among the edible cultivars, human selection pressures have yielded characteristics that hinder the cultivar's ability to persist in the wild (e.g., large, fleshy, non-bitter fruits). Consequently, most cultivars (e.g., pumpkins, zucchinis, crooknecks, etc.) do not survive as long-lived escaped populations in wild or weedy habitats. Although the supposition has yet to be tested, the occurrence of wild-type characteristics in ornamental gourds has led to the hypothesis that feral populations of *C. pepo* have been principally derived from ornamental gourd escapes (Asch and Asch 1992).

WHAT IS KNOWN?

There is little or no evidence that the introduction of pest resistance genes could increase the ability of any of the Old World cucurbits to become established as a noxious weed species. It is unlikely that New World pests would have affected introduced Old World species and prevented their ability to become established as feral species, as these Old World species were cultivated in highly favorable environments. However, new pests continue to be identified, and pest problems on Old World species in the US have become production-limiting over time as production systems matured. In response to emerging pests, resistance genes have been identified and transferred to acceptable cultivars to maintain production in the face of pressure from pest populations.

Dudaim melon became a feral species before genes from any known dudaim accession were used for resistance in cantaloupe or honeydew. Dudaim can easily intercross with all the other melon groups (Robinson and Decker-Walters 1997). There were 41,000 acres (16,400 ha) of melons grown across the US in 1991 (FAO 1992). Dudaim melon remains a minor weed species and is not a problem in production fields in Arizona, California, or Texas. There were ca. 82,000 acres (32,800 ha) of watermelons grown in the US in 1991 (FAO 1992). Citron was brought to the New World for cultivation and became feral over time in the southern US. Citron can easily intercross with watermelon (Robinson and Decker-Walters 1997).

There is no anecdotal or experimental evidence to suggest that pests have a significant effect on Old World feral cucurbit populations; therefore it is feasible that they are susceptible to the same pests as their cultivated cousins. The consequences of one or more pest resistance traits moving from the crop to their feral cousins are unknown.

The situation for the New World *Cucurbita* is different. Recent studies have concluded that genes will escape from transgenic crops into cross-compatible wild populations (Hancock *et al*. 1996). The environmental risk of this gene exchange creating aggressive weeds is believed to be dependent on whether or not the transgene is selectively advantageous in native populations. In evaluating the potential hazards of the transgenic, viral-resistant squash 'Freedom II' *(Cucurbita pepo* ssp. *ovifera),* researchers concluded that the risk of increased weediness caused by spread of transgenic resistance into

wild populations would be minimal because wild-habitat populations were not limited by viral infections (Grumet and Gifford 1998). Not sufficiently tested, however, were weedy-habitat populations of *C. pepo*, which have been serious pests in the agricultural fields of other crops (e.g., soybean, cotton, and corn) in eastern United States for the last 10 to 50 years. Given their agricultural habitat, which promotes high population density and may be within reach of pests and diseases in nearby cultivated fields of cucurbits, it is more likely that weedy populations of *C. pepo* are under various pest and disease pressures. Consequently, escape of transgenic resistance into these populations could increase their success as aggressive weeds.

Recent experiments under cultivated field conditions (D. Gonsalves, pers. comm. 1999) have confirmed that transgenes (i.e., genes from transgenic constructs) for viral resistance will pass from transgenic hybrids (i.e., wild x transgenic squash) into wild squash genotypes via natural pollen dispersal, and that viral resistance is advantageous to the wild material when this material is exposed to high viral pressure. Yet to be tested is the fate of introduced viral resistant transgenes in wild or weedy populations themselves. It is particularly important to test the impact of transgene transmission on weedy populations since the habitats that these plants occupy are more likely to be subject to viral pressures.

Although past researchers have not generally been interested in or looked for the occurrence of viruses in free-living populations of *C. pepo*, there are at least two reports of possible viral infection in weedy-habitat populations. Pathologist Doug Boyette (pers. comm. 1999) saw unconfirmed signs of viral infection in a weedy population near Hope, Arkansas in the 1980s. An herbarium label of a plant (T. C. Andres *et al*. #293, Cornell University Herbarium, 1994) collected from a weedy population in Issaquena County, Mississippi on November 7, 1994 noted, " . . . in a harvested cotton field. A serious weed problem. One young vine was still green with some slight virus symptoms . . . " This putative viral symptom was not confirmed by biological or laboratory assay.

Also not sufficiently examined in earlier experiments with transgenic squash was the risk posed by spread of transgenic viral resistance to some wild-type cultivars (e.g., ornamental gourds) in which the resistance could increase the ability that these cultivars already have to become successful escapes. Whereas most of the edible cultivars do not survive as long-lived escaped populations in the wild, some persistent weedy populations in Illinois and Kentucky exhibited isozymic and morphological evidence of having originated as ornamental gourd escapes. The cultivation of wild-type ornamental gourds throughout northeastern United States threatens to produce future weedy populations. Those weedy populations that find homes in the agricultural fields of other crops may become more aggressive if they possess resistance to agricultural diseases and pests.

WHAT IS NEEDED?

Little specific information exists about the two major Old World weed taxa (citron and dudaim melon) or the wild and weedy *Cucurbita* populations. Table 4 lists specific types of data desired in order to develop a complete assessment of the consequences of gene flow from cultivated to weedy cucurbits. The taxa of interest for such studies include: *Cucurbita pepo* ssp. *ovifera* var. *ozarkana*, *Cucurbita pepo* ssp. *ovifera* var. *texana*, *Cucurbita pepo* ssp. *fraterna*, *Citrullus lanatus* var. *citroides*, and *Cucumis melo* subsp. *melo* Group Dudaim.

Transgenic plants of *Cucurbita pepo* ssp. *ovifera* var. *ovifera* (summer squash) have been released for commercial production in the US. Certain site-specific, eco-geographic data and samples (Table 5) for free-living populations of this species would assist in the risk assessment of a crop becoming a weed or serving as a source of genes for its weedy relatives.

The seed samples suggested in Table 5 would be increased for long term storage in the National Seed Storage Laboratory (NSSL) and for working storage at the appropriate Regional Plant Introduction Station. The increased seeds would be available for genetic diversity analysis. Currently, the USDA possesses germplasm of only nine populations in Texas, ten in

Mississippi, and one probable escape from California, although such populations have been reported from 75 counties in eight states (Smith *et al*. 1992). This information, seed samples, and diversity analyses are not suggested to be required for the regulatory process involved in the approval of transgenic releases.

No known characteristics of the crop exist that affect our ability to extrapolate from small-scale field tests to large-scale use in terms of evaluating its establishment, persistence, and spread.

The following are important objectives for future research that might be considered given the limited information now available from previous risk assessment studies on the release of transgenic cucurbits, genetic information known about *C. pepo*, and systematic information that identifies weedy and wild populations of *C. pepo*.

1. Determine the nature of the pathogen load (particularly viruses), as well as other pests, in weedy populations of *C. pepo.*
2. Determine the differential susceptibility of different *C. pepo* populations to virus infection.
3. Determine the competitiveness of weedy squash into which transgenic virus resistance has been introgressed.
4. Evaluate the ecological and genetic diversity among free-living (wild or weedy) populations of *C. pepo* in the United States.
5. Evaluate cultivars of *C. pepo* for their ability to become persistent escapes from cultivation.

SUMMARY

Herbicide resistance has not been identified as a priority for any of the cucurbits although they are affected by the typical warm season weed species found in many of our crops.

Some cucurbits pose problems as weeds in agricultural systems. Except for citron, which is cross compatible with watermelon, no weedy cucurbits exist in cucurbit crop fields. Some cucurbits may be weeds in other crops, but there is little evidence of their role as a major weed species in the US. An exception is *Cucurbita pepo* ssp. *ovifera* var. *ozarkana*, which is an aggressive weed in fields of soybean and cotton in Arkansas, Louisiana, and Mississippi.

Pest resistance genes may move from cucurbit crops to their weedy relatives. Although this movement will not likely be a problem for cucumber and melon, there may be consequences in relatively limited production fields of watermelon in which citron is a weed. Where *C. pepo* ssp. *ovifera* var. *ozarkana* is an aggressive weed, squash production poses a potential problem to the extent that these weedy populations are pollinated by bees from production fields of *C. pepo*.

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Table 4. Information needed to assess feral and wild native species populations.

Item	Description
Weediness	Degree of aggressiveness, genetic similarity with wild and cultivated species (degree of introgression)
Distribution	Position characterization to include global positioning, altitude, and orientation to urban population centers
Ecological requirements	Abiotic (environmental) and biotic (common plant and animal relationship) constraints
Sympatry: cultivated and wild species	Relationship and degree of interaction among species
Reproductive biology – crossability	Constraints for reproduction, relative fecundity
Pests of wild species	Frequency and degree of interaction
Pest resistance in wild species	Type, frequency and relative stability of host, and host-pest interactions

Table 5. Site specific data and samples needed for risk assessments.

Type	Description
Global positioning coordinates	Precise characterization of position
Aspect	Clarification of plant position with regard to slope, directional position (N, S, W, E, etc.), relationship to adjoining landmarks (lakes, rivers, etc.)
Soil sample	Standardization of number, frequency and depth of sampling
Taxonomic inventory of associated plant species	Voucher specimens, frequency, and species associations
Animal species	Description of type, frequency, and species associations
Pests (insects, nematodes, pathogens)	Description of type, frequency, and species associations
Seed samples for deposit in the gene banks	Coordination with regional and national seed storage facilities National Plant Germplasm System before and after collection

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REPORT OF THE GRAINS WORKING GROUP (RICE, SORGHUM AND WHEAT)[1]

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CROP TO WEED GENE FLOW

The grains working group identified three crops which have sexually compatible weedy relatives likely to be subject to gene flow in US agricultural systems (Table 1). The ease of cross pollination and the successful production of a fertile hybrid vary with each case. If the selective advantage of an introduced trait is positive, however, introgression of the new trait into an existing weed population is possible. The risk of ecological harm is then dependent upon the habitat of the weed. In the crop-weed complexes considered here, in which the habitat of the weedy relative is limited to agricultural systems, the chance that a new trait may threaten natural ecosystems is not likely.

Crop-companion weed complexes often have a common progenitor and a parallel evolution (Harlan 1992). None of our three examples are native to North America; the cultivated crop and the weed relative were introduced into US agriculture at the same time. Rice and wheat seed imported for planting also contained seed of the weed. Sorghum, johnsongrass, and sudan grass were independently introduced into US agriculture as forage crops. See Annex 1 for more detailed background information on each of the crop and weed complexes.

Wheat and rice in the US have a 150 year history. The original seeds were often land races from Europe and Asia. These land races were grown in different areas of the US, and seed of those that produced a sustainable crop was saved. For the first half of the 20th century, genetics-based plant breeding aimed for high yielding varieties. In the last 50 years, improved varieties have been introduced that include pest resistance genes derived from related germplasm collections, and dwarf varieties derived from induced mutations. Changes in plant stature have been important for the mechanization of agriculture.

As agricultural management practices change, crop-companion weeds are subjected to strong selection pressures. Addition of certain new genes transferred from a crop relative could enhance the adaptability of the weed species. To date, however, there has been no evidence that the introgression of a pest resistance trait has exacerbated a weed problem in a sorghum, rice, or wheat agricultural system. Modern agriculture does have experience with weed populations developing resistance to herbicides. Intensive use of herbicides in continuous cropping operations, combined with little rotation among different herbicide chemistries, has resulted in the selection for resistant individuals in weed

[1] Group Report from the "Workshop on Ecological Effects of Pest Resistance Genes in Managed Ecosystems," in Bethesda, MD, January 31 – February 3, 1999. Sponsored by Information Systems for Biotechnology.

populations. Selection for resistance can be very fast when herbicides with long residual activity are in continuous use (Heap 1998). The impact of herbicide resistance has been managed by changing agricultural practices. The working group did not see potential adverse effects outside agricultural systems because the companion weeds remain contained within the cultivated system, with the exception of wheatgrasses in rangelands and conservation areas.

BREEDING FOR PEST RESISTANCE TRAITS IN THE US

Rice

Fungal pathogens cause the most important diseases of rice in the US (Table 2). In other parts of the world, bacterial and viral diseases have a greater impact on rice culture. Blast (*Pyricularia orzyzae* Cav.) is the most important rice disease worldwide and the key fungal disease in the US. There are many races of the blast pathogen, and blast resistant rice germplasm is primarily race specific (McKenzie *et al*. 1994). Members of the gene family conferring blast resistance, identified by the rice genomics project, are being used to study the mechanism of plant response to pathogens (Ronald 1997). It may not be long before genes for both race-specific and more general disease resistance will be available for evaluation in rice.

Wheat

The Proceedings of the 9th International Wheat Genetics Symposium provides an extensive list of genes for disease resistance in use by wheat breeding programs (Table 3). The information may be accessed at: http://www.extension.usask.ca/Publications/Ulearn/wheat_genetics_symp.html. It was noted that wheat already contains disease resistance genes for chitinase and glucanase, but increased expression or alternative timing may be achieved by rDNA technology.

In a reference document prepared by the OECD (Cook *et al.* 1993), the section on wheat cites eyespot resistance. The *Pch* gene for eyespot resistance was transferred to hexaploid wheat by a wide cross from *Aegilops ventricosa*. By outcrossing, the *Pch* gene could be transferred from wheat to jointed goatgrass, *A. cylindrica*, a related weed that is a host for eyespot fungus. According to this document, if jointed goatgrass acquired the *Pch* gene from wheat, it would not become a more important weed, but rather become a less important host for eyespot disease. However, members of our working group questioned what is really known of the negative impacts of disease on jointed goatgrass populations. The second statement in the OECD reference document may simply be opinion and is not documented by ecological studies.

Sorghum

The working group could not identify any effort to use biotechnology to improve pest resistance in sorghum. The USDA has not reported any authorizations for rDNA sorghum field trials as of the end of 1998. Current efforts to increase resistance to pests are based upon plant breeding and germplasm (Table 4).

GENERAL FINDINGS ON THE IMPACT OF PEST RESISTANCE TRAITS

- There is no evidence for these crops or weeds that introduced traits affect the establishment, persistence, or ability to spread.
- All these crop/weed complexes are subject to integrated weed management programs; thus, the negative impact is minimized.
- Wheatgrass presents a special case if fields are adjacent to conservation preservation areas and range lands.

The working group asked the question, “Can we apply the successful strategies learned from monocultured crop/weed complexes in managed agro-systems to weeds in the wild?” The consensus was No; these sets of data do not exist and would require difficult and lengthy research to gather. On the other hand, there are inferential data sets that may be derived from floristic survey and weed census reports. For example, in a review for the OECD background paper on plant breeding (Cook *et al.* 1993), the authors note that easily recognized wheat traits such as red coleoptile and pubescent leaves have never been reported in populations of jointed goat grass.

Table 1. Crop-weed complexes considered by the working group.

Crop	Weed	Relative likelihood of outcrossing
Sorghum *(Sorghum bicolor)*	Johnsongrass (*S. halepense)*, Shattercane (*S. bicolor)*, Sudan grass (*S. bicolor)*	High
Rice (*Oryza sativa)*	Red rice (*Oryza sativa)*	Moderate
Wheat (*Triticum aestivum)*	Jointed goatgrass (*Aegilops cylindrica)*, Volunteer rye (*Secale cereale)*, Wheatgrasses (*Agropyron sp., Elymus sp)*.	Low

Table 2. Important pests of rice in the US and current efforts to provide resistance.

Rice pests in US	Approach to resistance
Blast (*Pyricularia orzyzae* Cav.)	Germplasm and genomics; Enhancement of native resistance mechanisms via molecular biology
Sheath blight (*Rhizoctonia sloani* Kuhn)	Germplasm limited
Stem rot (*Sclerotium oryzae* Catt.)	Related species
Rice water weevil *(Lissorhoptrus oryzophillus* Kushel)	Germplasm
Bacterial leaf blight *	rDNA
Rhizoctonia*	rDNA
Insect resistance for lepidopteran and coleopteran pests*	rDNA for potato proteinase inhibitor II and various Bt toxins.

* Genetically engineered pest resistant rice field tested in the US as of 1998.

Table 3. Important pests of wheat in the US and current efforts to provide resistance.

Wheat pests in US	Approach to resistance
Leaf rust (*Puccinia recondita)* Stem rust (*P. graminin)*	Related germplasm, *Lophopyron* and *Triticum triaristatum*
Wheat powdery mildew (*Erysiphe graminis)*	Germplasm
Fusarium, *Septoria* and general fungal resistance*	rDNA for chitinase and glucanase genes
WSMV and BYDV*	rDNA

* Genetically engineered pest resistant wheat field tested in the US as of 1998.

Table 4. Important pests of sorghum in the US and current efforts to provide resistance.*

Sorghum pests in US	Approach to resistance
Smut (*Sphacelotheca sorghi)*	simple recessive
Leaf anthracnose (*Colletotrichum graminicolum)*	simple dominant
Red stem rot (*Colletotrichum graminicolum)*	simple dominant
Rust (*Puccinia purpurea)*	simple dominant
Sugar-cane mosaic virus (SCMV)	simple dominant
Chinch-bug (*Blissus leucopterus)*	possible simple dominant
Corn leaf aphid (*Rhopalosiphum maidis)*	possible simple dominant

* *Sorghum bicolor* resistances reported from traditional breeding (Doggett, 1988)

RECOMMENDATIONS ON INFORMATION NEEDED TO ASSESS RISK

Genes From the Same Gene Pool Are Low Risk.

Low risk gene pools, represented by conventional breeding and genomics programs, are generally characterized and predictable.

More Information is Needed on Genes From Diverse Sources.

Information should be acquired on the action of the resistance gene and its range of target organisms. Studies are needed to screen the weed population for pests, e.g., employ breeders' disease nursery, or compile field observations concerning pest infestations of the weed. If resistance is already present in the weed, the potential risk from gene flow is low. If resistance is not present, researchers must determine pest impacts on weed population dynamics. If no impact is found, then again the risk is low. If the pest does have an impact on weed dynamics, then the number of individuals in the population with and without the pest should be measured and fitness traits suitable for the weed should be scored. If fitness or population dynamics are affected to a large extent, then hybrid studies may be conducted.

Post Commercial Release Studies Can Provide Valuable Information.

The working group recognized that the first five years of a commercial release provide a unique opportunity for risk assessment on a larger scale. The group recommended that the USDA Risk Assessment Research Grants Program fund post-commercial release studies to identify and collect data in the first five year period. The data should be specific for the crop/weed/trait combination and include input from ecologists, weed scientists, and breeders. Important parameters to measure are changes in both number and distribution of weed populations. Once the appropriate data is identified, it should be communicated to target researchers, especially those in extension, crop associations, conservation staffers, and plant breeders who will be working with the new crops. Such information will prove useful for guiding the design of future releases and research.

ANNEX I. BACKGROUND INFORMATION FOR EACH CROP-WEED COMPLEX

The Sorghum – Johnsongrass Complex.

(Prepared by Paul Arriola)

Three compatible relatives of crop sorghum grow in the US, *Sorghum sudanense, S. almum, and S. halepense.* Of these three, *S. halepense,* or johnsongrass, is of greatest concern because of its aggressive weedy habit. Johnsongrass is considered to be one of the world's ten worst weed pests (Holm *et al.* 1977). A native of the southeastern Mediterranean region and Eurasia, it was introduced to the southeastern US as a forage crop sometime before 1830 (McWhorter 1971). Johnsongrass has since developed into an aggressive colonizing weed that has spread throughout most of the continental United States. It is a noxious weed pest for North American agriculture and is reported to be commonly found in crops such as maize, grain sorghum, soybean, cotton, grapes, potato, and sugar cane (Bridges and Baumann 1992). Johnsongrass has continued to spread throughout the southern and western United States, and over-wintering ecotypes are expanding northward into Canada (Warwick 1990).

The biology of johnsongrass has been well described. Although it reproduces and spreads principally by rhizomes, johnsongrass can reproduce sexually by producing selfed, or outcrossed seed with nearby compatible relatives (see Warwick and Black 1983). It is generally tetraploid (2x=40), and may be an allopolyploid result of past hybridization between *S. bicolor* and *S. propinquum* (Paterson *et al.* 1995). North American johnsongrass populations are often believed to contain pools of stable introgressants of wild plants and modern cultivated sorghum (Doggett 1988). In fact, Harlan (1992) suggested that crop-to-wild hybridization has likely been the key to the continued success and aggressiveness of johnsongrass in the United States, though there is no empirical evidence to support this idea.

The gene pools of the wild and cultivated sorghums can be described as unique, but not exclusive due to their common ancestry. Compatibility between these congeners has been well documented (see Hadley 1958). The likelihood of gene flow from crop to weeds is generally considered to be high. Crop sorghum outcrosses at rates as high as 15% (Ellstrand and Foster 1983), and the range of johnsongrass overlaps that of crop sorghum in all areas of cultivation in the US. Arriola and Ellstrand (1996) reported spontaneous weed x crop hybrid formation in the field at rates ranging from 0 - 12% and at distances of 0.5 - 100 meters. Subsequent measurements of hybrid fitness demonstrated no apparent fitness costs to the wild x crop hybrids when compared to the non-hybrid weeds under field conditions (Arriola and Ellstrand 1997). Although hybridization is variable, in this system one can regard the crop sorghum/johnsongrass complex as having a high probability of stable gene transfer in the wild.

The Rice – Wild Rice/Red Rice Complex

(Prepared by David Gealy and Donna Mitten)

The two rice relatives, "wild rice" and "red rice" can mimic the cultivated crop and are considered to be weed problems in various parts of the world. Wild rice, *O. rufipogon*, is a separate species from domestic rice and is included on the USDA Federal Noxious Weed List. It has only been identified in the United States as a single patch in the Everglades, Florida and does not exist in any of the rice production regions (Vandiver 1992).

The second weed, red rice, is a variant or ecotype of domestic rice, *O. sativa*. It does not share the perennial nature of *O. rufipogon* and persists in cultivated rice fields primarily by having highly dormant seed. Seed banks of red rice can be long lived and management of the weed is often based upon depletion of the seed bank. The species can compete with commercial rice and, if not controlled, is considered a weed problem (Craigmiles 1979; Noldine 1998). Red rice mimics crop behavior and often causes reductions of crop yield and quality through the admixture of red grains with the harvest. Red rice has been described as a dominant competitor; in competition studies, as many as three crop rice plants were required to impact yield as much as one red rice plant (Pantone and Baker 1991).

Historically, it is believed red rice originated in the cultivated fields of India where both red and white rice were grown. Its introduction into the US is attributed to a seed mixture imported from the East Indian Seed Company. Red rice was established in the rice fields of the American colonists; in 1850, the USDA published reports that listed four red rice types (Craigmiles 1979). Strict quality standards for seed rice, combined with agricultural practices designed to deplete the red rice seed bank, have eliminated red rice populations from California and sections of the southeastern production area (Hill *et al*. 1994). Red rice populations continue to hybridize naturally with cultivated rice. Gene flow travels predominately from the cultivated crop into the weedy red rice population. Cultivation of early maturing commercial varieties provides a partial hybridization barrier to the later maturing red rice populations (Langevin *et al*. 1990). Although the pollination periods for red rice may be later than most of our currently cultivated rice varieties, red rice exhibits an uneven maturity in the panicle and can produce some seed capable of rapid germination. Thus red rice, allowed to produce seed in a commercial rice field, can shatter mature grains in advance of even the early harvested varieties.

Red rice can express a long seed dormancy when submerged and buried in the soil. In field studies of five red rice populations buried at three locations, red rice survived more than 6.5 years, however the length of seed survival varied with location and population (Goss and Brown 1939).

The wheat – jointed goatgrass/crested wheatgrass complex

(Prepared by Marie Jasieniuk)

Wheat (*Triticum aestivum*) is a hexaploid (2n = 42) with genomes A, B, and D (Kimber and Sears 1987). Although originally believed to be allopolyploid, polyploid wheats are more auto- than allopolyploid and behave cytologically like diploids, thus maintaining a high level of fertility and stability.

Jointed goatgrass (*Aegilops cylindrica*) is a major weed of winter wheat in the western United States (Dewey 1996). The species is believed to be indigenous to southern Europe and Russia (Gunn 1958; Donald and Ogg 1991). It was probably first introduced into the United States in contaminated winter wheat seed brought by settlers from the eastern Mediterranean region. Goatgrass is a tetraploid (2N=28) with genomes C and D (Donald and Ogg 1991). Jointed goatgrass and wheat share the D genome in common. The shared genome allows hybridization between the species in the field (Zemetra *et al*. 1998). Hybrids were once believed to be sterile, but two recent studies found hybrids in the field with viable seed (Mallory-Smith *et al.* 1996; Seefeldt *et al.* 1998). Hybrids were not self-fertile. Rather, hybrid plants exhibited a low level of female fertility (approximately 2%) that allowed for natural backcrossing to occur in the field (Zemetra *et al*. 1998). Greenhouse experiments indicated that percent seed set was similar with wheat or jointed goatgrass as the pollen parent, but that seed set and self-fertility in second generation backcrosses favored jointed goatgrass as the recurrent parent. Based on these results, only two crosses in the field after hybrid formation appear to be sufficient to recover partial self-fertility with jointed goatgrass as the recurrent parent (Zemetra *et al.* 1998). Thus, if the wheat that produced the hybrid carried a pest resistance gene on the D genome, it would be possible for the pest resistance trait to transfer to jointed goatgrass after only two backcross generations.

In addition to jointed goatgrass, intergeneric hybridization between spring wheat and crested wheatgrasses, *Agropyron* Gaertn. (*sensu stricto*), has been reported (Chen *et al.* 1989, 1990). The crested wheatgrasses constitute a perennial cross-pollinating complex of roughly 10 species with diploid (2n = 14), tetraploid (2n = 28), and hexaploid (2n = 42) forms built on what appears to be one basic genome, P (Dewey 1984; Chen *et al*. 1990). The species are indigenous to Eurasia but are now widely grown as economically important forage on arid rangelands in United States and Canada (Dewey 1983).

Intergeneric hybridization between wheat and four crested wheatgrasses, *Agropyron mongolicum* (2n = 14), *A. cristatum* (2n = 28), *A. desertorum* (2n = 28), and *A. michnoi* (2n = 28) has been documented (Chen *et al*. 1989, 1990). Hybrid seed set varied among wheatgrass species

but was always low, ranging from 0.24 to 2.87% seed set. Crossability of diploid species was lower than that of polyploid species. Although most hybrid plants died of hybrid necrosis, a few plants were successfully established. Hybrid necrosis occurred at varying frequencies with different plant combinations suggesting that crossability varies among plants and accessions of a species. Backcross progeny of wheat x *A. cristatum* and wheat x *A. michnoi* hybrids were obtained by embryo rescue. Whether hybrids and backcross progeny occur naturally in the field is unknown.

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REPORT OF THE TURFGRASSES WORKING GROUP[1]

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GENERAL INFORMATION

Over 30 species of grasses are utilized for turf (Huff 1998), while others are important in agriculture as forage crops. The commercial value of this group of plants makes them attractive for improvement through modern genetic engineering techniques (Johnson and Riordan, *in press*). Because of the diversity of species and the consequent differences in biology among them, broad generalizations regarding the ecological effects of pest resistance genes introduced into these crops cannot be made. Rather, questions regarding the potential for pest resistance genes must be directed toward specific cases in which the species and the particular introduced gene are known. In keeping with this approach, particular attention at this meeting was paid to the turfgrasses—in particular creeping bentgrass (*Agrostis palustris* Huds.) and Kentucky bluegrass (*Poa pratensis* L.)—since these are the two grass species that have had transgenic lines tested in the field, and therefore are the species that present the greatest likelihood of being commercialized in the near future.

Major Pests and Diseases

The major pests and diseases that attack turfgrasses are listed in the table below (T. Riordan, pers. comm.). A comprehensive description of turfgrass diseases is published by the American Phytopathological Society (Smiley *et al*. 1992).

Traits Introduced by Breeding

Breeding for disease resistance, greater adaptability to environmental conditions, and turf quality, while maintaining or improving seed yield (in seeded species), have been the main goals of turfgrass breeders. These improvements are typically accomplished through traditional plant breeding, which usually involves the crossing of domesticated genotypes and subsequent selection of cultivars that display the desired trait. Often the genetic control of these traits is not clear and the degree of resistance is not complete (P. Johnson, pers. comm.). Traits that have been introduced by breeding into commercial cultivars include resistance to stem rust and leaf rust (*Puccinia* spp.), brown patch (*Rhizoctonia solani* Kuehn), summer patch (*Magnaporthe poae* Landschoot and Jackson), chinch bugs (*Blissus* spp.), and SAD (Panicum mosaic) virus. In addition, tolerance to heat, salt, and cold has been bred into various turfgrass cultivars.

Traits Introduced by Genetic Engineering

Genetic transformation of turfgrass species is reviewed briefly by Johnson and Riordan (*in press*) as well as by Spangenberg *et al*. (1998). The first trait to be introduced into turfgrasses by genetic engineering was resistance to the herbicide glufosinate in creeping bentgrass. Subsequently, resistance to another herbicide, glyphosate, has also been introduced, as have genes conferring resistance to fungi, viruses, and insects, or tolerance to stresses such as drought,

[1] Group Report from the "Workshop on Ecological Effects of Pest Resistance Genes in Managed Ecosystems," in Bethesda, MD, January 31 – February 3, 1999. Sponsored by Information Systems for Biotechnology.

salt, and aluminum (references in Johnson and Riordan, *in press*; Information Systems for Biotechnology, 1999).

Weeds of Turfgrasses

With the exception of bermudagrass, turfgrass species are not known to be weeds of other agricultural crops. Among turfgrasses, the most problematic weeds are other species or varieties of turfgrass. In particular, *Poa annua* is an important weedy species (P. Johnson, pers. comm.), and *Eleusine indica* (L.) Gaertn. presents a problem in some areas (J. Neal, pers. comm.).

Degree of Domestication

The turfgrasses varieties used in lawns and golfcourses are an extremely domesticated group compared with their wild progenitor species. The agricultural varieties of creeping bentgrass most likely originated from pastures in northern Europe, while bluegrasses probably came from central Europe (P. Johnson, pers. comm.). They have been selected for their ability to survive close mowing and intense management. Turfgrasses are relatively slow-growing, small in stature, and quickly shaded or out-competed by most plants (Johnson and Riordan, *in press*). In general, the traits selected by breeders have been those that are deleterious to the ability of these species to survive in an unmanaged environment.

Crop Management

Management of turfgrasses is labor intensive, and management practices select for varieties with specialized traits. Soil composition varies among particular areas of golf courses (P. Johnson, pers. comm.); fairways and tees are normally constructed from native soil, but golf course greens are usually constructed with sandy soil mixes (95% sand, 5% peat). Since the water-holding capacity of the greens is low, frequent watering is necessary, especially in warm and dry weather. During warm periods when temperatures on golf greens can exceed 120°F, daily watering is common, and small amounts of additional water are applied during mid-day to cool the plants. Mowing is frequent (6-7 times per week), since the height of the plants is kept at 1/10-1/4". Soil nutrient levels are carefully monitored; nitrogen and potassium levels are maintained at 2-7 lbs/1000 sq. ft./year. Specialized cultivation practices are also employed (P. Johnson, pers. comm.). Greens, tees, and fairways are “core aerified.” During this procedure, cores of soil measuring 2-4" long, 1/4-3/4" in diameter, and spaced 3/4-1" apart, are pulled from the turf surface. The resulting holes are filled with sand. Many of the new cultivars of creeping bentgrass are frequently mowed vertically; blades that are held perpendicularly to the soil on a rapidly spinning shaft are used to cut stolons and reduce thatch buildup in the turf. This is done regularly, varying from every day on some greens to twice a year on some fairways.

Weed Management

Weeds are controlled by a variety of methods (P. Johnson and J. Neal, pers. comm.). Physical measures to control such weeds as *Poa annua* include hand picking, careful water management, fertility management, cultivation as described in the previous section, and mowing. In addition, growth regulators and herbicides (e.g., “Prograss” [Ethofumesate]) are employed. The careful attention given to weed control places a high value on new transgenic varieties that are herbicide resistant.

WEEDINESS POTENTIAL OF THE CROP

The working group considered evidence that introduction of a pest resistance trait could increase the ability of the crop to become established, persist, or spread. Diseases are clearly a factor in the distribution of turfgrasses as crops. Thus, disease resistances have enabled more extensive planting of turfgrasses within the range in which they are used. Examples of traits that have allowed more extensive planting are resistance to summer patch, Phythium blight (*Pythium* spp.), brown patch, and snow mold. It is important to note that turfgrass spread is due to human planting rather than any inherent ability conferred on these crops to spread on their own as a consequence of disease resistance. In the same way, tolerance to heat, salt, and other environmental stress tolerance have expanded the range in which these crops can be grown.

ROLE OF PESTS IN LIMITING CROP-RELATED WEEDS

To evaluate potential ecological effects of introduced pest resistance genes, the first step is to examine evidence that pests have a significant effect on populations of plant species that are sexually compatible with the crop. The working group was unaware of any evidence that introduction of a disease or pest resistance trait had resulted in the release of turfgrasses or their sexually compatible relatives from any control exerted by those diseases or pests. However, the potential utility of studying endophytes was discussed. Turfgrasses are known to obtain significant benefits from endophytic fungi, which confer greater overall vigor on the plants and therefore might provide greater tolerance to pests or disease. In this respect, evidence obtained from the comparative study of plants with and without endophytes may provide insight into effects of genes that confer resistance to pests, pathogens, or environmental stress. The effect of endophytes might also be useful as a model of the effects of broad pest resistance genes on the fitness and potential weediness of the crop species.

As with the lack of evidence concerning the effect of pest resistance genes on turfgrasses, there was also a lack of knowledge regarding the similarity of pests and pathogens attacking crops and their sexually compatible relatives. It was assumed that the pests affecting the crops also affected sexually compatible species. However, this lack of information was seen as an important gap that needs to be filled.

CONSEQUENCES OF PEST RESISTANCE GENE FLOW

The working group began its consideration of this issue with a discussion on the potential effects of herbicide tolerance. It was concluded that herbicide tolerance would probably not make these crops more invasive. Creeping bentgrass and Kentucky bluegrass possess several traits that render these species ill-adapted for unmanaged situations; hence, the single trait of herbicide resistance was judged to be insufficient to cause these species to become weedy. However, the effect that engineering glyphosate resistance in bentgrass could have on annual bluegrass was raised as a concern. In this case, the effect would not be caused by the transfer of the herbicide resistance trait from bentgrass to annual bluegrass; rather, use of glyphosate on the bentgrass would raise the selection pressure exerted upon annual bluegrass. The emergence of resistance within this species would be accelerated, leading to the consequent loss of glyphosate as a weed management tool in golf course greens. The net result would be reversion to the present situation in which control of annual bluegrass in bentgrass by spraying with glyphosate is not possible. Despite this concern, the potential benefits of glyphosate resistance (reduced use of herbicide, a more environmentally friendly herbicide) was judged to counteract the concern presented.

After further discussion of the consequences of pest resistance, the group concluded that an assessment of the potential effects of pest resistance traits required a case-by-case evaluation. Important considerations in this assessment included the type and mechanism of resistance. In particular, broad spectrum pest resistance was viewed as being of special concern. Therefore, a hypothetical example was considered in which broad spectrum pest resistance was engineered into creeping bentgrass and subsequently transmitted to the sexually compatible species, redtop (*Agrostis gigantea*). For this specific example, it was judged unlikely that pests or pathogens limited populations of bentgrass or redtop. Therefore, even the addition of broad spectrum pest resistance would be unlikely to convert either species to a weed. Additionally, bentgrasses are perennials that do not display many traits seen for typical weeds. Based on these reasons, the addition of pest resistance was seen to be of little concern in the cases of bentgrass and redtop. On the other hand, it was recognized that the conclusion could be different for a species such as buffalograss, which is more likely to have populations controlled by pests or pathogens.

INFORMATION NEEDED FOR RISK ASSESSMENT

The group concluded that we are currently lacking important information for evaluating the

effect of pest resistance genes on the establishment, persistence, and spread of the crop or its sexually compatible relatives. Basic information on the natural history and biology of turfgrasses, as well as weeds in general, was seen as important in evaluating the effect on weediness of an introduced resistance gene in the crop or sexually compatible relatives. Specific areas where information should be obtained or compiled are:

- The life history and invasiveness of the various turfgrass species.
- The geographic range of related species, as well as the cross-compatibility of those species with crop species. Some information on crossing relationships is already known (see for example, Johnson and Riordan, *in press*). However, local variations in genotype and ploidy will result in different rates of transmission of a transgene to sexually compatible relatives.
- The range of pests and pathogens that attack the sexually compatible relatives.
- The factors (including pests and pathogens) that limit populations of sexually compatible relatives.
- The rate of increase of populations of sexually compatible relatives, and the factors that control them.
- A greater understanding of the characteristics of weeds in general. A more thorough study of the characteristics that predispose plants to becoming weeds is needed.

SOURCES OF INFORMATION AND EXPERIMENTAL APPROACHES

The information listed above can be obtained from a number of sources or through experimentation. The committee discussed the following sources and general approaches:

1. Manuals/Literature. Much pertinent information already exists and should be compiled from the literature to provide a useful database for risk assessment. With respect to pathogens infecting sexually compatible species of turfgrasses, information exists from surveys such as those conducted on fungi (Roane and Roane 1994, 1996, 1997) or maize dwarf mosaic virus (Rosenkranz 1981). Such information can also be found on the internet, for example at: http://biology.anu.edu.au/research-groups/MES/vide/refs.htm.

2. Introduction experiments. Introduction of transgenic plants into wild populations of sexually compatible relatives may be a useful approach to consider. Monitoring the ability of various transgenes to confer fitness advantages to plants in these populations would provide information on their potential to cause or enhance weediness.

3. Simulation experiments. Provide a particular genotype with an advantage by artificially increasing the input of seed into an experimental area. This experiment can be conducted with defined genotypes of non-transgenic plants.

4. Experimental crosses. Produce hybrids between selected transgenic crop species and sexually compatible relatives that may have weediness potential. These crosses may then be characterized in experimental plots or greenhouse experiments to assess their weediness.

EXTRAPOLATING FROM SMALL-SCALE FIELD TESTS TO LARGE-SCALE USE

Extrapolation from small to large scale was not seen to present as great a problem in turfgrasses as it may in other crops. In the case of creeping bentgrass, releases will be on a relatively small scale, since golf courses are typically only 100-200 acres, and bentgrasses make up even a smaller proportion of that area (about three acres). Consequently, any information that might be obtained in small scale risk assessment studies could be readily extrapolated to commercial-scale release. The fact that management practices are relatively uniform throughout the range of commercial releases also increases the applicability of data obtained from small scale tests to wide-scale releases. However, certain factors could affect the ability to extrapolate from small scale to large scale:

Region

The region where transgenic turfgrasses are used may affect the applicability of data extrapolated from small to large scale. Regional differences that might affect this include climatic differences and the distribution of sexually compatible relatives.

Scale

Although the original releases of transgenic turfgrasses will be in the commercial market (golf courses), development of transgenic varieties for the homeowner market will involve larger scale releases.

Pollen Spread

As a result of turfgrass crop management and the production of seed for that crop, much of the potential for pollen production and gene flow will be reduced. For the typical end user (golf courses), frequent mowing ensures that plants rarely go to seed. Therefore, transmission of transgenes to sexually compatible relatives should be greatly reduced compared to what would occur if the crop were allowed to flower and produce seed. There is an economic incentive for the producer to prevent cross-pollination during seed production, therefore, isolation of production plots from each other and from sexually compatible wild relatives will also be well controlled, as are production fields of any other crop.

Isolation

However, gene flow might still be frequent and commonplace. Stray plants at edges of fairways or on abandoned golf courses would produce seed, as would plants growing from seeds dropped or scattered during resowing. Though this might not occur on a large scale, the effect might be significant. In production fields where plants are allowed to flower, grass pollen can move several hundred meters or more. Therefore, as with any other crop, complete isolation cannot be assured during seed production.

Effects of Gene Flow

Although there are routes for gene flow between transgenic turfgrasses and their wild relatives, it is unclear whether a transgene would spread once it escapes and what its effect may be if it does spread. These questions can only be answered on a case-by-case basis.

The use of small scale trials to predict performance on a large scale is a standard tool of plant variety development. In plant breeding, there is a long history of extrapolating performance based on small plot trials. In the case of turfgrasses, knowledge of performance is gained through National Turfgrass Evaluation Trials. Although the information obtained from these trials does not usually address the issue of wild relatives becoming weeds, considerable observational data on the weediness potential of the crops themselves could be gathered from these types of trials.

Table 1. Pests And Pathogens Of Turfgrass

Kentucky bluegrass
Ascochyta leaf blight (*Ascochyta* spp.)
Billbug (*Sphenophorus* spp.)
Chinch bugs (*Blissus* spp.)
Curvularia blight (*Curvularia* spp.)
Dollarspot (*Sclerotinia homoecarpa*)
Fall armyworm (*Spodoptera frugiperda*)
Fusarium blight (*Fusarium roseum* and *F. tricinctum*)
Greenbug (*Schizaphis graminum*)
Leafspot (*Drechslera poae*)
Necrotic ringspot (*Leptosphaeria korrae*)
Powdery mildew (*Erysiphe graminis*)
Rust (*Puccinia* spp.)
Sod webworm (Pyralidae)
Stripe smut (*Ustilago striiformis*)
Summer patch (*Magnaporthe poae*)
White grubs *(*Scarabaeidae*)*

Creeping bentgrass
Ataenius (*Ataenius spretulus*)
Brownpatch (*Rhizoctonia solani)*
Curvularia blight (*Curvularia* spp.)
Cutworms (Noctuidae)
Dollarspot (*Sclerotinia homoecarpa*)
Fusarium blight (*Fusarium roseum* and *F. tricinctum*)
Gray snowmold (*Typhula* spp.)
Pink snowmold (*Fusarium nivale*)
Pythium (*Pythium* spp.)

St. Augustinegrass
Brownpatch (*Rhizoctonia solani*)
Chinch bugs (*Blissus* spp.)
Curvularia blight (*Curvularia* spp.)
Fire ants
Gray leafspot (*Piricularia grisea*)
Mole crickets (*Scapteriscus* spp.)
Pythium (*Pythium* spp.)
St. Augustine Decline (SAD virus)

Tall fescue
Ascochyta leaf blight (*Ascochyta* spp.)
Ataenius (*Ataenius spretulus*)
Billbug (*Sphenophorus* spp.)
Brownpatch (*Rhizoctonia solani*)
Chinch bugs (*Blissus* spp.)
Curvularia blight (*Curvularia* spp.)
Cutworms (Noctuidae)
Fall armyworm (*Spodoptera frugiperda*)
Fusarium blight (*Fusarium roseum* and *F. tricinctum*)
Greenbug (*Schizaphis graminum*)
Leafspot (*Bipolaris* spp.)
Net blotch (*Helminthosporium* spp.)
Rust (*Puccinia* spp.)
Sod webworm (Pyralidae)
White grubs (Scarabaeidae)

Perennial ryegrass
Ascochyta leaf blight (*Ascochyta* spp.)
Billbug (*Sphenophorus* spp.)
Brownpatch (*Rhizoctonia solani*)
Fusarium blight (*Fusarium roseum* and *F. tricinctum*)
Gray snowmold (*Typhula* spp.)
Pink patch (*Limonomyces roseipellis*)
Pink snowmold (*Fusarium nivale*)
Pythium (*Pythium* spp.)
Red thread (*Laetisaria fuciformis*)
Rust (*Puccinia* spp.)
Sod webworm (Pyralidae)
White grubs (Scarabaeidae)

Bermudagrass
Curvularia blight (*Curvularia* spp.)
Fire ants
Mole crickets (*Scapteriscus* spp.)
Scale (*Odonaspis ruthae*)
Spring dead spot
Stunt Mites (*Aceria neocynodonis*)

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REPORT OF THE POPLAR WORKING GROUP[1]

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BACKGROUND BIOLOGY

Poplars consist of all species of the genus *Populus*, including cottonwoods, aspens, and the many interspecies hybrids in common use (Dickmann and Stuart 1983). Our working group focused on the fungal pathogens, arthropod herbivores, and weed competitors of *Populus* in the United States. However, bacterial and viral diseases of *Populus* are significant in Europe, and genetic engineering approaches toward their control or management are being studied. The key aspects of poplar biology important to understanding the use of pest resistance genes are described below.

Mating Biology

Poplars are almost exclusively dioecious (separate male and female trees), and thus are obligately outcrossing. In addition, their large size when reproductively active (beginning at 4-15 years of age) and potential for wide distribution of both pollen and seeds enable long distance gene dispersal. Pollen is wind dispersed, and seeds are embedded in a matrix of cotton-like fibers that provides flotation, enabling them to be carried long distances via wind and water.

Seed Ecology

Poplar seeds are small and rapidly lose viability. They must find sites with abundant water and sunlight shortly after dispersal or they will not survive. Therefore, poplars do not produce seed banks. In addition, competition from herbaceous weeds soon after germination precludes or greatly reduces survival. For aspens on upland or northern-temperate to boreal sites, successful seedling establishment usually requires fire or a comparable intensive disturbance that exposes mineral soil and reduces competition. Cottonwoods in arid zones usually require riparian areas with newly deposited alluvial soils and little competition from herbaceous vegetation. In mesic areas, cottonwoods require exposed mineral soil with high moisture and light, and little competition. Because of their high intolerance of shade, poplars do not invade forest or herbaceous stands with a closed canopy.

Vegetative Regeneration

Because of the stringent conditions for reproduction by seed, vegetative reproduction is often more common than sexual reproduction for local dispersion. All poplars tend to sprout vigorously from stumps after trees are cut or fall from natural causes. Thus, genotypes can persist on sites for long time periods beyond the longevity of single trees (which itself is approximately 50-300 years). The aspens (section *Populus*) are particularly vigorous root sprouters even in the absence of disturbance, enabling clones to spread widely over the course of many years (Mitton and Grant 1996). In addition, other tissues can serve as effective vegetative propagules. Boles, branches, and short-shoots of cottonwoods can break off and

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float down streams and establish new trees. The extent of vegetative vs. sexual reproduction varies widely depending on species, environment, and disturbance history.

Breeding and Plantations

Poplar plantations are predominantly established as clonal blocks using unrooted cuttings or other vegetative propagules. In the northern and western United States, first generation interspecific hybrids of wild cottonwoods are cloned in this manner. In the southern United States, selected clones of natural *P. deltoides* are predominantly planted. Thus, cottonwoods are domesticated only to a very small extent with all plantations being only one to two generations removed from wild trees. They can therefore readily cross with the wild populations that commonly grow near poplar plantations. Because of the limited size of plantations compared to wild stands in most areas, plantation-derived propagules are usually greatly diluted with propagules from wild stands, including those located a short distance from plantations (S. DiFazio, unpubl. data). Hybrid breakdown and maladaptation are expected to limit the ability of hybrid progeny to invade established areas of wild poplar stands. However, when wild stands are small compared to hybrid plantations, introgression may be observed after long periods of time, as has been detected at low levels among wild stands of *P. nigra* in Europe (e.g., Heinze 1997; Arens *et al*. 1998; Winfield *et al*. 1998).

Amenability to Biotechnology

The amenability of poplars to transformation via *Agrobacterium* (Han *et al*. 1996) and the possibility of map-based cloning because of their small genomes (Bradshaw 1996) make genetic engineering for pest resistance and other traits feasible. A large number of genome markers and marker technologies are available for genome analysis. Transgenic elite clones require limited field testing and can be rapidly deployed without further breeding to stabilize transgenic traits.

Concept of "Genetic Inertia"

There is likely to be strong resistance of wild poplar stands to significant introgression from plantations due to the combination of poplar traits discussed above—delayed flowering, tree longevity, vegetative persistence, extensive wild stands, dilution of plantation-derived propagules by those from wild stands, stringent habitat requirements, and inability to establish under existing vegetation. Thus, except when a gene is employed that has a dramatic impact on tree fitness in the wild (none of which are known or appear on the horizon—see below), the impacts of pest resistance genes are expected to be localized and slight for many decades. In the future, however, if transgenic trees become prominent in the landscape compared to wild stands *and* large areas become suitable for regeneration through natural or human causes (e.g., large scale conversion of agricultural fields to forests), then genetic impacts could be more substantial and rapid. However, under near-term conditions, risk assessment and ecologically based analyses can focus on the consequences of new stands established very close to plantations and on the effects of numerically rare long-distance gene flow. This situation is radically different from that of crops with significant agricultural weeds as relatives, whose populations undergo rapid annual turnover and are subject to strong selection pressures from anthropogenic causes (e.g., herbicides). The extended time frame required for large-scale ecological impact makes risk assessment problematic because other major variables, particularly changes in genetic technology, human land-use, pest evolution, alterations to riparian systems (e.g., flood control), invasion of exotic organisms, and climate change, are expected to have far larger and overriding effects compared to those of transgenes.

PATHOGEN RESISTANCE GENES

Disease is believed to be the most important factor limiting adoption and productivity of poplar plantations (Royle and Ostry 1995). Poplars are susceptible to many pathogens (Newcombe 1996), and intensive culture has triggered changes in pathogen populations. Changes in North America have included the introduction of Eurasian pathogens (Newcombe 1996), the movement of regional pathogens within North America (Newcombe 1998b; Newcombe and Callan 1997), and hybridization between exotic and native species of the leaf rust pathogen *Melampsora* (Newcombe, unpubl. data). Leaf rust is the most important disease of

Populus worldwide. Host resistance has been the only widespread and economical control method for which both pathotype-specific and non-specific types of resistance are known (Newcombe 1996). Exotic species of *Populus* frequently are resistant to native pathogens (Newcombe 1998a), and resistance is often simply inherited in F_1 interspecific hybrids. Genome analysis methods have allowed mapping of the genes for resistance to races E1, E2, and E3 of *Melampsora larici-populina* (Cervera *et al*. 1996) and the *Mmd1* gene for resistance to *Melampsora medusae* (Newcombe *et al*. 1996). The *Mmd1* gene is expected to be physically isolated and transformed into a susceptible genotype in the near future, demonstrating the feasibility of genetically engineering disease resistance using native genes.

Attempts to increase resistance using heterologous genes have so far given poor results, but work has been limited (e.g., Strauss *et al*. 1988). The prospect of a heterologous resistance gene having broad and durable effectiveness against major pathogens, without negative pleiotropic effects on fitness, appears remote using current and foreseeable technology. These transgenes therefore do not appear to have the potential to significantly impact poplars in wild systems via introgression of transgenes. Moreover, transgenes that might give a useful degree of resistance in a well-tended genetic monoculture such as a clonal plantation are unlikely to be comparably important to pathogen resistance in genetically and environmentally diverse wild populations. Simple alterations in expression of native poplar or pathogen genes, such as by inducing constitutive overexpression or cosuppression, were also considered unlikely to be of significant ecological consequence. The transfer of unmodified resistance genes between *Populus* species is commonplace in conventional poplar breeding and should bring about similar risks if accomplished via gene isolation and genetic transformation. This should apply equally to leaf rust (Newcombe 1996) and other diseases of *Populus*.

INSECT RESISTANCE GENES

Insect damage is a major limitation to plantation viability and productivity in many regions (Ostry *et al*. 1988). Currently, the primary control method uses pesticides rather than resistant genotypes. Genetically based resistance is known but is often either incomplete or would require major alterations of breeding programs to accommodate, such as the use of different species as hybrid parents, with a consequent reduction in genetic improvement of other traits. The cottonwood leaf beetle (CLB) is the major pest of poplars in the United States and is believed to be largely restricted to poplars and other species in the same family (Salicaceae). The cry3a toxin from Bt (*Bacillus thuringiensis*) is highly toxic to CLB when applied topically or expressed in transgenic poplars (Strauss *et al*. 1998).

Other chrysomelid leaf beetles are also locally important. Lepidopteran defoliators are episodically significant, many have broad host ranges (e.g., gypsy moth and forest tent caterpillar), and most are sensitive to Cry1A Bt toxins (e.g., Kleiner *et al*. 1995). Wood borers of several taxa can be important pests in specific areas; because they are hard to reach with topical pesticides, the use of transgenes could be an important control option. Insect damage in wild stands is sporadic in space and time though rarely results in genotypic mortality because of poplar's resprouting capability. Thus, invasion of established stands by progeny of insect resistant transgenic trees is expected to be very slow. Field trials of transgenic poplars with beetle- and caterpillar-active Bt transgenes are underway in several areas (e.g., Ellis and Raffa 1997; Yingchuan *et al*. 1993; Strauss *et al*. 1998), most notably China, France, and the northwestern United States. Other than Bt, work with alternative insect resistance transgenes has been limited. Proteinase inhibitors expressed in poplars have given either modest levels of resistance or none at all (e.g., Leplé *et al*. 1995; Confalonieri *et al*. 1988) and thus do not appear to be under consideration for commercial use. Genes with different modes of action, but as effective as Bt against poplar pests, are unknown.

The most important consideration when using Bt transgenes is the significant potential for development of Bt-resistant insect biotypes if the extensive transgenic poplar plantations are established without accompanying resistance

management considerations (Raffa *et al*. 1997). High levels of CLB resistance have readily been bred in laboratory colonies under Cry3A selection (L. Bauer, pers. comm.). For most poplar plantations, wild stands are expected to provide large refugia that can slow resistance development and may obviate the need for planted refugia. However, the role of natural stands in the dispersal and mating behavior of target pests in areas where transgenic trees are being deployed should be studied. The working group considered that the potential for resistant biotype development from plantation use was a far greater concern than the risk of Bt transgenes providing a significant fitness advantage in wild trees after introgression. Sterility or other strategies for stringent gene containment were therefore not viewed as essential for use of pest resistance transgenes.

HERBICIDE RESISTANCE GENES

High levels of weed control for the first one to three years are essential for obtaining high rates of survival and tree growth in poplar plantations (Tuskan 1998). Plantation managers in many parts of the US believe that herbicide resistance (HR), particularly to glyphosate, can significantly reduce weed management costs and increase tree growth by providing more effective weed control and increasing moisture availability to trees (W. Schuette and J. Finley, pers. comm.). Because of the common use of poplars as windbreaks between agricultural fields and the future likelihood of their increased use for biofiltration plantings near streams in agricultural areas, HR poplars resistant to spray drift may be important components of agroecosystems dominated by glyphosate tolerant crops. Transgenic poplars with high levels of field resistance to glyphosate and phosphinothricin herbicides have been demonstrated in field trials (e.g., Strauss *et al*. 1998).

If transgenes are allowed to spread via seed, sprouting of HR poplars could complicate their control (Strauss *et al*. 1997). In some systems, poplars are considered "mild" weeds; examples include perennial crops (e.g., conifers), rights of way, and drainage ditches. Spread of HR trees would remove certain herbicides as control options, which could be an important loss in systems that must rely on one or few herbicides for control. HR trees also may complicate plantation management in significant ways, such as making the "volunteers" from seed produced in flowering stands more difficult to control in regenerating stands and requiring use of other chemicals for killing resprouts from stumps after harvest.

LIMITS TO EXTRAPOLATION FROM SMALL TO LARGE SCALE TRIALS

Poplars and other trees present substantial difficulties for extrapolating from small trials to large-scale effects for several reasons:

- The scale of *potential* impact of transgenic poplars is large because of their extensive dispersal of pollen and seed.
- Nearly all pre-commercial field trials do not permit trees to flower to avoid environmental release of transgenes, limiting opportunities for study of transgene movement and impact on a small scale.
- Because of the large size of trees and the need to study their growth over several years, tests using trees are costly in space and time. As a result, most trials are smaller than is optimal for obtaining information relevant to commercial use and for assessing ecological impacts; trials are of shorter duration than commercial releases.
- Significant impacts due to gene escape can accrue in poplars and other forest trees over multiple generations ("genetic inertia," see above). Therefore, risk assessments are required that span decades to hundreds of years and use complex predictive models, which are necessarily speculative and imprecise.

RESEARCH NEEDED

The working group identified seven research areas important to regulators and needed to improve overall scientific risk assessment (see Table 1). None of the knowledge gaps were considered so large that they should preclude commercial uses (i.e., none of the areas were rated as "urgent" under the regulatory decisions category in the Table), however, this conclusion

presumes that reasonable research and monitoring are done as part of commercialization.

The most important research area identified was the continued acquisition of highly effective pest resistance genes. The transgenes presently available either do not provide sufficiently strong resistance to disease or, in the case of insect resistance, may not provide the functional diversity needed to adequately deter evolution of resistant pest biotypes. Use of multiple transgenes with different mechanisms for toxicity is highly desirable. Although engineered sterility systems (Strauss *et al*. 1995) are not considered essential to prevent ecological impacts, the group recognized that such systems would simplify scientific and regulatory assessments and avoid some important agronomic factors that would impact plantation management. Therefore, research on sterility mechanisms was considered as important as obtaining new resistance genes.

Intermediate priority was given to learning more about the following: reproductive biology, particularly rates of gene flow through pollen, seed, and vegetative spread; the degree of hybrid fertility; and factors limiting the spread of hybrid-derived genes into wild populations. The group felt that too little is known about the impacts of herbivores and diseases in wild populations, the nature and genetic variation of resistance mechanisms, and the way in which changes in resistance genetics might directly and indirectly affect species interactions and ecosystem processes. Interdisciplinary, long-term studies are required for advances in knowledge in these areas.

The group also concluded that too little socioeconomic and environmental impact data are available to assess the value of pest resistance transgenes on a broad basis. Studies are needed to identify the kinds of land uses and landowners that might be economically impacted by the spread of pest or herbicide resistant transgenes and quantify the extent of that impact. This information is also important for assessing the need for sterility systems. Finally, information is needed on the broader impacts of transgenic poplars to help society and government assess their socioeconomic and environmental importance. For example, what degree of economic and environmental values are expected in the medium term on farm, landscape, and regional levels for trees with multiple functional transgenes (e.g., herbicide resistance, insect resistance, sterility, and disease resistance)? If these transgenic technologies are used wisely, the economies they provide farmers and industries may significantly increase the representation of poplars in agroecosystems in place of annual crops, with multiple environmental benefits. This economic aspect needs to be understood on a regional and national level to guide research, policy, and regulatory decisions.

Table 1. Priority research concerning pest resistance genes in poplars needed to inform scientific analysis and regulatory decision making.

Research Area	Regulatory[1] Decisions	Scientific Needs
1. Isolation of additional kinds of insect and disease resistance genes	2	1
2. Gene containment methods (engineered tree sterility) and analysis of their importance	2	1
3. Information to support resistance management (e.g., insect dispersal, refugia design)	2	2
4. Poplar reproductive biology, seed and pollen dispersal, hybrid fertility	2	2
5. Ecology of natural resistance mechanisms in relation to species interactions and ecosystem function in the wild	3	2
6. Evaluation of legal/social/economic impacts of transgene spread	3	2
7. Analysis of the contributions of transgenic poplars to economic and environmental sustainability	3	2

[1] Rating system: 1 = urgent, 2 = important, 3 = desirable

GENERAL HYPOTHESES TO GUIDE ECOLOGICAL ASSESSMENTS

The group considered several broad hypotheses frequently encountered when considering the risks of transgenic poplars and other plants. For each hypothesis we accepted, refuted, or qualified the stated hypotheses.

1. *Introduction of pest or herbicide resistance genes into poplars presents* significantly greater ecological risks *than traditionally bred pest resistance or other common pest control practices*.
   REFUTED. The risks are not zero, but are similar in kind and degree to those routinely encountered in plantation management using insect and disease resistant varieties, and topical herbicides and pesticides.

2. *Pest resistant or herbicide resistant plantations are likely to cause* significant ecological problems *due to spread of their offspring.*
   REFUTED. The main risks of these plantations are not primarily ecological but agronomic. Fertile herbicide resistant trees will produce progeny for which the target herbicide is no longer useful in managed systems; the insect or disease resistant trees may accelerate the emergence of pests resistant to the transgenic control mechanism, requiring new clones in plantations. Ecological impacts on wild populations via spread of pest resistance genes in progeny of transgenic trees are expected to be limited by comparison.

3. *Genetically engineered sterility is* essential *for reducing the ecological risks of pest or herbicide resistant poplars*.
   REFUTED. Sterility is an important genetic engineering goal because it will simplify ecological and regulatory assessments, however, because of "genetic inertia" and other factors discussed above, the transgenes currently being considered for commercial use are not expected to have important ecological impacts on wild stands.

4. *Vegetative propagation and vegetative persistence of poplars present significant concerns for the use of pest or herbicide resistant transgenic poplars*.
   QUALIFIED. The pattern of dispersal will be highly constrained and slowed in the absence of sexual reproduction, but some spread of riparian transgenic cottonwoods is expected and will be hard to track. Even if numerically limited, transgenics that become established will be hard to eliminate due to vegetative persistence.

5. *The* environmental *benefits provided by herbicide or pest resistant poplars are likely to be overshadowed by their adverse ecological impacts*.
   QUALIFIED. Positive environmental benefits within plantations are expected from use of transgenes (e.g., reduced use of undesirable pesticides or herbicides), however, transgene dispersal into wild stands creates the possibility of undesirable, even if limited, environmental effects. Engineered sterility, by containing transgene impacts, would minimize these concerns.

6. *The* scientific *need for large-scale studies of pest resistance management factors and the large* costs *of these tests require study as part of commercialization.*
   ACCEPTED. The large scale studies required for pest resistance development make the inferences from lab models tenuous. The resources needed to conduct large studies are beyond the means of most researchers, so working closely with industry during early stages of commercial use is likely to be the best means for assessing the effectiveness of resistance management strategies.

7. *Exotic species and their associated risks are good models for evaluating release of transgenic organisms.*
   REFUTED. In contrast to transgenic organisms, which differ in one or a few highly defined traits, exotic organisms represent new co-adapted gene complexes with new modes of development and thus have the potential to occupy new ecological

niches. They are effectively "super-resistant" to pests because they are often introduced without most of the diseases and herbivores present in their native range. Transgenic organisms are relatively precise and limited in their phenotypic changes and thus highly predictable by comparison.

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INTRODUCTION

Cultivated sunflower (*Helianthus annuus*) is grown in many temperate, semi-dry regions of the world, often in rotation with small grain cereals such as wheat. The largest areas of sunflower cultivation in the US are in the northern plains (North and South Dakota) and southern, high plains (western Nebraska and Kansas, plus areas of Colorado and Texas) where the growing season is often too dry and/or too short for profitable soybean and corn production. Most commercial sunflower is the oilseed type; in addition, the crop is grown for confectionery seed and is common as an ornamental in home gardens throughout the US.

The US is the center of diversity of the ancestral species of cultivated sunflower (Heiser 1954). The crop is capable of hybridizing with its wild progenitor, wild *H. annuus*, but most crosses with other *Helianthus* species such as *H. petiolaris* are unsuccessful or yield infertile F_1 progeny (Rieseberg *et al.* 1999). Cultivated sunflower also occurs as a volunteer weed. Although volunteer domesticated plants can represent a significant portion of the weeds infesting subsequent crops (Auwarter and Nalewaja 1976; Gillespie and Miller 1984), they do not persist for more than one or two years under most cropping systems and are not known to spread. For these reasons, the working group focused on the consequences of gene flow to wild *H. annuus*.

Wild *H. annuus* is an outcrossing annual that occurs in disturbed sites and is widespread throughout much of the US, reaching its greatest abundance in midwestern states (Heiser 1954). Wild sunflower occurs at elevations ranging from sea level to 3,000 meters and in a variety of habitats that include roadsides, agricultural fields, abandoned fields, construction sites, and rangeland. Populations are typically patchy and ephemeral, relying on the soil seed bank and long-distance dispersal for opportunities to become established in available clearings. This species occurs as a common but manageable weed of wheat, cultivated sunflower, corn, soybean, sugarbeet, sorghum, safflower, and other crops (Al-Khatib *et al.* 1998; Geir *et al.* 1996; Irons and Burnside 1982; Schweitzer and Bridge 1982; Teo-Sherrell 1996).

Pollen from cultivated sunflower is certain to spread to adjacent wild populations by the movements of foraging insects, especially bees. Commercial sunflower seed companies are required to have 1.6-2.4 km of isolation between hybrid seed production fields and wild sunflower and/or other cultivated sunflower to prevent contamination by "foreign" pollen (e.g., Smith 1978; Schneiter 1997). The extent of pollen movement from the crop to wild sunflowers is greatest at the crop edge, where up to 42% of

[1] Group Report from the "Workshop on Ecological Effects of Pest Resistance Genes in Managed Ecosystems," in Bethesda, MD, January 31 – February 3, 1999. Sponsored by Information Systems for Biotechnology.

seeds can be crop-wild hybrids, diminishing to nearly zero at distances of 800-1,000 m (Arias and Rieseberg 1995; Whitton *et al*. 1997). F_1 crop-wild hybrids are fertile and capable of backcrossing with nearby wild plants, but they typically produce fewer flower heads per plant than purely wild genotypes (Snow *et al*. 1998). Once crop genes enter wild populations, they can spread farther by both pollen and seed dispersal. Seeds can be transported inadvertently by farm equipment and as contaminants of hay, manure, topsoil, and seed lots. Whitton *et al*. (1997) and Linder *et al*. (1998) have documented long-term persistence of crop genes in populations of wild sunflower.

PEST RESISTANCE GENES IN CULTIVATED AND WILD SUNFLOWERS

Common pests of cultivated sunflower are listed in Table 1 below and described further in Seiler (1992) and Schneiter (1997). Cultivated sunflower is susceptible to several economically important fungal diseases, and genes that confer disease resistance have been obtained through both conventional and transgenic breeding programs. Conventional breeding has produced commercial sunflower hybrids that are resistant to several races of rust and downy mildew. Resistance to other important diseases such as *Sclerotinia* (wilt, stalk rot, and head rot) has not been achieved, but transgenic expression of oxalate oxidase shows promise for enhancing resistance to *Sclerotinia* (Lu *et al*. 1998; transgenes were obtained from wheat).

The most damaging insect pests of cultivated sunflower are those that infest developing seed heads (weevil, moth, and midge larvae) and those that transmit disease (e.g., stem weevils that transmit phoma black stem). In wild relatives of *H. annuus*, insect resistance is either absent or polygenic, and efforts to introgress strong resistance into the crop have been unsuccessful (Seiler 1992). A high priority for transgenic commercial hybrids is resistance conferred by Bt toxins, which are specific to different groups of insects such as Lepidoptera (moths, butterflies), Coleoptera (weevils, beetles), or Diptera (flies, midges). Bt-induced resistance to Coleoptera was first field-tested in the US in 1996 and resistance to Lepidoptera was approved for field-testing in 1999 (http://www.isb.vt.edu; note that VanderHave sunflower trials now take place primarily in the Netherlands). Broad-spectrum resistance involving multiple Bt genes and other genes for insect resistance (e.g., Stewart 1999) could also be developed.

POSSIBLE IMPACTS OF PEST RESISTANCE GENES ON WILD POPULATIONS

No studies have been conducted to determine whether gene flow from conventionally bred sunflowers has caused wild populations to become more abundant, although we suspect that traditional genes have had little impact on wild populations. Pest resistance genes have probably spread to sexually compatible wild relatives in the past, but in several cases these traits were derived from wild relatives in the first place (Seiler 1992; Snow *et al*. 1998, 1999). It is interesting to note that the frequency of rust resistance genes varies both within and among wild sunflower populations (Seiler 1992; Snow *et al*. 1998 and references therein).

Transgenic sunflower has not yet been released commercially, but several pest resistance traits may be introduced in the near future. Resistance to *Sclerotinia* is currently under development, prompting us to ask whether this trait could benefit wild genotypes, which are also susceptible to *Sclerotinia*. Dr. Gerald Seiler (USDA) has surveyed hundreds of wild sunflower populations without detecting *Sclerotinia* symptoms in mature plants. If this is true for most populations and for earlier life stages, we suspect that *Sclerotinia* is unlikely to regulate or limit the abundance of wild genotypes in the field. In contrast, transgenic resistance to insect seed predators might be beneficial to wild plants, which sometimes lose as many as 20-30% of their seeds to these insects (Pilson 1999 and unpublished data). Since transgenic insect resistance is now under development, this trait is the focus of our recommendations for further research.

Table: The most common pests of cultivated sunflower in the US[1]

Diseases	Insects
****Wilt, middle stalk rot, and head rot (mainly *Sclerotinia sclerotiorum*)	****Sunflower moth (*Homeosoma electellum*)
***Downy mildew (*Plasmopara halstedii*)	***Banded sunflower moth (*Cochylis hospes*)
***Stem canker (*Phomopsis helianthi=Diaporthe helianthe*)	***Red sunflower seed weevil (*Smicronyx fulvis*)
***Rust (*Puccinia helianthi*)	***Sunflower beetle (*Zygogramma exclamationis*)
***Verticillium wilt (*Verticillium dahliae*)	***Sunflower midge (*Contarinia schulzi*)
**Head rots (*Rhizopus arrhizus, R. stolonifera, Botrytis cinerea)*	***Sunflower stem weevil (*Cylindrocopturus adspersus*)
**Phoma black stem (*Phoma macdonaldii*)	*Cutworms (*Euxoa messoria, E. ochrogaster, F. jaculifera*)
*Alternaria leaf and stem spot (*Alternaria helianthi* or *A. zinniae*)	*Gray sunflower seed weevil (*Smicronyx sordidus*)
*Septoria leaf spot (*Septoria helianthi*)	Sunflower bud moth (*Suleima heliantha*) [2]
*Charcoal rot (*Macrophominia phasiolina*)	Sunflower head-clipping weevil (*Haplorhynchites aenes*) [2]
***Bacterial Infections	
*Powdery Mildew (*Erysiphe cichoracearum*)	

[1] **** Designates most important economically, * Designates least important, based on recommendations of our discussion group and Schneiter 1997.
[2] Species that occur on wild sunflower but are not economically important to the crop.

RESEARCH RECOMMENDATIONS

The working group outlined a series of questions that should be addressed for each new type of transgene that confers resistance to insects or disease (volunteer sunflowers are not discussed because they are not known to persist as free-living populations). If the answer to any of these questions is "no" based on adequate empirical evidence, it is logical to conclude that the risk associated with a given type of transgene is minimal. This "decision tree" approach is similar to those described previously in Tiedje *et al*. (1989) and Rissler and Mellon (1996). We consider several scales that should be studied, including individual plants, local populations, and regional metapopulations.

Is the transgene inherited as a stable, Mendelian trait when it is artificially crossed into wild plants?

Beginning at the scale of individual wild plants, we need to know whether a particular introgressed transgene is inherited as a dominant Mendelian trait. In addition, it will be essential to determine whether the transgene is expressed under a wide range of environmental conditions, and whether the anticipated phenotype (e.g., resistance to Coleoptera) is realized. Presumably, previous screening by crop breeders will ensure that a particular transgene is stable and predictable, but this should be confirmed in experiments involving backcrossed wild plants.

Do insects or diseases that are targeted by the transgene occur in populations of wild sunflower and, if so, how common are they?

By targeted species, we refer to organisms that would be killed or deterred by the effects of the transgene, including species that occur on wild plants but are not considered to be serious pests of the crop. Surprisingly little is known about the prevalence of insect pests and diseases in weed populations. Multi-year, multi-region surveys are

needed to determine the frequencies of insect and disease damage in wild sunflowers. Surveys that focus on mature plants could miss mortality or damage from insects or diseases that affect seeds, seedlings, or young plants, as is the case with many soil-borne pathogens. Likewise, if a disease is sporadic yet severe, it may kill the host population and escape being detected. Despite these problems, it is better to have quantitative baseline data from surveys than to evaluate risks based solely on anecdotal evidence.

When the transgene has introgressed into wild plants, will these plants exhibit greater survival or fecundity than their nontransgenic counterparts?

This question could be approached in two ways. To test for effects of insects or diseases on wild plants, these pests could be removed with insecticides or fungicides in field experiments. Examples of pesticide application experiments with wild plants can be found in Waloff and Richards (1977), Louda (1982), Simms and Rausher (1989), Louda and Potvin (1995), Louda and Rodman (1996), and Guretzky and Louda (1997). If broad-spectrum pesticides do not benefit wild plants (and are not harmful to plant growth), then further experiments to test for impacts of specific groups of insects are not necessary.

Alternatively, plant breeders could artificially introgress the transgene into wild genotypes to study characteristics of the backcrossed generations in the field (pending approval from APHIS). We recommend that APHIS encourage such projects if appropriate precautions will be taken. Field experiments can be used to quantify the ecological consequences of the transgene in backcrossed progeny that segregate for the presence or absence of the transgene. Survival and lifetime seed production could be compared to test for fitness differences between transgenic and nontransgenic plants. These experiments should be carried out at several sites where pest populations are known to occur. The level of insect damage seen in nontransgenic plants should be compared to natural levels that have been documented in baseline surveys from other regions and years in order to evaluate whether the experimental conditions were representative of commonly occurring field conditions.

If the transgene leads to greater survival or fecundity, will this cause wild populations to become more troublesome weeds?

This is a difficult question that will require a combination of field experiments and modeling. Field experiments can be used to determine whether populations are “seed-limited” on a local scale. In other words, we need to know how the addition of seeds affects seedling recruitment and population size (this very basic question has rarely been studied empirically). Carefully designed seed addition experiments should be carried out at a variety of sites for multiple years. Using these results, models could be used to examine the larger scale consequences of an increased seed production, taking into account the numbers of “unoccupied” sites in a region, the rate at which seeds disperse to and colonize these sites, and the rate at which sunflowers are killed by weed management practices or displaced by other species. In wild sunflower, recruitment from the seed pool in the soil may be delayed for many years. This aspect of their population dynamics is very important, as most populations are ephemeral and are out-competed by other species. Eventually, however, tilling or other soil disturbance in an area allows recruitment from dormant seeds. Scattered, temporary populations in a region are often referred to as constituting a metapopulation, the dynamics of which can be explored using mathematical models.

A good introduction to this approach can be found in a paper by Rees and Paynter (1997) titled “Biological control of Scotch broom: Modeling the determinants of abundance and the potential impact of introduced insect herbivores.” Models of metapopulation dynamics can be very instructive, especially when good empirical data are available to use as the main parameters of the model. Modeling efforts are needed to extend our understanding of population dynamics beyond the context of small-scale experiments to include regional changes in the abundance of wild sunflower. At the very least, models can help identify the specific conditions necessary for wild sunflowers to become more invasive in both managed and unmanaged ecosystems. This approach can be used to make informed decisions about the possible effects of genes for

pest resistance and is more reliable than decisions based on intuition and opinion.

OTHER ISSUES

Further research could include efforts to model the rate at which transgenes with different fitness benefits are expected to spread among populations and persist in seed banks. It will also be important to consider how quickly target insect pests will evolve resistance to Bt toxins and other transgenic types of pest resistance. Wild sunflowers could provide a refuge for Bt-targeted pests, at least initially (before the transgene has spread), and this might delay the evolution of resistance to Bt in insects.

Although not thoroughly discussed by our group, the potential impact of transgenic herbicide tolerance is as important as transgenic insect or disease control. Resistance to herbicides can evolve spontaneously in wild sunflower populations (e.g., Al-Khatib *et al.* 1998), or it can be acquired via crop-to-wild hybridization, including hybridization with transgenic sunflower. Here we present some of the issues that should be part of future dialogue on this topic.

Herbicide tolerance has the potential of being introduced into the crop as the transgenic trait of interest or it may be incorporated indirectly as the selectable marker for the transformation "cassette." In the latter case, the herbicide tolerance is intended to serve as a tool for plant breeders to identify the absence or presence of the closely linked transgene of interest. Even though the herbicide tolerance is not the primary trait, it is still present and has the potential to move to the wild species via pollen flow. This issue can be minimized by using selectable markers that are not herbicides or by developing transformation systems that do not utilize selectable markers.

As with herbicide tolerance in other crops, the tolerance is specific to a given herbicide and does not confer resistance to all herbicides. Therefore if the tolerance genes are expressed in wild species, it should still be possible to control wild sunflowers possessing the transgene with other herbicides. Presently there is a wide array of herbicides available to control sunflower in rangeland and cropland systems. Transgenic insect or disease tolerance typically will not impact other crops in a farming system, since the insects and diseases are specific to sunflower, but a unique feature of transgenic herbicide tolerance in sunflower is its impact on other crops in a farm rotation with the same herbicide tolerance gene. This situation may be problematic for farmers, but may be managed by 1) selecting crops with different herbicide tolerant genes to avoid the increase of herbicide tolerant wild sunflower, 2) tank mixing two herbicides, or 3) choosing not to grow herbicide tolerant sunflower (however this option does not consider the impact of neighboring farms which may be using herbicide tolerant sunflower).

CONCLUSIONS

In the short-term, the first types of transgenic sunflowers to be released may pose few environmental risks. To be confident of this, we recommend that risks associated with pest resistance transgenes be evaluated as outlined above. At present, the most urgent need for further research is an evaluation of how transgenes for insect resistance could affect the abundance of wild populations. A worst case scenario would be that transgenic wild plants would produce 20-30% more seeds per plant, perhaps leading to larger pools of dormant seeds in the soil and more successful colonization of disturbed sites in natural and agricultural areas, thereby exacerbating existing weed problems. Alternatively, empirical studies may show that effects of transgenic pest-resistance traits are negligible, especially in the case of narrow-spectrum Bt transgenes. We recommend a combination of baseline surveys, field experiments, and modeling of metapopulation dynamics to permit informed assessments of the risks associated with novel transgenes.

Taking a longer-term view, we expect that commercial sunflower hybrids with strong resistance to herbivores, diseases, herbicides, and even drought- or frost-induced stress (see Kasuga *et al.* 1999) could be developed for commercialization. Multiple transgenes could be "stacked" within the same cultivar, perhaps as tightly linked traits that would be transferred

together or by simply entering wild populations as separate transformation events. The combined effects of multiple fitness-related transgenes on wild/weedy populations should be carefully considered prior to their commercial release to avoid undesirable increases in the abundance of weedy sunflowers.

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