PEER REVIEW REPORT
FOR
BIOTECHNOLOGY REGULATORY SERVICES
DRAFT PROGRAMMATIC ENVIRONMENTAL IMPACT STATEMENT

USDA Animal and Plant Health Inspection Service

Biotechnology Regulatory Services

Riverdale, MD

July, 2007

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Reviewer Report 1

Introduction

Following the USDA/APHIS 'Charge to Peer Reviewers of Influential Scientific Information Contained in the APHIS BRS Draft Programmatic Environmental Impact Statement, this review report addresses the following parameters: Completeness, Currency, Accuracy, Uncertainty, Objectivity, and Clarity and ends with an overall conclusion. Detailed comments and suggestions have been provided to USDA-APHIS in a 'track changes' commented version of chapter 4.1.

Completeness:

This draft EIS identifies the potential factors and relevant issues raised with the development and commercial use of GE organisms relevant to human health and the environment.

Currency: For the most part, the information presented reflects current scientific thinking on the subject. Additional information has been presented in the draft document using track changes and comments. The main short-coming of this draft EIS is in meeting its intent "to provide non-expert readers with sufficient context and background with which to understand the biological and environmental science used to by the APHIS to evaluate potential impacts on the human environment arising from possible changes to APHIS regulations for GE organisms." Two examples are offered below in addition to examples offered as edits and comments in the text.

Accuracy: The information presented is scientifically accurate; or comments and edits have been offered in the text where there is reason to question accuracy. Some of the conclusion or summary sections do not read like conclusions or summaries; rather, they seem to bring out more information that could more appropriately been covered under a different heading or folded into the text. Suggestions for where this might be done are made as edits and comments in the text.

Uncertainty: This draft EIS could offer more context and background in dealing with the "scientific uncertainty" questions raised by GE organisms. The many examples from the extensive experiences with conventional plant breeding and cultural practices already provided are very helpful, but more examples are needed to meet the stated intent of this document, namely "provide non-expert readers with sufficient context and background with which to understand the biological and environmental science used to by the APHIS to evaluate potential impacts on the human environment arising from possible changes to APHIS regulations for GE

organisms." Additional suggestions are made as edits and comments in the text.

Objectivity: As far as it goes, the presentation of the body of scientific knowledge is balanced and objective. There are sections that seem to focus selectively on plant-insect interactions where examples from plant-microbe interactions would seem more appropriate, but I see no evidence that references are selectively cited or discussed in such a way as to introduce bias into the document.

Clarity: Suggestions have been made in the text where the information as written might not be understandable for a non-expert audience with a modest understanding of biological and environmental sciences have been, or where the information struck this reviewer as vague or ambiguous.

Conclusion:

The Charge to Peer Reviewers referred to above, requests the peer reviewers to explicitly answer the following question: **Does this scientific information presented accurately and objectively provide non-expert readers with a broad base of knowledge to understand the aspect of the biological and physical environment that is likely to be affected by the regulations currently administered by APHIS BRS and by possible changes of those regulations?**

My response/recommendation with regard to this question is that this chapter is acceptable after revisions have been made to address some weaknesses, specified in the above report and illustrated in the 'track changes' version of the report.

As indicated in the comments above, the scientific information presented in this document is, for the most part, accurate and objective. However, it should offer more information to help the reader with context and background, i.e., to "provide non-expert readers with a broad base of knowledge to understand the aspect of the biological and physical environment that is likely to be affected by the regulations currently administered by APHIS BRS and by possible changes of those regulations."

Without changing the focus of this document on GE organisms, it would seem particularly helpful for the non-expert reader to be made more aware of historic as well as recent advances in crop improvement using other methods of genetic modification, or using gene splicing methods to move genes between varieties of the same crop species which could well be the main use of GE in the future.

Examples of ways to provide this additional context and background information are offered as edits and comments in the text. However, here I offer two particularly relevant examples that I realize might be considered inappropriately addressing regulatory and policy implications but which I believe are relevant to context and background. The examples are derived from my own familiarity with wheat and barley in the U.S. Pacific Northwest.

The first example is the tolerance to the herbicide imazamox, developed in wheat using BASF patented technology and marketed under the trade name Clearfield®. Unlike Roundup tolerance, which involves introduction by plant transformation of a gene coding for a glyphosate-insensitive variation of the enzyme 5-endopyruvylshikimate-3-phosphate (EPSP) synthase, tolerance to the acetolactate synthase (ALS)-inhibiting family of herbicides involves the use of mutagenesis of the plant's gene for production of of a variation on ALS. Four Clearfield®-type varieties of winter wheat have been developed thus far by PNW wheat breeders. Of these, three are herbicide-tolerant selections of existing varieties, i.e., 'Madsen', 'Stephens', and 'Coda', produced by exposing mutagenized seed of these varieties to lethal doses of the ALS-inhibiting herbicide imazamox and picking the survivors. The fourth, developed by Oregon State University by conventional plant breeding and released as 'ORCF-101,' is the product of a three-way cross involving the herbicide-tolerant CV9884 as the donor, produced in France by mutagenesis of the French variety 'Fidel,' and the PNW winter wheat varieties 'Madsen'and a 'Malcom'/'Stephens' hybrid (see Peterson, C.J. 2003. Release of the new OSU Clearfield wheat variety. http://cropandsoil.oregonstate.edu/wheat/reports/OWC_CF_1.

Other examples of crop varieties produced by mutagenesis could just as easily be presented, in the context of what we have learned or assume with these varieties with respect to health and environmental safety questions that should now help guide or predict with more certainty what can be expected with GE varieties developed for herbicide tolerance..

The second example is the recent report based on a collaborative project between Washington State University and the University of Minnesota, namely the transformation of barley for resistance to stem rust using a gene for stem rust resistance from barley (Hovarth, H., Rostoks, N., Brueggeman, R., Steffenson, B. von Wettstein, D., and Klienhofs, A. 2003. Genetically engineered stem rust resistance in barley using the Rpg1 gene. Proc. Nat. Acad. Sci. 100:364-369.) The widely held belief among non-experts and somewhat reinforced by information presented in this document is that genetic engineering is about moving genes between unrelated organisms, even between kingdoms. However, the greater use of this technology in the future will be expediting the breeding process by splicing genes from one variety into another variety of the same crop plant species. In the case of disease resistance genes, it will be possible to update popular varieties by splicing in or stacking genes for resistance as needed in response to changing virulence in the pathogen population. This can also now be done such that the new phenotype contains only the gene of interest, without the genetic marker. Here is where context and background are especially important, since implications for human health or the environment of varieties modified with a single gene from another variety introduced by GE should be no different than that of a line isogenic for the same gene but derived using backcrossing.

In addition to these two examples relevant to the development and commericial use of GE crops, I offer the following brief comment on the draft document **Biological Control of Pests.**

This document is well written but is focused on traditional and historic use of biological control of insect pests and weeds. The title of the document would more accurately be **Biological Control of Insect Pests and Weeds** since there is no reference to biological control of plant pathogens. The document could easily set biological control of plant pathogens aside in the

introduction by clarifying that plant pathogens, being microorganisms, require the use of other microorganisms (antagonists) for their biological control, and that any use of a microorganism introduced as a product for pest or pathogen control requires registration by the EPA as a "microbial pesticide" under authority of the U.S. Federal Insecticide, Fungicide and Rodenticide Act

For more information on the science and practice of biological control of plant pathogens the readers could be referred to Baker and Cook, 1974 and Cook and Baker, 1983[1]. For a comprehensive discussion and scientific framework on the use and evaluation of the safty of microorganisms intended for pest and plant disease control, the reader could be referred to Cook et al., 1996.[2]

[1] These two books provide the scientific framework for biological control of plant pathogens and take the concepts into quite different directions from that of insects and weeds. For example, lowering the pathogen population would be nice but is not necessary for successful biological control of plant pathogens, if the antagonist keeps the plant healthy. These books are: Baker, K. F. and Cook, R. J. 1974. Biological Control of Plant Pathogens, W. H. Freeman and Co., San Francisco, 433 pp. (Book, reprinted in 1982, American Phytopathological Society, St. Paul, MN. Cook, R. J., and Baker, K. F. 1983. The Nature and Practice of Biological Control of Plant Pathogens. American Phytopathological Society, St. Paul, MN. 539 pp.

2 This journal article is written by a team of insect pathologists, weed scientists, nematologists, and plant pathologists that worked on this for more than two years. It lays a common framework for the safe use or microorganisms intended for pest control. I led this team motivated by using the experienced I gained from production of the OECD document on scale up of crop plants developed by biotechnology. The article is: Cook, R. J., et al. Safety of microorganisms intended for pest and plant disease control: framework for scientific evaluation. Biol. Control 7:333-351. 1996.

Hopefully these comments and suggestions will be helpful. This is a very comprehensive chapter already, and needs only the additional examples from traditional and modern breeding and management practices as context and background to make it an excellent document.

APHIS: Amended to this Reviewer Report below is the Reviewer's "Track Changes" edits to the draft EIS chapter.

Chapter 4

Environmental Consequences

Introduction

Genetically engineered (GE) organisms have been field tested in the U.S since the 1980s and GE plants have been grown commercially on millions of acres in the US since the mid-1990s. Developers and researchers monitor field trials while growers, extension agents, agribusiness economists and researchers all scrutinize commercially grown GE crops. APHIS is not aware of any verifiable reports of environmental harm or harm to human health resulting from such field trials or from commercial use of GE plants. The system of safeguards and controls imposed by APHIS on all GE organisms within the scope of authority has been highly effective in ensuring that releases are conducted in accordance with the conditions set by APHIS and that crops which are de-regulated pose no greater risk than conventionally bred crops.

Comment: This is an interesting comment that no doubt has merit and assures the public that someone is "minding the store." However, the claim could also be disputed on the basis that a) this same safety record would have been the case with or without APHIS regulations (there is no check) and b) this same safety record can be cited for conventional breeding that has gone on over the past century with mechanisms of review and approvals developed and implemented by breeders and research instituions with little APHIS oversight apart from phytosanitary certification of imported germ plasm. It would be revealing to have information on the percentage of submissions denied.

The agency recognizes, however, new technological trends have the potential to result in novel types of GE organisms being developed, which may have a greater propensity for environmental impact, both positive and negative, than those field-tested to date. Therefore, even though the past environmental releases have been conducted safely, and commercial products are being safely grown and consumed, APHIS will continue to rigorously scrutinize new scientific developments as well as the potential environmental impacts of any proposed changes in the regulations.

Deleted: that in a scientific approach, it cannot make general conclusions about the safety of GE organisms. In addition,

Chapter 4 examines the ways in which the implementation of current APHIS biotechnology regulations and possible changes might impact the quality of the environment.

Section 4.1 provides general background information for non-specialist readers to better understand the discussions in subsequent chapters regarding potential environmental impacts, and illustrates with some examples how such environmental impacts are typically assessed.

Section 4.2 describes in general the regulatory features of APHIS's current system and how these features control the environmental interactions described in the first section in order to reduce the likelihood of significant negative impacts.

Section 4.3 describes the impacts of the "no change option" with respect to 10 specific issues. For each issue the "no change" option is followed by an analysis which compares one or more options for new approaches under consideration.

Chapter 4

Chapter 4, Section 1: Impacts of Genetically Engineered Organisms

A. Introduction to biological factors that may be affected

This section briefly introduces the general ways in which plants, animals, insects, and micro-organisms affect the environment. Given that GE plants currently form the bulk of the releases into the environment, this section also gives a general introduction to Plant Biology, Crop Improvement, Seeds Biology and Commercial Seed Production.

Plants

Plants engage in numerous physical and biochemical processes which affect humans and the environment. Plants produce food and fiber for humans and for animals, both domesticated and wild. Plants alter the atmosphere, removing carbon dioxide from the air and adding oxygen. They modulate air and soil temperature and create microenvironments for other organisms. Plants modify soil structure through root growth and stabilize soil, reducing erosion, and plants add organic matter to the soil, which feeds microorganisms and improves soil quality. Plants also interact with each other, competing for sunlight, water, and soil nutrients, and weeds are plants which can compete so effectively with crop plants to cause economic harm to the crop. Lastly, plants produce a large variety of chemical substances that may affect the local environment or provide economic value to humans. Genetic engineering, like any other method of genetic modification, can alter the value of a plant to humans and may also affect one or more of the physical and biological interactions between plants and their environment.

Animals and Insects

Many insects and other animals are intimately associated with plants. These associations can be harmful to the plant, as in the case of animals which feed on plants and cause harm ranging from economic damage to death of the plant. There are also positive associations such as animals like bees and hummingbirds, which pollinate plants, and ladybugs, which eat harmful insect pests. In other cases, the association may be neutral: the animal may simply live on or near the plant. GE traits in plants may alter these associations, or create new ones.

Microorganisms

Plants also have a variety of interactions with microorganisms. Certain soil microbes, like Rhizobium bacteria and some fungi, associate with plant roots and provide additional nutrition to the plants, via various mechanisms. On the other hand, there are hundreds of negative associations: many microorganisms bacteria, fungi, and

viruses, among others, cause serious plant diseases, resulting in enormous economic losses. There are also neutral associations: many yeasts, for example, live on plant leaves without causing any harm to the plant, and other microorganisms help decompose dead plant material in the soil. Creating disease resistant plants through genetic engineering could change some of these negative associations, but other GE traits such as those affecting nutritional quality or plant structure could alter other plant-microbe interactions.

Plant Biology and Crop Improvement

Introduction

Plants exist in agricultural, or managed, ecosystems and wild, or unmanaged, ecosystems, and they interact with the environment in both types of ecosystems (Janick *et al.* 1981). This section summarizes these interactions in three contexts – physical, physiological, and ecological. The discussion is necessarily brief and broad but provides a basis for understanding how plants function in the environment and why plant breeders are attempting to modify those functions. Generally, breeders are attempting to enhance plant performance, which relates to a plant's ability to benefit from its positive interactions with the environment while suffering minimally from negative interactions (Allard 1964). Should traits enhancing plant performance move into wild plants, one might find increased fitness in these plants as well. For this discussion, genetic engineering is considered a tool available to plant breeders, available as another method to add a desirable trait to a plant variety.

Physical Environment

Except for parasitic plants, which grow partially within other plants, and epiphytic plants, which grow on other plants, most plants grow partially embedded in soil or in or on water. Many plants are capable of limited directional growth, but most plants cannot move large distances (Wareing and Phillips 1981). Therefore they are forced to obtain nutrients and water from nearby sources. A wild plant which is more able to exploit limited resources may be more likely to survive and reproduce, while a highly efficient crop plant may be more likely to produce large yields.

Terrestrial plants produce roots to absorb water and nutrients from the soil and to physically anchor themselves in the soil, but roots also directly affect the soil. Roots create spaces in soil for the passage of air, water, and soil organisms. In addition to these physical changes, roots release organic acids, which alter nutrient availability and accelerate soil development. As roots die and decompose, they contribute organic matter to the soil, improving its texture and its ability to retain water and nutrients. Plant roots also anchor soil particles and reduce soil erosion (Brady 1974).

Plant breeders are frequently interested in developing varieties with robust growth, including root growth. There are few GE traits currently under APHIS oversight whose purpose is to alter plant morphology, however APHIS anticipates that altered morphology traits may be developed by researchers increasingly in the future. Root

growth traits may alter plant drought tolerance but may also affect soil water distribution and irrigation practices and possibly soil stability and erosion.

Because adequate water is essential for survival and growth, plants have developed elaborate systems to absorb, transport, and retain water (Janick *et al.* 1981). Although roots can grow towards sources of soil water, soil water generally can move in the soil faster than roots can grow to reach it. Plants therefore use their own tissues to store water when it is readily available, and plants use various means such as waxy leaf coatings to restrict water loss when water is not available (Esau 1977). Some water loss is unavoidable however, and through the process called transpiration, plants lose water from aboveground surfaces and thereby convey water from the soil into the air. For field crops, this water loss can equate to several inches of rainfall during the crop season.

Depending on the environment to which a plant is adapted, too much or too little water may kill the plant. Some plants have adapted the means to temporarily withstand flooding or drought, and plant breeders are actively working on developing these traits in crop plants in order to enable crop production in areas with suboptimal water availability. Drought tolerance may increase the range of environments where a crop or wild plant can grow successfully and alter water management practices for growers.

Green plants have a profound affect on the Earth's atmosphere. As a result of photosynthesis, plants remove carbon dioxide from the air, combine it with water, and produce carbohydrates, which are used by the plant as its primary form of stored energy and to increase biomass. Oxygen is produced as a byproduct of photosynthesis and is released by plants back into the atmosphere. Plants also reverse this process when carbohydrates are utilized for energy, producing carbon dioxide and water, while using up oxygen (Bidwell 1974). However, while plants both produce and use up carbohydrates and oxygen, the net balance is in favor of the release of oxygen into the atmosphere and the removal of carbon dioxide from the atmosphere.

Plants have anatomical, morphological, and physiological adaptations to allow the exchange of internal oxygen and carbon dioxide with gases in the atmosphere while conserving water to maintain a healthy water balance. Traits which modify these plant characteristics could affect photosynthesis, water efficiency, and irrigation practices.

Light provides the energy driving the photosynthetic process. During periods of inadequate light, plants cannot produce new carbohydrates and are forced to use stored carbohydrates to survive. When light is limited, such as when plants grow in shade, the plants which best exploit the available light may outcompete less efficient plants (Janick *et al.* 1981). Plants use both structural means, such as producing larger leaves or growing taller then their neighbors, and physiological means, such as producing more chlorophyll, to better utilize limited amounts of available light (Bidwell 1974). Plant breeders exploit these adaptations to produce crop varieties that make the most of available light. Plants able to better exploit sunlight may grown successfully in environments previously unsuitable for crop production. A crop which

uses light more efficiently may be grown at higher density, *i.e.*, more plants per acre, thereby changing some crop management practices.

Physiological Environment

In general terms, photosynthesis consists of three processes: the absorption and retention of energy from sunlight; the conversion of light energy into chemical energy; and the stabilization of chemical energy into stored energy in the plant (Bidwell 1974). The process of photosynthesis bas been studied for decades and scientists know that this process can be accomplished in several subtly different ways. Variations in photosynthetic processes have evolved that enable adaptation to specific environmental conditions such as low light or restricted water. These adaptations may be biochemical or anatomical, resulting from one or more genetic changes in the plant. Although breeders have been trying to alter photosynthetic efficiency for many years, the complexity and number of genes that must be expressed to coordinate efficient photosynthesis has limited breeders' ability to significantly improve photosynthetic efficiency. Alterations to photosynthetic efficiently may increase yields but may also require additional water and fertilizer to achieve higher yeild. Increased photosynthetic efficiently may also result in increase overall environmental fitness, which could affect both crops and wild plants bearing the traits.

Although plants produce carbohydrates to be used as energy via photosynthesis, plants are still reliant on the soil as a source of mineral nutrition. Nitrogen, phosphorous, potassium, iron, magnesium, and other elements must be absorbed by plants roots and transported to tissues where they participate in myriads of biochemical reactions necessary for plant survival and growth. For the most part, these minerals are either already present in the soil or have been added by a grower in the form of fertilizer. Plants simply rely on the fact that soil water dissolves the minerals and makes them available for uptake by the roots (Van der Have 1979). It may be possible to produce plants through genetic engineering that are able to better take up minerals from the soil or that are able to use soil minerals more efficiently. Such traits could increase plant fitness and possible alter crop management practices, specifically how much fertilizer a grower may use for optimal yield.

Certain symbiotic soil microorganisms associate with plant roots to increase nutrient availability. For example, *Rhizobium* bacteria associate with the roots of some plants, mostly legumes, take nitrogen from the air, which cannot be used directly by plants, and convert it into ammonium, which can be taken up by plant roots. Certain soil fungi, called mycorrhizal fungi, associate with plant roots, making some soil nutrients, like phosphorous, more available for uptake (Brady 1974). Breeders are interested in increasing the number of plant species able to associate with these microorganisms and other researchers are working with the microorganisms themselves to improve their efficiency as nitrogen-fixing symbionts. Increasing the number of plants able to benefit from symbiotic relationships with soil microbes, either through genetic changes in the plant or genetic changes in the symbiont, may increase plant fitness, increase the geographic ranges of some crops and possibly change soil fertility management practices.

In addition to carbohydrates, fats, and proteins made by plants for growth, plants also make a wide variety of substances whose purpose is either unknown or is unrelated to growth. These substances are called *secondary metabolites*, and although they do not appear necessary for growth, many have important functions in such areas as disease resistance, reproduction, and herbivory reduction (Verpoorte *et al.* 2002). Many of these substances are of interest to breeders, either because the secondary metabolite makes the plant more tolerant of environmental stress or because the metabolite is valuable to humans for pharmacological or other purposes. Altering secondary metabolite production may result in a plant that is more fit due to enhanced environmental stress tolerance, or it may result in a plant with higher value as a crop because the metabolite itself is useful to humans.

Ecological environment

In most environments where plants grow, one or more resources, *i.e.*, light, water, nutrients, and space, are in limited supply, and plants growing together in the same location are generally competing with each other for the same resources. When the plants are a managed crop, the grower attempts to supply limited resources to the crop so that the individual plants are not competing with each other and are each growing at or near full potential (Janick *et al.* 1981). However, other plants growing with the crop can also benefit from the resources provided by the grower and take them from the crop. These plants are considered weeds and are removed when possible to reduce unnecessary competition and waste of resources intended for the crop. Breeders are always looking to develop crops that make more efficient use of resources to reduce competition and reduce inputs from the grower. In unmanaged environments, plants also compete for resources, but because no grower is supplementing the supply of resources for wild plants, plants with more competitive adaptations and more efficient resource use will tend to grow better and reproduce more than their less competitive neighbors.

Two positive interactions between plants and other organisms, *Rhizobium* bacteria and mycorrhizal fungi, were discussed above. There are other examples of positive associations between plants and other organisms. Plants are pollinated by insects and hummingbirds; many animals assist plants by disseminating their fruits and seeds; still other animals, such as ladybugs, help plants by eating insect pests, like aphids. Other associations appear neutral, as far as the plant is concerned. In some cases, plants provide a beneficial habitat for the organism, for example, when a spider builds a web, using a plant as support. Another kind of neutral association between plants and other organisms occurs after plants die. Dead plant material provides food to a wide variety of organisms from vertebrate and invertebrate animals to thousands of microorganisms, which all feed on the plant debris until it is completely broken down. APHIS is unaware of research into GE traits altering these types of positive and neutral interactions between plant and other organisms, but APHIS anticipates that such traits, if developed, could affect plant fitness and may also affect the associated organisms as well as non-target organisms.

There are many associations between plants and other organisms in which the plant suffers some harm. The organism may eat the plant or feed from the plant.

Caterpillars, aphids, and nematodes all get nutrition from plants, at the plant's expense. Organisms, such as bacteria, fungi, and viruses cause plant diseases that can either kill the plant or weaken it so that it cannot reproduce or compete with weeds. The organism may use the plant to launch an attack on other plants, as a means of completing its life cycle, or as a place to overwinter. Obviously, breeders care a great deal about minimizing the occurrence and intensity of these negative interactions and focus significant efforts to develop disease resistant crops (Fehr 1987). Complicating the use of disease resistant crops is the development of new strains of disease organisms that can overcome (defeat) the plant's resistance. Disease and insect resistance derived by either genetic engineering or conventional breeding are traits with which APHIS is very familiar, and APHIS anticipates continued interest in the development of these traits. Such traits could be expected to increase plant fitness, change crop management practices, especially in regards to pesticide use, and potentially raise questions of impacts on non-target organisms and development of resistance within pest populations.

In general, APHIS expects plant breeders to continue to improve crop performance and value using traditional breeding and GE traits. APHIS currently examines the potential impact of the trait on the health of the plant and on the environment with which the plant interacts.

Seed Biology and Commercial Seed Production

Seed has a dual character. From the standpoint of the plant, seed is a means to reproduce, but humans use seed as a product in the form of grain. When seed is used to reproduce plants, it has genetic and other quality characteristics that differentiate it from grain. This section will look at the biological nature of seeds and will briefly describe how seeds are produced for commerce.

Biology of Seeds

Seeds produced by plants have been the foundation of agricultural development by the human race for well over 10,000 years. During this time, man has progressively transformed selected plant species from wild progenitors into highly specialized crops. Seeds today are used as a source of energy and nourishment for human and animal consumption. They produce fibers used in clothing and construction. They are a source of raw materials for manufacturing an ever-broadening array of commercial products. And they are becoming an increasingly valuable source of renewable energy. The market value of agricultural seeds produced for planting each year is tens of billions of dollars worldwide. The world production of major grains and oilseeds produced from seed comes to about 2.5 billion tons, worth more than a half trillion dollars.

The role of seeds in plant reproduction

From the plant's perspective, seeds have a very different function. Simply stated, they are created to sustain the species. They contain the genetic instructions passed down

from their parents, and serve as the conduits for transferring that genetic information to the next generation.

Plants have developed a wide array of mechanisms to increase the chances of successfully passing genetic information on to the next generation. Most crop plants reproduce sexually, which increases variation among the offspring and has advantages in natural evolution, but requires a carefully-orchestrated interaction between male and female gametes. Successful mechanisms involve various flowering forms, mechanisms of pollen dispersal, self-incompatibility, and sensitivity to environmental cues. Knowledge of these reproductive strategies has enabled man to transform wild progenitors into agronomically useful crops through many generations of crossing plants followed by the selection of superior individuals in the progeny. These same reproductive strategies, however, can create challenges for maintaining genetic purity of seeds, particularly in crops that utilize natural environmental conditions to aid pollen and seed dispersal.

Although there are many physical variations, all crops produce flowers with the same basic anatomical parts. The stamen contains the male reproductive parts (anthers) which produce pollen. The pistil contains the female reproductive parts (ovules) which house the egg cells. 'Complete' flowers contain both male and female reproductive parts. Crop plants with perfect (complete) flowers are largely self-pollinated, and the majority of crop plants are self-pollinating. It is possible for pollen from another plant to cause fertilization in these self-pollinating plants, but the probability is very low because pollination typically occurs even before the flower petals open.

The flowers of some crop plants require cross pollination (*i.e.*, pollination by another flower). Maize tassels, for example, produce flowers that do not usually develop female structures, and produce only pollen. The flowers on the rachis (ear) do not develop male floral structures, and require pollen from the tassel for pollination. This mechanism of separating male and female flower parts increases the probability of cross pollination. The flowers of some crop species cannot pollinate themselves, which maximizes mixing of genes between plants.

Pollen and seed dispersal

Forcing cross pollination in crops with perfect self-pollinated flowers, such as soybean and wheat, requires that that either pollen be inactivated or anthers be physically removed before they mature and release pollen. In either case the pollen from another plant is delivered to the stigma.

In crops that are self-incompatible or have separate male and female flowers, pollen must be delivered to the female flowers by wind dispersal, animals, or, in most cases, insects. Corn pollination, for example, relies on wind dispersal of pollen. This reproductive strategy requires the corn plant to produce a large abundance of pollen and release it in close synchrony with maturation of female flowers, and it forces the pollen to travel through dry air.

Crop plants that rely on wind dispersal of pollen for pollination create challenges for those concerned with genetic purity: breeders, seed producers, grain growers and

sometimes consumers. With few exceptions, the female flowers of corn will accept pollen from any corn plant. The seed industry continues to refine isolation standards and develop novel genetic, physical, and chemical mechanisms to minimize cross pollination (for corn, see Beck 2004). Fortunately, the predicable nature of flowering dynamics and improved methods for modeling pollen dispersal are making it possible to quantify loss of genetic purity under field conditions (Fonseca *et al.* 2004).

Seed development, maturation, and long term viability

After fertilization, the developing seed becomes the primary recipient for water and photosynthetic products of the plant, rapidly gaining weight due to embryo development. The seed must store the chemicals that will be used to feed the growing seedling at the early stages of seed germination. The chemical composition of a seed is determined by genetic and environmental factors. Carbohydrates, fats and oils, and proteins, are among the most important seed stored compounds.

Once the seeds have matured, they may or may not be dispersed. Mechanisms of seed dispersal are relevant to the discussion of gene flow into unmanaged environments and specifically into the wild relatives of crop plants. Seed dispersal mechanisms are mainly used by plants in the wild. Crop plants have been developed to hold their seed until harvest. Some crops do not do this perfectly and some grain is lost prior to harvest.

After physiological maturity the seeds of many species dehydrate, which helps seeds survive cold winters and dry periods. These seeds have the ability to dehydrate to very low moisture content while remaining viable, even though their moisture content is 8 percent to 12 percent (well below the 70 percent water that makes up all living tissues in plants). Not all seeds, however, will undergo dehydration: seeds from plants adapted to tropical environments usually do not dehydrate as much as those from temperate climates. At low temperatures and moisture content, the seed metabolism diminishes and the aging process of the seed is slowed. Depending on seed composition, original seed quality, and storage conditions, seeds can be stored for several months to several years in an insect-free, low-temperature and dry environment.

Accumulation of storage materials

Seeds, primarily cereals and legumes, make up 70 percent of the food consumed in the world. Seeds store large amounts of chemical substances not stored in any other parts of the plant. These are proteins, carbohydrates, lipids (fat and oils), and they provide food to the seedling at the early stages of germination and growth.

Proteins are an important stored food component of many seeds, especially legumes. In many countries of the world legumes are the main source of protein in the diet, replacing proteins from animal origin. Most protein is broken down to amino acids in digestion and reassembled to make new proteins. Man does not produce all of the amino acids and some of these important building blocks must come from plants. The role of proteins as a source of energy in the human diet is secondary.

Carbohydrates are the most important storage compounds in the seeds of cereal crops. Starch and hemicellulose are the two main forms of carbohydrates stored in seeds, and are the source of simple sugars needed for germination. Starch is the principal stored carbohydrate in seeds and is stored in the endosperm in two forms, amylose and amylopectin. Hemicellulose is primarily a structural carbohydrate that is located in the cell wall, but in certain species is stored as reserve food. Carbohydrates are primarily a source of energy in human food.

Lipids or fats serve as energy storage within the seed and are an important part of all cell membranes. They are also used in food and animal feed and for industrial uses. Fatty acids from seeds contain larger amounts of unsaturated fatty acids (those containing one or more double-bonds within their molecule) than lipids of animal origin, and these plant lipids are used increasingly in processed foods.

DNA is a natural component of all seeds, as it is in all plant tissues; it provides the biochemical instructions for germination, growth and development of the new plant. DNA is broken down by digestion when eaten, and its genetic information content has no impact on human health.

Deleted: present in

Deleted: . The DNA content of the seed is vital because

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Comment: I understand why this paragraph is here; because of the high percentage of people that do not know or believe that DNA is in their food. However, maybe the changes I have made will make this paragraph seem not quite so elementary to the more sophisticated reader.

Opportunities for genetic modification

Nutritional studies also indicate that seeds are important sources of vitamins, antioxidants, and phytohormones. During the past two decades, there have been major advances in the understanding of biosynthetic processes controlling the synthesis and accumulation of these products in seeds. This knowledge, in concert with development of molecular biological techniques, has made it possible to modify seeds from crop plants to improve human health and produce raw materials for non-food uses. Transforming seeds for these purposes involves the addition of genes not currently present in plants. Numerous studies to date indicate that seeds of some crops can be induced to synthesize and accumulate various novel compounds. It is likely the programming for accumulation of normal seed storage components will need to be modified as well if seeds are to accumulate new compounds in sufficient quantity. For crops that will be produced in large scale such transformations need to be made with minimal effect on seed development, seed physical characteristics, and viability. A new genotype with poor agronomic characteristics and low capacity for seed production will not survive long in the seed industry.

Commercial Seed Production

Trade and value

Before the 19th century, farmers generally produced their own seed, and seed commerce was usually limited to the replacement of stocks that had become mixed or degraded. The advent of modern plant breeding methods has led to the importance of seed as a commercial product valued for its particular trait purity and quality components. The increased sophistication of plant breeding to produce crops meeting very specialized needs and market niches has in recent decades increased the need for

high standards of seed genetic purity in order to assure identity preservation in increasingly diversified markets.

Comment: Equally or more importantly, many plant diseases that depend on seed transmission of the pathogen have been controlled by use of seed produced under conditions and with practicies to certifiy it as pathogen free. Control of fungal, bacterial,, and viral pathogens dependent on seed transmission to produce disease is considered one of the major accomplishmes in plant health management in the 20th centurity {Reference Omitted}

Seeds are internationally traded commodities. The U.S. is the largest producer and consumer of seeds in the world. An estimated $5.7 billion worth of commercial seeds are produced annually in the U.S., which is a 19 percent share of the $30 billion world seed market. Maize seed is the largest segment of the U.S. domestic planting seed market, valued at $2.2 billion. Annual U.S. seed exports and imports are estimated roughly at $800 million and $400 million respectively thus providing a net trade surplus. The U.S. exports seeds mainly to Mexico, Canada, Italy, Japan and Argentina, and imports are mainly from Canada, Chile, the Netherlands and China.

In the U.S., farmers purchase a large portion of seeds from commercial sources, and the commercial sector is engaged in production, conditioning, distribution and marketing of seeds. Government policies and regulations impact interstate movement of seeds within the U.S., and have an even greater effect on international seed commerce. These laws, policies and regulations control plant variety protection (PVP), variety registration, truthful labeling, phytosanitary certification, and seed certification. Science-based policies and regulations are vital to the harmonization of the protocols for import and export among countries to promote global seed trade especially when seeds of GE varieties are traded.

Seed Purity

One of the principal seed quality concerns is genetic purity. To discuss genetic purity it is useful to divide crops into self-pollinated and outcrossing, because genetic purity is linked to these modes of seed fertilization. Of course all combinations of intermediates and some unusual specific cases exist.

The production of self-pollinating crop seeds is procedurally less complex than for outcrossing crops. When a crop is exclusively self pollinated, there is no need for pollen control in seed production, because little pollen is released into the air around the flower. Seed mixing therefore tends to dominate purity issues. The scale up of seed production of self-pollinated crops starts with the production of so-called "breeder" seed by self-pollination in research nurseries, followed by repeated cycles of seed production in plots and then fields, with plots or fields being checked during the growing season for off-types. Self-pollinated crops tend to be well adapted to being inbred, and it is less likely that hybrids will show strong hybrid vigor. As a result self-pollinating crops are less likely to be used as commercial hybrids.

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Comment: Off-types can result from more than mixtures, e.g., continued albeit rare segration at some low frequency within the population.

Deleted: resulting from mixtures

In outcrossing crops, the chain of breeding and production steps includes opportunities for both pollen flow and mixing. Insect pollination of outcrossing crops is common, and when it occurs it adds complexity to pollen control. Adaptation of outcrossing crops to the hybrid condition makes advantages for hybrid varieties larger and more common than in self-pollinated crops.

Hybrid seed production involves well defined production steps, and much of the structure of the commercial hybrid seed industry is influenced by the difficulty and

cost of pollen control, which control and limit, but do not eliminate, outcrossing and mixtures. In the production field, the sequence of seed increases generally follows a pattern where:

- Reproduction of core stocks of breeder seed occurs by hand controlled crosses;
- Breeder seed is increased using hand crossing with intense observation
- Foundation seed is produced from breeder seed by open pollination in controlled isolation in small isolation fields allowing intense observation to continue; and
- Hybrid seed is produced by controlled crossing between male sterile or emasculated female plants and pollen shedding male plants.

Crop improvement through traditional breeding

Delivery of genetic improvements is one of the most important roles of the seed industry. Conventional breeding, at its most basic, is a process in which differences in plants are observed in small plots, the differences are compared with the needs of the person doing the selection, and the plots that most fit the selector's needs are saved and perpetuated. Other variants are eliminated from the selected gene pool.

Breeder Seed

Breeder seed is usually produced in research nurseries where individual plants can be inspected and where pollination control can be maintained. Intensive observation of individual plants allows high levels of purity to be maintained. The use of breeder seed keeps the seed system from accumulating unintended genes indefinitely over time. For some non-commercial and traditional varieties, there may not be an effective breeder's seed system.

Foundation and Certified Seed

Foundation seed is produced directly from breeder seed or other foundation seed under conditions designed to maintain specific genetic purity and identity of the seed. Certified seed is produced from breeder or foundation seed under conditions designed to maintain satisfactory genetic purity and identity. Certified seed is the highest grade of seed ordinarily planted by farmers for commercial use or for production of their own "bin-run" or "saved" seed.

For outcrossing crops, unintended crossing during open pollination is the major source of unintended off-type pollinations in the field. Isolation and borders effectively limit the level of unintended off-types in the final product. Their use is supported by decades of industrial experience. Experimentation has shown that pollination of outcrossing crops declines rapidly with increasing distance from the source. The vast majority of unintended outcrosses and of off-type plants come from adjacent fields. The advent of GE crop plants and DNA detection technology provides a sensitive means for monitoring gene transfer and has led to recent controversies over inadvertent trait occurrence which, in turn, has lead to tightening of production standards and practices for all seed production systems. Regardless, for an open-pollinated crop, outcrossing at low frequencies will always be a possibility.

Comment: It is not clear what is meant by this statement; breeders' seed should always be of the highest quality since this is the start of the scale up process and typically involves hand selection of the desired plant types.

Deleted: Lower grades of seed are produced from breeder seed.

Comment: It would be useful here to give the number of distinct varieties of crop plants that are in various stages of seed increase in the U.S. each year. This information is collected by an organization at N.C. State (I do not recall the name). Most people have no idea that some 3000 varieties of wheat are in various stages of seed scale up each year, or that it takes some 1000 varieties to produce the U.S. wheat crop each year. I suspect that even larger numbers apply to soybeans.

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The other possible source for unintended presence of off-type genotypes in seed of both outcrossing and self-pollinated crops is commingling. In outcrossing crops, industry experience leads to the conclusion that field contamination is a more frequent source of off-type genotypes than is mixing in planting, harvest, transport and processing. Careful application of the procedures for field production, transport, and processing of corn normally results in the production of both hybrid and self-pollinated seed that is 99 percent or more pure in many crops.

Seed Quality and Regulation

The primary quality characteristics are: physical purity, presence of other crop seeds and weed seeds, especially noxious weeds, germination, varietal purity, disease status, and moisture. Special germination tests for difficult conditions may add important information. However, the customer can not readily observe the quality of the seed that is purchased. Seed laws provide assurance that the seed merchant is providing accurate information. Both Federal and State governments have seed laws, and the International Seed Testing Association provides global standards for germination testing for international commerce. All official seed certifying agencies belong to the Association of Official Seed Certifying Agencies (AOSCA), which establishes minimum standards for each crop. Individual certifying agencies may set higher standards than AOSCA, but not lower.

Many countries have customer protection regulations that require varieties meet performance standards. Varieties that meet the standards are described, registered and are then eligible for certification. The U.S. has fewer regulations of this type. Certification by the Organization for Economic Cooperation and Development provides international mutual recognition of certification. In the U.S. both varieties and genes can be patented. Varieties can also be protected under plant breeder rights. The International Union for the Protection of New Varieties of Plants coordinates a simplified plant breeder's rights system with standardized claims.

Technical Innovation and Seed

Breeding makes changes by combining great numbers of genes and sorting out useful changes by selecting among progeny. Genetic engineering starts with a copy of a specific DNA sequence (gene) from one organism and makes it produce its gene product in a different organism. The changes made by genetic engineering are minor in comparison to the amount of DNA in the plant, typically 1 or 2 genes inserted among the approximately 30,000 genes that account for the plant's phenotype (although the changes in the plant phenotype can be significant). After a trait has been successfully incorporated, it can be transferred to other varieties of the same species by conventional breeding techniques.

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The emergence of specialized food crops (*e.g.*, zero trans fat crops) and non-food varieties (pharmaceutical and industrial crops) increases the need to consider heightened standards for preventing pollen outflows and seed mixing in specialty seed production and brings up the special need to isolate non-food varieties from food varieties.

The Future of Plant Agricultural Biotechnology

The first decade of commercial plant agricultural biotechnology has seen remarkable growth from a mere 6 million acres in GE crops in 1996 to over 220 million acres in 21 countries in the 2005 growing season. The year 2005 also marked the point where cumulatively over one billion commercial acres of GE crops had been grown world wide. This rapid growth has more recently slowed due to Three issues: 1) ; 2) and 3) lack of an existing commercial marketing mechanism for plants with unique non-traditional (output) traits and uses. This situation is further exacerbated by the fact that the vast majority of GE crop plants only carry two production-oriented traits, glyphosate herbicide resistance and insect resistance due to the production of various Bt toxins, and some regional markets for these traits may be reaching near saturation.

Comment: With respect to input traits, two issues account for why adoption of biotech-derived varieties have slowed in recent times: market saturation for the two traits that account for 99+% of the 220 million acres, namely glyphosate resistance and Bt (as mentioned below but which should be mentioned first); and failure to obtain approvals in the export markets, namely Japan and Europe for other crops, e.g., wheat, with these same traits or even other events of the currently approved crops (corn and soybean) with variations on use of Bt for example. The failure of the New Leaf and New Leaf Plus Russet Burbank potato in the market place was due to the fast food industry led by McDonalds refusing these potatoes for making fries because of non acceptance in their European market. RR wheat is on the shelf because of no approval in the Japanese market. This has nothing to do with "a number of health issues" and to imply such would be blantantly false!

Deleted: a number of issues

Deleted: of health and environmental concern and

Comment: Put this statement as 1).

Not withstanding these concerns, a continued world-wide expansion in the use of GE plants is likely. This is exemplified by the activities of government regulators around the globe who are working to create regulatory regimes which allow GE plants and plant products to the market place while assuring the products' human and environmental safety. Concomitant with this governmental activity, the academic, non-profit, and corporate communities are working on creating new Identity Preservation, Quality and Trait Assurance Programs and market channeling mechanisms to allow GE plant products to flow in commerce without the inadvertent contamination of other products.

Four areas of GE crop trait development that may experience rapid development and significant world-wide commercialization in the next decade are: traits to address environmental stress on plants, plant derived biofuels, plant-produced proteins, and non-traditional industrial chemicals. Due to a decreasing supply of high-quality crop production land, drought, desertification, salinization, and global warming, there is a critical need for culturally acceptable food, fiber, and feed plants that can flourish under these environmental stresses. There is a broad scientific effort to identify and introduce traits that will allow plants to deal with environmental stress, especially for use in developing nations. With the rapid escalation in petroleum and fossil fuel prices, a renewed and significant scientific effort is being placed on renewable plant-derived fuels. The most interesting GE plant-derived biofuels from an intermediate-term development period of 10-15 years are ones that may be grown, extracted and utilized without further modification or with limited modification, much like existing bio-diesel products. Members of the plant kingdom are the synthetic giants of the planet and fully capable of producing large, complex proteins in significant quantities. The next ten years will see an increase in the development of GE plant-derived protein products such as vaccines, enzymes, biologicals, nutraceuticals, immunological proteins, and new custom-designed therapeutic proteins to treat cancer, birth defects and chronic ailments. The fourth area that seems destined for increased development and commercialization in the next decade is non-traditional industrial chemicals such as adhesives, improved or unique plant-derived fiber, lubricants, pharmaceuticals, nutritional supplements, and anti-disease food-derived health supplements.

B. Assessing effects on the environment.

Introduction

Chapter 4.2 and 4.3 below examine the way in which the implementation of current APHIS biotechnology regulations and possible changes might impact the quality of the environment.

To better understand chapters 4.2 and 4.3, this section B gives a general introduction to how potential effects of GEOs on the environment are typically assessed, and section C gives some specific examples. These assessments are done on a case-by-case basis as the agency makes decisions. It is emphasized, however, that this EIS does not contain risk assessments of specific GEOs. The aim of the EIS is to assess potential effects of any changes in regulations.

Since the advent of biotechnological methods, a wealth of experience with risk assessment has been accumulated worldwide resulting in a robust international consensus on the general principles and methodology for risk assessments regarding GE organisms.

Deleted: GEOs

Risk assessments for GE organisms:

- Are scientifically sound and transparent
- Are conducted on a case by case basis
- Are comparative – i.e. risks associated with GE Organisms are considered in the context of the risks posed by the (non-modified) recipients
- Are explicit in addressing uncertainties

Comment: Stay consistent with terminology

Deleted: GEOs

The overall methodology for risk assessment, typically follows a number of steps:

1. *Hazard identification* - An identification of any novel genotypic and phenotypic characteristics associated with the GE organism that may have adverse effects in the potential receiving environment;

2. *Likelihood estimation* - An evaluation of the likelihood of these adverse effects being realized, taking into account the level and kind of exposure of the likely potential receiving environment to the GE organism;

3. *Consequence evaluation* - An evaluation of the consequences should these adverse effects be realized,

4. *Overall risk estimation* An estimation of the overall risk posed by the GE organism based on the evaluation of the likelihood and consequences of the identified adverse effects being realized;

5. *Risk management* – A recommendation as to whether or not the overall risks are acceptable or manageable, including, where necessary, identification of strategies to manage these risks, including monitoring.

In the process of conducting the steps outlined above, *risk assessment takes into account* the relevant characteristics of:

- The recipient organism, host organism or parental organisms.
- Inserted genes and sequences and related information about the donor(s) and the transformation system.
- The resulting GE organism
- Detection and identification of the GE organism
- The intended use (e.g. the scale of the activity - field trial or commercial use)
- The likely receiving environment.

The sections below discuss:

- General topics relevant to risks assessments
- Examples of assessing potential impacts of GE plants on the environment.

C. *General topics relevant to risks assessments*

Potential changes in weediness and invasiveness

Introduction

A key consideration in assessing the potential risks of GE plants is whether or not changes in weediness and/or invasiveness have occurred or are likely to occur as a result of the genetic modification. In this context "weedy" refers to plants that are growing where they are not wanted, typically in managed ecosystems. "Invasive" refers to species that tend to spread aggressively, typically of concern in unmanaged ecosystems.

Comment: Excellent paragraph; very clear distinction of weediness and invasiveness.

Genetically Engineered Crop Plants and Weediness

Plants can evolve into weeds in three basic ways: 1) wild plants can, through unintentional selection in managed settings, gain the ability to invade managed habitats; 2) genes can be exchanged between cultivated crops and wild (free-living) relatives such that the wild relatives become weeds; and 3) weedy traits can be selected in crop plants such that the crop itself becomes a weed. It has been suggested that certain traits introduced through genetic engineering of crop plants might confer weedy characteristics to the plants, thereby creating new weeds in managed areas.

Comment: For context and background, also mention the use of gene transfers from weeds into crop plants, e.g., by chromosome insertions and manipulations. Alien genes from wild relatives of crop plants have been used extensively and safely in crop variety development for more than 50 years. E.g., see Jones, S.S., Murray, T. D., and Allan, R. E. (1995) Use of alien genes for the development of disease resistance in wheat. Annu. Rev. Phytopathol. 33:429-443. The genes transferred to wheat are from diploid and tetraploid weedy relatives of wheat and have not caused the new varieties of wheat to be weedy.

There are many common definitions of a weed, most not involving a specific biological feature, but rather how weeds are regarded by people (Booth, Murphy, and Swanton 2003; King 1966). The term *weed* is commonly defined as a plant growing where it is not wanted, due to interference with human activities or human welfare (Anderson 1977). Therefore, for the purposes of this DEIS, the weediness of GE plants and wild relatives with acquired GE traits in agro-ecosystems and other areas managed by humans will be discussed separately from the invasiveness of these plants into unmanaged ecosystems. There are also several definitions "invasive species" in the scientific literature (Richardson *et al.* 2000; Colautti and MacIsaac 2004; Pyšek *et al.* 2004). In this DEIS, an "invasive species" is defined as an introduced species that has a substantial or transformative impact in the unmanaged environment.

Comment: Do you want to introduce this word, in place of managed ecosystems? Again, it is a matter of being consistent in use of terms. Or define how agro-ecosystem fits within but is different from managed ecosystem.

Crop plants can in some cases be considered weeds, and some crops have more weedy characteristics than others. All crop plants are weeds when they carry over as volunteers (self sown) from seed left in the field after harvest. However, using common definitions such as the one given above, it is not possible to know whether any crop, GE or not, will be considered a weed in some particular instance. APHIS approaches this dilemma by comparing the biology of the GE plant to its non-engineered counterpart, usually the same plant without the GE trait.[3] In this way conclusions

Comment: The volunteer corn plants in the soybean field that killed the Texas biotech company (name escapes me) was a weed in that context. The most important weed for no-till wheat and barley is volunteer wheat and barley that serve as hosts (green bridge) for root pathogens between harvest of one crop and planting the next crop {Reference Deleted}

[3] APHIS has developed and made available on the Internet (via the APHIS website) a list of biological characteristics that petitioners for non-regulated status should address in their data set. The list is found in Appendix II of the Canada/U.S. 2001 Bilateral Agreement on Agricultural Biotechnology.

can be drawn as to whether a GE plant is different than its non-engineered counterpart in its basic phenotypic characteristics and life history. It may be difficult to predict, based on phenotype, whether or not a GE crop would become a weed, but any significant change in environmental fitness might trigger the need for heightened scrutiny. However to date, the incorporation of GE traits in crop plants has not resulted in the creation of novel weeds.

Wild Relatives With Acquired Genetically Engineered Traits as Weeds

Many of the concepts and proposed mechanisms by which transgenes might increase the fitness and consequently the weediness of crops would also apply to their wild relatives (Jenczewski, Ronfort, and Chèvre 2003). An important difference, however, is that crop plants often are themselves not very weedy and have a low propensity for persistence when not managed in an agricultural context, whereas wild relatives by their nature may have weedy characteristics and an ability to persist in the environment. Hybridization of many species of traditional crop plants with their wild relatives is well established, and it has been suggested that the resultant gene flow may contribute to the evolution of weediness (Ellstrand, Prentice, and Hancock 1999).

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In classic studies on the origin and evolution of weeds, Baker (1965, 1974) listed characteristics typically associated with weedy plants. The following is that list as adapted by Rissler and Mellon (1996):

1. Seeds germinate in many environments.
2. Seeds remain viable a long time.
3. Plants grow rapidly through vegetative phase to flowering.
4. Plants produce seeds continuously as long as growing season permits.
5. Flowers are self-compatible, but not obligatorily self-pollinated.
6. Pollen from flowers that are cross-pollinated is carried by nonspecialized flower visitors (usually insects) or by wind.
7. Plants produce large numbers of seeds in favorable environmental circumstances.
8. Plants produce seeds in a wide range of environmental circumstances.
9. Plants are adapted for both long-distance and short-distance dispersal.
10. If perennials, the plants have vigorous vegetative reproduction or regeneration from fragments.
11. If perennials, the plants are brittle near the soil line to prevent easy withdrawal from the soil.

Comment: This first property is vague; seed germination is controlled in the environment primarily by the occurrence of suitable temp and moisture. What does "many environments"mean? From tropics to temperate or alpine? Many environments can provide the right temp and moisture but so what? If this first point is adapted from Risler and Mellon, I suggest you stick with the original list developed by Baker.

Comment: As an aside, the introduction of the dwarfing gene into wheat and rice greatly increased the number of seeds produced by these varieties, by changing the harvest index from roughly 0.3 to 0.5 grain to biomass ratio. Have these genes moved to wild relatives and if so, contributed to weediness of these relatives, e.g.. goat grass and red rice, respectively? There is no evidence in the case of wheat.

12. Plants compete by special means, such as forming rosettes, choking growth, or producing toxic chemicals.

Comment: Points 1-9 describe wheat.

Keeler (Keeler 1985) reviewed the evolution of weeds from crop plants focusing on the characteristics described by Baker that may distinguish weeds. She listed characteristics associated with weediness in certain species and noted that many of these characteristics are known to be controlled by single genes. Her work showed uneven distribution of such characteristics among crops, weeds and other plants. While the most serious weeds had an average of ten or twelve weedy characteristics, other randomly surveyed plants averaged seven, and crop plants averaged only five. Thus it seemed unlikely that most crops would acquire enough of these characteristics to become weedy, even if the traits could be inherited as single loci.

Comment: This statement is confusing, but more importantly, why cite it if there are limitations to the study? The paragraph ends nicely with the above sentence.

Deleted: While noting several limitations to her study, she concluded that GE crops with "low weediness" and no weedy relatives are as likely to be the source of significant weed populations as are their non-engineered counterparts.

However, Williamson, studying invasiveness, concluded that neither those traits listed by Baker nor any others can accurately predict which plants could become weeds (Williamson 1993). He proposed rather that any such list of characteristics would have to be specific for groups of closely related species and noted that small genetic changes can sometimes spur large ecological changes. He concluded that GE plants have the potential to become weeds because the genetic changes may have unexpected environmental effects; however, he also concluded that the proportion of GE plants that will become weeds is very small (Williamson 1993). This conclusion was based on an earlier study of invasive species which had led to his formulation of the "10:10 rule." According to this model, approximately ten percent of introduced species will become established and truly naturalized, and ten percent of those will become pest species. Hence for introduced species, as a rough predictor, only one percent 1 percent will become pest plants. This model could be applied equally well, and with equal validity, to traits that have been introduced using conventional breeding, such as pest resistance, or those which can be acquired naturally or introduced through conventional breeding efforts, such as herbicide resistance. Thus, if the model is correct, APHIS can rely on its experience with these types of traits in assessing the risks of GE traits.

It has been suggested that the release of organisms with novel phenotypes bears similarities to the introduction of non-native species (Marvier 2001). However, the usefulness of such a model for evaluating the risks of GE crops has been questioned (Hancock and Hokanson 2001). The argument against exotic plant species as a useful analogy is that many of them are already good colonizers in their native habitats and carry an array of traits associated with weediness. Thus when they are introduced into a new environment where there are few or none of the factors which may have limited their numbers in their native environment, populations can sometimes explode to fill an ecological niche. There are examples of non-native plants, such as field bindweed, quackgrass and Canada thistle in intensely managed habitats, and kudzu, purple loosestrife and cheatgrass in less managed or unmanaged habitats, becoming weeds and causing significant impact to the environment and resulting in huge economic costs. This is in contrast the antecedents of GE crops which are generally poor colonizers outside of the agro-ecosystem designed for their cultivation. The traits selected for in domestication and the ongoing development of most crop plants typically have made them less fit than their undomesticated counterparts in situations

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where the crop plants are not managed (Gepts 2004). Also, as discussed above, crop plants generally have relatively few weediness traits apart from their almost-universal ability to grow as volunteers in the field after harvest. Thus, there are multiple and complex constraints that coalesce to limit the weediness and invasiveness of typical agronomic crops, and in most cases, only one such constraint would be removed by the addition of a single gene through genetic engineering, depending on the gene. Hancock and Hokanson (2001) concluded that the risk of deploying GE plants can be effectively determined by considering the phenotype conferred by the transgene and the invasiveness of the antecedent crop.

Crawley *et al.* (2001) performed one of the few studies of GE plants where potential weediness and invasiveness were measured directly. This was done by monitoring different habitats for ten years following the cultivation of four different GE crops, namely herbicide-tolerant sugar beet, maize and rape, and potato producing either a Bt toxin or pea leptin. In none of the cases did the researchers find the GE crops to have increased fitness over that of the conventional controls, and no unintended effects for the particular crops were identified. The most important factor to consider in interpreting these results, however, is that the particular traits studied would not be expected to increase fitness, except for the Bt toxin under certain conditions. The authors noted that the results might be different for other types of traits, such as drought tolerance or certain pest resistance genes that might confer a fitness advantage under field conditions.

A more thorough consideration of increased fitness may be warranted if there is reason to believe that the trait could increase fitness. Pest resistance genes have been the focus of much attention in this regard. Virginia Tech's Information Systems for Biotechnology, with support from USDA, sponsored a workshop on Ecological Effects of Pest Resistance Genes in Managed Ecosystems (Traynor and Westwood 1999). Many participants felt that the types of pest resistance traits being tested or released commercially were not fundamentally different from those bred in conventionally and as such, would present similar ecological risks, no different from the risks managed effectively over past decades with pest resistance bred into crop plants conventionally. However some participants disagreed and contended that some transgenes could have a much greater impact on weediness. Most participants agreed that gene stacking (*i.e.*, insertion of multiple transgenes) to confer a broad spectrum of pest resistance would be less predictable with respect to ecological consequences than single-trait resistance.

Comment: There is also extensive experience with multiple disease resistances bred into crop plants, eg. Wheat in the Pacific NorthWest with resistance to two rust diseases, eyespot foot rot, and common smut, or in Kansas with resistance to three rust diseases, soilborne mosaic virus, Hessian fly, and greenbug, with no issues related to weediness. I suggest that more be stated in this section on the long history of breeding crop plants for mainly disease but also insect resistance, all combined (stacked) into the same varieties.

Snow *et al.* (Snow *et al.* 2003) reported field studies of wild sunflower populations carrying a Bt *cry1Ac* transgene acquired via experimental hybridization to a non-commercial GE crop line and backcrossed into the wild-type plants. They observed decreased insect pest damage and increased fecundity (seed production) for the experimental unmanaged populations carrying the transgene versus those without it. This suggests the possibility that the transgene, by conferring increased fitness, could have an ecological impact on wild sunflower populations, either increasing the number of modified plants within a population, creating more such populations, and/or creating more extensive seed banks of such plants.

Comment: As is brought out near the end, in the section on GE resistance to viruses, but which also should be mentioned here, weeds are also a major source of pest infestations and pathogen inoculum and therefore any transfer of resistance to theses crop relatives could also help reduce the potential for new epidemics or epizooitics within the crop.

Genetically Engineered Crops and invasiveness

Introduction

In addition to the development of weediness, there is concern that GE crops may escape cultivation and persist to a significant degree in unmanaged ecosystems. It is also conceivable that a transgene from a GE crop could be transferred via cross-pollination to a wild relative of the crop, producing hybrid offspring containing the transgene that could themselves persist in the environment, or through introgression (by repeated natural backcrossing), result in the incorporation of the transgene in the genome of the wild relative. In the previous section, the primary focus is the potential for weediness of GE plants and their crossing with wild relatives in agro-ecosystems and other managed areas.

Gene Flow via outcrossing from Genetically Engineered Crops

For a GE crop to become established in an unmanaged habitat, seeds or other propagative structures must be transported from cultivated land to the habitat. This can occur via seed spillage during the movement of harvesting equipment between cultivated fields or during the transport of harvested seed, or seed can be moved by animal activity, wind, or water. Non-seed propagative plant material such as stolons or rhizomes could be moved via mowing equipment or by animal activity, wind, or water. However, the movement of seeds or other structures is independent of any transgenes in the crop genomes with which APHIS is familiar, so the escape of a GE crop is not inherently more likely than the escape of any other crop (Keeler 1985). Although it is conceivable that transgenes increasing seed dispersal rates could be engineered into crop plants, it is highly unlikely that this would be done. A primary goal for crop variety development is the prevention of seed loss via seed dispersal mechanisms (Frary and Doğanlar 2003), since the seed or fruit is usually the plant part with the highest value. However, if seed dispersal genes were to be altered in a crop plant, the resulting GE plants would merit increased scrutiny to verify that gene flow was not increased in ways causing significant environmental effects.

Gene Flow via Hybridization with Wild Relative

The exchange of genes between crop plants and sexually-compatible wild plants has occurred ever since plants were first domesticated. It is possible that a transgene could be established in the genome of a wild relative of the GE crop as a result of an initial hybridization between a GE crop and its wild relative, followed by introgression of the transgene into the wild relative's genome (Gealy, Mitten, and Rutger 2003) and rapeseed (Halfhill *et al.* 2004; Légère 2005). For a transgene to become incorporated in a wild crop relative, crop pollen carrying the gene would first need to be carried via wind or insects or other pollinators to a plant present in the crop field as a weed or present in a nearby unmanaged habitat. Conversely, pollen from a wild crop relative in the unmanaged habitat could be carried via wind or insects or other pollinators to a crop plant growing in a cultivated field. Hybrid seed produced in the crop field would have to be harvested along with the crop and be spilled onto non-cultivated land as discussed above, or dispersed by an animal, whereas the movement of crop pollen

Comment: Something is missing here.

onto uncultivated land could result in the production of hybrids with no seed movement necessary.

Hybridization between a GE crop and a wild relative is dependent on several key factors: simultaneous flowering, sexual compatibility, and proximity sufficiently close to allow pollen movement between the two plants (Ellstrand, Prentice, and Hancock 1999). The first two factors are determined by the specific crop and wild relative, and can result in little or no outcrossing as in the case of wheat or frequent outcrossing as in the case of rice (Ellstrand, Prentice, and Hancock 1999). However, even when a crop can hybridize with a wild relative, the plants must be close enough together to allow pollination to occur. Again, this factor is different for every crop plant and depends on a variety of characteristics including whether the crop is pollinated by wind, insects, or other pollinators, to what extent the crop is self-pollinated, how long-lived the pollen is, and how the crop is cultivated. However, these parameters have been studied in depth in many agronomically important crops, and the Association of Official Seed Certifying Agencies has established standard growing conditions for crop seed production which result in very low levels of outcrossing (AOSCA 2003).

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Invasiveness Potential

Only a small fraction of introduced species become successfully invasive (Ellstrand and Schierenbeck 2000), and there is no evidence that crops improved via genetic engineering are more likely to be invasive than crops improved via conventional plant breeding. The potential for a GE crop or a GE crop/wild-relative hybrid to become invasive depends first on the ability of the plant to become established in the environment, and second on the ability of the plant to successfully persist and thrive in the environment. Very few crops have been shown to be persistent and invasive outside of cultivation (Hancock and Hokanson 2001) and maintain their presence in the environment (Brown and Mitchell 2001; Ellstrand and Schierenbeck 2000; Mitchell and Power 2003). The particular transgene introduced into the crop may also have some effect on the plant's survival. Because the weediness and invasiveness of a particular crop is known throughout the U.S. range where the crop is produced, the invasiveness of a GE crop possessing one or more transgenes can be estimated by evaluating the environmental fitness impacts of the individual introduced genes (Hancock and Hokanson 2001).

Comment: If there are any, it would be helpful to name it (them); persistent would be easy, e.g., potatoes volunteering in the second and not just the first crop after potatoes in the rotation, but invasive??? Maybe cereal rye, but even the continued volunteering of cereal rye has been an issue of persistence and not invasiveness. This senetence needs to clarify the distinction as a minium; examples would help even more.

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In the case of GE crop/wild hybrids, establishment will depend on the fertility and overall vigor of the hybrid plants and their progeny (Vacher *et al.* 2004) as well as on the nature of the transgene. For example, naturally occurring hybrids between wheat and its distant relative jointed goatgrass are occasionally found, but the hybrids are usually self-sterile, due to a lack of proper chromosome pairing (Guadagnuolo, Savova-Bianchi, and Felber 2001; Morrison *et al.* 2002; Seefeldt *et al.* 1998). However, it is also possible for interspecific and intergeneric crosses to exhibit enhanced fitness through heterosis, an increase of genetic diversity caused by hybridization (Vacher *et*

Deleted: [4] Initial establishment of a crop plant will depend on the crop's ability to survive without any human intervention. This includes successfully competing with other plants for nutrients, water, pollinators, and sunlight; surviving attacks by diseases, insects, and other herbivores; and producing sufficient progeny or propagative structures to

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al. 2004). A hybrid may possess a novel combination of traits making it more able to adapt to an ecological niche than either of the parents (Ellstrand and Schierenbeck 2000). In other words, each type of hybrid may exhibit unique and possibly unexpected characteristics. For example, hybrids between oilseed rape and wild radish are much more fit when wild radish is the maternal parent (Gueritaine *et al.* 2002), but even so fitness is very low and dependent on the particular environmental circumstances (Al Mouemar and Darmency 2004; (Gueritaine *et al.* 2002). Fortunately, years of cultivation and plant breeding have resulted in an extensive and growing body of information regarding the likelihood of hybridization between crops and their wild relatives and the fitness and fertility of these hybrids (Arriola 1997; Stewart, Halfhill, and Warwick 2003).

Comment: This is the kind of statement needed in a few more places in this document. Keep GE in context with what has been done already with far more unknowns, yet so little has happened by way of unmanageable issues pertaining to weediness and invasiveness.

Persistence of Genetically engineered Plants in Natural Environments

The likelihood for a GE plant to persist in the environment depends primarily on the plant species and on the ecosystem in question, including competing species, diseases and herbivorous pests, and the physical environment. One factor that can be analyzed experimentally is whether the GE version of a crop plant has better field performance, *i.e.*, is more fit, or persists longer than a conventionally bred version of the crop. A recent study asked this question using GE and conventional varieties of corn, oilseed rape, sugar beet, and potato growing for ten years in twelve natural habitats in Britain. The transgenes studied were for herbicide resistance, Bt toxin, and pea lectin (Crawley *et al.* 2001). The study found that none of the GE crops were more fit or persisted longer in the environment than the conventional crop counterparts. Establishment of seedlings of the herbicide resistant corn and rapeseed was significantly lower than for the conventional versions of the crops, and survival of the GE potato lagged behind that of conventional potato (Crawley *et al.* 2001). However, it must be noted that none of the transgenes at issue in the study were intended to increase plant fitness in natural habitats.

There is little evidence that beneficial agronomic traits moved into crops via conventional breeding have led to the development of invasiveness in crop plants (Duvick 1999). Similarly, it is highly unlikely that the mere entry of a GE crop plant into an unmanaged ecosystem will result in the permanent establishment of the plant in that ecosystem. To evaluate the environmental impact of a GE crop, researchers begin with the body of knowledge developed through years of cultivating the non-GE version of the crop, including any information about its weedy or feral tendencies. It is then possible to superimpose any effects of the transgene on the already familiar traits of the non-GE crop (Parker and Kareiva 1996). A systematic experimental approach, where the field performance of a GE crop and its non-GE counterpart are thoroughly compared in the greenhouse and in the field, should indicate whether the transgene has any unexpected effects on characteristics that could contribute to invasiveness (Wang *et al.* 2003).

The transgene may or may not confer any advantage to the GE plant, depending on the nature of the gene, the ecosystem, and the presence of human intervention or other factors that may provide sporadic or continuously-acting selection pressure such as herbicide application, disease, insect attack, or environmental stress. Without this

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pressure, the transgene's effects would not be expected to manifest themselves, and the GE plant would be expected to be phenotypically indistinguishable from its non-GE counterparts in that particular environment (Vacher *et al.* 2004). For example, a transgene conferring herbicide tolerance would not increase fitness for the recipient plant unless the natural habitat was regularly treated with the appropriate herbicide (Metz, Stiekema, and Nap 1998). Lacking such management, the GE plants would not be expected to be any more fit than conventional plants of the same species (Gueritaine *et al.* 2002). If, however, regular herbicide applications were used, the GE individuals could have a significant advantage over their non-GE counterparts. However, not every transgene would be expected to respond to selection pressure. For example, a crop containing a transgene that alters a food quality trait is unlikely to have any effect on plant fitness, since there is unlikely to be a corresponding selection pressure for the trait (Parker and Kareiva 1996).

If the transgene confers insect or disease resistance, the recipient plant may gain a fitness advantage, but only if the insect pest or disease organism ordinarily acts to control the normal distribution or role of that plant in that particular environment (Parker and Kareiva 1996). In that case, it would be expected for the GE plant, whether crop or wild-relative hybrid, to have a fitness advantage over other plants in the environment (Vacher *et al.* 2004). The greater the impact of the insect pest or disease on the vigor and reproductive potential of the plant population, the more likely it is for the GE plant to have a fitness advantage over non-GE counterparts (Parker and Kareiva 1996). Over the course of many generations, with continuous selection pressure from the insect pest or disease, the GE plant could become invasive, in the case of a crop plant, or could replace the non-GE population, in the case of a GE wild relative.

Comment: Would it be appropriate to go so far as to say "evolutionary time," to reinforce the point that the period of time over which natural selection pressure gives rise to population shifts in unmanaged ecosystems are very very long?

If the transgene is question confers an agronomic characteristic such as drought tolerance or increased photosynthetic efficiency, the recipient plant could become invasive or replace its non-GE counterpart, but only if a corresponding environmental stress consistently acts to control the plant populations in that ecosystem. APHIS anticipates that as plant genetic engineering technology advances, applicants will propose with greater frequency field trials of plants with traits such as increased photosynthetic efficiency and tolerance to various environmental stresses. Such traits, either singly or in combination, could contribute to the invasiveness of a GE crop or crop/wild-relative hybrid or introgressed progeny. However, given that most crop plants are not naturally invasive, and given that most cultivated crops possess several domestication traits (Frary and Doğanlar 2003; Gepts 2002), such as dwarfing, non-shattering seed heads, and larger fruits, which usually are disadvantageous in unmanaged ecosystems, it has been proposed that a single plant would have to possess several transgenes conferring improved fitness characteristics before that plant would become invasive (Hancock and Hokanson 2001). The insertion of multiple genes affecting fitness in a single plant, so-called "stacking," is more likely as genetic engineering technology advances, and plants with such gene stacks would receive additional scrutiny to determine their potential for weediness or invasiveness.

A single instance of gene flow to an unmanaged ecosystem or a transgene into a wild relative is very unlikely to result in the development of an invasive population of GE

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plants (Ellstrand and Schierenbeck 2000; Siemann and Rogers 2001). Even if the initial introduction succeeds, a lag time of several generations may be necessary during which time the introduced species may undergo genetic adaptation, ultimately making the plants better able to survive in their new environment than other species or non-GE populations of the same species (Siemann and Rogers 2001; Willis, Memmott, and Forrester 2000). Multiple introductions via repeated instances of gene flow may be necessary before a potentially invasive species can become established (Ellstrand and Schierenbeck 2000). Delays in the development of invasiveness may also depend on the crop or crop relative in question (Ellstrand and Schierenbeck 2000). For example, trees, shrubs, and other perennial plants with long reproductive cycles may take decades or longer to develop invasiveness, assuming no human intervention, while annual plants or short-lived perennials may become invasive only a few years after an inadvertent instance of gene flow (Ellstrand and Schierenbeck 2000). Minimizing the size and frequency of transgene flow to unmanaged ecosystems is therefore the most direct way to minimize the development of invasiveness both in the short term and over long lag periods.

Potential Effects of Genetically Engineered Crops on Soil

Introduction

The plant-soil matrix is a complex milieu of interactions between abiotic and biotic components. These interactions can be considered on a small scale: seeds germinate within the soil, and the resulting seedlings and plants interact with the soil, soil microorganisms, and soil water resources to obtain nutrients growth, development and reproduction. The soil is then enriched through plant decomposition by scavengers and other soil dwelling organisms. Interactions also exist on a large scale: traditional agricultural practices, including tillage, irrigation and herbicide and pesticide use have significant and predominately detrimental environmental impacts. Both scales of interactions should be considered when evaluating potential effects of GE plants on soil and water environments.

The second edition of *Soil Taxonomy* (NRCS 1999) defines soil as the solids (minerals and organic matter), liquid and gases that occur on the land surface and are characterized by the presence of horizons, or layers, that are distinguishable from the primordial material as a result of additions and losses, transfers and transformations of energy and matter, and the ability to support rooted plants in a natural environment. Soil is a much more dynamic environment than indicated by this definition. It may be the most diverse and populous habitat on the earth; a single gram of soil contains millions of individual organisms, with several thousand species of bacteria alone (Torsvik *et al.* 1994). These organisms function in decomposition leading to soil formation, aeration, and nitrogen fixation and disease suppression; and aid in root function (Giller *et al.* 1997). The immense number of organisms and the complicated and poorly understood relationships between these organisms, the environment, and plants complicate the analysis of the potential effects of introducing GE plants and other organisms. This section will detail factors that should be

Deleted: Plants and the soil and water environments in which they reside are inarguably intertwined.

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Comment: This is a very important point that needs more comment. Consider, for example, the impact of crop rotation on soil organisms. We know a great deal about the changes in both pathogens and beneficial rhizosphere microorganisms in response to crop monocultures versus crop rotations. Below is a quote from an article in the proceedings of NABC 15 {Reference deleted}.

"…our understanding of environmental impacts [of new varities] and the effort to reduce them through science, education, regulations, and innovations have been based on two principles.

•*It is the management used to grow the variety, i.e., the intensity of tillage, pesticide use, planting date, irrigation, etc., and not the variety itself that has impact on the environment; and*

•*Each new crop variety usually leads to changes in the management system used to grow that crop.*

A new high-yielding variety may require more fertilizer or pesticides to attain its full yield potential, as was the case with the IR-8 semi-dwarf variety of rice released by the International Rice Research Institute (IRRI) in the 1960s. In this example, *management*, especially fertilization and pesticide application, changed in response to needs of the variety. The breeding programs that followed up on this breakthrough in yield potential, and that continue to this day, have worked largely to develop replacement varieties that maintain the high yield potential but also have genes for resistance to the diseases and pests as well as improvements in other agronomic traits and quality measures. The replacement varieties may have no higher yield potential but rather, and often just as important, they allow the crop to be produced with management that is both more economical and has less impact on the environment. In the example of direct seeding to protect soil, water and air resources by preventing soil erosion, the change in management happened first, and is now being followed by the development of varieties that fit the management, such as soybeans with herbicide-tolerance."

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considered when evaluating the potential effects of particular genes on the soil and groundwater environments.

Accumulation and Persistence

Some traits added to plants via genetic engineering involve the production of one or more substances that the plant would normally not produce. Novel chemical substances produced by GE plants may enter the environment from leaf shedding, root exudates and decomposition (Donegan *et al.* 1997). If these substances do not dissipate at a rate at least equal to the rate of the products entry to the soil system, bioaccumulation and biomagnification may result. Bioaccumulation is the increase in concentrations of chemicals in biological systems over time as compared to the chemical's concentration in the environment. This occurs when a chemical becomes more and more concentrated as it moves up the food chain.

Additionally, herbivorous animals that feed on these plants and subsequently die, either due to natural causes or due to consuming a pesticidal substance such as a Bt toxin, may also add these novel chemical substances to the soil environment. The substances in the plant may not necessarily be in the same form in the insect: for example, the Bt protoxins made by insect-resistant GE plants are modified in the guts of susceptible insects (Höfte and Whiteley 1989).

Bt crops offer the best studied example to date of accumulation, persistence and residual toxicity within the soil (Höfte and Whiteley 1989; Saxena *et al.* 1999). Crop residues from plants genetically modified to express *cry* genes from Bt have been shown to decompose more slowly in soil, possibly due to a higher lignin content compared to their non-GE counterparts (Stotzky 2004). The binding of chemical substances by soil particles is also a factor. The Bt toxins adsorb and bind rapidly (< 30 minutes) to clays and organic matter within the soil, allowing the Bt toxins to persist and also to remain toxic to insect larvae (Stotzky 2000, 2002). In non-flooded soils, the Bt toxins released from root exudates and biomass of Bt corn were bound to soil particles and remained larvicidal for at least 180 days (Tapp and Stotzky 1998) and toxins remained detectable in the biomass of Bt corn three years after incorporation into soil (Saxena and Stotzky 2003). The Bt endotoxin associated with Bt crops appears to degrade rapidly in water, with a half-life between four to ten days, depending on the presence of microorganisms (Douville *et al.* 2005). This result suggests that the Bt accumulation and persistence in water bodies adjacent to land planted with Bt-engineered crops is not a significant concern but more studies need to be done to further evaluate accumulation in soil and sediments in water bodies. Obviously, soil and natural bodies of water are not sterile environments and there are a number of abiotic and biotic factors that will affect accumulation and persistence, such as soil type, aeration, water movement, and soil biota activity.

GE plants may add more than novel chemical substances into the soil. DNA is also released into the soil as organisms decompose. Clay minerals bind DNA molecules and can prevent vertical movement of DNA within the soil and delay DNA degradation by protecting free DNA from degradation (Greaves and Wilson 1970). The presence of DNase in the soil can also affect the accumulation and persistence of DNA in soil

Comment: As an aside, wheat straw from crops treated with fungicides are also slower to decompose compared to straw from crops not treated with fungicides, making it more difficult to manage the high residue loads in no-till systems. This is a particular problem in Europe, possibly accounting in part for the slow adoption of no-till in western Europe compared to North America, Australia, and South America. However, there does not seem to be an issue of Bt corn slowing the adoption of no-till practice with corn because the residue breaksdown more slowly. On the other hand, Bt corn stays green longer, mostly because of less stalk rot caused by Fusarium. Bt corn has also been shown to have less of an issue with mycotoxins in the grain. This last point should be mentioned somewhere in this text. I believe the work was done by Munkvold when he was still at Iowa State University.

Comment: Saxena, D. and Stotzky, G. (2001) Bacillus thuringiensis (Bt) toxin released from root exudates and biomass of Bt corn has no apparent effect on earthworms, nematodes, protozoa, bacteria, and fungi in soil. Soil Biology and Biochemistry, 33: 1225-1230. The main point of this report as captured in the title should be brought out in this paragraph. By comparison, standard farming practices such as crop rotation and tillage have major effects on earthworms, nematodes, protozoa, bacteria, and fungi in soil.

(Blum, Lorenz, and Wackernagel 1997; and Dunfield and Germida 2004). Studies so far have demonstrated persistence of GE DNA from several days (Widmer *et al.* 1997) to at least two years (Gebhard and Smalla 1999) in the soil. As explained in the introduction to risk assessment, these data should be compared with corresponding data from the host plant modified by conventional breeding, mutagenesis, or other methods.

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Biomagnification and bioaccumulation of products released by GE organisms should be considered and compared with similar, potentially cumulative effects from traditional crops. APHIS is unaware of any studies or data demonstrating bioaccumulation or biomagnification as a result of planting GE crops. In cases where , biomagnification and bioaccumulation is likely, expression of transgenes in GE organisms can be manipulated in ways that may mitigate any biomagnification and bioaccumulation. New techniques that limit the expression to specific plant parts rather than the whole plant or prevent expression except in the presence of specific environmental stimuli can significantly limit how much of these products enter the soil.

Comment: Here, or somewhere in this section, it would be useful to introduce the concept of base line, ie. What we know or assume regarding the impact of current practices (rotations, tillage, planting date effects, use of pesticides) on nontarget organisms and then, taking these collective effects as the baseline, answer the question of what additional or different effects come from or can be expected from the addition of a specific transgene in the system.

Water and movement away from the site

Water can move products from GE organisms away from the immediate site of entry into the soil environment. Precipitation, run-off and irrigation will provide transport for these products through the soil column. These products can enter groundwater, where they may be transported to larger underground reservoirs used for drinking water or to neighboring streams via underground conduits. Soil factors affecting how water will move GE products include, but are not limited to, soil type, texture, permeability, and the depth of the water table.

Besides interactions on the small scale, such as increasing the number of organisms that are exposed as products of GE crops are carried by water out of the rhizosphere, there are large scale impacts that should be considered. For example, currently available GE glufosinate and glyphosate-tolerant crops allow farmers to replace triazine and chloroacetanilide herbicides with less environmentally damaging glufosinate- and glyphosate-based herbicides. These herbicides are readily degraded by soil-dwelling bacteria and fungi and therefore have a lower potential to reach water resources and also exhibit reduced human toxicity. Combined, this can lead to reduced risk in drinking water contamination and improvements in the water quality of vulnerable watersheds (Wauchope *et al.* 2001).

Comment: Excellent point. This is the kind of information that I am suggesting be introduced in more places, to keep the big picture and GE in context.

Interactions with Soil Organisms

Soil- and plant-associated microbial communities and their interactions are not completely understood, and modern agricultural cropping systems can affect these interactions (Dunfield and Germida 2004). Soil communities are incredibly diverse and include beetles, springtails, mites, worms, spiders, nematodes, fungi, bacteria and other organisms. Only a small portion of species within the classes of soil organisms have been described. For example, the number of identified soil-dwelling fungi is 18,000-35,000 species? Propagules per gram of soil?, while the projected number is

greater than 100,000 (Hawksworth 1991). These organisms improve the entry and storage of water, soil mixing resistance to erosion, plant nutrition, and breakdown of organic matter (Giller *et al.* 1997).

Plants develop mutually beneficial relationships with soil organisms in the rhizosphere, and these relationships are heavily affected by metabolites released by plants into the soil. Root exudates have been proposed to be the most important factor in the development of the rhizosphere microflora (Lynch and Whipps 1991). Genetic modifications in crops may alter these root exudates and affect the associated microflora. The incorporation of crop residues into the soil may affect organisms that are not directly associated in a mutualistic relationship with the plant but serve indirectly beneficial functions, *i.e.*, scavengers and decomposers, or which protect plants from detrimental microorganisms (Bashan and Holguin 1998). The degree to which these effects result in measurable changes to soil ecosystems may be difficult to assess. Additionally, the origin of the transgene should be considered. For example, soil-dwelling organisms are likely to have had previous exposure to proteins from *Bacillus thuringiensis*, a naturally-occurring soil bacterium, which potentially mitigates the impact of Bt crops on the soil ecosystem.

It is difficult to summarize the results of studies evaluating the impact of GE plants on soil-dwelling organisms since these impacts are tightly associated with the crop, the engineered trait, and the environment (Griffiths, Geoghegan, and Robertson 2000). The specific soil-associated community is also difficult to characterize and make generalizations about. The microbial community found at one field site may be entirely different from that at another field site (Dunfield and Germida 2001; Blackwood and Buyer 2004; Muchaonyerwa *et al.* 2004; Wu *et al.* 2004; Castaldini *et al.* 2005; Saxena and Stotzky 2001; Donegan *et al.* 1996; Duan *et al.* 2004; and Milling *et al.* 2004). The results of studies evaluating soil effects from GE crops thus far suggest that the impact on the microbial and invertebrate communities of the GE crops as compared to conventional crops was minor or not measurable? when compared to other factors such as seasonal, environmental and crop management? effects (Milling *et al.* 2004) and this appears to be the case for other soil-bound organisms.

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Interactions between the plant and soil organisms may also change due to unintended effects on plant traits and defense abilities, leading to reduced plant fitness. For example, studies have suggested that the application of glyphosate increased populations of various fungi in the soil (Brammall and Higgins 1987), and that glyphosate applications also suppressed natural plant defenses and enhanced disease susceptibility in crops and weeds (Wrather, Stienstra, and Koenning 2001; and Myers *et al.* 1999). However, further studies have been unable to link glyphosate resistance with disease susceptibility (Sanogo, Yang, and Schrem 2000). Certainly non-GE crops are not immune to changes in the soil community and disease prevalence due to unpredictable environmental events, and it is not clear that genetic modification necessarily makes plants more susceptible to deleterious soil organisms.

Large scale interactions, resulting from standard agricultural practices, could influence the incorporation of products from GE plants into the soil and the exposure of soil organisms. For example, the tillage system employed by the grower could

influence the amount of interaction that occurs between the novel proteins and the microbial community (Angle 1994). Direct-seed (no-till) cropping systems preserve fertile soil and reduces the amount of sediment that enters streams adjacent to the farmland, a major pollutant in streams. A report released by the Conservation Technology Information Center identified the largest growth in no tillage practice occurring where herbicide-tolerance technology is utilized, achieving weed control through the application of herbicides without damaging the crop (Fawcett and Towery 2002). No-till systems keep the GE plant material at the surface, limiting contact with soil organisms to those at the surface. On the other hand, with conventional tillage the GE plant material will be incorporated into the soil, potentially diluting the products, but also increasing the number of organisms exposed.

Horizontal Gene Transfer

Horizontal gene transfer (HGT) is the natural transfer of genetic material from one organism (the donor) to another organism (the recipient) that is not sexually compatible with the donor (Gay 2001). Though HGT is thought to be extremely rare, the transfer of chromosomal DNA between bacterial species is considered to represent a significant mechanism for their evolution (Nielsen, Bones, Smalla, van Elsas 1998). Plant DNA can persist in the soil (Gebhard and Smalla 1999), triggering concern that transgenes from GE plants may spread horizontally to bacteria. HGT is relevant to the assessment of risks to the soil environment because of the nature of some of the genes used to develop GE organisms. For example, there is concern that bacterial antibiotic resistance markers, used in the process of selecting GE cells, encode resistance to clinically useful antibiotics (Metz and Nap 1997). Theoretically, resistance could spread to recipient micro-organisms in soil or in the digestive tracts of humans and livestock (Dröge, Pühler, and Selbitschka 1998). Although there is general consensus that such HGT from plant tissue to micro-organisms would happen at extremely low frequencies and has not been observed under natural (i.e. in the absence of heavy selection pressure), this issue is considered on a case by case basis, whereby the nature of the antibiotic resistance and its occurrence in the environment are taken into account.

The plant surface and the immediately surrounding environment have been shown to be active areas of HGT between bacteria, likely due to nutrient availability, high humidity and proximity of colonizing bacteria on the limited growth surface (Björklöf *et al.* 1995). Studies of the uptake of free DNA into competent bacteria have been made of HGT in terrestrial and aquatic habitats by viruses (Kidambi, Ripp, and Miller 1994) and by natural transformation, the uptake of free DNA into competent bacteria (Bertolla *et al.* 1999). Particular elements within the rhizosphere and certain plant exudates can affect transformation frequencies (Nielsen and van Elsas 2001). However the rate of natural transformation in soil is extremely low; in sterile soil, natural transformation has been shown to occur at rates below 10^{-7} transformants per recipient (Nielsen, van Elsas, and Smalla 2000) and is estimated to be as low as 10^{-10} and 10^{-11} transformants per recipient in nonsterile soil (Smalla, Borin, Heuer, Gebhard, van Elsas, and Nielsen 2000). There are a number of mitigating factors that suggest that HGT is not a significant concern when examining the potential effects of GE plants on soil-dwelling organisms.

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Comment: As another aside, {Reference Deleted}

"Direct seeding, also known as "no-till farming," saves time, energy and steel by allowing annual crops to be produced with a sprayer (for weed control), planter [that simultaneously and precisely places seed and fertilizer in the same seed furrow in a single pass over the field (figure 1)] and combine harvester, thereby eliminating the need for plows, cultivators, disks, and harrows to till the soil. As an example of the economic benefits, it takes about one-fourth as much diesel fuel (approximately 5 L/T of grain compared to 20 L/T of grain) to produce wheat with direct seeding compared to wheat production using the standard and multiple tillage operations. More importantly than just the greater use of fuel, four times the amount diesel must be visualized in terms of the additional time and steel needed to use that much more diesel fuel.

In addition to the economic benefits, leaving all residue of the previous crop on the soil surface protects against evaporative loss of water needed to grow the next crop, essentially stops soil loss from wind and water erosion, results in a buildup (sequestration) of soil carbon as organic matter, and the stubble with all weeds seeds left on the soil surface provides habitat and food for birds and other wild life. Indeed, direct-seed cropping systems can provide the same ecosystem services expected of natural ecosystems, including mitigation of floods and droughts, purification of water and air, recycling of nutrients, and protection of biodiversity. North American agriculture has been moving slowly but steadily in this direction since the days of the Dust Bowl of the 1930s."

While I am citing the above as ... [1]

Comment: I note that the perspective comes later, but would it not be preferable to make the point here as to the extremely low probability (again, likely to be in "evolutionary time," meaning thousands or hundreds of thousands of years) that a microbe will acquire and integrate funtional DNA into its genome from the substrate that it grows upon. The gene for production of chalco ... [2]

Comment: Corerct as modified? Makes more sense this way

First, several events would have to occur successfully for HGT to occur. In order for natural transformation to occur in the soil environment, free DNA needs to be available. The persistence of DNA in the soil was discussed above. There must also be bacteria in close proximity to the free DNA which are capable of taking up the DNA. Some bacteria, called "naturally competent," have evolved the ability to transport free DNA from outside the bacterial cell into the cytoplasm; however, not all bacterial species are competent, and not all competent bacteria are competent all of the time. The transgene must then be incorporated and maintained by the recipient organism (Gebhard and Smalla 1998). Maintenance of the transgene requires that the alteration is non-detrimental to the fitness of the organism and therefore will not be negatively affected by selection. Transfer of a plant gene to a bacterium does not equal functionality in the bacterium; regulatory sequences (promoters, enhancers) may not work and introns may not be recognized in the recipient (Conner, Glare, and Nap 2003). As the risk of HGT from GE plants is considered, it is necessary to bear in mind that the transgene portion of a GE plant's DNA is a very small part of the total DNA. Therefore the likelihood that a piece of native DNA undergoes HGT is significantly greater than a given piece of transgene DNA (Conner, Glare, and Nap 2003).

Comment: Again, it would seem better to introduce the information in these three paragraphs as the opener to this section and then go into why there are still concerns and specifically what are those concerns or issues.

Comment: Is it possible to say how small?

Second, the antibiotic resistance genes commonly used have limited use in treating infections. The most popular selectable marker gene, *nptII*, has limited therapeutic value as an antibiotic and hygromycin, another antibiotic used in molecular biology, is too toxic an antibiotic for therapeutic use (Conner, Glare, and Nap *2003*). New genetic transformation methods rely less on antibiotic resistance marker genes, either by using other types of markers or by eliminating marker genes entirely. As a result, the potential HGT of antibiotic resistance genes is likely to continue to diminish in significance.

Third, although HGT between bacteria has been extensively demonstrated in natural systems (Nielsen *et al.* 1998), no evidence has been found of plant DNA moving to native soil microorganisms (Badosa, Moreno, and Montesinos *2004*; Heinemann and Traavik *2004*; and Maynard Smith, Dowson, and Spratt 1991). The lack of information on the abundance of naturally competent bacteria in the environment, frequencies of transformation processes and environmental factors triggering these processes impairs predictions of horizontal gene transfer from plants to bacteria (Gebhard and Smalla 1999). However, the probability of HGT is extremely low and the evidence thus far indicates that HGT does not pose a significant risk for the transfer of traits from GEOs. For example, Bt crops are currently grown in many countries, in many diverse environments, covering millions of acres, and there is still no evidence that Bt genes have moved, via HGT, to new organisms.

Conclusion

Comparing the risks of GE crops and their non-GE counterparts for the management of physical, chemical and biological aspects of soil ecology are important for sustainability of agroecosystems. The dynamic nature of the soil and its components and the lack of comprehensive research on soil interactions make it difficult to draw any definitive conclusions about the effects of products derived from GE crops entering the system. Traditional agriculture and its associated activities have

Comment: I suggests deletion of this Conclusion section. Connection to sustainability of agroecosystems in not clear and the rest is mostly generalizations or platitudes.

significant, often detrimental, impacts on soil's abiotic and biotic components, and it is thus far unclear that introducing GE materials increase or possibly alleviate these detrimental impacts.

Genetically engineered Crops and potential Impacts on Human Health

Introduction

The term "genetic modification" can be used to describe various methods of altering a plant's genetic makeup resulting in a plant that expresses different traits or characteristics than its parent (NRC 2004). In fact, crop plants have been genetically modified throughout history to produce plants with desired traits (Day 1996; Kessler *et al.* 1992; NRC 2004). The basic principles or steps in the process are: find or create useful variation for the trait or traits of interest and then transfer that trait or those traits into an agronomically adapted/acceptable variety with the desired end-use properties. Methods such as mutagenesis, chromosome transfer, genetic engineering and conventional breeding by hybridization typically are used together to acehieve the desired variety. For example, a useful trait cloned and transferred into a recipient line by genetici engineering, or induced by mutagenesis, will then typically be used to create the new variety using conventional breeding. Plant breeders introduce many new crop varieties intended for food and feed into commercial production every year that may have characteristics such as insect resistance, higher yield or improved nutritional attributes (Fuchs and Astwood 1996; Kessler *et al.* 1992; Metcalfe, Astwood *et al.* 1996). Each method of genetic modification, including the two most common methods currently employed, traditional breeding and genetic engineering, requires intense human intervention (NRC 2004). Traditional breeding techniques have been employed for centuries, while genetic engineering only became a prominent method of plant genetic modification late in the 20th century (Kessler *et al.* 1992; NRC 2004).

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All methods of genetic modification have the potential to alter a plant's genetic makeup resulting in changes in characteristics such as plant color, flavor, nutrient content, disease resistance and environmental stress tolerance (Kessler *et al.* 1992). While many of the potential changes in the plant's genetic makeup can be considered intentional and/or improvements, a number of unintentional changes can also occur (Fuchs and Astwood 1996; Kessler *et al.* 1992; Metcalfe, Astwood *et al.* 1996). These unintentional changes can have positive as well as negative effects on human health. In most instances, phenotypic changes such as plant color, growth, and production can be identified during the plant breeding and selection process, while unintentional changes such as changed levels of allergens, toxicants, and antinutrients are less apparent without additional analysis.

Traditionally, new varieties of whole foods have not been subjected to extensive chemical, toxicological or nutritional evaluation prior to marketing (CODEX 2003). The regulatory agencies that oversee the safety of GE plants do not evaluate new, traditionally-bred crop varieties for human or animal health safety (NRC 2004). The exception is in cases where the food may represent a substantial part of the diet of a specific group (*e.g.*, infants), where some evaluation is performed (CODEX 2003).

While most crops naturally produce some level of allergens, toxins, and antinutrients, standard plant breeding practices allow for monitoring the levels of potentially hazardous substances (NRC 2004). The history of crop development has shown that except in very rare instances, these standard crop development techniques include steps that make it possible to identify potential hazards in crop lines developed for commercialization (Kessler *et al.* 1992; NRC 2004; Pastorello *et al.* 1998).

Unlike traditional breeding, genetic engineering gives developers the ability to move genes from organisms that may not have a history of prior exposure in the diet of humans and animals into crop plants. Given this potential, there is some concern about the possibility of moving a gene whose gene product may be allergenic or toxic (NRC 2004). While the ability to move genes, the respective products of which are potentially toxic or allergenic is not unique to genetic engineering, biotechnology techniques can enable the movement of genes between a broader group of species (*e.g.*, plants, insects and microorganisms).

Deleted: genes

In the U.S., each GE crop undergoes an extensive food safety and environmental safety review prior to commercialization, using the most current and available scientific data and information (Fuchs and Astwood 1996; Kessler *et al.* 1992; Metcalfe, Astwood *et al.* 1996). To address the issue of GE crop safety, the U.S. government is guided by a Coordinated Framework (51 FR 23302, 1986) in which three different agencies, the Animal Plant Health Inspection Service (APHIS), Environmental Protection Agency (EPA) and Food and Drug Administration (FDA) each have a role. While the regulatory authority for APHIS focuses on plant health, both the EPA and FDA have responsibility to ensure the safety of human food and animal feed derived from GE crops. The EPA is responsible for the human/animal health and environmental safety of any pesticidal substance produced in these crops. The FDA is responsible for the safety of the whole food product and seeks to ensure that GE crops are as safe as their traditional counterparts.

Comment: Is it the crop or is it the product of the transgene that undergoes extensive review? My impression is that the gene product scaled up in a fermentor using E. coli or other delivery system accounts for most of the evaluation.

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To meet their responsibility to insure the safety of the food products, EPA and FDA use similar approaches to evaluate the potential allergenicity or toxicity of the gene product produced by expression of the transgene in the GE crop. In addition, FDA uses a multidisciplinary approach to assess changes in nutritional quality of food products derived from these GE crop plants. Both EPA and FDA have worked with members of industry and academia to develop guidance and methods for safety assessment, based on sound scientific principles, that help predict potential adverse effects of GE crop plants (EPA 2000, 2000; FDA 2002).

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This section will briefly address some of the prominent issues associated with the safety of GE crops used as human food and animal feed. The issues addressed in this section, from potential allergenicity and toxicity to nutritional quality, are more thoroughly reviewed in numerous publications and guidance documents that are publicly available. Organizations such as the National Research Council (NRC), Codex Alimentarius Commission (CODEX), and Food and Agriculture Organization/World Health Organization (FAO/WHO) have developed guidance for assessing the safety of GE foods (CODEX 2003, 2003; FAO/WHO 2000, 2001; NRC 2004). In addition, numerous other publications are available from researchers and

others, that address the issue of GE food safety (Astwood and Fuchs 1996; Fuchs and Astwood 1996; Fuchs and Goodman 1998; Lehrer, Horner, and Reese 1996; Mendelsohn *et al.* 2003; Metcalfe, Fuchs *et al.* 1996; Metcalfe 2003).

Potential Allergenicity of Proteins Expressed by Genetically engineered Plants

Background

Agricultural crops that are common components of our food supply contain thousands of different proteins that become part of human and animal diets. Some of these proteins have been well-characterized, while others have undergone little or no scrutiny. However, humans have been exposed to a huge variety of foods for thousands of years, and consumption of the vast majority of proteins found in foods presents little or no risk of adverse reactions (Metcalfe, Fuchs *et al.* 1996).

Deleted: Proteins are a common component of the human and animal diet.

However, there are a number of proteins that have been identified and/or characterized for their ability to induce allergic reactions, and the majority of allergic reactions triggered by these proteins involve IgE-mediated immune responses (Fuchs and Astwood 1996). While more than 160 foods and food-related substances have been associated with allergic reactions, a small group of foods, including eggs, milk, fish, crustaceans, mollusks, peanut, soybean, wheat and tree nuts are responsible for greater than 90 percent of allergic reactions in adults (Fuchs and Astwood 1996). Overall, only a small portion of the adult population - less than 2 percent - is considered to have food allergies (Kimber, Lumley, and Metcalfe 1997). While the severity of food allergy can vary significantly, when an allergy to a food item is confirmed, individuals are usually allergic to only a few specific proteins in one or two specific foods (Metcalfe, Fuchs *et al.* 1996). The percentage of children with food allergies is slightly higher than adults, with children being most frequently allergic to milk and eggs. Most food allergies in children disappear by adulthood (Kimber, Lumley, and Metcalfe 1997; Metcalfe, Fuchs *et al.* 1996).

Scientific methods used to assess allergenic potential continue to evolve. However, there is no one test that can be used to assess the allergenic potential of a protein. Because of this, the safety of a protein is typically assessed based upon the source of a protein and its structural characteristics, compared to the structural characteristics of known allergens. These structural characteristics include the stability of the protein to enzymatic degradation, such as an artificial digestive system; stability to heat and/or acid and enzymatic treatment; and amino acid sequence similarity of the protein to known allergens (CODEX 2003). The use of animal models for allergenicity determination has been an area of significant focus over the past decade, but no adequate model has been developed.

Approaches to Allergenicity Assessment

In addition to several reviews that focus on engineered foods and food allergy published by members of industry and academia (Astwood and Fuchs 1996; Astwood, Leach, and Fuchs 1996; Gendel 1998, 1998; Lehrer, Horner, and Reese 1996; Sampson and Metcalfe 1992), in 1996, the International Food Biotechnology Council

(IFBC) in collaboration with the Allergy and Immunology Institute (AII) of the International Life Sciences Institute (ILSI) published a peer-reviewed report that proposed an approach to evaluating allergenicity of proteins in bioengineered foods (Metcalfe, Fuchs *et al.* 1996). The approach taken by the scientists participating in this effort used a decision tree for the assessment of potential allergenicity.

In 2000, the Food and Agricultural Organization of the United Nations (FAO) and World Health Organization (WHO) convened a Joint Expert Consultation on Foods Derived from Biotechnology and published a report on the safety aspects of bioengineered crops that included a discussion of allergenicity (FAO/WHO 2000). The 2000 FAO/WHO report supported the approach to allergenicity assessment described in the 1996 ILSI/IFBC report and adopted a slightly modified version of the 1996 ILSI/IFBC decision tree.

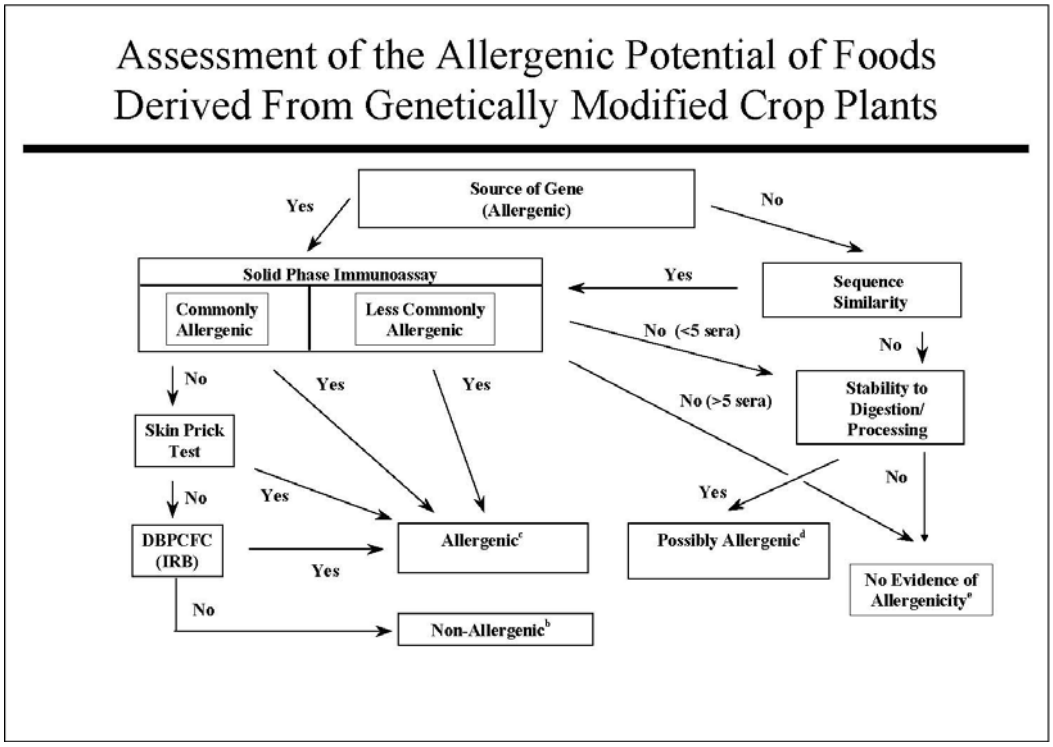

Figure 1 Decision tree for the assessment of the allergenic potential of foods derived from GE crop plants Source: Joint FAO/WHO Expert Consultation on Allergenicity of Foods Derived from Biotechnology, 2000.[5]

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[5] (a) The figure was adapted from decision-tree approach developed by International Food Biotechnology Council and Allergy and Immunology Institute of the International Life Sciences Institute (Metcalfe *et al.*, 1996).
(b) The combination of tests involving allergic human subjects or blood serum from such subjects would provide a high level of confidence that no major allergens were transferred. The only remaining uncertainty would be the likelihood of a minor allergen affecting a small percentage of the population allergenic to the source material.
(c) Any positive results obtained in tests involving allergenic human subjects or blood serum from such subjects would provide a high level of confidence that the novel protein was a potential allergen. Foods containing such novel proteins would need to be labeled to protect allergic consumers.
(d) A novel protein with either no sequence similarity to known allergens or derived from a less commonly allergenic source with no evidence of binding to IgE from the blood serum of a few allergic individuals (<5), but that is stable to digestion and processing should be considered a possible allergen. Further evaluation would be necessary to address this uncertainty. The nature of the tests would be determined on a case-by-case basis.

In January 2001, the Joint FAO/WHO Consultation on Foods Derived from Biotechnology was convened specifically to provide scientific advice in relation to the assessment of allergenicity of bioengineered foods (FAO/WHO 2001). The consultation focused on several items, including the general issues of allergenicity of foods from GE crop plants, the reevaluation of the decision tree for the assessment of allergenicity of foods from GE crop plants developed in the 2000 FAO/WHO report (FAO/WHO 2000), and the development of standardized procedures for the use of the decision tree. After consideration of the current status of scientific information and extensive discussion, these scientists developed a new decision tree.[6] This decision tree builds upon previous approaches to examining allergenicity but also includes several additional strategies. These strategies are: targeted serum screening of proteins from sources with no known history of allergenicity; targeted serum screening of protein from sources with no sequence homology to known allergens; the use of animal models; and the elimination of human testing.

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Comment: Again, avoid introducing a new term…bioengineered food, since typically it is not the food itself that is bioengineered, depending on the product produced.

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In 2003, the Codex Ad Hoc Intergovernmental Task Force on Foods Derived from Biotechnology finalized documents describing "Principles for the Risk Analysis of Foods Derived from Modern Biotechnology" and "Guidelines for the Conduct of Food Safety Assessment of Foods Derived from Recombinant-DNA Plants." This latter document included an Annex on the Assessment of Possible Allergenicity (Codex 2003). In the allergenicity annex, the Task Force acknowledges that there is no definitive test that can be relied upon to predict allergic response in humans to a protein new to the food supply, and recommended a “weight of evidence” approach.

This approach begins with an initial data set consisting of the source of the newly introduced protein; any significant similarity between the amino acid sequence of the protein and that of known allergens; and its structural properties including but not limited to its susceptibility to acidic or enzymatic degradation and its stability to heat. Specific serum screening is recommended for proteins that originate from a source known to be allergenic or have sequence homology with a known allergen. These data, as well as additional factors including predicted levels of exposure and the effects of relevant food processing, contribute to an overall conclusion regarding human health risk. Targeted serum screening and animal models are also discussed in the allergenicity annex. However, neither is recommended for inclusion in an allergenicity assessment until fully developed and validated as predictive for human allergic response.

(e) A novel protein with no sequence similarity to known allergens and that was not stable to digestion and processing would have no evidence of allergenicity. Similarly, a novel protein expressed by a gene obtained from a less commonly allergenic source and demonstrated to have no binding with IgE from the blood serum of a small number of allergic individuals (>5 but <14) provides no evidence of allergenicity. Stability testing may be included in these cases. However, the level of confidence based on only two decision criteria is modest. The Consultation suggested that other criteria should also be considered such as the level of expression of the novel protein.

[6] http://www.who.int/entity/foodsafety/publications/biotech/en/ec_jan2001.pdf

Digestibility and Stability

Stability to digestion, in addition to stability to heat and other food processing conditions are among the characteristic properties that were first considered to be common among food allergens. Simulated gastric and simulated intestinal fluids are used as *in vitro* models for assessing the digestibility of proteins (CODEX 2003; FAO/WHO 2000, 2001; Metcalfe, Astwood *et al.* 1996). Proteins rapidly broken down into single amino acids and peptides smaller than 3.5 kDa are considered to be readily digestible (FAO/WHO 2001).

The rationale for the use of digestion stability is based on initial information that food allergens were not readily digestible (Taylor 1987). However, more recent studies comparing the digestion stabilities of food allergens and other proteins have shown that some allergens are as digestible as non-allergens and allergens are not necessarily more stable than non-allergenic proteins. In addition, it has been shown that major food allergens are not necessarily more stable to digestion than minor allergens (Astwood, Leach, and Fuchs 1996).

Another rationale to support the use of digestion stability as a criterion for allergenicity assessment is that stability to the conditions of the human digestive system is a key requirement to sensitize for food protein allergenicity. One study suggests that an allergenic protein must be intact to sensitize a person (Hanson *et al.* 1993). However, degradation of food allergens may not prevent elicitation of allergic reactions in individuals that have previously been sensitized (Maynard, Jost, and Wal 1997; Nilsson *et al.* 1999). While there are few data in the literature to support the assertion that stability in human digestive tract is required for food allergenicity, a consensus exists among scientists that the likelihood of small peptides to sensitize an individual is low.

Because allergic reactions involve the interaction between an entire protein molecule or specific three-dimensional parts (called “epitopes”) of the protein with various components of the immune system, the easier a protein is degraded by digestion, the less likely the three-dimensional structure necessary for the interaction will be retained. In this respect, stability to digestion may be one useful parameter to measure the likelihood of a protein to interact with the immune system when ingested and therefore its ability to sensitize. However, it may not be useful to prevent entry into the marketplace of those proteins that cause human sensitization through routes other than ingestion (*e.g.*, respiration or skin contact) or proteins that cross-react with existing proteins that have already resulted in the sensitization of some individuals. In these cases, rapidly digested proteins, or previously degraded proteins, may still elicit allergic responses in individuals who have already been sensitized (Nilsson *et al.* 1999).

Because the correlation between the allergenicity and the digestibility of a protein has not yet been established definitively, it is difficult to relate, with confidence, the outcome of the stability/digestibility studies to the allergenic potential of a protein. However, a protein that degrades rapidly is less likely to interact with the immune

system and thus less likely to be allergenic. A protein that is resistant to digestion is more likely to interact with the immune system but is not necessarily an allergen.

Sequence Homology and Similarity

Amino acid sequence analysis is an important tool for identifying similarity between a new protein and a known allergenic protein (CODEX 2003; FAO/WHO 2000, 2001; Metcalfe, Astwood *et al.* 1996). However, there are no standardized rules that can be applied to how sequence comparisons are performed, nor are all allergenic proteins included in databases. Searches are also limited to examining linear sequence homology with known allergens that have already been sequenced. Further, the usefulness of sequence homology comparison is limited by a number of other factors, including the algorithms and strategies used for the search, and the criteria for evaluating the search, in addition to the composition, completeness, and design of the database. When sequence homology to a known allergen is detected, additional evaluation of that protein should be performed, including immunoassay using sera from individuals sensitive to the allergen in question.

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Animal Models

Because no one characteristic property of food allergens is predictive of potential allergenicity of novel proteins, there has recently been much emphasis on the development of animal models. Several animal models for food allergy are currently under development, employing Brown Norway rats (Penninks and Knippels 2001); Balb/c mice (Dearman and Kimber 2001); C3H/HeJ mice (Li *et al.* 2001); and Beagle dogs (Ermel *et al.* 1997). These models differ with respect to route of sensitization, route of challenge, symptoms exhibited, and responses evaluated.

In the context of protein allergenicity, the production of specific IgE antibodies is the most relevant response in the vast majority of instances. Many of the rodent models have been developed in strains that are genetically disposed to react with specific serum IgE to various test proteins. The genetic predisposition of these animals is believed to mimic the susceptible human population. In addition to providing important information for understanding the mechanism of allergenicity, animal models may be useful in the prediction of the allergenicity of a novel protein and in risk assessment. With appropriate positive and negative controls, animal models may be important in determining the threshold of sensitization, the dose-dependent effects in sensitization and challenge, as well as the ranking of allergenic potency among proteins.

There are numerous limitations to current animal models for the assessment of food allergenicity. Because no animal models have been validated at this time, assessment of protein allergenicity using models can be made only in association with other data and information. Few models have been tested for the ability to rank the allergenic potency of proteins using proteins of known allergenicity, a key step in validating a model. In addition, one factor common to both human and animal allergenicity is genetic predisposition. However, it is unlikely that a single animal model can adequately reflect the genetic variability of humans, in particular, the predisposition to

respond to all potential allergens. Furthermore, published data show that the diet of animals must be free of the test protein for at least two generations prior to testing for potential allergenicity (Knippels, Penninks, and Houben 1998).

Serum Testing

Immunoreactivity with sera from sensitized individuals is another measure used to evaluate the potential allergenicity of bioengineered foods. Serum testing is an important element of current approaches for allergenicity assessment, because specific IgE against food antigens is often, but not always, associated with clinical allergy. An indication that serum IgE has reacted to a new protein suggests a cautious approach should be taken with the development of a GE crop. This is based on the fact that IgE is implicated in immediate hypersensitivity reactions that are consistent with food allergies. In addition, serum testing is a minimally invasive and low risk procedure (CODEX 2003; FAO/WHO 2000, 2001; Metcalfe, Astwood *et al.* 1996).

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To date, serum testing has been recommended for proteins derived from known allergenic sources or for proteins exhibiting sequence homology with known allergens. Under these circumstances, it has been possible to obtain sera from individuals sensitive to the particular allergenic substances in order to conduct the testing. Consistently positive results among several individual sera may indicate that further study is necessary to determine the clinical significance of the reaction. Inconsistent positive serological results from an enzyme-linked immunosorbent assay (ELISA) may be confirmed by other methods such as competitive inhibition with free antigen or Western blot.

However, while positive results are suggestive of potential allergenicity, the presence of IgE antibodies does not always correlate with clinical allergy. In addition, negative results may be inconclusive. Furthermore, human sera for less commonly allergenic substances may not be available. However, despite its limitations, specific serum testing is currently one of the most useful methods for screening proteins derived from known allergenic sources or for proteins exhibiting sequence homology with known allergens.

In the case of proteins derived from non-allergenic sources, the choice of which sera to use is problematic. In that situation, no known human serum is available to serve as a positive control and the results of such testing would be difficult to interpret. A negative result would always be subject to the question of whether a sufficient number of sera were used. In addition, the criteria for the quality and quantity of 'normal' sera to be used have not been established.

Potential Toxicity of Proteins Expressed by Genetically engineered Plants

Background

As previously discussed, crop plants contain thousands of proteins and other substances that have become a normal part of the human and animal diet.

Fortunately, the vast majority of substances found in crop plants do not cause adverse health effects in humans and animals (Fuchs and Astwood 1996; Kessler *et al.* 1992; Metcalfe, Astwood *et al.* 1996). However, all foods, regardless of their source or method of development, can potentially contain toxins and other dangerous substances. Because of familiarity gained during development of crop plants over many centuries and the inclusion of these crops in mammalian diets, much is known about the toxins, antinutrients, and other undesirable substances that can occur naturally in crop plants (CODEX 2003; NRC 2004). In addition to information available in published literature, OECD has published a number of consensus documents for a variety of crop plants that provide details about crop biology, including the presence of potentially harmful substances (OECD 2004).

While it is important to know what harmful substances can be found in our food crops, it is also important to have knowledge about what levels of these substances are considered acceptable, and do not pose a threat to human or animal health (CODEX 2003; NRC 2004). This knowledge gives plant breeders the ability to identify breeding lines containing elevated levels of these toxins relatively early in the breeding process and therefore avoid introducing these potentially harmful crops into the food (NRC 2004). This same knowledge is also valuable to crop developers who use genetic engineering as a method of plant genetic modification. An important consideration for GE crop developers is not only what toxins may naturally occur in a plant, but also whether any unintended changes occurred in the plant as a result of the transformation process that potentially introduces new harmful substance or increases levels of those substances that occur naturally in the plant (CODEX 2003; NRC 2004).

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Once an assessment is completed on the plant itself to identify potential unintended changes resulting from the transformation, the safety assessment should then consider the donor, chemical nature and function of the newly expressed substance (CODEX 2003; NRC 2004). If the GE plant expresses a specific protein, then the toxicity assessment may include an amino acid sequence similarity comparison to known toxins and anti-nutrients; stability to heat, processing and digestion; acute oral toxicity tests using surrogate animals; and previous exposure in human or animal diets (CODEX 2003; EPA 2000; NRC 2004). When the GE plant expresses a substance that is not a protein, the toxicity assessment should be handled on a case-by-case basis, using appropriate tests to characterize the expressed substance (CODEX 2003). Further, the assessment should ensure that genes coding for known toxins or anti-nutrients that may be present in the donor organism are not transferred to the GE plant (CODEX 2003; NRC 2004).

Exposure

As mentioned, it is not only important to consider if a substance found in a plant is potentially harmful, but it is also important to consider the level at which exposure to the substance is considered unsafe (Day 1996; NRC 2004). This can either be accomplished by considering available data based on prior exposure to the substance in the diets of humans or animals, or by using surrogate animal testing that will be discussed later in this section (CODEX 2003; NRC 2004). If the substance has

previously been a part of the human or animal diet, then information should be available about what levels of exposure are considered safe or if there have been reports of any adverse effects resulting from exposure to the substance. If there has been no previous exposure then consideration should be given to how much and in what form exposure to the expressed substance will occur and whether the crop is normally consumed raw or if it is normally processed or cooked. If the crop is to be consumed raw, a determination should be made regarding whether the substance is stable to digestion; if the food is normally processed food, a determination should be made whether the processing affects stability (CODEX 2003; NRC 2004).

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Amino Acid Sequence Similarity

Most proteins and other substances that are known to be mammalian toxins have been well studied (Ecobichon 1993; EPA 2000; Majak 1995). For toxic proteins, their amino acid sequences have been elucidated and entered into publicly available databases. These databases are powerful tools that can be used as part of the safety assessment for substances expressed in GE plants. The amino acid comparison can be used to identify structural and functional relatedness of a protein to known toxins and antinutrients (CODEX 2003; EPA 2000). However, while the amino acid sequence comparison can provide valuable information about the relatedness of a protein to a known toxin, relatedness, or lack thereof would only be considered to be part of the assessment (CODEX 2003; NRC 2004).

Digestibility

Many of the substances are well characterized and others remain unknown or uncharacterized. For proteins found in the mammalian diet, the assumption is that these proteins are broken down into amino acids or peptides as part of the digestion process (EPA 2000). As discussed in the section on allergenicity, stability of a protein to digestion can be assessed using in vitro methods in which the protein is subjected to simulated gastric fluid, and then examined by gel electrophoresis (CODEX 2003; EPA 2000; NRC 2004). While in vitro digestion alone is not a sole determinant of the potential of a protein to be a toxin, it can contribute to the overall characterization of a protein (CODEX 2003; EPA 2000; NRC 2004).

Comment: Repetious; in fact, this entire section seems repetitious with sections above.

Deleted: Crop plants contain numerous proteins and other substances.

Surrogate Animal Testing

Unlike the allergenicity assessment for proteins, surrogate animal testing can be performed when warranted to assess the potential toxicity of a protein or other expressed substance. The use of animal models is often a significant part of the risk assessment of substances, such as proteins, expressed in GE crops (CODEX 2003; EPA 2000; NRC 2004). The use of surrogate animals allows for determination of whether a substance is potentially a mammalian toxin and at what exposure levels a potential toxin can induce adverse effects upon consumption. However, there are limitations to the use of animal models, such as when they are used to assess the safety of whole foods (CODEX 2003). Feeding surrogate animals certain whole foods, or diets comprised entirely of one food can induce adverse effects on the animal that

are not related to the test substance itself (CODEX 2003). Therefore, the use of surrogate animals may not be appropriate when attempting to identify any potential unintended effects that may occur as a result of a transformation.

Nutritional Quality of Genetically engineered Plants Compared to their Traditional Counterparts

Background

Agricultural food crop development through the use of modern biotechnology techniques has provided the potential to improve the amount and quality of our food supply (Fuchs and Goodman 1998; Kessler *et al.* 1992; Metcalfe, Astwood *et al.* 1996). Crops have been developed to resist damage caused by insects and microorganisms that in turn allows for a reduction in the amount of chemical pesticides used on these crops. Other food crops have been developed that may provide improved nutritional quality, or produced improved oils purified from those crops. In addition to crops GE for use as food and feed, some agricultural crops have been developed to produce pharmaceutical or industrial products (ISB 2004).

Food and feed crops have been and will continue to be engineered to intentionally alter the nutritional quality of that crop. In such cases, additional analysis is required to determine what impact the intentional composition change may have (CODEX 2003; Kessler *et al.* 1992; NRC 2004). Beyond the food safety aspects of GE crops, the nutritional quality of these crops should be analyzed to ensure that they are as nutritious as the same crop that was developed through traditional methods and grown under the same conditions (NRC 2004). When performing the overall assessment of a GE crop, a determination is made about whether the engineered crop is substantially equivalent to its traditional counterpart (NRC 2004). Substantial equivalence in terms of plant composition, along with the other safety testing, helps address the issue of whether there has been any unintended changes resulting from the transformation process (NRC 2004). It should be seen as a key step in the safety assessment process although it is not a safety assessment in itself; it does not characterize hazard, rather it is used to structure the safety assessment of a GE food relative to a conventional counterpart. (OECD 2000)

Comment: As all of the varieties of that crop developed by traditional breeding? The most nutritious varieties of that crop developed by traditional breeding? The recipient variety not engineered? This requirement seems absurd. Traditionally- bred varieties of any given crop are highly unlikely to be exactly the same as sources of nutrients, but so what? The range of variation has not been an issue. Citation of the NRC 2004 study does not validate the stance expressed in this statement. Cite experience with traits induced by mutagenesis? If ever there is a method to change the nutrient status of the variety, it would be mutagenesis. This paragraph needs major modification.

Composition

Each line of crops developed via genetic engineering should be analyzed to determine the composition of the transformed plants. In others words, the concentrations of key components (*e.g.* nutrients, anti-nutrients, metabolites, toxins and allergens) normally found in a particular crop should be determined and compared to what are considered to be normal levels of these components in parental and/or non-transformed lines of the same crop (CODEX 2003; NRC 2004). CODEX describes key nutrients and key anti-nutrients as those found in a particular food that may have a substantial impact on the overall diet (CODEX 2003). If this analysis identifies any differences in composition of statistical significance, then an analysis should be performed to determine if the differences have biological significance (CODEX 2003; NRC 2004).

Examples of assessing potential impacts of GE plants on the environment.

Genetically Engineered Insect-Resistant crop plants

Introduction

In general, all plants have the ability to repel, destroy or mitigate pests. While the mechanisms of plant pest resistance remain a mystery in most cases, all plants are resistant to most pests. In other words, plant pest susceptibility is generally the exception (CAST 1998). For centuries, farmers and plant breeders have used insect and disease resistance genes from wild relatives to improve crop plants, an ongoing process because often insects overcome the resistance. Agricultural biotechnology has increased the number of ways in which crops can made resistant to pests. Since the early 1990s, many biotech companies and public institutions (*e.g.*, government and universities) have invested considerable research and development efforts on GE crops resistant to insect pests. To date, only insect-resistant plants expressing genes from *Bacillus thuringiensis* (Bt) have been deregulated by the United States Department of Agriculture Animal Plant Health Inspection Service (USDA/APHIS) and registered for commercial use by the EPA. Bt proteins have been used for more than 40 years as microbial insecticides, which are sprayed on crop plants. However, their use in commercial agriculture has been limited because the proteins are short-lived in the environment, and sprays can protect only above-ground portions of the plant. Genetic engineering of plants that contain Bt proteins in all tissues continuously throughout the growing season has overcome many of the limitations of Bt microbial insecticides.

Bacillus thuringiensis is a naturally occurring gram-positive bacterium found in many environments including soil, insects, stored-product dust, and deciduous and coniferous leaves. There are two current types of Bt proteins used as insecticides: "crystal" proteins and "vegetative insecticidal" proteins. Crystal proteins, called Cry toxins or delta-endotoxins, form within the spores of Bt bacteria. When ingested by a susceptible insect, these proteins readily bind to receptors on the midgut, insert into its membrane (Gill, Cowles, and Pietrantonio 1992; Schnepf *et al.* 1998), and form pores causing destruction of cells, leading to starvation, gut paralysis, septicemia (blood poisoning) and death of the insect (Schnepf *et al.* 1998).

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Commercialization of Bt crops has resulted in fewer insecticide applications and thus lower management costs (Fitt 2000; Schnepf *et al.* 1998). Also, one notable advantage of Bt crops over conventional insecticides is their high specificity, such that potential toxic effects on non-target insects are minimal (Betz, Hammond, and Fuchs 2000; Macintosh *et al.* 1990). Bt crops may also reduce the need for synthetic insecticides which, in turn, would decrease risks to the environment and effects on non-target organisms including beneficial insects.

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Assessment of environmental effects

Issues that are typically considered in risk assessment of Bt crops include:

- Potential effects on non target organisms
- Potential unintended effects on the target organism
- Potential changes in toxicity and allergenicity.

Potential effects on non-target organisms[i]

As the inserted genes code for insecticidal proteins, there is reason to consider in the risk assessment the question of potential effects on non-target organisms, including beneficial organisms. The scenarios that would be considered are (1) direct effects in the case of other insects or other animals eating the GE plants with the Bt gene, and (2) indirect effects in the case of other animals that consume the target insects [delete here to end of sentence?] due to (a) indirect consumption of the Bt protein or (b) reduced numbers of prey. In the cases of the GE crops with Bt genes to date, the gene products are well known to specifically target a small group of Lepidoptera. The likelihood of those Lepidoptera insects being directly affected by the Bt toxin depends on the size of crop, i.e., in cases of small scale field trials, any impact at the population level of affected Lepidoptera insects is very unlikely. In cases of large scale commercial use, the estimation of likelihood should consider the presence and feeding behavior of those Lepidoptera insects, which depends on those insects and on the crops involved. When those insects are not present in the area of planting or do not use the crop involved as main source of food, then an impact at the population level of those insects is very unlikely. When they are present and do use the crop involved as main source of food, [end here?] additional testing may be required.

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Comment: For what? Food? Habitat? Is this about the Monarch butterfly issue and corn pollen on leaves of milkweed? This issue seems like a real stretch. The implication of this argument reminds me of the British work published in *Science* 3 or so years ago reporting that a certain speices of field birds (larks?) were negatively impacted by HT sugarbeet because weed control was so good it left too few weed seeds in the field for the birds. Surley plant breeders will not need to answer these kinds of questions for APHIS. And since the great majority of U.S. crops are naturalized alien plant species, what did those insects do before that crop was introduced for commercial use? What would they do when fewer acres of the crop are planted because of economic considerations, e.g., as is happening in the U.S. of late with wheat.

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Potential unintended effects on the target organism

The continuous production of Bt proteins may increase the potential for target insects to become resistant to Bt proteins through constant selection pressure upon target and non-target susceptible insects. This type of sustained exposure can increase the probability for pest resistance to develop. These concerns resulted in the requirement of insect resistance management (IRM) strategies for good stewardship of these crops, and EPA has been the lead government agency regulating IRM for Bt crops. Written reports on various aspects of IRM are submitted to EPA to aid in the evaluation of the success of resistance management for Bt crops. Although information is often shared between EPA and USDA/APHIS, most of the IRM materials and reports are not submitted to or reviewed by APHIS as part of deregulation.

An insect resistance management strategy is developed by incorporating (1) knowledge of Bt proteins, their targets, and their alternative modes of action, (2) knowledge of pest ecology and biology, (3) appropriate dosages for Bt proteins, (4) appropriate refuge design, and integrated pest management (IPM) of the refuge and Bt crop, (5) plans for monitoring, reporting and mitigating incidents of insect resistance, and (6) communication and educational strategies on the use of the product (SAP 1995, see Office of Pesticide Program (OPP) docket, OPP-00401) into a single plan to delay resistance of target pests to Bt crops.

Thorough knowledge of pest biology is essential to the effective use of crops expressing Bt proteins and to the management of insect resistance to Bt proteins. For example, feeding behavior of the target pest may influence the optimal location within the plant for Bt protein expression, as well as dosage expression (see 'Dose of Bt Insecticidal Protein' section). Larval and adult movement (within and between fields, and overwintering habitats) may affect the types, sizes, and management of refuges developed for IRM (see 'Refuges' section). Reproduction (egg-laying habits, mating preferences, and generations per year) will also influence the development of resistance management plans, particularly when implemented to encourage random mating of insects residing in Bt and non-Bt crops (see 'Refuges' section below for further discussion).

Another important component of IRM is determining the effective and appropriate dosage of Bt protein. The February, 1998 FIFRA Scientific Advisory Panel (SAP) Subpanel on *Bacillus thuringiensis* (Bt) Plant-Pesticides and Resistance Management determined that a high dose strategy (together with a refuge strategy, see 'Refuges' below) is necessary to mitigate resistance of stalk boring Lepidoptera (e.g., moths) in Bt corn (meeting held on February 9-10, 1998. Docket # OPPTS-00231). A "high dose" is defined as 25 times the protein dose necessary to kill all susceptible lepidopteran insects (EPA-SAP 1998). For coleopteran (beetle)-active Bt products, the definition of a high dose has not been determined, nor has it been concluded that a high dose is necessary to mitigate resistance.

The August 2002 SAP suggested that it is adequate to differentiate between high dose and non-high dose products when determining an effective IRM. A high dose strategy would involve killing a high proportion of the target pest population, whereas a non-high dose has limited impact on the fitness, survival and selection pressure of the target pest. Products with a non-high dose may not require a refuge and may have a longer period of durability. In general, IRM plans for a non-high dose product are not well understood. With a non-high dose event, it may be difficult to sufficiently control the pest while limiting effect on its fitness (http://www.epa.gov/scipoly/sap/1998/february/finalfeb.pdf).

Comment: Non-high dose is not clear. Does it mean low dose? Please define

Refuge development is another significant component of an IRM program. Structured refuges are non-Bt host plants that are managed to provide sufficient Bt-susceptible adult insects to mate with potentially Bt-resistant adult insects. These matings result in Bt-susceptible offspring, which decreases the number of resistant insects and dilutes the frequency of resistance genes.

Refuge size, proximity to the GE crop and refuge management are believed critical for resistance management. Refuge size and location must be structured to maximize the potential for mating between susceptible insects (from the refuge) and possible resistant survivors (from the Bt field). Currently, refuges are planted with a similar hybrid, in close proximity to, and concurrently with, the Bt crop. Refuges are treated as needed to control insect pests with non-Bt insecticides or other appropriate IPM practices but otherwise managed by the same practices used in the field planted to the Bt crop.

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Consideration should also be given to the use of temporal, alternative host, and spatial refuges to delay resistance. Adjusting the planting time and/or availability of alternative host-plants, may increase the attractiveness of the Bt field by manipulating the insects' life history strategies. Currently, there is no evidence that refuges can be temporally implemented to delay resistance.

As more Bt products are commercialized, it is theoretically possible for insect pests to come into contact with multiple Bt insecticidal proteins during their development. If the insecticidal proteins produced by the Bt plants all have similar modes of action, pests may develop cross-resistance (resistance to all proteins using that mode of action) (Tabashnik *et al.* 1994). One potential method to circumvent or delay cross-resistance is to plant two or more Bt crops, each of which produces a Bt protein with a mode of action different from the others. The theory behind spatial refuges is that it is very unlikely that a pest population would develop resistance to multiple unrelated proteins. However, for many pests, a single individual will only experience a single plant, and therefore a single Bt protein (mode of action), during its development. Because many pest larvae do not move from plant to plant, and would not be exposed to multiple Bt proteins, spatial refuges have not been implemented.

Comment: An even better method is to stack two Bt genes with different mechanisms in the same plant, making it necessary for the insect pest to defeat both genes to overcome this resistance. This approach has become standard practice for cotton in Australia using what is call "twin gene" control. However, it is necessary to use both genes stacked form the beginning; if done sequentially, i.e., first one gene and then the second after the pest has defeated the first, and that new biotype allowed to build up first, then the advantage of stacked genes is no different than using the two genes in separate plant genotypes.

Monitoring and Mitigation

Identifying populations of resistant insects through a comprehensive resistance monitoring plan is one method to test the effectiveness of resistance management programs and detect the onset of resistance before widespread crop failure occurs. However, monitoring and detecting pest resistance to a Bt protein is a difficult and imprecise task requiring a high level of sensitivity and accuracy. Appropriate resistance monitoring requires baseline susceptibility data prior to initiation of a monitoring program. In addition to baseline susceptibility data, information is needed to determine how many individuals need to be sampled and in how many locations. The chances of finding resistant larvae in a Bt crop depend on the level of pest pressure, the frequency of resistant individuals, the location and number of samples that are collected and the sensitivity of the detection technique.

Because there have been no confirmed instances of pest resistance to Bt crops currently planted, there has been no need to implement mitigation measures, and their success has not been evaluated. Mitigation may involve informing customers and extension agents in the affected areas of suspected or confirmed resistance, increasing monitoring in the affected areas, implementing alternative means to reduce or control target pest populations in the affected areas, implementing a structured refuge in the affected areas, and cessation of Bt seed sales in the affected and bordering counties until an effective local management plan has been implemented.

Grower Stewardship

Growers are responsible for planting refuges according to guidelines, and for monitoring fields for unexpected pest damage. Therefore, an education program that informs growers why IRM is needed and provides guidance how to implement appropriate strategies is necessary. Growers are required by the EPA to sign a technology use agreement that outlines IRM requirements and acknowledges the

Deleted: are an essential element for the implementation and success of an IRM plan as they are

growers responsibility to comply with them. The agreement states that growers received a Product Use Guide provided by the company selling the Bt seed. Technical bulletins, grower guides, sales materials, training sessions, websites, toll-free numbers for questions or further information and educational publications have been recommended as tools to educate growers. Educational materials should be consistent and reflect the most current resistance management guidelines to help ensure compliance with IRM requirements. It takes time and money to comply with IRM requirements, and there is a concern that if IRM requirements are too complex or time consuming, growers may avoid planting Bt crops or not adhere to IRM strategies.

Potential changes in toxicity and allergenicity to Mammal, Avian and Aquatic Organisms

EPA-registered Cry proteins have been considered safe because the intestinal walls of mammals do not have the endotoxin receptor necessary for the toxic effect, and the proteins are degraded quickly in the stomach. Vegetative insecticidal proteins (VIPs) are secreted proteins derived from the vegetative growth stage of Bt. When ingested, the protein binds to midgut cells, attacks the epithelial layer of the midgut, and eventually causes death (Lee *et al.* 2003). VIPs have a similar mode of action as Cry proteins, but VIPs associate with different midgut binding sites (Cao-Guo *et al.* 1997; Lee *et al.* 2003; Yu *et al.* 1997). Both Bt Cry proteins and VIPs have been deregulated by the USDA and Cry proteins have been registered for commercial use by EPA.

As part of the ecological risk assessment, the EPA also considers potential risks to mammal, avian and aquatic (e.g., fish) organisms. Although wildlife may be exposed to Bt protein, there is no evidence to date that shows toxicity to wild or domesticated mammals, fish, or avian species, and there are no reports of adverse effects from the commercial poultry industry after several years of using Bt corn in poultry feeds. Accidental aquatic exposure from Bt crops is extremely small, and there is no evidence for sensitivity of aquatic species to Bt proteins (EPA-BPPD 2001). USDA/APHIS, as part of their ecological risk assessment, also considers potential risks of GE crops to migratory birds under the Migratory Bird Act and threatened and endangered species under the Threatened and Endangered Species Act.

Genetically engineered drought and saline tolerant crop plants

Introduction

There is intense public and private interest in the development of drought- and salinity-tolerant plants. The size of the human population is increasing, creating a need for increased agricultural production. At the same time, most prime farmland is already under cultivation, and growers are considering the use of more marginal lands. Decreasing availability of fresh water and changes in rainfall patterns due to climate change provide some of the impetus to modify plants for drought-tolerance. Soil salinity is increasing in irrigated cropland, with approximately 20 percent of irrigated cropland experiencing salt stress at some level (Yamaguchi and Blumwald 2005; Yeo 1998). The increased probability of salt-stress conditions is promoting the development of salt-tolerant plants. The objectives of much biotechnological research, therefore, is to obtain plants that can be grown under dry conditions and on marginal,

Deleted: All plants have in various degrees tolerances against abiotic stresses such as drought, and salinity. However, while some specialized wild plants such as succulents can have high tolerances against stresses, most crop plants have low tolerances to drought and salinity. ¶

saline land, resulting in increase in production and reduction of the use of water in agriculture, which is of particular importance to developing countries.

Assessment of environmental effects

As the appearance of abiotic stress tolerant GE plants is a relatively new development in biotechnology, this section is only a sampling of the potential effects, and does not exhaustively address risk assessment.

Issues that would be considered in risk assessment of drought or saline tolerant crop plants include:

- Potential effects on plant insect interactions
- Potential changes in weediness

Potential effects on plant-insect interactions

When plants undergo the effects of an abiotic stress, a multitude of physiological changes occur. For example, when plants undergo the effects of drought and salinity stress, protein metabolism and amino acid synthesis are impaired (Hsiao 1973). Under such conditions, existing proteins may be broken down increasing the pool of available nitrogen and amino acids (Brodbeck and Strong 1987); (Bohnert, Nelson, and Jensen 1995; Delauney and Verma 1993). These stress-induced changes in plant physiology are widely thought to positively influence insect herbivores due to favorable modifications in plant nutrient content, particularly increases in amino acids and nitrogen (Bentz and Townsend 2001; Busch and Phelan 1999) or increase its concentration in food resources (Chen and Welter 2002; Richardson *et al.* 2002). These stress-induced changes can also predispose the plant to more severe root, stem and crown diseases caused by certain opportunistic necrotropic pathogens

However, not all stress-induced changes in plant physiology will positively affect insect herbivores. Defensive chemical compounds, collectively called "allelochemicals," are produced by the plant to affect insect herbivores in a negative manner, such as decreasing survival (Brodbeck and Strong 1987; White 1993; Gershenzon 1984; Inbar, Doostdar, and Mayer 2001; Mattson and Haack 1987, 1987). Different insects feed on plants in different ways: for example, some chew plant tissues, while others suck plant fluids from vascular tissue. Because allelochemicals are much less concentrated in vascular tissue compared to leaf tissue (Raven 1983), insect species that feed in vascular tissue (sap-feeding insects such as aphids) may respond more positively to plant stress than chewing insects, such as caterpillars, that feed on leaf tissue with higher levels of defensive chemicals (Larsson 1989). As plants are engineered to become tolerant of abiotic stress, there is a need to consider the complex interactions between genetically-engineered [why just GE?] stress-tolerant plants and insect herbivores.

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Comment: Many plant diseases are favored by water or other abiotic stresses that predispose the plant to more aggressive attack by the pathogen. Fusarium root and crown rot of wheat is one such disease, and is managed in the Pacific NorthWest by use of practices and varieties that avoid or tolerate plant water stress and not by use of a resistance trait directly inhibitory to the pathogen. {Reference Deleted}

Comment: Insects are constantly foraging for just the right food source, but do not always succeed owing to the natural cycles of food supply. Is the concern that the physiological change(s) may result in greater susceptibility of the GE crop to the insect as a pest? This is not a new issue for plant breeding [It has happened many times that the release of a variety improved for one trait revealed after scale up that it was vulnerable to some different hazard] but the issue has not previously been brought under APHIS regulatory authority, if that is the intent of this discussion. If the concern is that a new pest problem could arise, this needs to be made clear.

Potential changes in weediness

A primary reason behind stress tolerance traits is to enable crop survival in areas or under conditions under which it could not survive before. Therefore, one of the key questions for the risk assessments of these traits will be whether they will also change the potential for crop plants to become weeds or cause the crop to become more invasive. The weediness or invasiveness of a plant depends on many different characteristics, such as persistence, reproductive strategy, and dispersal and other factors such as the receiving environment and its climate. In general, it is not very likely that a change in one particular trait would coordinately make a plant become more weedy. However, it is theoretically conceivable that a change in abiotic stress resistance may incrementally increase the weediness potential of a crop that already had a number of weedy tendencies. Whether or not this may be the case will depend on the characteristics of the crop itself, the phenotypic changes, and the receiving environment.

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Genetically engineered crop plants tolerant to low levels of nutrient availability.

Introduction

Another stress-tolerant phenotype being developed is the ability of plants to withstand low levels of nutrient (fertilizer) availability through increased efficiency in nutrient assimilation and/or utilization. Increases in nitrogen-use efficiency of crop plants have the potential to decrease fertilizer costs to farmers by decreasing the nitrogen applications required to achieve the full yield potential of the variety, as well as reducing nitrogen run-off and subsequent water contamination (Oliveira *et al.* 2002). Tobacco modified to increase ammonium assimilation and thus increase nitrogen efficiency resulted in plants with greater biomass and leaf soluble protein compared with the non-GE tobacco plants (Oliveira *et al.* 2002), indicating that increased nitrogen-use efficiency is possible by genetic manipulation. Use of phosphorus is also being examined as a potential modification for crops plants. Over 30 percent of crop land experiences phosphorus deficiency (Vance, Uhde-Stone, and Allan 2003). GE tobacco plants modified for increased phosphorus use-efficiency exhibited significantly greater growth and higher phosphorus concentrations in phosphorus-deficient conditions than non-transformed plants (Lung *et al.* 2005) and *Arabidopsis* (Xiao *et al.* 2006). Thus, genetic modifications that alter the assimilation and utilization efficiency of nutrients can result in increased nutrient content of the plant.

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Assessment of environmental effects

Issues that would could? be considered in risk assessment of drought or saline tolerant crop plants include:

- Potential effects on plant-insect interactions
- Potential changes in weediness

Potential effects on plant-insect interactions

GE plants engineered for stress tolerance show an increase in nutrient content, either nitrogen or phosphorus, through either an accumulation of nitrogen-based osmoprotectants during drought and salinity or an increased nutrient-use efficiency. Although nitrogen has traditionally been recognized as a limiting nutrient for insect herbivores (Mattson 1980; McNeill and Southwood 1978; White 1993), from the few studies that have investigated the effects of phosphorus limitation on insects, there is evidence showing that phosphorus availability can be an important determinant of survivorship (Ayers *et al.* 2000; Clancy and King 1993), fecundity (Popp *et al.* 1989), body size (Busch and Phelan 1999; Janssen 1994), oviposition (Skinner and Cohen 1994), growth rate (Perkins *et al.* 2004) and population density (Schade *et al.* 2003). Thus, genetic modifications that result in plants increasing in either nitrogen or phosphorus content may potentially increase pressure from? insect herbivore populations on stress-tolerant plants because of the Responsiveness? [sensitivity] of insect herbivores to nitrogen and phosphorus content.

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Comment: Again, it is not clear if the concern is for greater pest pressure from herbiviorous insects. Would not the same response occur by increasing the N or P content in the plant by normal fertilization practices? It would seem that the variability in available soil N and P for plant uptake would totally override variability in use-effciency of the variety as a determinant of insect activity. Should the same concern be raised for use of mycorrhizae? My point is, offer more context and background based on what is the norm or baseline.

Potential changes in weediness

Because plants frequently inhabit environments where water and nutritional resources are limited, the ability to more efficiently exploit these resources may enable a plant to outcompete its neighbors. This could result in the development of plants with invasive or weedy characteristics, and the assessment of the impacts from these characteristics would need to include considerations of the impacts of the movement of stress-tolerance traits to the wild relatives of GE crop plants.

Conclusion

Comment: The first two para of this section do not read like conclusions; these statements really belong at the opening of the section.

Of the more than 11,000 permits and notifications that have been issued, acknowledged or are pending over the last 18 years, only 315 applications include plants that are GE for stress-tolerance. As this area of biotechnology research continues to moves from strictly experimental to product development, stress-tolerant phenotypes and the mechanisms underlying the tolerance will become more refined and better understood. Subsequently, because insect herbivores show such significant sensitivity toward even minute changes in plant nutrient content, future field studies involving nutrient efficient or stress-tolerant plants and other GE phenotypes that change plant nutrient or defensive chemical content may have the ability to establish any positive or negative relationships between these GE plants and their insect herbivores.

Comment: The repeated emphasis on plant-insect interactions in relation to changes in plant physiology is curious. I am not suggesting that the points made here be extended to plant-microbe interactions, but I will point out that plant pathogens are just as responsive if not more responsive to these changes than are insects. Obligate parasites such as rust fungi are less active on stressed than non stressed plants while facultative parasistes and saprophytes are more able to produce more disease on stressed than non stressed plants. This is outlined in {Reference Deleted} As mentioned in comment 44, Fusarium root and crown rot of wheat is managed by managing water stress, such that the predisposing effects of the stress on the disease are mitigated.

Genetically Engineered Virus Resistant Crop Plants

Introduction

Plant viruses represent a significant threat to global agriculture because of their ability to reduce the quality and, more important, the yield of food and fiber crops (Hull 2004; Pappu 1999). Hundreds of plant viruses have been described, affecting a wide range of plants and trees. In general, most plant viruses consist of genetic material – either RNA or DNA – enclosed by a protective coat. This coat, which is made from many individual protein molecules ("coat proteins") plays an important role in protecting the genetic material, as well as determining how the virus moves within the plant or spreads from plant to plant via insect vectors (Hull 2004; OECD 1996). Additional means of plant virus transmission include fungal transmission, seed transmission, mechanical transmission and/or grafting (Hull 2004; OECD 1996).

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Common control or management strategies for plant viruses have included the use of pesticides for control of insect vectors; cultural practices such as a change in planting date; removal of infected plants and plant material serving as sources of viruses; use of virus-free planting material; and/or use of resistant varieties (Gooding 1985; Khetarpal *et al.* 1998; OECD 1996; Superak *et al.* 1993; Swiezynski 1994). While the use of these control strategies has been effective in parts of the world, the overall effectiveness of these strategies can vary significantly from crop to crop and year to year (Hadidi, Khetarpal, and Koganezawa 1998; OECD 1996; Pappu 1999).

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Another control strategy shown to be effective is cross protection (Gonsalves 1998; Gooding 1985; Hull 2004; Sherwood 1987). Cross protection involves the ability of a mild strain of a virus to prevent or delay infection by a second more virulent strain of virus (Culver 2002; Gooding 1985; Hull 2004; Sherwood 1987). Cross protection has been attributed to various mechanisms (Culver 2002; Goregaoker, Eckhardt, and Culver 2000); (Beachy 1999; Culver 2002; Goregaoker, Eckhardt, and Culver 2000; Sherwood 1987). Coat protein mediated cross protection, for example, relies upon the coat protein to properly associate with and block disassembly of the virulent virus (Culver 2002). While cross protection has proven to be effective with some viruses, because of the labor and time needed to infect plants with the mild virus strain, cross protection is generally not a practical means of controlling virus disease in large scale or agricultural systems.

In recent years, much of the research and development for plant virus disease control has focused on development of virus resistant plants using genes from the virus expressed in the plant as transgenes. Using the concept of pathogen-derived resistance (Sanford and Johnston 1985) and cross protection, genetic modifications of host plants and trees are made that allow for expression of viral genes or proteins [proteins redundant?] in the plant and tree tissue. Plant expression of viral genes or proteins often acts to delay or prevent infection by the same or related viruses. This form of pathogen-derived resistance was first accomplished in 1986 by Roger Beachy and colleagues (Abel *et al.* 1986) in which tobacco plants engineered to express tobacco mosaic tombusvirus (TMV) coat protein were resistant to TMV infection.

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Numerous other virus resistant plants have been developed and field tested (ISB 2004; Tepfer 2002). Most of the virus resistance is based on so-called "pathogen-derived resistance," most often using viral coat protein (VCP) or VCP gene expression as the basis for resistance (ISB 2004; Tepfer 2002). Over the past 15 plus years, nearly 900 virus resistant plants (including trees) have been authorized by USDA–APHIS for field testing in the U.S. In addition, several virus-resistant crop plants have been deregulated by APHIS and have been grown commercially. GE virus resistant plants deregulated by APHIS to date include those that express viral coat protein genes (*e.g.*, papaya ringspot virus resistant papaya) or the replicase protein gene of potato leafroll virus resistant potato (EPA 1998; Gonsalves 1998; ISB 2004).

Comment: To my knowledge, the only commercial use of this technology today for virus control is with papaya ringspot virus and the viruses of squash; all the rest are on the shelf, including for potato leaf roll virus. This situation has nothing to do with health or environmental concerns as I have pointed out above, but rather, refusals to accept these products in the export market place, namely Europe and Japan. CP-resistance to bushy dwarf virus of raspberry, which was developed by ARS at Oregon State, is on the shelf because of the importance of the Japanese export market to OR and WA raspberries, with no one working at the present time to obtain approval in Japan, to my knowledge.

Assessment of environmental effects

While the development and deployment of genetically-modified plants has proven to be highly effective in controlling targeted virus diseases, some concern has been raised about the potential risks associated with agricultural use of genetically-modified virus-resistant plants (NRC 2002, 2000). The safety of these plants has been the subject of numerous scientific meetings and workshops, as well as scientific articles written by members of the U.S. government, academia and industry that address potential risks associated with these plants (AIBS 1995; Falk and Bruening 1994; Miller, Koev, and Mohan 1997; OECD 1996; Tepfer 2002).

Potential adverse effects that have been identified and studied in detail include:

- The development of new virus diseases, through either:
  - Heterologous Encapsidation (transcapsidation) – the phenomenon where the coat protein of one virus is able to enclose ("encapsidate") the nucleic acid of a separate virus. When heterologous encapsidation occurs, there is some potential for altered phenotypes and/or host range.
  - Virus Recombination – exchange of the genetic material between two or more different viruses. If recombination is possible, there is some potential for the generation of new viruses.
- Synergy – increase in severity of infection or symptoms when two or more viruses infecting the same plant. If synergy occurs, the potential result is increased virus disease severity.
- Change in Weediness due to gene flow between cultivated crops and weeds, there is some potential for a weedy relative to acquire virus resistance from the crop plant.

While the technology, as well as the analysis of potential adverse effects, continues to evolve, currently available scientific data have been used to study these risks (AIBS 1995; OECD 1996; Tepfer 2002). These data and information will be discussed in this section to briefly explore the possible risks and other concerns that have been raised with regard to large-scale deployment of genetically-modified virus-resistant plants.

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Many of the issues that will be discussed in this section are similar for both RNA and DNA plant viruses. However, to date, the development of GE virus resistant plants has been mostly limited to plants developed for resistance to RNA viruses (ISB 2004; Tepfer 2002), and most GE virus resistant plants that have been commercialized express the VCP's or VCP genes. Therefore, the focus of this section will be GE plants expressing VCP's or VCP genes from RNA viruses. Over 30 different species of plants have been developed for virus resistance and subsequent field testing, with many developed to express genes other than VCP, including: replicase protein; nuclear inclusion protein; movement protein; nucleocapsid protein; N gene; helper component; as well as other virus-specific proteins (ISB 2004). As crops continue to be developed to express proteins/genes other than VCP, the agency will assess the safety of these crop/gene combinations on a case by case basis, using the same level of scrutiny that has been used for VCP's.

Development of new virus diseases through Heterologous Encapsidation

There are many instances in nature and in agricultural settings where a single host plant is infected by two or more viruses. In fact, some reports about naturally infected plants have identified individual plants that were infected by as many as six different viruses (Abdalla, Desjardins, and Dodds 1985; Falk *et al.* 1995). Viruses in a naturally-occurring mixed infection could interact in a number of scenarios. One potential scenario that can occur in plants co-infected with two or more viruses is a phenomenon involving the viral coat protein known as heterologous encapsidation (Falk *et al.* 1995; Miller, Koev, and Mohan 1997; Waterhouse and Murant 1983).

Heterologous encapsidation occurs when the coat protein of one virus is able to encapsidate the nucleic acid of a second virus (Figure 2). Heterologous encapsidation was first described by Rochow (1970) and has been the subject of numerous reviews (Falk and Duffus 1981; Falk *et al.* 1995; Miller, Koev, and Mohan 1997; Rochow 1977; Tepfer 2002. These interactions occur naturally in both agricultural crop and weed plants, and are a natural part of virus-virus and virus-plant interactions (Falk and Duffus 1981; Falk *et al.* 1995; Rochow 1977). In some cases, heterologous encapsidation is a specific interaction between two viruses that plays an important role in both virus biology and survival.

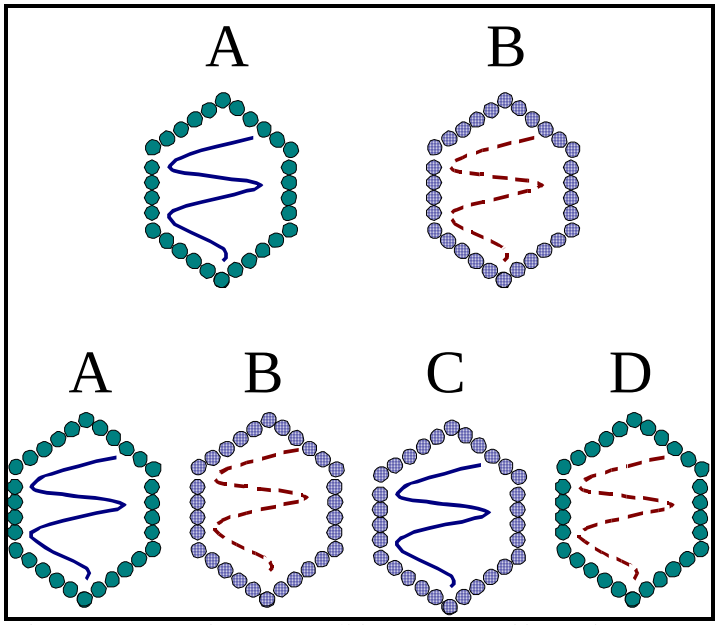

Figure 2 Heterologous Encapsidation. Possible outcomes of heterologous encapsidation interactions as previously described by Rochow 1977 and Falk 1995. A and B represent the parental viruses. When two viruses co-infect the same cell, the progeny can include virions that are identical to the parental viruses (A and B), or progeny that are composed of the capsid protein of one virus and the RNA of the second virus (C and D).

Because the viral coat protein may determine which insect vector is capable of transmitting a particular virus, when heterologous encapsidation occurs, the RNA of one virus, essentially acquires the phenotypic properties of the second virus for insect transmission (virions C and D in Figure 2). This observation means that the insect vector recognizes the coat protein, not the RNA inside the coat protein. In other words, the progeny viruses that are the result of heterologous encapsidation often temporarily exhibit new or altered biological properties differing from those of the parental viruses (Falk *et al.* 1995). The impact on vector specificity is likely limited because many of the heterologous encapsidation interactions that have been identified occur between viruses that are closely related enough that they are transmitted by the same vectors (Hull 2004).

Once heterologous encapsidation occurs, the potential exists for new or different "exposure" or host range for the RNA of the encapsidated RNA via insect transmission. If such a scenario occurs, the result may be one of the following:

1. The inoculated plant is not a host for the virus.

2. The inoculated plant is a host for virus, but there no insect vector is available to transmit the virus from that plant.

3. The inoculated plant is a host for virus and an insect vector is available to transmit the virus from that plant.

If scenario one occurs and the new plant is not a host for virus whose RNA is encapsidated, it represents a "dead-end" for the virus. This is because the virus will likely not be able to replicate and/or move in the new plant. Without the ability to establish an infection—due to its inability to move and/or replicate—the virus would not likely be transmitted from that plant. Heterologous encapsidation that occurs according to this scenario would not likely be of any ecological significance (Falk *et al.* 1995; OECD 1996).

If scenario two occurs, the virus whose RNA is encapsidated, could replicate and move within the plant to which it was transmitted, and would subsequently be encapsidated in its own coat protein. However, the virus would not be able to move from that plant via insect transmission because the plant would not be a typical host for an insect vector of this virus. In general, the significance of this scenario is likely only transient because once the encapsidated RNA is injected into the host plant, the biological properties of the virus will take over and determine its subsequent fate (Falk *et al.* 1995; OECD 1996).

If scenario three occurs, the result would be similar to a typical infection of the encapsidated virus, if the virus is transmitted to a normal host of the virus. Once the virus is inoculated into the plant, its normal replication mechanisms would take over and the virus would then produce its own coat protein. However, because the viral RNA was initially transmitted by a different insect vector, it is possible that the virus could be introduced into a host to which it has not been previously exposed. Whether or not the virus would be subsequently transmitted from this host plant would depend upon whether an insect vector was available to transmit the virus. In terms of virus biology, this scenario would not be significantly different from what naturally occurs, other than the fact that the primary inoculation occurred with virus RNA that was the product of heterologous encapsidation. Subsequent virus biology would reflect that of the naturally-occurring virus

Overall, despite the potential for heterologous encapsidation to readily occur in nature, there have only been a few cases in which heterologous encapsidation has been shown to be important in agricultural situations (Falk *et al.* 1995; OECD 1996). The likelihood of heteroencapsidation would not be significantly different in VCP-expressing plants (Hull 2004).

Helper-Dependent Transmission

There are a limited number of cases where heterologous encapsidation is a natural part of plant virus epidemiology (Falk *et al.* 1995). Helper-dependent transmission occurs when one virus exclusively relies upon another virus for heterologous encapsidation for subsequent insect transmission from mixed infections (Falk and Duffus 1981; Falk *et al.* 1995; Hull and Adams 1968; Rochow 1977). In cases such as carrot motley dwarf, groundnut rosette, and lettuce speckles, the virus diseases are caused by co-infection of the plant by two or more viruses (Falk, Duffus, and Morris

1979; Falk *et al.* 1995; Hull and Adams 1968; Waterhouse and Murant 1983). In each of these, and other virus disease complexes, one of the viruses is insect transmissible and the other is not independently insect transmissible. The non-independently transmissible virus relies upon the insect transmissible virus, via heterologous encapsidation, for insect transmission. In each of the complexes that has been characterized, the insect transmissible virus is able to replicate and move within the host plant in the absence of the non-insect transmissible virus. The non-insect transmissible virus is also able to replicate and move within the host in the absence of the insect transmissible virus, however, it lacks a coat protein and therefore must rely on the insect transmissible virus for encapsidation and insect transmission (Falk *et al.* 1995). While the non-insect transmissible virus is able to be spread by mechanical inoculation by itself, it benefits greatly by being associated with the insect transmissible virus that provides for more efficient dissemination and potentially a wider host range.

Given the crucial role that the coat protein plays in insect transmission and natural plant virus epidemiology, some concern has been raised about whether constitutive expression of viral coat protein in GE plants would increase heterologous encapsidation interactions. Because the amount of coat protein available in GE plants is so dramatically less than the amount of coat protein in virus infected plants, the potential for heterologous encapsidation is reduced

Reducing the Risk

Even if one assumes that there is risk associated with heterologous encapsidation interactions occurring in virus resistant GE plants, the potential exists to reduce ecological impact via modification of the viral coat protein gene (Tepfer 2002). Research has shown that mutations in the coat protein gene can result in loss of insect transmissibility of the virus (Tepfer 2002). By incorporating such mutations into the coat protein gene that is expressed in GE plants, the potential for insect transmission of viruses that may have been encapsidated by the plant-expressed coat protein can be eliminated, without affecting the effectiveness of the virus resistance.

Development of new virus diseases through Recombination

Plant virus recombination occurs when the exchange of genetic material between two, or more, different viruses results in production of a new virus (OECD 1996; Tepfer 2002; Worobey and Holmes 1999). Recombination between viruses in different taxonomic groups has played a significant role in virus evolution (AIBS 1995; OECD 1996; Roossinck 1997; Worobey and Holmes 1999). In terms of virus evolution, recombination would be considered to be a frequent event (Hull 2004). Nucleotide sequence comparisons of different/unrelated viruses have identified similar segments of nucleotide sequence, suggesting that recombination has occurred (Hull 2004).

Because of the potential for recombination to occur in GE plants, consideration should be given to whether recombinants arising from GE plants would be different from those that arise from mixed infections in non-GE plants, and whether the

recombinants are viable (Hull 2004). Factors affecting the rate of recombination include sequence between the two viruses, the location of the virus within the plant, and structural similarity between the nucleic acids (OECD 1996). The ability of a virus arising via recombination to persist in nature depends upon factors such as its ability to replicate, spread systemically and/or its ability to be transmitted to other host plants (Hull 2004; OECD 1996). A significant difference between the potential for recombination in non-GE plants with mixed infections versus the potential for recombination in GE plants is that the virus gene is constitutively expressed, i.e. is continuously produced in every cell in the GE plant, which allows for greater opportunity for interaction and hence recombination between the expressed gene and the infecting virus (Hull 2004).

Plant-Expressed Genes-Virus Recombination

Looking beyond virus-virus recombination, other studies have focused on whether viral transgenes present in virus resistant plants can either complement or recombine with viruses that infect the GE plant. In the mid-90's, Greene and Allison (1994, 1996) were able to show that such recombination could occur. Their experiments were the first to show the potential for recombination between plant expressed genes and viruses infecting that plant. It is not clear, however, from these and subsequent studies (*e.g.*, (Borja *et al.* 1999)) how closely these experiments performed under non-natural [artificial? Experimental?] conditions reflect what occurs in natural/agricultural settings with either GE or non-GE virus-resistant plants. Rubio *et al.* (1999) suggest that the levels of recombination seen in their experiments are orders of magnitude higher than would be expected in GE plants where virus replication is reduced or prevented.

Comment: Here and elsewhere in the document, there is no need to mention author names twice.

Deleted: (Greene and Allison 1994, 1996)

Deleted: Rubio *et al.*

Plant-Virus Recombination

Finally, other researchers have shown that over time, plant viruses in natural settings, have incorporated various plant cellular RNA's into their genomes as part of their evolutionary process (Karasev 2000; Masuta *et al.* 1992; Mayo and Jolly 1991). In some cases, it appears that once these cellular RNA's become incorporated into the viral genome via recombination, they are subsequently maintained as part of the viral genome.

Overall, given that mixed infections are common in nature, the opportunity for both related and unrelated viruses to interact in natural virus populations is high. Issues such as selection pressure, adaptation to changing environments, competition, fitness, etc., likely play significant roles in the various types of recombination that have been identified. These factors also help determine what role the resulting recombinants play in virus biology and epidemiology. Based upon currently available information, it appears that the potential for recombination in virus resistant GE plants (*i.e.*, virus-virus & plant-virus) would be similar to the natural occurrence of recombination in virus infected, non-GE plants (Falk and Bruening 1994; OECD 1996; Rubio *et al.* 1999).

Synergy – increase in severity of infection or symptoms when two or more viruses infect the same plant

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Synergy occurs when two independent viruses infect a plant simultaneously and the resulting disease symptoms are more severe than when either virus infects the plant individually (OECD 1996; Pruss *et al.* 1997; Tepfer 2002). Several naturally-occurring synergistic virus interactions have been described (OECD 1996; Pruss *et al.* 1997; Rochow and Ross 1955; Tepfer 2002; Vance 1991; Vance *et al.* 1995). Vance and colleagues have shown that when plants are co-infected with two different viruses, the disease symptoms are significantly worse than plants infected with either of the viruses alone (Vance 1991; Vance *et al.* 1995).

Subsequent studies have shown that the potyvirus helper-component-protease (HC-Pro) mediates the increase in PVX (Pruss *et al.* 1997; Tepfer 2002; Vance *et al.* 1995). Pruss, *et al.* also showed that the potyvirus HC-Pro can enhance pathogenicity and virus accumulation of other viruses including cucumber mosaic virus (CMV) and tobacco mosaic virus (TMV) (Pruss *et al.* 1997). Researchers continue to explore whether other viral proteins or genes play similar roles in virus synergy.

What, if any, risk synergy poses on the environment as a result of the use of GE virus resistant plants is not entirely clear. However, current scientific data suggests that any impact would be minimal for several reasons. The first consideration is that any effect of synergy associated with a particular GE crop will be limited to the GE plants themselves (OECD 1996). Additionally, it is not likely that the potential for synergy occurring in GE plants expressing virus genes would be greater than in natural mixed infections (Hull 2004). Consideration of this type of effect on the GE plant should be included as a part of product development by the plant developer (OECD 1996; Tepfer 2002). Potential synergistic interactions could be identified during development of a plant line by inoculating GE plants with widely prevalent viruses of that host plant.

Given the knowledge of the roles that different virus genes play in synergy, developers can also select only those genes that likely would not contribute to synergism, or include mutations in such genes so that their potential impact is limited. Genes such as the potyvirus HC-Pro should be avoided given what is known about its ability to enhance disease development and virus titer of some co-infecting viruses (AIBS 1995; Tepfer 2002). Other genes such as those that might aid in virus replication, movement and/or symptom severity might also be avoided because of their potential to facilitate virus disease development (AIBS 1995; Tepfer 2002).

Change in Weediness due to gene flow between cultivated crops and weeds

Weeds and other non-cultivated plants are one of the primary sources of genes for resistance to pests and pathogens. In general, most pest and pathogen resistance genes used in traditional breeding for resistance have been found in the centers of origin and areas of diversification of cultivated plants (Khetarpal *et al.* 1998). These are the areas in the environment where plants have been exposed to selective pressure

Comment: Weeds and other non-cultivated plants are also a source of pest infestation and pathogen inoculum when the weed is a relative and grows in close promity to the crop.

Deleted: resistance genes

of pests and pathogens over thousands of years and therefore have developed resistance as a mechanism of survival (Khetarpal *et al.* 1998).

The potential for introgression of a virus resistance transgene into a wild or weedy species is another possible outcome of large-scale agricultural use of GE virus resistant plants. The primary concern is whether transgene introgression would result in a wild or weedy species becoming invasive because introgression of the virus transgene has made the wild or weedy species resistant to a virus disease that normally plays a role in control of the species (Fuchs, Chirco, and Gonsalves 2004(a); Fuchs *et al.* 2004(b); Tepfer 2002). To consider the potential risk, several aspects of virus and plant biology should be considered.

As discussed earlier in this section, plant viruses cause significant problems by limiting the amount and quality of agricultural products. Most virus epidemics are the result of a virus and/or a vector moving from non-crop plants located adjacent to production areas into cultivated crops. Plant viruses are obligate parasites, and, as such, total destruction of their plant hosts would lead to the extinction of that virus. Therefore, it is assumed that there is a certain level of tolerance by some hosts - possibly wild or weedy hosts – that allow for persistence of the virus. In fact, many virus infections do not produce visible symptoms in weeds (Hull 2004). Because of this, there likely exists a number of wild or weedy plant species that contain resistance genes that allow these plants to survive virus infection and serve as reservoirs for the virus (Raybould *et al.* 1999).

This is somewhat different than the relationship between cultivated crops and plant viruses. Most of the major crop species used in today's agriculture (*e.g.* soybean, rice, wheat, beans) have been subjected to intensive artificial selection over centuries and only have low survival under most natural conditions. The vast majority of the crops used in agriculture are much less fit, under natural conditions, than wild or weedy plants. Because of this, the impact of virus infection on crop plants is potentially more severe than on many wild or weedy plants.

It is known that gene flow from cultivated agricultural crops to wild and weedy species has occurred since the domestication of a particular crop when sexually compatible wild or weedy species are present (Fuchs, Chirco, and Gonsalves 2004(a); Stewart, Halfhill, and Warwick 2003). It is also known that gene flow occurs between virus resistant GE crops and non-GE crops (Fuchs, Chirco, and Gonsalves 2004(a))). What is not as well understood is how much gene flow from GE virus resistant plants to wild or weedy relatives results in introgression of the gene(s), and what ecological consequence this introgression would have. Stewart and others discuss the basic difference between gene flow, mediated via pollen or other mechanisms, and introgression of genes, as well as the frequency of introgression and impacts on the frequency (Fuchs, Chirco, and Gonsalves 2004(a); NRC 2000; Stewart, Halfhill, and Warwick 2003). According to Stewart, there have been a relatively low number of confirmed cases of introgression (Stewart, Halfhill, and Warwick 2003).

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However, there is no clear evidence to indicate that the introgression of a virus resistance transgene into a wild or weedy species would be any different than

introgression of a naturally-occurring virus resistance gene from a non-GE plant (Fuchs, Chirco, and Gonsalves 2004(a); Tepfer 2002). Further, there is no evidence indicating that a weedy plant would become more competitive, if it gained virus resistance via gene flow from VCP-expressing plants.

Whether or not introgression is considered to be a significant issue with a particular crop or crops, there are steps that can be taken to reduce any potential risk. These include use of crops for which there are no sexually-compatible relatives present in the geographical region; use of crops that have been identified to exhibit low levels of gene flow/introgression; and further development of transgene containment strategies (Stewart, Halfhill, and Warwick 2003).

Current knowledge and data suggests that gene flow from a GE virus resistant plant to a wild or weedy plant is not likely to provide different exposure from that which occurs under natural agricultural and environmental settings.

Herbicide Tolerant crop plants

Introduction[7]

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 proportion of pesticides used in the US that are herbicides continues to grow and is now close to 75 percent of the crop protection pesticide market. While some persistent herbicides can have serious negative impacts on the environment, and in particular on soil and aquatic ecosystems, some of the newer, non-selective and non persistent herbicides are less hazardous to the environment.

Crops made resistant to post-emergence, non-selective herbicides, like glyphosate and glufosinate, by biotechnology are being widely adopted in North America and other parts of the world. These products allow the farmer to more effectively use reduced- or no-tillage cultural practices, eliminate use of some of the more environmentally harmful herbicides, and use fewer herbicides to manage nearly the entire spectrum of weed species.

Comment: As context and background, but also to update this document, mention tolerance to imazamox developed by mutagensis and marketed by BASF as Clearfield technology. See comment in this reviewer's report. Add imazmox to the table 1 or change the heading to clarify HT crops developed by GE.

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 with the concern has been raised regarding 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 section below discuses some of the issues.

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 has been documented. This problem has not yet reached the severity of insecticide resistance, but in isolated cases the impact has been severe. 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.

[7] See: WEED MANAGEMENT: IMPLICATIONS OF HERBICIDE RESISTANT CROPS, Dr. Stephen O. Duke, USDA-ARS-Natural Products Utilization Research Unit

Over the past few years, several HRCs, both transgenic and non-transgenic, have become available in North America (see Table 1). Of these, glyphosate- and glufosinate-resistant crops have been widely adopted. 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. HRCs have accounted for nearly one-third of field tests conducted under USDA authority.

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*

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. 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).

Reliance 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. A 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, possibly resulting in ecological impacts.

[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.

[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.

GE crop plants producing pharmaceuticals and vaccines

A new development in biotechnology is the production of pharmaceuticals and vaccines in plants ('Plant Made Pharmaceuticals'- PMPs). This area of research has expanded over the years, because PMPs may have advantages in terms of production scale, production costs, ease of storage, and distribution. In addition, the production of pharmaceuticals and vaccines in plants may avoid one of the major disadvantages of pharmaceuticals produced in animal cells, namely, the risk of pathogens such as human and animal viruses in the mammalian cells that are traditionally used to produce vaccines.

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While these potential advantages are generally recognized, there is also recognition that this development poses new challenges, which require that adequate confinement and segregation measures are in place. This is particularly important where there is a possibility of commingling with crops for the food or feed chain. It is not only the growing the crop itself – be it in field trials or commercially - but also the likely impact it has on surrounding crops and crops planted later in the rotation cycle that must be taken into account. Which measures are taken to ensure appropriate confinement and segregation is decided in a case by case basis.

Silviculture

Introduction

The United States forest products industry employs 1.6 million people and ranks among the top ten manufacturing employers in 46 states. The industry produces products valued in excess of $230 billion each year which includes $23 billion in exports. One third of the United States is forested which is about 747 million acres. Of this amount, 350 million acres represent commercial timberland. Over 270 million acres of federal land have been set aside for use as wildlife refuges, parks and wilderness areas[8].

Approximately 2.6 million acres are planted annually in the United States. Approximately 1.6 billion trees are produced, harvested, and shipped by forest tree nurseries annually. Forest product nurseries produce 852 million trees, private nurseries produce 366 million trees, state nurseries produce 348 million trees, and federal nurseries produce 38 million trees.

Currently most forest tree breeding programs are only in the third or fourth generation of tree improvement. The more advanced genetic improvement programs were begun in the late 1940s early 1950s. The species receiving most of the attention in the U.S. has been loblolly pine in the southern U.S., but there is considerable research with other species such as slash pine, hybrid poplar and cottonwood. In the western U.S. the dominant species is Douglas fir.

Breeding programs have historically been primarily conducted using recurrent selection where the best parents are selected to establish seed orchards. The best trees, called "mother" trees, are planted in a seed orchard and are allowed to cross among each other. The resulting seeds that are produced are of higher genetic value than seeds collected in the wild. Over time the performance of the progeny are evaluated and trees lacking the desired traits can be removed from the orchard. Most trees resulting from tree breeding programs are destined for the lumber and paper pulping industries, and so most of the traits that have been selected are related to growth and form. Trees with rapid growth and good form that have increased volume are selected to create the next breeding generation. Wood quality has been examined in some species, most notably in wood density for pulp yield. Also traits such as disease and insect resistance are selected. For example in loblolly pine, trees are selected for resistance to southern fusiform rust, while *Populus* (poplar) species, are selected for resistance to fungal diseases such as *Septoria* leaf spot or *Melampsora* leaf rust. Resistance to white pine blister rust in pine species in the western U.S. is another important trait selected for by breeders. Trees that are not intended for timber, but for landscape use or production of tree fruits, also are improved through

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[8] Source of Statistics: American Forest & Paper Association

breeding programs in a similar manner, however the desirable traits being selected are different.

Because most tree breeding programs have been in existence only three or four generations and because little has been done to "domesticate" these species, they are not far removed from their wild progenitors when compared to agronomic crops, and many can intercross freely with their wild relatives. For example, species of *Pinus* and *Populus* are indigenous to the continental United States and have been selected from the wild for tree breeding programs.

Current forest tree breeding programs are moving towards so-called "clonal" forestry, in which all of the trees planted are genetically identical. Clonal programs are well advanced in some genera, such as hybrid poplar and cottonwood, and are under development in others, such as loblolly and slash pine. By selecting superior clones, substantial genetic gains in volume can be achieved, thus allowing more wood to be grown on less land.

Trees, like other plants, are GE using established biotechnological methods which result in stable transgene incorporation. However, tree breeding methods differ in some ways from those used with annual crops, and these differences may affect the deployment of products from GE trees. In the immediate future it is most likely that any deregulated GE trees will be deployed as clones. Assessing the stability of transgenes in GE trees over multiple generations may be more difficult since breeding cycles take several years. Also since generation times are long, it will be a number of years before breeding with GE trees occurs and new cultivars are produced. Varieties with stacked traits will initially be produced by multiple genetic transformations.

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Tree breeders are currently not using GE trees as either pollen or seed parents, and, production seed orchards using GE trees for the production of GE seeds could be decades away. This also means that all of the GE trees that are produced for deployment in the near term will be produced by vegetative propagation. This will most likely be done through tissue and cell culture or rooted cutting propagation. Therefore plantations will be established by transplanting vegetative propagules into the landscape.

The life span of hardwood and softwood trees are decades or centuries. Thus the duration of a field test of GE trees can span a number of years. Depending on the trait being measured, it could require several years of testing to gather meaningful data.

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Many forest trees are wind pollinated and the pollen can travel large distances. Pollen from some tree species can live a long time compared to many crop species. Therefore pollen from GE trees could potentially travel for miles from one plantation to another or from managed plantations to unmanaged areas.

In many forest trees seed dormancy is common. Seeds of some species can remain dormant for years and in some species stratification, *i.e.*, a cold treatment, is required

for germination. Therefore seeds from GE trees could lay dormant for years following harvest and germinate in subsequent years.

Genetically improved trees are usually grown in plantations. These plantations cover hundreds to thousands of acres. Plantations are planted in large blocks or mosaics and are harvested when the trees are mature. In a plantation the rotation can range from 8-12 years for a genus like eucalyptus to 80 years for long-lived conifers such as spruce or fir. Therefore a "crop" can last for decades.

The control of flowering by genetically engineering sterility is under consideration. Some stakeholders have expressed the opinion that all GE trees will have to be sterile before they can be deregulated. Questions arise as to whether sterility is necessary or desirable. It is anticipated that this will be evaluated on a case by case basis. Questions also arise as to whether sterility over a long period of time is achievable and whether redundant methods will be required to insure long term stability of a sterile trait.

Tree traits under development

Forest trees have a number of insect pests. Some insects attack young trees and others attack older trees. Annual crops engineered for insect resistance using Bt toxins are grown with refugia to delay the development of resistant insects. For trees engineered for insect resistance, refugia may be useful when grown in a plantation setting. For some species, these resistance genes will need to be effective in plantations for 20 or more years. This could argue for incorporating multiple mechanisms for resistance into these trees. The potential impact of species that feed on insects that are no longer present in resistant plantations may also have to be considered.

Forest trees are also subject to a number of devastating diseases. For example, in 1900, the American chestnut was deemed the "Redwood of the East Coast," standing 100 feet tall and comprising approximately thirty percent of the eastern seaboard forests. A fungal pathogen, accidentally introduced in the late 1800's, resulted in the death of approximately 3.5 billion trees, and relegated the species to low growing sprouts, unusable as timber. Resistance to tree diseases, such as fusiform rust in southern pines, is being researched using genetic markers and genomics. There is also a potential for engineering resistance to disease using existing genes. Projects are underway to engineer resistance to blight in American chestnut and Dutch elm disease in American elm.

Comment: A disease is a process, with a beginning and an end, and results from the interaction of at least two organisms.

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Comment: Blight is the name of the disease and not the pathogen. It was the pathogen that was introduced and thereafter caused the blight.

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Modification of lignin levels and types through genetic engineering is well on its way in forest trees. These projects are aimed at improving pulping traits or solid wood properties. This will result in a change in wood chemistry and secondary compounds. These changes could affect resistance to insects and diseases or the ability of the trees to respond to adverse environments. These alterations could also accelerate or slow wood decay rates. This would not likely be an issue in a plantation where almost all the wood is harvested but could be an issue if the gene were to escape into native stands.

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Lignin is an integral part of the cell walls of plants, providing strength. When trees are used for paper production, lignin must be removed from pulp before manufacturing the paper. This extra step is costly both economically and environmentally (Pilate et al. 2002); thus, poplar trees have been genetically-engineered to reduce lignin content (Baucher et al. 1996; Van Doorsselaere et al. 1995). Lignin also has a secondary function as a plant defensive chemical; it reduces the leaf digestibility in insect herbivores and functions as a barrier to some pathogens. By decreasing lignin content in GE trees, a possible outcome could be an increase in insect herbivore pest populations (Van Frankenhuyzen and Beardmore 2004) as well as an increase in disease incidence (Pinçon et al. 2001). However, a small plot study investigating herbivore and pest pressures on trees genetically-engineered for reduced lignin content found no change in insect abundance, a similar variety of insect species within the GE and non-GE plots, and no difference in phytopathogen occurrence (Pilate et al. 2002).

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Forest certification

Large amounts of forest land are bought and sold between companies and other institutions every year. If APHIS adopts a conditional approval process and required data to be provided once large acreages of GE trees are planted, there will need to be some mechanism to monitor long-term tracking of GE trees as they move from one owner to another.

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There are different organizations that currently certify forestry operations. Currently there is no one industry standard and often programs compete with each other. Organizations such as Forest Stewardship Council (FSC, http://www.fscus.org), Sustainable Forestry Initiative (SFI, http://www.aboutsfi.org), Pan European Forest Certification (PEFC, http://www.perf.org), and Canadian Forest Certification System (CFCS) are the more prominent. These competing organizations have differing certification standards. In particular some certification programs allow GE trees and others do not. For example the FSC (Forest Stewardship Council) does not allow the planting of any GE trees, even for testing purposes, on certified plantations. SFI has no such restriction. This could cause problems both within the U.S. and between the U.S. and other countries, if certified and non-certified products were to become co-mingled.

Currently no premium is given for products with certification by FSC, SFI, PEFC, CFCS and others. However, more and more companies are looking at purchasing forest products with a “seal” of certification that can have an effect on the price of a resource or product. Increasingly, outlets such as Staples and Home Depot are indicating that they will stock products only that have one or the other seal of approval. Consequently mills that have a seal will be the preferred supplier for these outlets. More and more outlets, producers and forest-based companies are taking the position that they will deal only in goods from certified forest operations.

Currently the number and size of field tests of GE forest trees are small compared to other crops. The current acreages of field tests are small but the duration of the field tests cover multiple years. APHIS currently has allowed a limited number of GE trees

in field tests to flower. The number of trees that are field tested and the size of field trials is limited by the ability to monitor for flowering. It is also limited in that few companies or institutions are at the point of producing large numbers of GE trees for testing.

No forest tree product has been deregulated. The possible time frame anticipated for the agency to be approached for deregulation of a forest tree engineered in the United States is within 3 to 7 years. However, entities outside the United States could possibly approach APHIS earlier for deregulation. Worldwide over 210 field trials with GE trees have taken place, mainly on species such as *Eucalyptus*, *Populus*, and *Pinus*. With the exception of China, none have been deregulated. China has reported the commercial release of GE poplar, with approximately 1.4 million insect-resistant trees planted on 300-500 ha[9] (FAO 2004).

Summary

The major difference between forest trees and crop plants is that most of the tree species that are being considered for deregulation and deployment in the United States are not far removed from their wild progenitors. Many can intercross freely with their wild relatives, thus gene flow can occur from plantations into surrounding forests.

There are a limited number of studies that examine how far pollen can move within and from a tree plantation, and there are few studies that have looked at potential gene flow from GE trees in a plantation to trees in native forests; however there are some studies that have looked at the movement of non-GE markers from an established field test. Therefore the best available data APHIS will have to evaluate the potential for transgenes to move into native forests may be gene flow models from other perennial species, like grasses. APHIS will need to evaluate the applicability of these data on a case by case basis, when considering deregulation or conditional approval of GE trees.

For some of the traits that are being engineered into trees, it may not be possible to gather data on the effect of the trait on the environment over many years. A good example would be genes for lignin modification. It will take years to produce such data. Therefore for some traits, APHIS may need to consider whether particular assumptions can be made without hard data when petitioned for deregulation.

[9] Food and Agriculture Organization: Preliminary review of biotechnology in forestry, including genetic modification, (Forest Genetic Resources Working Papers, FRG/59E), Rome: FAO: 2004.

Appendices

Appendix B

Glossary

Abiotic stress. Stress due to non-living, environmental factors such as cold, heat, drought, flooding, salinity, toxic substances, and ultraviolet light.

Adventitious presence, AP. Low-level and intermittent occurrence in commerce of biotechnology-derived material that has not completed all applicable reviews.

Agrobacterium tumefaciens. A bacterium that causes crown gall disease in some plants. The bacterium characteristically infects a wound, and incorporates a piece of its own DNA into the host plant genome, causing the host cell to grow into a tumor-like structure. This DNA-transfer mechanism is commonly exploited in the genetic engineering of plants.

***Agrobacterium tumefaciens*-mediated transformation.** The process of DNA transfer from *Agrobacterium tumefaciens* to plants, that occurs naturally during crown gall disease, and can be used as a method of transformation.

Allele. One of several alternate forms of a gene occupying the same location on the chromosome.

Allelochemical. A chemical produced by a plant of one species that has a detrimental effect on plants of other species.

Allergen. Any substance that causes an allergic reaction.

Antibiotic resistance marker gene. Genes (usually of bacterial origin) used as selection markers in transformation, because their presence allows cell survival in the presence of normally toxic antibiotic agents.

Anti-nutritional compound. A compound in food or animal feed that has a negative impact on nutrition or the absorption of nutrients.

Antisense DNA. The DNA strand complementary (hence "anti") to the mRNA, *i.e.* the non-transcribed strand.

Antisense gene. A gene that produces an mRNA complementary to the transcript of a normal gene, usually constructed by inverting the coding region relative to the promoter. See also **antisense RNA**.

Antisense RNA. An RNA sequence that is complementary to all or part of a functional mRNA molecule, to which it binds, thereby blocking its translation.

Arabidopsis thaliana. A small plant in the mustard (Brassicaceae) family. *Arabidopsis* is used as a model for studying plant genetics.

Backcrossing. Crossing an individual with another organism that is genetically identical to its parent. The offspring of such a cross are referred to as the backcross generation or backcross progeny.

***Bacillus thuringiensis* (Bt).** A common soil bacterium, notable for its ability to produce proteins which are toxic to certain categories of insects. See also ***Cry* proteins**.

Bioaccumulation. The increase in the concentration of a chemical in biological systems over time as compared to the chemical's concentration in the environment.

Biological control agent, biocontrol agent. Any enemy, antagonist, or competitor used to control a plant pest or noxious weed (Plant Pest Act of 2000).

Biolistics. A technique to generate genetically engineered cells, in which DNA-coated microscopic metal particles, usually tungsten or gold, are propelled by various means fast enough to puncture target cells (a "gene gun"). Provided that the cell is not killed, the DNA may be taken up by the cell and incorporated into the cell's genome. *Synonym*: microprojectile bombardment.

Biomagnification. The process that results in the accumulation of a chemical in an organism at higher levels than are found in its food; occurring when a chemical becomes more and more concentrated as it moves up the food chain.

Biotechnology. Techniques based on molecular biology, such as gene manipulation, gene transfer, DNA typing and cloning of plants and animals, for making specific modifications to the genome of an organism.

Breeding. The process of sexual reproduction and production of offspring.

Breeder Seed. Seeds of a particular plant variety maintained by a plant breeder, usually at a very high level of purity, which serves as the source for all subsequent generations of seed production. See also **foundation seed**.

Bt proteins, Bt toxins. See ***Cry* proteins**.

Certified Seed. Seed produced to specific standards to assure purity, freedom from weed seeds and seedborne pathogens, and used for commercial production of the crop.

Codex Alimentarius Commission. An international food safety standard setting body (part of the Food and Agriculture Organization of the United Nations) responsible for defining a set of international food standards. The Commission periodically determines, then publishes a list of food ingredients and maximum allowable levels (the *Codex Alimentarius*) deemed to be safe for human consumption.

Coding sequence. That portion of a gene which directly specifies the amino acid sequence of its product. Non-coding sequences of genes include introns and control regions, such as promoters, operators, and terminators.

Competent bacteria. Bacteria able to take up and stably maintain foreign DNA.

Conservation tillage. A broad range of soil tillage systems that leave crop residue on the soil surface, substantially reducing the effects of soil erosion from wind and water.

Constitutive expression. Describing a gene that is expressed (i.e. "turned on") at a relatively constant level in all cells of an organism without regard to cell environmental conditions.

Construct. An engineered piece of DNA designed to be transferred into a cell or tissue. Typically, the construct comprises a gene or genes of interest, a marker gene and appropriate control sequences, often from different organisms, as a single package. A repeatedly used construct may be called a "cassette."

Cross protection. Resistance to one disease that also confers resistance to other diseases.

Comment: This is not correct. The original usage was within plant virology where inoculation of the plant with a mild strain of the pathogen (not the disease) resulted in less or no symptoms produced by an otherwise severe strain of the pathogen. Check with a plant virologist for this definition.

***Cry* proteins.** A class of crystalline proteins produced by strains of the soil bacterium *Bacillus thuringiensis*, and engineered into crop plants to give resistance against insect pests. These proteins are toxic to certain categories of insects (*e.g.* corn borers, corn rootworms, mosquitoes, black flies, armyworms, tobacco hornworms, some types of beetles, etc.), but are harmless to mammals and most beneficial insects. *Synonyms*: delta endotoxins, *Bt* toxins.

Dicot. A flowering plant with two embryonic seed leaves. Examples include oaks, maples, roses, beans, mustards, and cacti. See also **monocot**.

Dietary toxicity. The toxicity of a substance when eaten.

Dietary toxicity tests. Methods used to determine whether and how much a substance is toxic if eaten.

Digestion stability. The ability of a substance to resist degradation by acids and digestive enzymes. A property often associated with allergens.

Disease resistance. The genetically determined ability of the plant to prevent the ingress and/or reproduction of a pathogen, thereby allowing the resistant individual to remain healthy.

Deleted: Some resistances operate by pathogen exclusion, some by preventing pathogen spread, and some by tolerating pathogen toxin.¶

DNA (deoxyribonucleic acid). A nucleic acid that carries the genetic information of a cell. The structure of DNA is two long chains of chemical building blocks called 'nucleotides,' twisted into a double helix. The order of nucleotides determines hereditary characteristics.

Donor. An organism that provides a gene or gene fragment used in the genetic transformation of another organism, which is called the "recipient."

Down regulation. The action of a DNA sequence or other chemical compound that causes a given gene to express less of the protein that it normally would.

ELISA (enzyme-linked immunosorbent assay). A sensitive assay that is used to detect a specific protein.

Encapsidation. The process by which the genetic material of a virus is enclosed in a protein coat (the capsid).

Endospermatic seed. Seed having an endosperm, the nutritive tissue surrounding the seed embryo.

Epistatic effects. The result of one gene suppressing the effect of a different gene.

Expression. The means by which a gene's information stored in DNA (or RNA in some viruses) is turned into biochemical information such as RNA or protein.

Fecundity. The capacity for producing offspring.

Flanking region. The DNA sequences extending on either side of a specific sequence.

Foundation seed. Seed of a particular plant variety that is produced from breeder seed and is then planted to produce certified seed used for commercial production. See also **breeder seed** and **certified seed**.

Deleted: in

Deleted: order to generate enough quantity for commercial sale

Gene. The basic unit of heredity transmitted from generation to generation during sexual or asexual reproduction; an ordered sequence of nucleotide bases, comprising of a segment of DNA. A gene contains the sequence of DNA that encodes an individual RNA or protein.

Gene expression. The process by which a gene produces mRNA and protein, and hence exerts its effect on the phenotype of an organism.

Gene flow. The spread of genes from one population to another, by the movement of individuals, gametes, or spores.

Gene gun. See **biolistics**.

Gene insertion. The incorporation of one or more copies of a gene into a chromosome.

Gene product. A RNA or a protein (*e.g.* an enzyme), the production of which is directed by the corresponding gene.

Gene silencing. Loss of gene expression either through an alteration in the DNA sequence of a structural gene or its regulatory region; or because of interactions between its transcript and other mRNAs present in the cell. See also **antisense RNA**.

Gene splicing. The enzymatic attachment of one gene or gene fragment to another.

Genetic engineering. Modifying the genotype, and hence phenotype, of an organism by genetic transformation.

Genetically engineered organism. An organism that has been modified by genetic engineering.

Gene stacking. The use of plant breeding to combine two or more genetic traits into a single plant variety.

Genetic marker. A gene that is a reliable indicator that a particular organism possesses a specific trait of interest. Markers may be used to select certain individual organisms, *e.g.*, cells that have inherited resistance to an antibiotic will be the only ones in a population that survive an antibiotic treatment.

Genetic transformation. See: transformation.

Genome. All of the hereditary material in a cell, including DNA present in the cell nucleus as well as in other locations, such as plant chloroplasts and mitochondria.

Genomics. The study of the entire genome of an organism, often in comparison to the entire genome of another organism (*i.e.* comparative genomics).

Genotype. The total genetic makeup that an individual receives from its parents.

Halophyte. A plant adapted to living in very salty soils.

Herbicide resistance/tolerance. The ability of a plant to remain unaffected by the application of what would otherwise be a toxic dose of a herbicide.

Heterologous encapsidation. The phenomenon where the coat protein of one virus is able to encapsidate (*i.e.* package) the nucleic acid of a different virus. See also *encapsidation*.

High dose. Twenty-five times the dose necessary to kill all susceptible insects.

Homologous recombination. The physical exchange of genetic material between two closely-related or similar genetic sequences.

Horizontal gene transfer. The transfer of genetic material from one organism (the donor) to another organism (the recipient) which is not sexually compatible with the donor.

Hybrid. The offspring of two genetically dissimilar plant varieties.

Industrial Gene. A gene whose effect on an organism or product is primarily of industrial use, as opposed to an agricultural or nutritional purpose.

Inserted gene. A piece of DNA that has been inserted into an organism using recombinant DNA technology and that contains sufficient heritable information to direct the production of a particular gene product in that living organism.

Instar. A stage in the development of an insect between two successive molts.

Interfertile. Two plants or groups of plants capable of interbreeding and producing offspring.

Introgression. The introduction of genes from one species into the gene pool of another, via sexual crossing. The process begins with hybridization between the two species, followed by repeated backcrossing to one of the parent species.

LD_{50}. Median lethal dose, or dose needed to kill 50 percent of a population of organisms.

$LD_{99.9}$. The dose that kills 99.9 percent of a population of organisms.

Macronutrient. An element or compound required in a relatively high proportion for normal growth and development. In animals, the term refers to carbohydrates, fats, and proteins. In plants, the term refers to elements like nitrogen, phosphorous, and potassium.

Marker gene. A gene of known function or known location that is inherited in Mendelian fashion and facilitates the study of inheritance of a trait or a linked gene.

Marker-assisted selection. The use of DNA markers to select the organisms that possess genes for a particular phenotype desired for subsequent breeding/propagation. This allows selection without having to screen for the performance trait itself, which may be difficult.

Monocot. A flowering plant with only one embryonic seed leaf. Examples include grasses, irises, lilies, and onions. See also **dicot**.

Mutagen. A chemical or dose of radioactivity capable of producing a genetic mutation, by causing changes in the DNA of living organisms.

Mutagenesis. Induction of heritable change(s) in the genetic constitution of a cell through alterations to its DNA, most often via treatments with chemicals or ionizing radiation.

Non-homologous recombination. The physical exchange of genetic material between two unrelated or dissimilar genetic sequences.

Non-propagative. See **non-viable plant material**.

Non-viable plant material. Broadly speaking, all plant tissues other than viable propagules, such as seeds, bulbs, tubers, etc. That is, all tissues dead or alive which cannot directly result in the propagation of a new plant.

Noxious weed. Any plant or plant product that can directly or indirectly injure or cause damage to crops (including nursery stock or plant products), livestock, poultry, or other interests of agriculture, irrigation, navigation, the natural resources of the United States, the public health, or the environment (Plant Protection Act of 2000).

Obligate parasite. A parasite that cannot live independently of its host.

Open reading frame (ORF). A sequence of nucleotides in a DNA molecule that has the potential to encode a peptide or protein. The term is generally applied to sequences of DNA for which no function has yet been determined. The number of ORFs provides an estimate of the number of genes transcribed from the DNA sequence.

Osmoprotectant. Compounds accumulated by plants to reduce water stress, such as caused by drought.

Outcrossing. The tendency of a plant species to produce offspring that result from the mating of two different individual plants. Compare to **self-pollinated**.

Pathogen-derived resistance. Resistance to a disease conferred by something derived from the disease-causing agent itself. Example: coat protein mediated viral resistance.

Performance-based standards. A form of regulation in which required *outcomes* are defined by regulation, but the actions or conditions necessary to attain the outcomes are not defined by regulation. Contrast with a *prescriptive* standard, which specifies actions or conditions that must be followed to attain the required outcome.

Phenotype. The appearance or other characteristics of an organism, resulting from the interaction of its genetic constitution with the environment.

Phytoremediation. The use of plants to remove or reduce pollutants in soil by production of compounds that stimulate their degradation or by uptake through roots and accumulation in plant tissues.

Plant incorporated protectants (PIPs). The term PIP was designated by the Environmental Protection Agency (EPA) to describe substances that are incorporated into plants to protect them from damage caused by insect pests and diseases. PIPs are defined as the pesticidal substance that is produced in a plant and the genetic material necessary for the production of those substances (Federal Register, Volume 66, No. 139, July 19, 2001).

Plant pest. Any living stage (including active and dormant forms) of insects, mites, nematodes, slugs, snails, protozoa, or other invertebrate animals, bacteria, fungi, other parasitic plants or reproductive parts thereof; viruses; or any organisms similar

to or allied with any of the foregoing; or any infectious agents or substances, which can directly or indirectly injure or cause disease or damage in or to any plants or parts thereof, or any processed, manufactured, or other products of plants (7 CFR 340.1).

Pleiotrophic effects. A phenomenon in which a single genetic alteration affects multiple phenotypic characteristics, such as a single gene affecting flowering, leaf shape, and growth rate.

Post-translational modification. The addition of specific chemical residues to a protein after it has been translated. Common residues are phosphate groups (phosphorylation) and sugars (glycosylation).

Primer. A short, single-stranded piece of DNA that, when annealed to a long template of single-stranded DNA, provides a doubled-stranded structure from which DNA polymerase will synthesize a new DNA strand to produce a duplex molecule.

Proline. An amino acid.

Promoter. A region of DNA location upstream of a gene that controls to what degree, where, and/or when a gene is expressed.

Propagules. A propagule is any part of a plant that can be detached from the organism and propagated in order for it to grow into a new plant in a new environment.

Proteomics. An approach that seeks to identify and characterize complete sets of protein, and protein-protein interactions in a given species.

Protoxin. A precursor of a toxin that requires additional modification before acquiring its toxic properties.

Pyramid. In the context of PIPs, the presence of multiple resistance genes that are targeting the same pests with possible overlap in the mode of action. For example, a corn or cotton plant containing a Cry1A protein and a Cry2A protein active against the same lepidopteran pest such as the European corn borer or tobacco budworm is termed a "pyramid."

Recombinant DNA organism. An organism in which the genetic material has been changed through gene splicing techniques, using recombinant DNA and direct injection of nucleic acid into cells or organelles.

Recombination. The physical exchange of genetic material between two genetic sequences that produces new combinations of genetic information. See also *homologous recombination* and *non-homologous recombination*.

Refuges. Non-Bt host plants that are managed to provide sufficient susceptible adult insects to mate with potentially Bt-resistant adult insects to decrease the number of resistant insects and dilute the frequency of resistance genes.

Regulated article. That which is subject to APHIS regulation under 7 C.F.R. Part 340.

Rhizosphere. The root surface together with that region of the surrounding soil in which the microbial population is affected by the presence of the root and root exudates.

Risk analysis. A process consisting of three components, risk assessment, risk management, and risk communication, performed to understand the nature of unwanted, negative consequences to human and animal health, or the environment.

Risk assessment. A scientifically based process consisting of the following steps: (i) hazard identification; (ii) hazard characterization; (iii) exposure assessment; and iv) risk characterization.

Risk communication. The interactive exchange of information and opinions throughout the risk analysis process concerning hazards and risks, risk-related factors, and risk perceptions, among risk assessors, risk managers, consumers, industry, the academic community, and other interested parties, including the explanation of risk assessment findings and the basis of risk management decisions.

Risk management. The process, distinct from risk assessment, of weighing policy alternatives, in consultation with all interested parties, considering risk assessment and other factors relevant for the health protection of consumers and for the promotion of fair trade practices, and, if needed, selecting appropriate prevention and control options.

RNA (ribonucleic acid). A nucleic acid composed of a long, often single-stranded chain of chemical building blocks called 'nucleotides.' RNA has multiple functions in the process of translating information stored in genes (DNA) into proteins.

Rotation. The number of years required to establish and grow trees to a specified size, product, or condition of maturity. A pine rotation may range from as short as 20 years for pulpwood to more than 60 years for sawtimber. Full rotation is the total time from planting to harvest. Half rotation would be approximately half the time to reach maturity or harvest.

Secondary metabolism. The production by living organisms of substances not essential for primary metabolic functions or physiology. Their role is associated with interaction with the environment, for example for defense, as elicitors, or as attractants. Some of these have useful pharmacological or nutritional properties, while others are toxic.

Self-pollinated. The tendency of a plant species to produce offspring that result from a flower pollinating itself. Compare to **outcrossing**.

Stratification. Seed stratification is a time for moist seeds to sit preparing to germinate. Many seeds require a cold stratification period in order to germinate while

some require a warm stratification prior to the cold stratification. Cold stratification is usually at the temperature of 34-41 degrees F., while warm stratification is at the temperature of 68-86 degrees F.

Stress tolerance gene. A gene which confers upon a plant an increased ability to withstand an environmental stress, such as drought, temperature extremes, or soil salinity.

Synergy. The interaction of two or more agents so that their combined effect is greater than the sum of their individual effects.

Trait. A characteristic of an organism that manifests itself in the phenotype. Traits may be the result of a single gene or may be polygenic, resulting from the simultaneous expression of more than one gene.

Transcription. The process by which a messenger RNA (mRNA) is created from the nucleotide sequence of a gene (DNA).

Transencapsidation. See **heterologous encapsidation.**

Transformant. A cell or organism that has been genetically altered through the integration of a transgene(s). A "primary" transformant is the first generation following the transformation event.

Transformation. The uptake and integration of DNA in a cell's genome, in which the introduced DNA is intended to change the phenotype of the recipient organism in a predictable manner.

Transgene. A gene that is inserted into the genome of a cell via gene splicing techniques.

Transgenic. An organism whose genome has been modified via the stable incorporation of a piece of foreign DNA (a transgene).

Translation. The process by which the sequence of nucleotides in a messenger RNA (mRNA) directs the sequence of amino acids in a new protein during protein synthesis.

Trophic. Relating to the feeding habits or food chain of different organisms in a food chain.

Up regulate. The action of a DNA sequence or other chemical compound that causes a given gene to express more of the protein than it normally would.

Vector. The agent, such as a plasmid, used by researchers to carry new genes into cells.

Viral coat protein. A protein produced by a virus that forms a protective layer, or *capsid*, around the genetic material of the virus.

Volunteer. Plants resulting from crop seed that escapes harvest and remains in the field until subsequent seasons, where it germinates along with the succeeding crop.

Weediness. The ability of a plant to colonize a disturbed habitat and compete with cultivated species.

Appendix H

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STUDIES ON BT TOXINS RECOUNTED

Biotech crops expressing Bt toxins have been used for a decade, and have allowed farmers all over the world to use reduced amounts of insecticides. The ubiquity of such crops has also allowed the Bt toxin class to become the most widely used in the world. What does this bode for regulation and safety assessment, now that more Bt crops are being developed, and will soon be released in the market?

Jorg Romeis and colleagues of the Swiss Federal Research Station for Agroecology and Agriculture offer their perspectives on "Transgenic Crops Expressing Bacillus thuringiensis Toxins and Biological Control." Their article appears in the latest issue of Nature Biotechnology.

The researchers look at studies already conducted on the safety of Bt crops by reviewing the results of those performed in the laboratory or glasshouse environment; experiments performed to assess the effects of the toxin on predators and parasitoids; and those conducted in a field or semi-field situation. They conclude that semi-field studies are likely to be the most accurate, as they offer some control over the environment, while at the same time keeping plants and predators in close-to-field conditions. They also perceive field studies to be less accurate, as such conditions are harder to keep under control, and their results thus cannot be interpreted conclusively.

In the studies reviewed, Bt plants have been shown to have no direct adverse effects on non-target species. Such data, however, cannot predict the effects of other insecticidal proteins in future GM crops. Thus, the writers recommend that: 1) researchers take great care in designing experiments, such that their results will be relevant for field situations; 2) insecticide treatments should be considered as the baseline for risk assessment; and 3) standards and protocols should be established regarding issues of non-target effects of Bt crops.

Subscribers to Nature Biotechnology can read the complete article at http://dx.doi.org/10.1038/nbt1180.

Reviewer Report 2

Introduction

As directed in the USDA/APHIS 'Charge to Peer Reviewers of Influential Scientific Information Contained in the APHIS BRS Draft Programmatic Environmental Impact Statement, this review report addresses the issues of completeness, currency, accuracy, uncertainty, objectivity, and clarity and ends with an overall conclusion. Detailed comments and suggestions have been provided to USDA-APHIS in a 'track changes' edited version of Chapter 4.1.

a. Completeness

In general, the chapter is comprehensive. Among several curious omissions, however, was any discussion of community diversity impacts of widespread use of GE (specifically herbicide-resistant) crops; there are several relevant papers from the multi-year British Farmscale Study that are not cited (Proc Roy Soc. B 272 (2001). Inasmuch as concerns have been raised, even a brief mention of impacts of transgenic plants on pollinators would also have been useful (the entire monarch/Bt corn controversy, more or less resolved in a series of six papers in PNAS 98 (2001), was effectively unmentioned as was the ongoing discussion about transgene product impacts on honey bees, reviewed in Malone et al. 2001 Apidologie, among other places.

b. Currency

Approximately three dozen references are less than two years old and the authors have made an effort to access a broad diversity of sources, including specialty journals, stringently peer-reviewed journals, government publications and webs sources. One place where currency is a severe problem is in the section on biological control of weeds; essentially a decade of literature documenting adverse impacts of biological control agents, including those introduced for weed control, on nontarget species. There are two reviews in Annual Review of Entomology germane to this subject (Louda et al. 2003; van Lenteren et al. 2006) that are not included in the discussion or reference list. The failure to cite or acknowledge this literature has implications for accuracy and uncertainty.

c. Accuracy

For the most part, the report is accurate. Several errors or misspellings are indicated by “track change” edits on the document. Perhaps as a consequence of an effort to make the text accessible, some imprecision was introduced in places (e.g., use of “animal” to mean “vertebrate”).

Again, the section on biological control of weeds is dogmatic in its assertion that there have been no adverse impacts of biocontrol agents on nontarget native plants; recent publications on *Rhinocyllus conicus* and Platte thistle provide just one glaring exception to these bald statements.

d. Uncertainty

There was little attention paid in the document to effects of human behavior on environmental impact and there is a literature to suggest that compliance (e.g., with providing refuges, Carriere et al. 2001), carelessness, or even deliberate flouting of regulations can change risk levels. As well, throughout the document, many outcomes were described as low-risk but no effort was

made to quantify probabilities (which may have been more effective at conveying risk level) (e.g., "a consensus exists among scientists that the likelihood of small peptides to sensitize an individual is low").

There is also greater certainty stated in the section on biological control of weeds than is justified by current literature; moreover, there is no acknowledgment or recognition that the degree of contemporary attention to risk assessment did not necessarily apply in the past and agents may have been introduced in the past (which are now established) that would not pass muster by contemporary standards.

Among the dogmatic and less than adequately documented statements are (see Track Change text for reference suggestions):

- "The host range of the biological control organisms that is observed in the field is always smaller than the host range that is observed in the laboratory because environmental factors reduce the number of species that may be attacked due to unfavorable environmental conditions".
- "long-term, extensive damage to a non-target plant population, which has never been documented from a biological control agent"

e. Objectivity

There were places where qualifying language, justified by the literature, was missing, which created an impression of bias. In the definition of Cry proteins, e.g., the statement is made that these "are harmless to mammals and most beneficial insects" but these proteins are broadly toxic within an order, so what kills target lepidopterans is likely to kill nontarget lepidopterans as well.

f. Clarity

See text for tracked changes where meanings weren't clear and where alternate wording has been suggested. Generally, the text was clearly written (although the document reads as if there were multiple authors—the first few sections were much less well-constructed than subsequent sections).

Conclusion

The Charge to Peer Reviewers requests the peer reviewers to answer the question, **Does this scientific information presented accurately and objectively provide non-expert readers with a broad base of knowledge to understand the aspect of the biological and physical environment that is likely to be affected by the regulations currently administered by APHIS BRS and by possible changes of those regulations?**

Referring to the review report and to the detailed comments and suggestions in the track-changes version, my answer to this question is **(2) Yes, but only after revisions have been made to address specific weaknesses.**

APHIS: Amended to this Reviewer Report below is the Reviewer's "Track Changes" edits to the draft EIS chapter.

Chapter 4

Environmental Consequences

Introduction

Genetically engineered (GE) organisms have been field tested in the U.S since the 1980s and GE plants have been grown commercially on millions of acres in the US since the mid-1990s. Developers and researchers monitor field trials while growers, extension agents, and researchers all scrutinize commercially grown GE crops. APHIS is not aware of any verifiable reports of environmental harm or harm to human health resulting from such field trials or from commercial use of GE plants. The system of safeguards and controls imposed by APHIS on all GE organisms within the scope of authority has been highly effective in ensuring that releases are conducted in accordance with the conditions set by APHIS and that crops which are de-regulated pose no greater risk than conventionally bred crops.

The agency recognizes, however, that in a scientific approach, it cannot make general conclusions about the safety of GE organisms. In addition, new technological trends have the potential to result in novel types of GE organisms being developed, which may have a greater propensity for environmental impact, both positive and negative, than those field-tested to date. Therefore, even though the past environmental releases have been conducted safely, and commercial products are being safely grown and consumed, APHIS will continue to rigorously scrutinize new scientific developments as well as the potential environmental impacts of any proposed changes in the regulations.

Chapter 4 examines the ways in which the implementation of current APHIS biotechnology regulations and possible changes might affect the quality of the environment.

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Section 4.1 provides general background information for non-specialist readers to better understand the discussions in subsequent chapters regarding potential environmental impacts, and illustrates with some examples how such environmental impacts are typically assessed.

Section 4.2 describes in general the regulatory features of APHIS's current system and how these features control the environmental interactions described in the first section in order to reduce the likelihood of significant negative impacts.

Section 4.3 describes the impacts of the "no change option" with respect to 10 specific issues. For each issue the "no change" option is followed by an analysis which compares one or more options for new approaches under consideration.

Chapter 4

Chapter 4, Section 1: Impacts of Genetically Engineered Organisms

A. Introduction to biological factors that may be affected

This section briefly introduces the general ways in which plants, animals, insects, and micro-organisms affect the environment. Given that GE plants currently form the bulk of the releases into the environment, this section also gives a general introduction to Plant Biology, Crop Improvement, Seeds Biology and Commercial Seed Production.

Plants

Plants engage in numerous physical and biochemical processes which affect humans and the environment. Plants produce food and fiber for humans and for animals, both domesticated and wild. Plants alter the atmosphere, removing carbon dioxide from the air and adding oxygen. They modulate air and soil temperature and create microenvironments for other organisms. Plants modify soil structure through root growth and stabilize soil, reducing erosion, and plants add organic matter to the soil, which feeds microorganisms and improves soil quality. Plants also interact with each other, competing for sunlight, water, and soil nutrients, and weeds are plants which can compete so effectively with crop plants to cause economic harm to the crop. Lastly, plants produce a large variety of chemical substances that may affect the local environment or provide economic value to humans. Genetic engineering can alter the value of a plant to humans and may also affect one or more of the physical and biological interactions between plants and their environment.

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Many insects and other animals are intimately associated with plants. These associations can be harmful to the plant, as in the case of animals which feed on plants and cause harm ranging from economic damage to death of the plant. There are also positive associations such as bees and hummingbirds, which pollinate plants, and ladybugs, which eat harmful insect pests. In other cases, the association may be neutral: the animal may simply live on or near the plant. GE traits in plants may alter these associations or create new ones.

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Microorganisms

Plants also have a variety of interactions with microorganisms. Certain soil microbes, such as *Rhizobium* bacteria and some fungi, associate with plant roots and provide additional nutrition to the plants, via various mechanisms. On the other hand, there are hundreds of negative associations: many microorganisms including bacteria, fungi, and viruses, among others, cause serious plant diseases, resulting in enormous economic losses. There are also neutral associations: many yeasts, for example, live on plant leaves without causing any harm to the plant, and other microorganisms help decompose dead plant material in the soil. Creating disease-resistant plants through genetic engineering could change some of these negative associations, but other GE traits such as those affecting nutritional quality or plant structure could alter other plant-microbe interactions.

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Plant Biology and Crop Improvement

Introduction

Plants exist in agricultural, or managed, ecosystems and wild, or unmanaged, ecosystems, and they interact with the environment in both types of ecosystems (Janick *et al.* 1981). This section summarizes these interactions in three contexts – physical, physiological, and ecological. The discussion is necessarily brief and broad but provides a basis for understanding how plants function in the environment and why plant breeders are attempting to modify those functions. Generally, breeders are attempting to enhance plant performance, which relates to a plant's ability to benefit from its positive interactions with the environment while suffering minimally from negative interactions (Allard 1964). Should traits enhancing plant performance move into wild plants, one might find increased fitness in these plants as well. For this discussion, genetic engineering is considered a tool available to plant breeders, available as another method to add a desirable trait to a plant variety.

Physical Environment

Except for parasitic plants, which grow partially within other plants, and epiphytic plants, which grow on other plants, most plants grow partially embedded in soil or in or on water. Many plants are capable of limited directional growth, but most plants

cannot move large distances (Wareing and Phillips 1981). Therefore they are forced to obtain nutrients and water from nearby sources. A wild plant which is more able to exploit limited resources may be more likely to survive and reproduce, while a highly efficient crop plant may be more likely to produce large yields.

Terrestrial plants produce roots to absorb water and nutrients from the soil and to physically anchor themselves in the soil, but roots also directly affect the soil. Roots create spaces in soil for the passage of air, water, and soil organisms. In addition to these physical changes, roots release organic acids, which alter nutrient availability and accelerate soil development. As roots die and decompose, they contribute organic matter to the soil, improving its texture and its ability to retain water and nutrients. Plant roots also anchor soil particles and reduce soil erosion (Brady 1974).

Plant breeders are frequently interested in developing varieties with robust growth, including root growth. There are few GE traits currently under APHIS oversight whose purpose is to alter plant morphology; however, APHIS anticipates that altered morphology traits may be developed by researchers more frequently in the future. Root growth traits may alter plant drought tolerance but may also affect soil water distribution and irrigation practices and possibly soil stability and erosion.

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Because adequate water is essential for survival and growth, plants have developed elaborate systems to absorb, transport, and retain water (Janick *et al.* 1981). Although roots can grow toward sources of soil water, soil water generally can move in the soil faster than roots can grow to reach it. Plants therefore use their own tissues to store water when it is readily available, and plants use various means such as waxy leaf coatings to restrict water loss when water is not available (Esau 1977). Some water loss is unavoidable however, and through the process called transpiration plants lose water from aboveground surfaces and thereby convey water from the soil into the air. For field crops, this water loss can equate to several inches of rainfall during the crop season.

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Depending on the environment to which a plant is adapted, too much or too little water may be deadly the plant. Some plants have adapted the means to temporarily withstand flooding or drought, and plant breeders are actively working on developing these traits in crop plants in order to enable crop production in areas with suboptimal water availability. Drought tolerance may increase the range of environments where a crop or wild plant can grow successfully and alter water management practices for growers.

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Green plants have a profound effect on the Earth's atmosphere. As a result of photosynthesis, plants remove carbon dioxide from the air, combine it with water, and produce carbohydrates, which are used by the plant as its primary form of stored energy and to increase biomass. Oxygen is produced as a byproduct of photosynthesis and is released by plants back into the atmosphere. Plants also reverse this process when carbohydrates are utilized for energy, producing carbon dioxide and water, while using up oxygen (Bidwell 1974). However, while plants both produce and use up

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carbohydrates and oxygen, the net balance is in favor of the release of oxygen into the atmosphere and the removal of carbon dioxide from the atmosphere.

Plants have anatomical, morphological, and physiological adaptations to allow the exchange of internal oxygen and carbon dioxide with gases in the atmosphere while conserving water to maintain a healthy water balance. Traits which modify these plant characteristics could affect photosynthesis, water efficiency, and irrigation practices.

Light provides the energy driving the photosynthetic process. During periods of inadequate light, plants cannot produce new carbohydrates and are forced to use stored carbohydrates to survive. When light is limited, such as when plants grow in shade, the plants which best exploit the available light may outcompete less efficient plants (Janick *et al.* 1981). Plants use both structural means, such as producing larger leaves or growing taller then their neighbors, and physiological means, such as producing more chlorophyll, to better utilize limited amounts of available light (Bidwell 1974). Plant breeders exploit these adaptations to produce crop varieties that make the most of available light. Plants able to better exploit sunlight may grown successfully in environments previously unsuitable for crop production. A crop which uses light more efficiently may be grown at higher density, *i.e.*, more plants per acre, thereby changing some crop management practices.

Physiological Environment

In general terms, photosynthesis consists of three processes: the absorption and retention of energy from sunlight; the conversion of light energy into chemical energy; and the stabilization of chemical energy into stored energy in the plant (Bidwell 1974). The process of photosynthesis has been studied for decades and scientists know that this process can be accomplished in several subtly different ways. Variations in photosynthetic processes have evolved that enable adaptation to specific environmental conditions such as low light or restricted water. These adaptations may be biochemical or anatomical, resulting from one or more genetic changes in the plant. Although breeders have been trying to alter photosynthetic efficiency for many years, the complexity and number of genes that must be expressed to coordinate efficient photosynthesis has limited breeders' ability to significantly improve photosynthetic efficiency. Alterations to photosynthetic efficiently may increase yields but may also require additional water and fertilizer. Increased photosynthetic efficiently may also result in increase overall environmental fitness, which could affect both crops and wild plants bearing the traits.

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Although plants produce carbohydrates to be used as energy via photosynthesis, plants are still reliant on the soil as a source of mineral nutrition. Nitrogen, phosphorus, potassium, iron, magnesium, and other elements must be absorbed by plants roots and transported to tissues where they participate in myriads of biochemical reactions necessary for plant survival and growth. For the most part, these minerals are either already present in the soil or have been added by a grower in the form of fertilizer. Plants simply rely on the fact that soil water dissolves the minerals and makes them available for uptake by the roots (Van der Have 1979). It

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may be possible to produce plants through genetic engineering that are able to better take up minerals from the soil or that are able to use soil minerals more efficiently. Such traits could increase plant fitness and possible alter crop management practices, specifically how much fertilizer a grower may use.

Certain symbiotic soil microorganisms associate with plant roots to increase nutrient availability. For example, *Rhizobium* bacteria associate with the roots of some plants, mostly legumes, take nitrogen from the air, which cannot be used directly by plants, and convert it into ammonium, which can be taken up by plant roots. Certain soil fungi, called mycorrhizal fungi, associate with plant roots, making some soil nutrients, such as phosphorous, more available for uptake (Brady 1974). Breeders are interested in increasing the number of plant species able to associate with these microorganisms and other researchers are working the microorganisms themselves. Increasing the benefit obtained from symbiotic relationships with soil microbes may increase plant fitness, increase the geographic ranges of some crops and possibly change soil fertility management practices.

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In addition to carbohydrates, fats, and proteins made by plants for growth, plants also make a wide variety of substances whose purpose is either unknown or is unrelated to growth. These substances are called *secondary metabolites*, and although they do not appear necessary for growth, many have important functions in such areas as disease resistance, reproduction, and herbivory reduction (Verpoorte *et al.* 2002). Many of these substances are of interest to breeders, either because the secondary metabolite makes the plant more tolerant of environmental stress or because the metabolite is valuable to humans for pharmacological or other purposes. Altering secondary metabolite production may result in a plant that is more fit due to enhanced environmental stress tolerance, or it may result in a plant with higher value as a crop because the metabolite itself is useful to humans.

Ecological environment

In most environments where plants grow, one or more resources, *i.e.*, light, water, nutrients, and space, are in limited supply, and plants growing together in the same location are generally competing with each other for the same resources. When the plants are a managed crop, the grower attempts to supply limited resources to the crop so that the individual plants are not competing with each other and are each growing at or near full potential (Janick *et al.* 1981). However, other plants growing with the crop can also benefit from the resources provided by the grower and take them from the crop. These plants are considered weeds and are removed when possible to reduce unnecessary competition and waste of resources intended for the crop. Breeders are always looking to develop crops that make more efficient use of resources to reduce competition and reduce inputs from the grower. In unmanaged environments, plants also compete for resources, but because no grower is supplementing the supply of resources for wild plants, plants with more competitive adaptations and more efficient resource use will tend to grow better and reproduce more than their less competitive neighbors.

Two positive interactions between plants and other organisms, *Rhizobium* bacteria and mycorrhizal fungi, were discussed above. There are other examples of positive associations between plants and other organisms. Plants are pollinated by a variety of insects birds and mammals; many animals assist plants by disseminating their fruits and seeds; still other animals, such as ladybugs, help plants by eating insect pests. Other associations appear neutral, as far as the plant is concerned. In some cases, plants provide a beneficial habitat for the organism, for example, when a spider builds a web, using a plant as support. Another kind of neutral association between plants and other organisms occurs after plants die. Dead plant material provides food to a wide variety of organisms from vertebrate and invertebrate animals to thousands of microorganisms, which all feed on the plant debris until it is completely broken down. APHIS is unaware of research into GE traits altering these types of positive and neutral interactions between plant and other organisms, but APHIS anticipates that such traits, if developed, could affect plant fitness and may also affect the associated organisms as well as non-target organisms.

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There are many associations between plants and other organisms in which the plant suffers some harm. The organism may eat the plant or feed from the plant. Caterpillars, aphids, and nematodes all get nutrition from plants, at the plant's expense. Organisms such as bacteria, fungi, and viruses cause plant diseases that can either kill the plant or weaken it so that it cannot reproduce. The organism may use the plant to launch an attack on other plants, as a means of completing its life cycle, or as a place to overwinter. Obviously, breeders care a great deal about minimizing the occurrence and intensity of these negative interactions and focus significant efforts to develop disease-resistant crops (Fehr 1987). Complicating the use of disease-resistant crops is the development of new strains of disease organisms that can overcome the plant's resistance. Disease and insect resistance derived by either genetic engineering or conventional breeding are traits with which APHIS is very familiar, and APHIS anticipates continued interest in the development of these traits. Such traits could be expected to increase plant fitness, change crop management practices, especially with regard to pesticide use, and potentially raise questions of impacts on non-target organisms and development of resistance within pest populations.

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In general, APHIS expects plant breeders to continue to improve crop performance and value using traditional breeding and GE traits. APHIS currently examines the potential impact of the trait on the health of the plant and on the environment with which the plant interacts.

Seed Biology and Commercial Seed Production

Seed has a dual character. From the standpoint of the plant, seed is a means to reproduce, but humans use seed as a product in the form of grain. When seed is used by humans to propagate plants, it has genetic and other quality characteristics that differentiate it from seed grown for use as grain. This section will examine the biological nature of seeds and will briefly describe how seeds are produced for commerce.

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Biology of Seeds

Seeds produced by plants have been the foundation of agricultural development by humans for well over 10,000 years. During this time, humans have progressively transformed selected plant species from wild progenitors into highly specialized crops. Seeds today are used as a source of energy and nourishment for human and domestic animal consumption. They produce fibers used in clothing and construction. They are a source of raw materials for manufacturing an ever-broadening array of commercial products. And they are becoming an increasingly valuable source of renewable energy. The market value of agricultural seeds produced for planting each year is tens of billions of dollars worldwide. The world production of major grains and oilseeds comes to about 2.5 billion tons, worth more than a half trillion dollars.

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The role of seeds in plant reproduction

From the plant's perspective, seeds have a very different function. Simply stated, they are created to sustain the species. They contain the genetic instructions passed down from their parents, and serve as the conduits for transferring that genetic information to the next generation.

Plants have developed a wide array of mechanisms to increase the chances of successfully passing genetic information on to the next generation. Most crop plants reproduce sexually, which increases variation among the offspring and has advantages in natural evolution, but requires a carefully-orchestrated interaction between male and female gametes. Successful mechanisms involve various flowering forms, mechanisms of pollen dispersal, self-incompatibility, and sensitivity to environmental cues. Knowledge of these reproductive strategies has enabled humans to transform wild progenitors into agronomically useful crops through many generations of crossing plants followed by the selection of desirable individuals in the progeny. These same reproductive strategies, however, can create challenges for maintaining genetic purity of seeds, particularly in crops that utilize natural environmental conditions to aid pollen and seed dispersal.

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Although there are many physical variations, all crops produce flowers with the same basic anatomy. The stamen contains the male reproductive parts (anthers) which produce pollen. The pistil contains the female reproductive parts (ovules) which house the egg cells. 'Complete' flowers contain both male and female reproductive parts. Crop plants with perfect (complete) flowers are largely self-pollinated, and the majority of crop plants are self-pollinating. It is possible for pollen from another plant to cause fertilization in these self-pollinating plants, but the probability is very low because pollination typically occurs even before the flower petals open.

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The flowers of some crop plants require cross pollination (*i.e.*, pollination by another flower). Maize tassels, for example, produce flowers that do not usually develop female structures, and produce only pollen. The flowers on the rachis (ear) do not develop male floral structures, and require pollen from the tassel for pollination. This mechanism of separating male and female flower parts increases the probability of

cross pollination. The flowers of some crop species cannot pollinate themselves, which maximizes mixing of genes between plants.

Pollen and seed dispersal

Forcing cross pollination in crops with perfect self-pollinated flowers, such as soybean and wheat, requires that that either pollen be inactivated or anthers be physically removed before they mature and release pollen. In either case the pollen from another plant is delivered to the stigma.

In crops that are self-incompatible or have separate male and female flowers, pollen must be delivered to the female flowers by wind dispersal, or, in most cases, insects. Corn pollination, for example, relies on wind dispersal of pollen. This reproductive strategy requires the corn plant to produce a large abundance of pollen and release it in close synchrony with maturation of female flowers, and it forces the pollen to travel through dry air.

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Crop plants that rely on wind dispersal of pollen for pollination create challenges for those concerned with genetic purity: breeders, seed producers, grain growers and sometimes consumers. With few exceptions, the female flowers of corn will accept pollen from any corn plant. The seed industry continues to refine isolation standards and develop novel genetic, physical, and chemical mechanisms to minimize cross pollination (for corn, see Beck 2004). Fortunately, the predicable nature of flowering dynamics and improved methods for modeling pollen dispersal are making it possible to quantify loss of genetic purity under field conditions (Fonseca *et al.* 2004).

Seed development, maturation, and long term viability

After fertilization, the developing seed becomes the primary recipient for water and photosynthetic products of the plant, rapidly gaining weight due to embryo development. The seed must store the chemicals that will be used to feed the growing seedling at the early stages of seed germination. The chemical composition of a seed is determined by genetic and environmental factors. Carbohydrates, fats and oils, and proteins, are among the most important seed stored substances.

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Once the seeds have matured, they may or may not be dispersed. Mechanisms of seed dispersal are relevant to the discussion of gene flow into unmanaged environments and specifically into the wild relatives of crop plants. Seed dispersal mechanisms are mainly used by plants in the wild. Many species of crop plants have been selected to hold their seed until harvest. Some crops do not do this perfectly and some grain is lost prior to harvest.

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After physiological maturity the seeds of many species dehydrate, which helps seeds survive cold winters and dry periods. These seeds have the ability to dehydrate to very low moisture content while remaining viable, even though their moisture content is 8 percent to 12 percent (well below the 70 percent water that makes up all living tissues in plants). Not all seeds, however, will undergo dehydration: seeds from plants adapted to tropical environments usually do not dehydrate as much as those from

temperate climates. At low temperatures and moisture content, the seed metabolism diminishes and the aging process of the seed is slowed. Depending on seed composition, original seed quality, and storage conditions, seeds can be stored for several months to several years in an insect-free, low-temperature and dry environment.

Accumulation of storage materials

Seeds, primarily cereals and legumes, make up 70 percent of the food consumed in the world. Seeds store large amounts of chemical substances not stored in any other parts of the plant. These are proteins, carbohydrates, lipids (fat and oils), and they provide food to the seedling at the early stages of germination and growth.

Proteins are an important stored food component of many seeds, especially legumes. In many countries of the world legumes are the main source of protein in the diet, replacing proteins of animal origin. Most protein is broken down to amino acids in digestion and reassembled to make new proteins. Humans do not produce all of the amino acids and some of these important building blocks must come from dietary sources, including plants. The role of proteins as a source of energy in the human diet is secondary.

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Carbohydrates are the most important storage compounds in the seeds of cereal crops. Starch and hemicellulose are the two main forms of carbohydrates stored in seeds, and are the source of simple sugars needed for germination. Starch is the principal stored carbohydrate in seeds and is stored in the endosperm in two forms, amylose and amylopectin. Hemicellulose is primarily a structural carbohydrate that is located in the cell wall, but in certain species is stored as reserve food. Carbohydrates are primarily a source of energy in human food.

Lipids or fats serve as energy storage within the seed and are an important part of all cell membranes. They are also used in food and animal feed and for industrial uses. Fatty acids from seeds contain larger amounts of unsaturated fatty acids (those containing one or more double-bonds within their molecule) than lipids of animal origin, and these plant lipids are used increasingly in processed foods.

DNA is present in all seeds, as it is in all plant tissues. The DNA content of the seed is vital because it provides the biochemical instructions for germination and growth of the new plant. DNA is broken down by digestion when eaten, and its genetic information content has no impact on human health.

Opportunities for genetic modification

Nutritional studies also indicate that seeds are important sources of vitamins, antioxidants, and phytohormones. During the past two decades, there have been major advances in the understanding of biosynthetic processes controlling the synthesis and accumulation of these products in seeds. This knowledge, in concert with development of molecular biological techniques, has made it possible to modify

seeds from crop plants to improve human health and produce raw materials for non-food uses. Transforming seeds for these purposes involves the addition of genes not currently present in plants. Numerous studies to date indicate that seeds of some crops can be induced to synthesize and accumulate various novel compounds. It is likely the programming for accumulation of normal seed storage components will need to be modified as well if seeds are to accumulate new compounds in sufficient quantity. For crops that will be produced in large scale such transformations need to be made with minimal effect on seed development, seed physical characteristics, and viability. A new genotype with poor agronomic characteristics and low capacity for seed production will not survive long in the seed industry.

Commercial Seed Production

Trade and value

Before the 19th century, farmers generally produced their own seed, and seed commerce was usually limited to the replacement of stocks that had become mixed or degraded. The advent of modern plant breeding methods has led to the importance of seed as a commercial product valued for its particular trait purity and quality components. The increased sophistication of plant breeding to produce crops meeting very specialized needs and market niches has in recent decades increased the need for high standards of seed genetic purity in order to assure identity preservation in increasingly diversified markets.

Seeds are internationally traded commodities. The U.S. is the largest producer and consumer of seeds in the world. An estimated $5.7 billion worth of commercial seeds are produced annually in the U.S., which is a 19 percent share of the $30 billion world seed market. Maize seed is the largest segment of the U.S. domestic planting seed market, valued at $2.2 billion. Annual U.S. seed exports and imports are estimated roughly at $800 million and $400 million respectively thus providing a net trade surplus. The U.S. exports seeds mainly to Mexico, Canada, Italy, Japan and Argentina, and imports are mainly from Canada, Chile, the Netherlands and China.

In the U.S., farmers purchase a large portion of seeds from commercial sources, and the commercial sector is engaged in production, conditioning, distribution and marketing of seeds. Government policies and regulations affect interstate movement of seeds within the U.S., and have an even greater effect on international seed commerce. These laws, policies and regulations control plant variety protection (PVP), variety registration, truthful labeling, phytosanitary certification, and seed certification. Science-based policies and regulations are vital to the harmonization of the protocols for import and export among countries to promote global seed trade especially when seeds of GE varieties are traded.

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Seed Purity

One of the principal seed quality concerns is genetic purity. To discuss genetic purity it is useful to divide crops into self-pollinated and outcrossing, because genetic purity

is linked to these modes of seed fertilization. Of course all combinations of intermediates and some unusual specific cases exist.

The production of self-pollinating crop seeds is procedurally less complex than for outcrossing crops. When a crop is exclusively self pollinated, there is no need for pollen control in seed production, because little pollen is released into the environment around the flower. Seed mixing therefore tends to dominate purity issues. Seed production of self-pollinated crops involves the production of so-called "breeder" seed by self-pollination in research nurseries, followed by repeated cycles of production of plots of seed with plots being checked for off-types resulting from mixtures. Self-pollinated crops tend to be well adapted to being inbred, and it is less likely that hybrids will show strong hybrid vigor. As a result self-pollinating crops are less likely to be used as commercial hybrids.

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In outcrossing crops, the chain of breeding and production steps includes opportunities for both pollen flow and mixing. Insect pollination of outcrossing crops is common, and when it occurs it adds complexity to pollen control. Adaptation of outcrossing crops to the hybrid condition makes advantages for hybrid varieties larger and more common than in self-pollinated crops.

Hybrid seed production involves well-defined production steps, and much of the structure of the commercial hybrid seed industry is influenced by the difficulty and cost of pollen control, which control and limit, but do not eliminate, outcrossing and mixtures. In the production field, the sequence of seed increases generally follows a pattern where:

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- Reproduction of core stocks of breeder seed occurs by hand-controlled crosses;
- Breeder seed is increased using hand crossing with intense observation
- Foundation seed is produced from breeder seed by open pollination in controlled isolation in small isolation fields allowing intense observation to continue; and
- Hybrid seed is produced by controlled crossing between male sterile or emasculated female plants and pollen shedding male plants.

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Crop improvement through traditional breeding

Delivery of genetic improvements is one of the most important roles of the seed industry. Conventional breeding, at its most basic, is a process in which differences in plants are observed in small plots, the differences are compared with the needs of the person doing the selection, and the plots that most fit the selector's needs are saved and perpetuated. Other variants are eliminated from the selected gene pool.

Breeder Seed

Breeder seed is usually produced in research nurseries where individual plants can be inspected and where pollination control can be maintained. Intensive observation of individual plants allows high levels of purity to be maintained. The use of breeder

seed keeps the seed system from accumulating unintended genes indefinitely over time. For some non-commercial and traditional varieties, there may not be an effective breeder's seed system.

Foundation and Certified Seed

Lower grades of seed are produced from breeder seed. Foundation seed is produced directly from breeder seed or other foundation seed under conditions designed to maintain specific genetic purity and identity of the seed. Certified seed is produced from breeder or foundation seed under conditions designed to maintain satisfactory genetic purity and identity. Certified seed is the highest grade of seed ordinarily planted by farmers.

For outcrossing crops, unintended crossing during open pollination is the major source of unintended off-type pollinations in the field. Isolation and borders effectively limit the level of unintended off-types in the final product. Their use is supported by decades of industrial experience. Experimentation has shown that pollination of outcrossing crops declines rapidly with increasing distance from the source. The vast majority of unintended outcrosses and of off-type plants come from adjacent fields. The advent of GE crop plants and DNA detection technology provides a sensitive means for monitoring gene transmission and has led to recent controversies over inadvertent trait occurrence which, in turn, has led to tightening of production standards and practices for all seed production systems. Regardless, for an open-pollinated crop, outcrossing at low frequencies will always be a possibility.

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The other possible source for unintended presence of off-type genotypes in seed of both outcrossing and self-pollinated crops is commingling. In outcrossing crops, industry experience leads to the conclusion that field contamination is a more frequent source of off-type genotypes than is mixing in planting, harvest, transport and processing. Careful application of the procedures for field production, transport, and processing of corn normally results in the production of both hybrid and self-pollinated seed that is 99 percent or more pure in many crops.

Seed Quality and Regulation

The primary quality characteristics are: physical purity, presence of other crop seeds and weed seeds, especially noxious weeds, germination, varietal purity, disease status, and moisture. Special germination tests for difficult conditions may add important information. However, the customer cannot readily observe the quality of the seed that is purchased. Seed laws provide assurance that the seed merchant is providing accurate information. Both Federal and State governments have seed laws, and the International Seed Testing Association provides global standards for germination testing for international commerce. All official seed certifying agencies belong to the Association of Official Seed Certifying Agencies (AOSCA), which establishes minimum standards for each crop. Individual certifying agencies may set higher standards than AOSCA, but not lower.

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Many countries have customer protection regulations that require varieties meet performance standards. Varieties that meet the standards are described and registered and are then eligible for certification. The U.S. has fewer regulations of this type. Certification by the Organization for Economic Cooperation and Development provides international mutual recognition of certification. In the U.S. both varieties and genes can be patented. Varieties can also be protected under plant breeder rights. The International Union for the Protection of New Varieties of Plants coordinates a simplified plant breeder's rights system with standardized claims.

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Technical Innovation and Seed

Breeding makes changes by combining great numbers of genes and sorting out useful changes by selecting among progeny. Genetic engineering selects a specific DNA sequence [?] initially and makes it work in a new place. The changes made by genetic engineering are minor in comparison to the amount of DNA in the plant, roughly 1 or 2 parts in 30,000 (although the changes in the composition of the plant can be larger). After a trait has been successfully incorporated, it can be added to other varieties of the same species by conventional breeding techniques.

The emergence of specialized food crops (*e.g.*, zero trans fat crops) and non-food varieties (pharmaceutical and industrial crops) increases the need to consider heightened standards for preventing pollen outflows and seed mixing in specialty seed production and brings up the special need to isolate non-food varieties from food varieties.

The Future of Plant Agricultural Biotechnology

The first decade of commercial plant agricultural biotechnology has seen remarkable growth from a mere 6 million acres in GE crops in 1996 to over 220 million acres in 21 countries in the 2005 growing season. The year 2005 also marked the point where cumulatively over one billion commercial acres of GE crops had been grown worldwide. This rapid growth has more recently slowed due to a number of issues of health and environmental concern and lack of an existing commercial marketing mechanism for plants with unique non-traditional traits and uses. This situation is further exacerbated by the fact that the vast majority of GE crop plants only carry two production-oriented traits, glyphosate herbicide resistance and insect resistance due to the production of various Bt toxins, and some regional markets for these traits may be reaching near saturation.

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Notwithstanding these concerns, a continued world-wide expansion in the use of GE plants is likely. This is exemplified by the activities of government regulators around the globe who are working to create regulatory regimes which allow GE plants and plant products to the market place while assuring the products' human and environmental safety. Concomitant with this governmental activity, the academic, non-profit, and corporate communities are working on creating new Identity Preservation, Quality and Trait Assurance Programs and market channeling

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mechanisms to allow GE plant products to flow in commerce without the inadvertent contamination of other products.

Four areas of GE crop trait development that may experience rapid development and significant world-wide commercialization in the next decade are: traits to address environmental stress on plants, plant derived biofuels, plant-produced proteins, and non-traditional industrial chemicals. Due to a decreasing supply of high-quality crop production land, drought, desertification, salinization, and global warming, there is a critical need for culturally acceptable food, fiber, and feed plants that can flourish under these environmental stresses. There is a broad scientific effort to identify and introduce traits that will allow plants to deal with environmental stress, especially for use in developing nations. With the rapid escalation in petroleum and fossil fuel prices, a renewed and significant scientific effort is being placed on renewable plant-derived fuels. The most interesting GE plant-derived biofuels from an intermediate-term development period of 10-15 years are ones that may be grown, extracted and utilized without further modification or with limited modification, much like existing bio-diesel products. Members of the plant kingdom are the synthetic giants of the planet and fully capable of producing large, complex proteins in significant quantities. The next ten years will see an increase in the development of GE plant-derived protein products such as vaccines, enzymes, biologicals, nutraceuticals, immunological proteins, and new custom-designed therapeutic proteins to treat cancer, birth defects and chronic ailments. The fourth area that seems destined for increased development and commercialization in the next decade is non-traditional industrial chemicals such as adhesives, improved or unique plant-derived fiber, lubricants, pharmaceuticals, nutritional supplements, and anti-disease food-derived health supplements.

B. Assessing effects on the environment.

Introduction

Chapter 4.2 and 4.3 below examine the way in which the implementation of current APHIS biotechnology regulations and possible changes might affect the quality of the environment.

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To better understand chapters 4.2 and 4.3, this section B gives a general introduction to how potential effects of GEOs on the environment are typically assessed, and section C gives some specific examples. These assessments are done on a case-by-case basis as the agency makes decisions. It is emphasized, however, that this EIS does not contain risk assessments of specific GEOs. The aim of the EIS is to assess potential effects of any changes in regulations.

Since the advent of biotechnological methods, a wealth of experience with risk assessment has been accumulated worldwide resulting in a robust international consensus on the general principles and methodology for risk assessments regarding GEOs.

Risk assessments for GE organisms:

- Are scientifically sound and transparent
- Are conducted on a case by case basis
- Are comparative – i.e. risks associated with GEOs are considered in the context of the risks posed by the (non-modified) recipients
- Are explicit in addressing uncertainties

The overall methodology for risk assessment, typically follows a number of steps:

1. *Hazard identification* - An identification of any novel genotypic and phenotypic characteristics associated with the GE organism that may have adverse effects in the potential receiving environment;

2. *Likelihood estimation* - An evaluation of the likelihood of these adverse effects being realized, taking into account the level and kind of exposure of the likely potential receiving environment to the GE organism;

3. *Consequence evaluation* - An evaluation of the consequences should these adverse effects be realized,

4. *Overall risk estimation* An estimation of the overall risk posed by the GE organism based on the evaluation of the likelihood and consequences of the identified adverse effects being realized;

5. *Risk management* – A recommendation as to whether or not the overall risks are acceptable or manageable, including, where necessary, identification of strategies to manage these risks, including monitoring.

In the process of conducting the steps outlined above, *risk assessment takes into account* the relevant characteristics of:

- The recipient organism, host organism or parental organisms.
- Inserted genes and sequences and related information about the donor(s) and the transformation system.
- The resulting GE organism
- Detection and identification of the GE organism
- The intended use (e.g. the scale of the activity - field trial or commercial use)
- The likely receiving environment.

The sections below discuss:

- General topics relevant to risks assessments

- Examples of assessing potential impacts of GE plants on the environment.

C. General topics relevant to risks assessments

Potential changes in weediness and invasiveness

Introduction

A key consideration in assessing the potential risks of GE plants is whether or not changes in weediness and/or invasiveness have occurred or are likely to occur as a result of the genetic modification. In this context "weedy" refers to plants that are growing where they are not wanted, typically in managed ecosystems. "Invasive" refers to species that tend to spread aggressively, typically of concern in unmanaged ecosystems.

Genetically Engineered Crop Plants and Weediness

Plants can evolve into weeds in three basic ways: 1) wild plants can, through unintentional selection in managed settings, gain the ability to invade managed habitats; 2) genes can be exchanged between cultivated crops and wild (free-living) relatives such that the wild relatives become weeds; and 3) weedy traits can be selected in crop plants such that the crop itself becomes a weed. It has been suggested that certain traits introduced through genetic engineering of crop plants might confer weedy characteristics to the plants, thereby creating new weeds in managed areas.

There are many common definitions of a weed, most not involving a specific biological feature, but rather how weeds are regarded by people (Booth, Murphy, and Swanton 2003; King 1966). The term *weed* is commonly defined as a plant growing where it is not wanted, due to interference with human activities or human welfare (Anderson 1977). Therefore, for the purposes of this DEIS, the weediness of GE plants and wild relatives with acquired GE traits in agro-ecosystems and other areas managed by humans will be discussed separately from the invasiveness of these plants into unmanaged ecosystems. There are also several definitions "invasive species" in the scientific literature (Richardson *et al.* 2000; Colautti and MacIsaac 2004; Pyšek *et al.* 2004). In this DEIS, an "invasive species" is defined as an introduced species that has a substantial or transformative impact in the unmanaged environment.

Crop plants can in some cases be considered weeds, and some crops have more weedy characteristics than others. However, using common definitions such as the one given above, it is not possible to know whether any crop, GE or not, will be considered a weed in some particular instance. APHIS approaches this dilemma by comparing the biology of the GE plant to its non-engineered counterpart, usually the same plant without the GE trait.[i] In this way conclusions can be drawn as to whether a GE plant is different from its non-engineered counterpart in its basic phenotypic characteristics and life history. It may be difficult to predict, based on phenotype, whether or not a

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GE crop would become a weed, but any significant change in environmental fitness might trigger the need for heightened scrutiny. However to date, the incorporation of GE traits in crop plants has not resulted in the creation of novel weeds.

Wild Relatives With Acquired Genetically Engineered Traits as Weeds

Many of the concepts and proposed mechanisms by which transgenes might increase the fitness and consequently the weediness of crops would also apply to their wild relatives (Jenczewski, Ronfort, and Chèvre 2003). An important difference, however, is that crop plants often are themselves not very weedy and have a low propensity for persistence when not managed in an agricultural context, whereas wild relatives by their nature may have weedy characteristics and an ability to persist in the environment. Hybridization of many species of traditional crop plants with their wild relatives is well established, and it is believed that the resultant gene flow may contribute to the evolution of weediness (Ellstrand, Prentice, and Hancock 1999).

In classic studies on the origin and evolution of weeds, Baker (1965, 1974) listed characteristics typically associated with weedy plants. The following is that list as adapted by Rissler and Mellon (1996):

1. Seeds germinate in many environments.
2. Seeds remain viable a long time.
3. Plants grow rapidly through vegetative phase to flowering.
4. Plants produce seeds continuously as long as growing season permits.
5. Flowers are self-compatible, but not obligatorily self-pollinated.
6. Pollen from flowers that are cross-pollinated is carried by nonspecialized flower visitors (usually insects) or by wind.
7. Plants produce large numbers of seeds in favorable environmental circumstances.
8. Plants produce seeds in a wide range of environmental circumstances.
9. Plants are adapted for both long-distance and short-distance dispersal.
10. If perennials, the plants have vigorous vegetative reproduction or regeneration from fragments.
11. If perennials, the plants are brittle near the soil line to prevent easy withdrawal from the soil.
12. Plants compete by special means, such as forming rosettes, choking growth, or producing toxic chemicals.

Keeler (Keeler 1985) reviewed the evolution of weeds from crop plants focusing on the characteristics described by Baker that may distinguish weeds. She listed characteristics associated with weediness in certain species and noted that many of these characteristics are known to be controlled by single genes. Her work showed uneven distribution of such characteristics among crops, weeds and other plants. While the most serious weeds had an average of ten or twelve weedy characteristics, other randomly surveyed plants averaged seven, and crop plants averaged only five. Thus it seemed unlikely that most crops would acquire enough of these characteristics to become weedy, even if the traits could be inherited as single loci. While noting several limitations to her study, she concluded that GE crops with "low weediness" and no weedy relatives are as likely to be the source of significant weed populations as are their non-engineered counterparts.

However, Williamson, studying invasiveness, concluded that neither those traits listed by Baker nor any others can accurately predict which plants could become weeds (Williamson 1993). He proposed rather that any such list of characteristics would have to be specific for groups of closely related species and noted that small genetic changes can sometimes spur large ecological changes. He concluded that GE plants have the potential to become weeds because the genetic changes may have unexpected environmental effects; however, he also concluded that the proportion of GE plants that will become weeds is very small (Williamson 1993). This conclusion was based on an earlier study of invasive species which had led to his formulation of the "10:10 rule." According to this model, approximately ten percent of introduced species will become established and truly naturalized, and ten percent of those will become pest species. Hence for introduced species, as a rough predictor, only one percent will become pest plants. This model could be applied equally well, and with equal validity, to traits that have been introduced using conventional breeding, such as pest resistance, or those which can be acquired naturally or introduced through conventional breeding efforts, such as herbicide resistance. Thus, if the model is correct, APHIS can rely on its experience with these types of traits in assessing the risks of GE traits.

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It has been suggested that the release of organisms with novel phenotypes bears similarities to the introduction of non-native species (Marvier 2001). However, the usefulness of such a model for evaluating the risks of GE crops has been questioned (Hancock and Hokanson 2001). The argument against exotic plant species as a useful analogy is that many of them are already good colonizers in their native habitats and carry an array of traits associated with weediness. Thus when they are introduced into a new environment where there are few or none of the factors which may have limited their numbers in their native environment, populations can sometimes explode to fill an ecological niche. There are examples of non-native plants, such as field bindweed, quackgrass and Canada thistle in heavily managed habitats, and kudzu, purple loosestrife and cheatgrass in less managed or unmanaged habitats, becoming weeds and causing significant impact to the environment and resulting in huge economic costs. This is in contrast the antecedents of GE crops which are generally poor colonizers outside of the agro-ecosystem designed for their cultivation. The traits selected for in domestication and the ongoing development of most crop plants

typically have made them less fit than their undomesticated counterparts in situations where the crop plants are not managed (Gepts 2004). Also, as discussed above, crop plants generally have relatively few weediness traits. Thus, there are multiple and complex constraints that coalesce to limit the weediness and invasiveness of typical agronomic crops, and in most cases, only one such constraint would be removed by the addition of a single gene through genetic engineering. Hancock and Hokanson (2001) concluded that the risk of deploying GE plants can be effectively determined by considering the phenotype conferred by the transgene and the invasiveness of the antecedent crop.

Crawley *et al.* (2001) performed one of the few studies of GE plants where potential weediness and invasiveness were measured directly. This was done by monitoring different habitats for ten years following the cultivation of four different GE crops, namely herbicide-tolerant sugar beet, maize and rape, and potato producing either a Bt toxin or pea leptin. In none of the cases did the researchers find the GE crops to have increased fitness over that of the conventional controls, and no unintended effects for the particular crops were identified. The most important factor to consider in interpreting these results, however, is that the particular traits studied would not be expected to increase fitness, except for the Bt toxin under certain conditions. The authors noted that the results might be different for other types of traits, such as drought tolerance or certain pest resistance genes that might confer a fitness advantage under field conditions.

A more thorough consideration of increased fitness may be warranted if there is reason to believe that the trait could increase fitness. Pest resistance genes have been the focus of much attention in this regard. Virginia Tech's Information Systems for Biotechnology, with support from USDA, sponsored a workshop on Ecological Effects of Pest Resistance Genes in Managed Ecosystems (Traynor and Westwood 1999). Many participants felt that the types of pest resistance traits being tested or released commercially were not fundamentally different from those bred in conventionally and as such, would present similar ecological risks. However some participants disagreed and contended that some transgenes could have a much greater impact on weediness. Most participants agreed that gene stacking (*i.e.*, insertion of multiple transgenes) to confer a broad spectrum of pest resistance would be less predictable with respect to ecological consequences than single-trait resistance.

Snow *et al.* (Snow *et al.* 2003) reported field studies of wild sunflower populations carrying a Bt *cry1Ac* transgene acquired via experimental hybridization to a non-commercial GE crop line and backcrossed into the wild-type plants. They observed decreased insect pest damage and increased fecundity (seed production) for the experimental unmanaged populations carrying the transgene versus those without it. This suggests the possibility that the transgene, by conferring increased fitness, could have an ecological impact on wild sunflower populations, either increasing the number of modified plants within a population, creating more such populations, and/or creating more extensive seed banks of such plants.

Genetically Engineered Crops and invasiveness

Introduction

In addition to the development of weediness, there is concern that GE crops may escape cultivation and persist to a significant degree in unmanaged ecosystems. It is also conceivable that a transgene from a GE crop could be transferred via cross-pollination to a wild relative of the crop, producing hybrid offspring containing the transgene that could themselves persist in the environment, or through introgression (by repeated natural backcrossing), result in the incorporation of the transgene in the genome of the wild relative. In the previous section, the primary focus is the potential for weediness of GE plants and their crossing with wild relatives in agro-ecosystems and other managed areas.

Gene Flow via outcrossing from Genetically Engineered Crops

For a GE crop to become established in an unmanaged habitat, seeds or other propagative structures must be transported from cultivated land to the habitat. This can occur via seed spillage during the movement of harvesting equipment between cultivated fields or during the transport of harvested seed, or seed can be moved by animal activity, wind, or water. Non-seed propagative plant material such as stolons or rhizomes could be moved via mowing equipment or by animal activity, wind, or water. However, the movement of seeds or other structures is independent of any transgenes in the crop genomes with which APHIS is familiar, so the escape of a GE crop is not inherently more likely than the escape of any other crop (Keeler 1985) unless there is a higher risk of deliberate human tampering or manipulation?. Although it is conceivable that transgenes increasing seed dispersal rates could be engineered into crop plants, it is highly unlikely that this would be done. A primary goal for crop variety development is the prevention of seed loss via seed dispersal mechanisms (Frary and Doğanlar 2003), since the seed or fruit is usually the plant part with the highest value. However, if seed dispersal genes were to be altered in a crop plant, the resulting GE plants would merit increased scrutiny to verify that gene flow was not increased in ways causing significant environmental effects.

Gene Flow via Hybridization with Wild Relative

The exchange of genes between crop plants and sexually-compatible wild plants has occurred ever since plants were first domesticated. It is possible that a transgene could be established in the genome of a wild relative of the GE crop as a result of an initial hybridization between a GE crop and its wild relative, followed by introgression of the transgene into the wild relative's genome (Gealy, Mitten, and Rutger 2003) and rapeseed (Halfhill *et al.* 2004; Légère 2005). For a transgene to become incorporated in a wild crop relative, crop pollen carrying the gene would first need to be carried via wind or insects or other pollinators to a plant present in the crop field as a weed or present in a nearby unmanaged habitat. Conversely, pollen from a wild crop relative in the unmanaged habitat could be carried via wind or insects or other pollinators to a crop plant growing in a cultivated field. Hybrid seed produced in the crop field would have to be harvested along with the crop and be spilled onto non-cultivated land as

discussed above, or dispersed by an animal, whereas the movement of crop pollen onto uncultivated land could result in the production of hybrids with no seed movement necessary.

Hybridization between a GE crop and a wild relative is dependent on several key factors: simultaneous flowering, sexual compatibility, and proximity sufficiently close to allow pollen movement between the two plants (Ellstrand, Prentice, and Hancock 1999). The first two factors are determined by the specific crop and wild relative, and can result in little or no outcrossing as in the case of wheat or frequent outcrossing as in the case of rice (Ellstrand, Prentice, and Hancock 1999). However, even when a crop can hybridize with a wild relative, the plants must be close enough together to allow pollination to occur. Again, this factor is different for every crop plant and depends on a variety of characteristics including whether the crop is pollinated by wind, insects, or other pollinators), to what extent the crop is self-pollinated, how long-lived the pollen is, and how the crop is cultivated. However, these parameters have been studied in depth in many agronomically important crops, and the Association of Official Seed Certifying Agencies has established standard growing conditions for crop seed production which result in very low levels of outcrossing (AOSCA 2003).

Invasiveness Potential

Only a small fraction of introduced species become successfully invasive (Ellstrand and Schierenbeck 2000), and there is no evidence that crops improved via genetic engineering are more likely to be invasive than crops improved via conventional plant breeding. The potential for a GE crop or a GE crop/wild-relative hybrid to become invasive depends first on the ability of the plant to become established in the environment, and second on the ability of the plant to successfully persist and thrive in the environment. Very few crops have been shown to be persistent and invasive outside of cultivation (Hancock and Hokanson 2001).[i] Initial establishment of a crop plant will depend on the crop's ability to survive without any human intervention. This includes successfully competing with other plants for nutrients, water, pollinators, and sunlight; surviving attacks by diseases, insects, and other herbivores; and producing sufficient progeny or propagative structures to maintain its presence in the environment (Brown and Mitchell 2001; Ellstrand and Schierenbeck 2000; Mitchell and Power 2003). The particular transgene introduced into the crop may also have some effect on the plant's survival. Because the weediness and invasiveness of a particular crop are known throughout the U.S. range where the crop is produced, the invasiveness of a GE crop possessing one or more transgenes can be estimated by evaluating the environmental fitness impacts of the individual introduced genes (Hancock and Hokanson 2001).

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In the case of GE crop/wild hybrids, establishment will depend on the fertility and overall vigor of the hybrid plants and their progeny (Vacher *et al.* 2004) as well as on the nature of the transgene. For example, naturally occurring hybrids between wheat and its distant relative jointed goatgrass are occasionally found, but the hybrids are usually self-sterile, due to a lack of proper chromosome pairing (Guadagnuolo, Savova-Bianchi, and Felber 2001; Morrison *et al.* 2002; Seefeldt *et al.* 1998). However,

it is also possible for interspecific and intergeneric crosses to exhibit enhanced fitness through heterosis, an increase of genetic diversity caused by hybridization (Vacher *et al.* 2004). A hybrid may possess a novel combination of traits making it more able to adapt to an ecological niche than either of the parents (Ellstrand and Schierenbeck 2000). In other words, each type of hybrid may exhibit unique and possibly unexpected characteristics. For example, hybrids between oilseed rape and wild radish are much more fit when wild radish is the maternal parent (Gueritaine *et al.* 2002), but even so fitness is very low and dependent on the particular environmental circumstances (Al Mouemar and Darmency 2004; (Gueritaine *et al.* 2002). Fortunately, years of cultivation and plant breeding have resulted in an extensive and growing body of information regarding the likelihood of hybridization between crops and their wild relatives and the fitness and fertility of these hybrids (Arriola 1997; Stewart, Halfhill, and Warwick 2003).

Persistence of Genetically engineered Plants in Natural Environments

The likelihood for a GE plant to persist in the environment depends primarily on the plant species and on the ecosystem in question, including competing species, diseases and herbivorous pests, and the physical environment. One factor that can be analyzed experimentally is whether the GE version of a crop plant has better field performance, *i.e.*, is more fit, or persists longer than a conventionally bred version of the crop. A recent study asked this question using GE and conventional varieties of corn, oilseed rape, sugar beet, and potato growing for ten years in twelve natural habitats in Britain. The transgenes studied were for herbicide resistance, Bt toxin, and pea lectin (Crawley *et al.* 2001). The study found that none of the GE crops were more fit or persisted longer in the environment than the conventional crop counterparts. Establishment of seedlings of the herbicide resistant corn and rapeseed was significantly lower than for the conventional versions of the crops, and survival of the GE potato lagged behind that of conventional potato (Crawley *et al.* 2001). However, it must be noted that none of the transgenes at issue in the study were intended to increase plant fitness in natural habitats.

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There is little evidence that beneficial agronomic traits moved into crops via conventional breeding have led to the development of invasiveness in crop plants (Duvick 1999). Similarly, it is unlikely that the mere entry of a GE crop plant into an unmanaged ecosystem will result in the permanent establishment of the plant in that ecosystem. To evaluate the environmental impact of a GE crop, researchers begin with the body of knowledge developed through years of cultivating the non-GE version of the crop, including any information about its weedy or feral tendencies. It is then possible to superimpose any effects of the transgene on the already familiar traits of the non-GE crop (Parker and Kareiva 1996). A systematic experimental approach, where the field performance of a GE crop and its non-GE counterpart are thoroughly compared in the greenhouse and in the field, should indicate whether the transgene has any unexpected effects on characteristics that could contribute to invasiveness (Wang *et al.* 2003).

The transgene may or may not confer any advantage to the GE plant, depending on the nature of the gene, the ecosystem, and the presence of human intervention or other factors that may provide sporadic or continuously-acting selection pressure such as herbicide application, insect or disease attack, or environmental stress. Without this pressure, the transgene's effects would not be expected to manifest themselves, and the GE plant would be expected to be phenotypically indistinguishable from its non-GE counterparts in that particular environment (Vacher *et al.* 2004). For example, a transgene conferring herbicide tolerance would not increase fitness for the recipient plant unless the natural habitat was regularly treated with the appropriate herbicide (Metz, Stiekema, and Nap 1998). Lacking such management, the GE plants would not be expected to be any more fit than conventional plants of the same species (Gueritaine *et al.* 2002). If, however, regular herbicide applications were used, the GE individuals could have a significant advantage over their non-GE counterparts. However, not every transgene would be expected to respond to selection pressure. For example, a crop containing a transgene that alters a food quality trait is unlikely to have any effect on plant fitness, since there is unlikely to be a corresponding selection pressure for the trait (Parker and Kareiva 1996).

If the transgene confers insect or disease resistance, the recipient plant may gain a fitness advantage, but only if the insect pest or disease organism ordinarily acts to control the normal distribution or role of that plant in that particular environment (Parker and Kareiva 1996). In that case, it would be expected for the GE plant, whether crop or wild-relative hybrid, to have a fitness advantage over other plants in the environment (Vacher *et al.* 2004). The greater the impact of the insect pest or disease on the vigor and reproductive potential of the plant population, the more likely it is for the GE plant to have a fitness advantage over non-GE counterparts (Parker and Kareiva 1996). Over the course of many generations, with continuous selection pressure from the insect pest or disease, the GE plant could become invasive, in the case of a crop plant, or could replace the non-GE population, in the case of a GE wild relative.

If the transgene is question confers an agronomic characteristic such as drought tolerance or increased photosynthetic efficiency, the recipient plant could become invasive or replace its non-GE counterpart, but only if a corresponding environmental stress consistently acts to control the plant populations in that ecosystem. APHIS anticipates that as plant genetic engineering technology advances, applicants will propose with greater frequency field trials of plants with traits such as increased photosynthetic efficiency and tolerance to various environmental stresses. Such traits, either singly or in combination, could contribute to the invasiveness of a GE crop or crop/wild-relative hybrid or introgressed progeny. However, given that most crop plants are not naturally invasive, and given that most cultivated crops possess several domestication traits (Frary and Doğanlar 2003; Gepts 2002), such as dwarfing, non-shattering seed heads, and larger fruits, which usually are disadvantageous in unmanaged ecosystems, it has been proposed that a single plant would have to possess several transgenes conferring improved fitness characteristics before that plant would become invasive (Hancock and Hokanson 2001). The insertion of multiple genes affecting fitness in a single plant, so-called "stacking," is more likely as genetic

engineering technology advances, and plants with such gene stacks would receive additional scrutiny to determine their potential for weediness or invasiveness.

A single instance of gene flow to an unmanaged ecosystem or a transgene into a wild relative may not result in the development of an invasive population of GE plants (Ellstrand and Schierenbeck 2000; Siemann and Rogers 2001). Even if the initial introduction succeeds, a lag time of several generations may be necessary during which time the introduced species may undergo genetic adaptation, ultimately making the plants better able to survive in their new environment than other species or non-GE populations of the same species (Siemann and Rogers 2001; Willis, Memmott, and Forrester 2000). Multiple introductions via repeated instances of gene flow may be necessary before a potentially invasive species can become established (Ellstrand and Schierenbeck 2000). Delays in the development of invasiveness may also depend on the crop or crop relative in question (Ellstrand and Schierenbeck 2000). For example, trees, shrubs, and other perennial plants with long reproductive cycles may take decades or longer to develop invasiveness, assuming no human intervention, while annual plants or short-lived perennials may become invasive only a few years after an inadvertent instance of gene flow (Ellstrand and Schierenbeck 2000). Minimizing the size and frequency of transgene flow to unmanaged ecosystems is therefore the most direct way to minimize the development of invasiveness both in the short term and over long lag periods.

Potential Effects of Genetically Engineered Crops on Soil

Introduction

Plants and the soil and water environments in which they reside are inarguably intertwined. The plant-soil matrix is a complex milieu of interactions between abiotic and biotic components. These interactions can be considered on a small scale: seeds germinate within the soil, and the resulting seedlings and plants interact with the soil, soil microorganisms, and soil water resources to obtain nutrients for vital functions, such as growth and reproduction. The soil is then enriched through plant decomposition by scavengers and other soil-dwelling organisms. Interactions also exist on a large scale: traditional agricultural practices, including tillage, irrigation and herbicide and pesticide use have significant and predominately detrimental environmental impacts. Both scales of interactions should be considered when evaluating potential effects of GE plants on soil and water environments.

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The second edition of *Soil Taxonomy* (NRCS 1999) defines soil as the solids (minerals and organic matter), liquid and gases that occur on the land surface and are characterized by the presence of horizons, or layers, that are distinguishable from the primordial material as a result of additions and losses, transfers and transformations of energy and matter, and the ability to support rooted plants in a natural environment. Soil is a much more dynamic environment than indicated by this definition. It may be the most diverse and populous habitat on the earth; a single gram of soil contains millions of individuals, with several thousand species of bacteria

alone (Torsvik *et al.* 1994). These organisms function in decomposition leading to soil formation, aeration, and nitrogen fixation; and aid in root function (Giller *et al.* 1997). The immense number of organisms and the complicated and poorly understood relationships between these organisms, the environment, and plants complicate the analysis of the potential effects of introducing GE plants and other organisms. This section will detail factors that should be considered when evaluating the potential effects of particular genes on the soil and groundwater environments.

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Accumulation and Persistence

Some traits added to plants via genetic engineering involve the production of one or more substances that the plant would normally not produce. Novel chemical substances produced by GE plants may enter the environment from leaf shedding, root exudates and decomposition (Donegan *et al.* 1997). If these substances do not dissipate at a rate at least equal to the rate of the products entry to the soil system, bioaccumulation and biomagnification may result. Bioaccumulation is the increase in concentrations of chemicals in biological systems over time as compared to the chemical's concentration in the environment. This occurs when a chemical becomes more and more concentrated as it moves up the food chain.

Additionally, herbivorous animals that feed on these plants and subsequently die, either due to natural causes or due to consuming a pesticidal substance such as a Bt toxin, may also add these novel chemical substances to the soil environment. The substances in the plant may not necessarily be in the same form in the insect: for example, the Bt protoxins made by insect-resistant GE plants are modified in the guts of susceptible insects (Höfte and Whiteley 1989).

Bt crops offer the best studied example to date of accumulation, persistence and residual toxicity within the soil (Höfte and Whiteley 1989; Saxena *et al.* 1999). Crop residues from plants genetically modified to express *cry* genes from Bt have been shown to decompose more slowly in soil, possibly due to a higher lignin content compared to their non-GE counterparts (Stotzky 2004). The binding of chemical substances by soil particles is also a factor. The Bt toxins adsorb and bind rapidly (< 30 minutes) to clays and organic matter within the soil, allowing the Bt toxins to persist and also to remain toxic to insect larvae (Stotzky 2000, 2002). In non-flooded soils, the Bt toxins released from root exudates and biomass of Bt corn were bound to soil particles and remained larvicidal for at least 180 days (Tapp and Stotzky 1998) and toxins remained detectable in the biomass of Bt corn three years after incorporation into soil (Saxena and Stotzky 2003). The Bt endotoxin associated with Bt crops appears to degrade rapidly in water, with a half-life between four to ten days, depending on the presence of microorganisms (Douville *et al.* 2005). This result suggests that the Bt accumulation and persistence in water bodies adjacent to land planted with Bt-engineered crops is not a significant concern but more studies need to be done to further evaluate accumulation in soil and sediments in water bodies. Obviously, soil and natural bodies of water are not sterile environments and there are a number of abiotic and biotic factors that will affect accumulation and persistence, such as soil type, aeration, water movement, and soil biota activity.

GE plants may add more than novel chemical substances into the soil. DNA is also released into the soil as organisms decompose. Clay minerals bind DNA molecules and can prevent vertical movement of DNA within the soil and delay DNA degradation by protecting free DNA from degradation (Greaves and Wilson 1970). The presence of DNase in the soil can also affect the accumulation and persistence of DNA in soil (Blum, Lorenz, and Wackernagel 1997; and Dunfield and Germida 2004). Studies so far have demonstrated persistence of GE DNA from several days (Widmer *et al.* 1997) to at least two years (Gebhard and Smalla 1999) in the soil. As explained in the introduction to risk assessment, these data should be compared with data from the non modified host plant.

Biomagnification and bioaccumulation of products released by GE organisms should be considered and compared with similar, potentially cumulative effects from traditional crops. APHIS is unaware of any studies or data demonstrating bioaccumulation or biomagnification as a result of planting GE crops. In cases where biomagnification and bioaccumulation are likely, expression of transgenes in GE organisms can be manipulated in ways that may mitigate any biomagnification and bioaccumulation. New techniques that limit the expression to specific plant parts rather than the whole plant or prevent expression except in the presence of specific environmental stimuli can significantly limit how much of these products enter the soil.

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Water and movement away from the site

Water can move products from GE organisms away from the immediate site of entry into the soil environment. Precipitation, run-off and irrigation will provide transport for these products through the soil column. These products can enter groundwater, where they may be transported to larger underground reservoirs used for drinking water or to neighboring streams via underground conduits. Soil factors affecting how water will move GE products include, but are not limited to, soil type, texture, permeability, and the depth of the water table.

Besides interactions on the small scale, such as increasing the number of organisms that are exposed as products of GE crops are carried by water out of the rhizosphere, there are large scale impacts that should be considered. For example, currently available GE glufosinate and glyphosate-tolerant crops allow farmers to replace triazine and chloroacetanilide herbicides with less environmentally damaging glufosinate- and glyphosate-based herbicides. These herbicides are readily degraded by soil-dwelling bacteria and fungi and therefore have a lower potential to reach water resources and also exhibit reduced human toxicity. Combined, this can lead to reduced risk in drinking water contamination and improvements in the water quality of vulnerable watersheds (Wauchope *et al. 2001*).

Interactions with Soil Organisms

Soil- and plant-associated microbial communities and their interactions are not completely understood, and modern agricultural cropping systems can affect these

interactions (Dunfield and Germida *2004*). Soil communities are incredibly diverse and include beetles, springtails, mites, worms, spiders, nematodes, fungi, bacteria and other organisms. Only a small portion of species within the classes of soil organisms have been described. For example, the number of identified soil-dwelling fungi is 18,000-35,000, while the projected number is greater than 100,000 (Hawksworth 1991). These organisms improve the entry and storage of water, soil mixing resistance to erosion, plant nutrition, and breakdown of organic matter (Giller *et al.* 1997).

Plants develop mutually beneficial relationships with soil organisms in the rhizosphere, and these relationships are heavily affected by metabolites released by plants into the soil. Root exudates have been proposed to be the most important factor in the development of the rhizosphere microflora (Lynch and Whipps 1991). Genetic modifications in crops may alter these root exudates and affect the associated microflora. The incorporation of crop residues into the soil may affect organisms that are not directly associated in a mutualistic relationship with the plant but serve indirectly beneficial functions, *i.e.*, scavengers and decomposers, or which protect plants from detrimental microorganisms (Bashan and Holguin 1998). The degree to which these effects result in measurable changes to soil ecosystems may be difficult to assess. Additionally, the origin of the transgene should be considered. For example, soil-dwelling organisms are likely to have had previous exposure to proteins from *Bacillus thuringiensis*, a naturally-occurring soil bacterium, which potentially mitigates the impact of Bt crops on the soil ecosystem.

It is difficult to summarize the results of studies evaluating the impact of GE plants on soil-dwelling organisms since these impacts are tightly associated with the crop, the engineered trait, and the environment (Griffiths, Geoghegan, and Robertson 2000). The specific soil-associated community is also difficult to characterize and make generalizations about. The microbial community found at one field site may be entirely different from that at another field site (Dunfield and Germida 2001; Blackwood and Buyer 2004; Muchaonyerwa *et al.* 2004; Wu *et al.* 2004; Castaldini *et al.* 2005; Saxena and Stotzky 2001; Donegan *et al.* 1996; Duan *et al.* 2004; and Milling *et al.* 2004). The results of studies evaluating soil effects from GE crops thus far suggest that the impact on the microbial and invertebrate communities of the GE crops as compared to conventional crops was minor when compared to other factors such as seasonal and environmental effects (Milling *et al.* 2004) and this appears to be the case for other soil-bound organisms.

Interactions between the plant and soil organisms may also change due to unintended effects on plant traits and defense abilities, leading to reduced plant fitness. For example, studies have suggested that the application of glyphosate increased populations of various fungi in the soil (Brammall and Higgins 1987), and that glyphosate applications also suppressed natural plant defenses and enhanced disease susceptibility in crops and weeds (Wrather, Stienstra, and Koenning 2001; and Myers *et al.* 1999). However, further studies have been unable to link glyphosate resistance with disease susceptibility (Sanogo, Yang, and Schrem 2000). Certainly non-GE crops are not immune to changes in the soil community and disease prevalence due to

unpredictable environmental events, and it is not clear that genetic modification necessarily makes plants more susceptible to deleterious soil organisms.

Large scale interactions, resulting from standard agricultural practices, could influence the incorporation of products from GE plants into the soil and the exposure of soil organisms. For example, the tillage system employed by the grower could influence the amount of interaction that occurs between the novel proteins and the microbial community (Angle 1994). No tillage practice preserves fertile soil and reduces the amount of sediment that enters streams adjacent to the farmland, a major pollutant in streams. A report released by the Conservation Technology Information Center identified the largest growth in no tillage practice occurring where herbicide-tolerance technology is utilized, achieving weed control through the application of herbicides without damaging the crop (Fawcett and Towery 2002). No-till systems keep the GE plant material at the surface, limiting contact with soil organisms to those at the surface. On the other hand, with conventional tillage the GE plant material will be incorporated into the soil, potentially diluting the products, but also increasing the number of organisms exposed.

Horizontal Gene Transfer

Horizontal gene transfer (HGT) is the natural transfer of genetic material from one organism (the donor) to another organism (the recipient) that is not sexually compatible with the donor (Gay 2001). Though HGT is thought to be extremely rare, the transfer of chromosomal DNA between bacterial species is considered to represent a significant mechanism for their evolution (Nielsen, Bones, Smalla, van Elsas 1998). Plant DNA can persist in the soil (Gebhard and Smalla 1999), triggering concern that transgenes from GE plants may spread horizontally to bacteria. HGT is relevant to the assessment of risks to the soil environment because of the nature of some of the genes used to develop GE organisms. For example, there is concern that bacterial antibiotic resistance markers, used in the process of selecting GE cells, encode resistance to clinically useful antibiotics (Metz and Nap 1997). Theoretically, resistance could spread to recipient micro-organisms in soil or in the digestive tracts of humans and livestock (Dröge, Pühler, and Selbitschka 1998). Although there is general consensus that such HGT from plant tissue to micro-organisms would happen at extremely low frequencies and has not been observed under natural (i.e. in the absence of heavy selection pressure), this issue is considered on a case by case basis, whereby the nature of the antibiotic resistance and its occurrence in the environment are taken into account.

The plant surface and the immediately surrounding environment have been shown to be active areas of HGT, likely due to nutrient availability, high humidity and proximity of colonizing bacteria on the limited growth surface (Björklöf *et al.* 1995). Studies have been made of HGT in terrestrial and aquatic habitats by viruses (Kidambi, Ripp, and Miller 1994) and by natural transformation, the uptake of free DNA into competent bacteria (Bertolla *et al.* 1999). Particular elements within the rhizosphere and certain plant exudates can affect transformation frequencies (Nielsen and van Elsas 2001). However the rate of natural transformation in soil is extremely low; in sterile soil,

natural transformation has been shown to occur at rates below 10^{-7} transformants per recipient (Nielsen, van Elsas, and Smalla 2000) and is estimated to be as low as 10^{-10} and 10^{-11} transformants per recipient in nonsterile soil (Smalla, Borin, Heuer, Gebhard, van Elsas, and Nielsen 2000). There are a number of mitigating factors that suggest that HGT is not a significant concern when examining the potential effects of GE plants on soil-dwelling organisms.

First, several events would have to occur successfully for HGT to occur. In order for natural transformation to occur in the soil environment, free DNA needs to be available. The persistence of DNA in the soil was discussed above. There must also be bacteria in close proximity to the free DNA which are capable of taking up the DNA. Some bacteria, called "naturally competent," have evolved the ability to transport free DNA from outside the bacterial cell into the cytoplasm; however, not all bacterial species are competent, and not all competent bacteria are competent all of the time. The transgene must then be incorporated and maintained by the recipient organism (Gebhard and Smalla 1998). Maintenance of the transgene requires that the alteration is non-detrimental to the fitness of the organism and therefore will not be negatively affected by selection. Transfer of a plant gene to a bacterium does not equal functionality in the bacterium; regulatory sequences (promoters, enhancers) may not work and introns may not be recognized in the recipient (Conner, Glare, and Nap 2003). As the risk of HGT from GE plants is considered, it is necessary to bear in mind that the transgene portion of a GE plant's DNA is a very small part of the total DNA. Therefore the likelihood that a piece of native DNA undergoes HGT is significantly greater than a given piece of transgene DNA (Conner, Glare, and Nap 2003).

Second, the antibiotic resistance genes commonly used have limited use in treating infections. The most popular selectable marker gene, *nptII*, is effective against kanamycin which? has limited therapeutic value as an antibiotic and hygromycin, another antibiotic used in molecular biology, is too toxic an antibiotic for therapeutic use (Conner, Glare, and Nap *2003*). New genetic transformation methods rely less on antibiotic resistance marker genes, either by using other types of markers or by eliminating marker genes entirely. Examples? As a result, the potential HGT of antibiotic resistance genes is likely to continue to diminish in significance.

Third, although HGT between bacteria has been extensively demonstrated in natural systems (Nielsen *et al.* 1998), no evidence has been found of plant DNA moving to native soil microorganisms (Badosa, Moreno, and Montesinos *2004*; Heinemann and Traavik *2004*; and Maynard Smith, Dowson, and Spratt 1991). The lack of information on the abundance of naturally competent bacteria in the environment, frequencies of transformation processes and environmental factors triggering these processes impairs predictions of horizontal gene transfer from plants to bacteria (Gebhard and Smalla 1999). However, the probability of HGT is extremely low and the evidence thus far indicates that HGT does not pose a significant risk for the transfer of traits from GEOs. For example, Bt crops are currently grown in many countries, in many diverse environments, covering millions of acres, and there is still no evidence that Bt genes have moved, via HGT, to new organisms.

Conclusion

Comparing the risks of GE crops and their non-GE counterparts for the management of physical, chemical and biological aspects of soil ecology are important for sustainability of agroecosystems. The dynamic nature of the soil and its components and the lack of comprehensive research on soil interactions make it difficult to draw any definitive conclusions about the effects of products derived from GE crops entering the system. Traditional agriculture and its associated activities have significant, often detrimental, impacts on soil's abiotic and biotic components, and it is thus far unclear that introducing GE materials increase or possibly alleviate these detrimental impacts.

Genetically engineered Crops and potential Impacts on Human Health

Introduction

The term "genetic modification" can be used to describe various methods of altering a plant's genetic makeup resulting in a plant that expresses different traits or characteristics than its parent (NRC 2004). In fact, crop plants have been genetically modified throughout history to produce plants with desired traits (Day 1996; Kessler *et al.* 1992; NRC 2004). Crop developers introduce many new crop varieties intended for food and feed into the market every year that may have characteristics such as insect resistance, higher yield or improved nutritional attributes (Fuchs and Astwood 1996; Kessler *et al.* 1992; Metcalfe, Astwood *et al.* 1996). Each method of genetic modification, including the two most common methods currently employed, traditional breeding and genetic engineering, requires some level of human intervention (NRC 2004). Traditional breeding techniques have been employed for centuries, while genetic engineering only became a prominent method of plant genetic modification late in the 20th century (Kessler *et al.* 1992; NRC 2004).

All methods of genetic modification have the potential to alter a plant's genetic makeup resulting in changes in characteristics such as plant color, flavor, nutrient content, disease resistance and environmental stress tolerance (Kessler *et al.* 1992). While many of the potential changes in the plant's genetic makeup can be considered intentional and/or improvements, a number of unintentional changes can also occur (Fuchs and Astwood 1996; Kessler *et al.* 1992; Metcalfe, Astwood *et al.* 1996). These unintentional changes can have positive as well as negative effects on human health. In most instances, phenotypic changes such as plant color, growth, and production can be identified during the plant breeding and selection process, while unintentional changes such as changed levels of allergens, toxicants, and antinutrients are less apparent without additional analysis.

Traditionally, new varieties of whole foods have not been subjected to extensive chemical, toxicological or nutritional evaluation prior to marketing (CODEX 2003). The regulatory agencies that oversee the safety of GE plants do not evaluate new, traditionally-bred crop varieties for human or animal health safety (NRC 2004). The

exception is in cases where the food may represent a substantial part of the diet of a specific group (*e.g.*, infants), where some evaluation is performed (CODEX 2003). While most crops naturally produce some level of allergens, toxins, and antinutrients, standard plant breeding practices allow for monitoring the levels of potentially hazardous substances (NRC 2004). The history of crop development has shown that except in very rare instances, these standard crop development techniques include steps that make it possible to identify potential hazards in crop lines developed for commercialization (Kessler *et al.* 1992; NRC 2004; Pastorello *et al.* 1998).

Unlike traditional breeding, genetic engineering gives developers the ability to move genes from organisms that may not have a history of prior exposure in the diet of humans and animals into crop plants. Given this potential, there is some concern about the possibility of moving a gene whose gene product may be allergenic or toxic (NRC 2004). While the ability to move potentially toxic or allergenic genes is not unique to genetic engineering, biotechnology techniques can enable the movement of genes between a broader group of species (*e.g.*, plants, insects and microorganisms).

In the U.S., each GE crop undergoes an extensive food safety and environmental safety review prior to commercialization, using the most current and available scientific data and information (Fuchs and Astwood 1996; Kessler *et al.* 1992; Metcalfe, Astwood *et al.* 1996). To address the issue of GE crop safety, the U.S. government is guided by a Coordinated Framework (51 FR 23302, 1986) in which three different agencies, the Animal Plant Health Inspection Service (APHIS), Environmental Protection Agency (EPA) and Food and Drug Administration (FDA) each have a role. While the regulatory authority for APHIS focuses on plant health, both the EPA and FDA have responsibility to ensure the safety of human food and animal feed derived from GE foods. The EPA is responsible for the human/animal health and environmental safety of any pesticidal substance produced in these crops. The FDA is responsible for the safety of the whole food product and seeks to ensure that GE crops are as safe as their traditional counterparts.

To meet their responsibility to insure the safety of the food products, EPA and FDA use similar approaches to evaluate the potential allergenicity or toxicity of a GE crop. In addition, FDA uses a multidisciplinary approach to assess changes in nutritional quality of these modified food products. Both EPA and FDA have worked with members of industry and academia to develop guidance and methods for safety assessment, based on sound scientific principles, that help predict potential adverse effects of GE crop plants (EPA 2000, 2000; FDA 2002).

This section will briefly address some of the prominent issues associated with the safety of GE crops used as human food and animal feed. The issues addressed in this section, from potential allergenicity and toxicity to nutritional quality, are more thoroughly reviewed in numerous publications and guidance documents that are publicly available. Organizations such as the National Research Council (NRC), Codex Alimentarius Commission (CODEX), and Food and Agriculture Organization/World Health Organization (FAO/WHO) have developed guidance for assessing the safety of GE foods (CODEX 2003, 2003; FAO/WHO 2000, 2001; NRC

2004). In addition, numerous other publications are available from researchers and others, that address the issue of GE food safety (Astwood and Fuchs 1996; Fuchs and Astwood 1996; Fuchs and Goodman 1998; Lehrer, Horner, and Reese 1996; Mendelsohn *et al.* 2003; Metcalfe, Fuchs *et al.* 1996; Metcalfe 2003).

Potential Allergenicity of Proteins Expressed by Genetically engineered Plants

Background

Proteins are a common component of the human and animal diet. Agricultural crops that are common components of our food supply contain thousands of different proteins. Some of these proteins have been well-characterized, while others have undergone little or no scrutiny. However, humans have been exposed to a huge variety of foods for thousands of years, and consumption of the vast majority of proteins found in foods presents little or no risk of adverse reactions (Metcalfe, Fuchs *et al.* 1996).

However, there are a number of proteins that have been identified and/or characterized for their ability to induce allergic reactions, and the majority of allergic reactions involve IgE-mediated immune responses (Fuchs and Astwood 1996). While more than 160 foods and food-related substances have been associated with allergic reactions, a small group of foods, including eggs, milk, fish, crustaceans, mollusks, peanut, soybean, wheat and tree nuts are responsible for greater than 90 percent of allergic reactions in adults (Fuchs and Astwood 1996). Overall, only a small portion of the adult population - less than 2 percent - is considered to have food allergies (Kimber, Lumley, and Metcalfe 1997). While the severity of food allergy can vary significantly, when an allergy to a food item is confirmed, individuals are usually allergic to only a few specific proteins in one or two specific foods (Metcalfe, Fuchs *et al.* 1996). The percentage of children with food allergies is slightly higher than adults, with children being most frequently allergic to milk and eggs. Most food allergies in children disappear by adulthood (Kimber, Lumley, and Metcalfe 1997; Metcalfe, Fuchs *et al.* 1996).

Scientific methods used to assess allergenic potential continue to evolve. However, there is no one test that can be used to assess the allergenic potential of a protein. Because of this, the safety of a protein is typically assessed based upon the source of a protein and its structural characteristics, compared to the structural characteristics of known allergens. These structural characteristics include the stability of the protein to enzymatic degradation; stability to heat and/or acid and enzymatic treatment; and amino acid sequence similarity of the protein to known allergens (CODEX 2003). The use of animal models for allergenicity determination has been an area of significant focus over the past decade, but no adequate model has been developed.

Approaches to Allergenicity Assessment

In addition to several reviews that focus on engineered foods and food allergy published by members of industry and academia (Astwood and Fuchs 1996; Astwood, Leach, and Fuchs 1996; Gendel 1998, 1998; Lehrer, Horner, and Reese 1996; Sampson and Metcalfe 1992), in 1996, the International Food Biotechnology Council (IFBC) in collaboration with the Allergy and Immunology Institute (AII) of the International Life Sciences Institute (ILSI) published a peer-reviewed report that proposed an approach to evaluating allergenicity of proteins in bioengineered foods (Metcalfe, Fuchs *et al.* 1996). The approach taken by the scientists participating in this effort used a decision tree for the assessment of potential allergenicity.

In 2000, the Food and Agricultural Organization of the United Nations (FAO) and World Health Organization (WHO) convened a Joint Expert Consultation on Foods Derived from Biotechnology and published a report on the safety aspects of bioengineered crops that included a discussion of allergenicity (FAO/WHO 2000). The 2000 FAO/WHO report supported the approach to allergenicity assessment described in the 1996 ILSI/IFBC report and adopted a slightly modified version of the 1996 ILSI/IFBC decision tree.

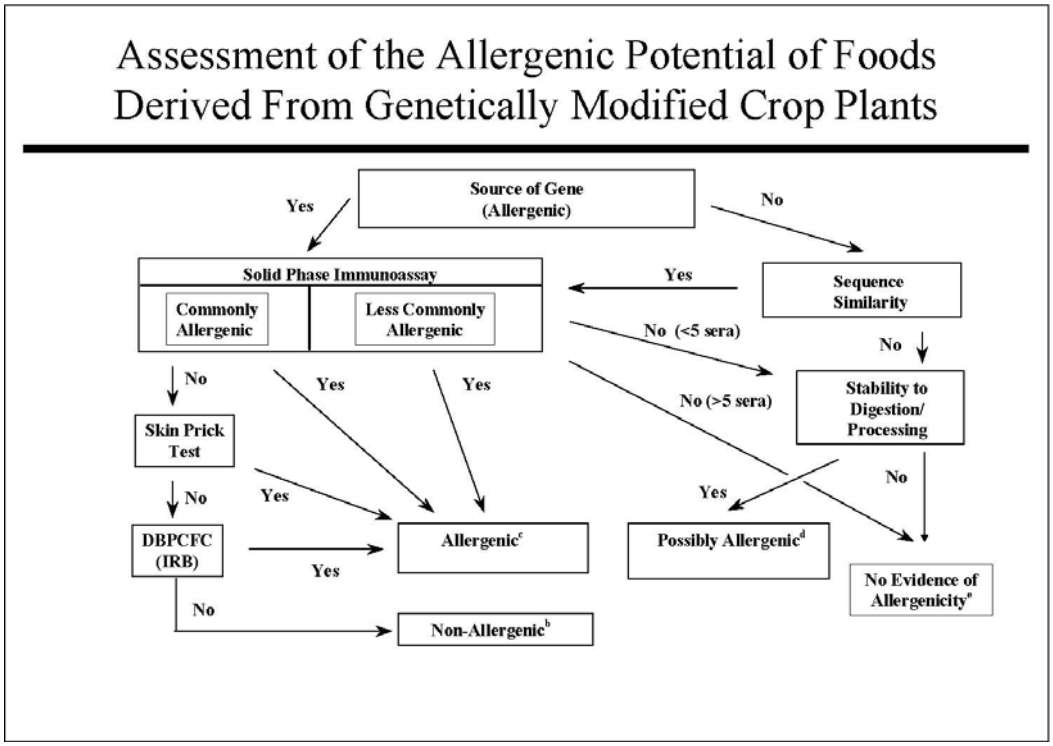

Figure Error! Main Document Only. Decision tree for the assessment of the allergenic potential of foods derived from GE crop plants Source: Joint FAO/WHO Expert Consultation on Allergenicity of Foods Derived from Biotechnology, 2000.[i]

In January 2001, the Joint FAO/WHO Consultation on Foods Derived from Biotechnology was convened specifically to provide scientific advice in relation to the assessment of allergenicity of bioengineered foods (FAO/WHO 2001). The consultation focused on several items, including the general issues of allergenicity of bioengineered foods, the reevaluation of the decision tree for the assessment of allergenicity of bioengineered foods developed in the 2000 FAO/WHO report (FAO/WHO 2000), and the development of standardized procedures for the use of the decision tree. After consideration of the current status of scientific information and extensive discussion, these scientists developed a new decision tree.[i] This decision tree builds upon previous approaches to examining allergenicity but also includes several additional strategies. These strategies are: targeted serum screening of proteins from sources with no known history of allergenicity; targeted serum screening of protein from sources with no sequence homology to known allergens; the use of animal models; and the elimination of human testing.

In 2003, the Codex Ad Hoc Intergovernmental Task Force on Foods Derived from Biotechnology finalized documents describing "Principles for the Risk Analysis of Foods Derived from Modern Biotechnology" and "Guidelines for the Conduct of Food Safety Assessment of Foods Derived from Recombinant-DNA Plants." This latter document included an Annex on the Assessment of Possible Allergenicity (Codex 2003). In the allergenicity annex, the Task Force acknowledges that there is no definitive test that can be relied upon to predict allergic response in humans to a protein new to the food supply, and recommended a “weight of evidence” approach.

This approach begins with an initial data set consisting of the source of the newly introduced protein; any significant similarity between the amino acid sequence of the protein and that of known allergens; and its structural properties including but not limited to its susceptibility to acidic or enzymatic degradation and its stability to heat. Specific serum screening is recommended for proteins that originate from a source known to be allergenic or have sequence homology with a known allergen. These data, as well as additional factors including predicted levels of exposure and the effects of relevant food processing, contribute to an overall conclusion regarding human health risk. Targeted serum screening and animal models are also discussed in the allergenicity annex. However, neither is recommended for inclusion in an allergenicity assessment until fully developed and validated as predictive for human allergic response.

Digestibility and Stability

Stability to digestion, in addition to stability to heat and other food processing conditions are among the characteristic properties that were first considered to be common among food allergens. Simulated gastric and simulated intestinal fluids are used as *in vitro* models for assessing the digestibility of proteins (CODEX 2003; FAO/WHO 2000, 2001; Metcalfe, Astwood *et al.* 1996). Proteins rapidly broken down into single amino acids and peptides smaller than 3.5 kDa are considered to be readily digestible (FAO/WHO 2001).

The rationale for the use of digestion stability is based on initial information that food allergens were not readily digestible (Taylor 1987). However, more recent studies comparing the digestion stabilities of food allergens and other proteins have shown that some allergens are as digestible as non-allergens and allergens are not necessarily more stable than non-allergenic proteins. In addition, it has been shown that major food allergens are not necessarily more stable to digestion than minor allergens (Astwood, Leach, and Fuchs 1996).

Another rationale to support the use of digestion stability as a criterion for allergenicity assessment is that stability to the conditions of the human digestive system is a key requirement to sensitize for food protein allergenicity. One study suggests that an allergenic protein must be intact to sensitize a person (Hanson *et al.* 1993). However, degradation of food allergens may not prevent elicitation of allergic reactions in individuals that have previously been sensitized (Maynard, Jost, and Wal 1997; Nilsson *et al.* 1999). While there are few data in the literature to support the

assertion that stability in human digestive tract is required for food allergenicity, a consensus exists among scientists that the likelihood of small peptides to sensitize an individual is low.

Because allergic reactions involve the interaction between an entire protein molecule or specific three-dimensional parts (called "epitopes") of the protein with various components of the immune system, the more easily a protein is degraded by digestion, the less likely the three-dimensional structure necessary for the interaction will be retained. In this respect, stability to digestion may be one useful parameter to measure the likelihood of a protein to interact with the immune system when ingested and therefore its ability to sensitize. However, it may not be useful to prevent entry into the marketplace of those proteins that cause human sensitization through routes other than ingestion (*e.g.*, respiration or skin contact) or proteins that cross-react with existing proteins that have already resulted in the sensitization of some individuals. In these cases, rapidly digested proteins, or previously degraded proteins, may still elicit allergic responses in individuals who have already been sensitized (Nilsson *et al.* 1999).

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Because the correlation between the allergenicity and the digestibility of a protein has not yet been established definitively, it is difficult to relate, with confidence, the outcome of the stability/digestibility studies to the allergenic potential of a protein. However, a protein that degrades rapidly is less likely to interact with the immune system and thus less likely to be allergenic. A protein that is resistant to digestion is more likely to interact with the immune system but is not necessarily an allergen.

Sequence Homology and Similarity

Amino acid sequence analysis is an important consideration for identifying similarity between a new protein and a known allergenic protein (CODEX 2003; FAO/WHO 2000, 2001; Metcalfe, Astwood *et al.* 1996). However, there are no standardized rules that can be applied to how sequence comparisons are performed, nor are all allergenic proteins included in databases. Searches are also limited to examining linear sequence homology with known allergens that have already been sequenced. Further, the usefulness of sequence homology comparison is limited by a number of other factors, including the algorithms and strategies used for the search, and the criteria for evaluating the search, in addition to the composition, completeness, and design of the database. When sequence homology to a known allergen is detected, additional evaluation of that protein should be performed, including immunoassay using sera from individuals sensitive to the allergen in question.

Animal Models

Because no one characteristic property of food allergens is predictive of potential allergenicity of novel proteins, there has recently been much emphasis on the development of animal models. Several animal models for food allergy are currently under development, employing Brown Norway rats (Penninks and Knippels 2001); Balb/c mice (Dearman and Kimber 2001); C3H/HeJ mice (Li *et al.* 2001); and Beagle

dogs (Ermel *et al.* 1997). These models differ with respect to route of sensitization, route of challenge, symptoms exhibited, and responses evaluated.

In the context of protein allergenicity, the production of specific IgE antibodies is the most relevant response in the vast majority of instances. Many of the rodent models have been developed in strains that are genetically disposed to react with specific serum IgE to various test proteins. The genetic predisposition of these animals is believed to mimic the susceptible human population. In addition to providing important information for understanding the mechanism of allergenicity, animal models may be useful in the prediction of the allergenicity of a novel protein and in risk assessment. With appropriate positive and negative controls, animal models may be important in determining the threshold of sensitization, the dose-dependent effects in sensitization and challenge, as well as the ranking of allergenic potency among proteins.

There are numerous limitations to current animal models for the assessment of food allergenicity. Because no animal models have been validated at this time, assessment of protein allergenicity using models can be made only in association with other data and information. Few models have been tested for the ability to rank the allergenic potency of proteins using proteins of known allergenicity, a key step in validating a model. In addition, one factor common to both human and animal allergenicity is genetic predisposition. However, it is unlikely that a single animal model can adequately reflect the genetic variability of humans, in particular, the predisposition to respond to all potential allergens. Furthermore, published data show that the diet of animals must be free of the test protein for at least two generations prior to testing for potential allergenicity (Knippels, Penninks, and Houben 1998).

Serum Testing

Immunoreactivity with sera from sensitized individuals is another measure used to evaluate the potential allergenicity of bioengineered foods. Serum testing is an important element of current approaches for allergenicity assessment, because specific IgE against food antigens is often, but not always, associated with clinical allergy. An indication that serum IgE has reacted to a new protein suggests a cautious approach should be taken with the development of a bioengineered crop. This is based on the fact that IgE is implicated in immediate hypersensitivity reactions that are consistent with food allergies. In addition, serum testing is a minimally invasive and low risk procedure (CODEX 2003; FAO/WHO 2000, 2001; Metcalfe, Astwood *et al.* 1996).

To date, serum testing has been recommended for proteins derived from known allergenic sources or for proteins exhibiting sequence homology with known allergens. Under these circumstances, it has been possible to obtain sera from individuals sensitive to the particular allergenic substances in order to conduct the testing. Consistently positive results among several individual sera may indicate that further study is necessary to determine the clinical significance of the reaction. Inconsistent positive serological results from an enzyme-linked immunosorbent assay (ELISA) may

be confirmed by other methods such as competitive inhibition with free antigen or Western blot.

However, while positive results are suggestive of potential allergenicity, the presence of IgE antibodies does not always correlate with clinical allergy. In addition, negative results may be inconclusive. Furthermore, human sera for less commonly allergenic substances may not be available. However, despite its limitations, specific serum testing is currently one of the most useful methods for screening proteins derived from known allergenic sources or for proteins exhibiting sequence homology with known allergens.

In the case of proteins derived from non-allergenic sources, the choice of which sera to use is problematic. In that situation, no known human serum is available to serve as a positive control and the results of such testing would be difficult to interpret. A negative result would always be subject to the question of whether a sufficient number of sera were used. In addition, the criteria for the quality and quantity of 'normal' sera to be used have not been established.

Potential Toxicity of Proteins Expressed by Genetically engineered Plants

Background

As previously discussed, crop plants contain thousands of proteins and other substances that have become a normal part of the human and animal diet. Fortunately, the vast majority of substances found in crop plants do not cause adverse health effects in humans and animals (Fuchs and Astwood 1996; Kessler *et al.* 1992; Metcalfe, Astwood *et al.* 1996). However, all foods, regardless of their source or method of development, can potentially contain toxins and other dangerous substances. Because of familiarity gained during development of crop plants over many centuries and the inclusion of these crops in mammalian diets, much is known about the toxins, antinutrients, and other undesirable substances that can occur naturally in crop plants (CODEX 2003; NRC 2004). In addition to information available in published literature, OECD has published a number of consensus documents for a variety of crop plants that provide details about crop biology, including the presence of potentially harmful substances (OECD 2004).

While it is important to know what harmful substances can be found in our food crops, it is also important to have knowledge about what levels of these substances are considered acceptable, and do not pose a threat to human or animal health (CODEX 2003; NRC 2004). This knowledge gives plant breeders the ability to identify breeding lines containing elevated levels of these toxins relatively early in the breeding process and therefore avoid introducing these potentially harmful crops into the food (NRC 2004). This same knowledge is also valuable to crop developers who use genetic engineering as a method of plant genetic modification. An important consideration for GE crop developers is not only what toxins may naturally occur in a plant, but also whether any unintended changes occurred in the plant as a result of the

transformation process that potentially introduces new harmful substance or increases levels of those that naturally occur in the plant (CODEX 2003; NRC 2004).

Once an assessment is completed on the plant itself to identify potential unintended changes resulting from the transformation, the safety assessment should then consider the donor, chemical nature and function of the newly expressed substance (CODEX 2003; NRC 2004). If the GE plant expresses a specific protein, then the toxicity assessment may include an amino acid sequence similarity comparison to known toxins and anti-nutrients; stability to heat, processing and digestion; acute oral toxicity tests using surrogate animals; and previous exposure in human or animal diets (CODEX 2003; EPA 2000; NRC 2004). When the GE plant expresses a substance that is not a protein, the toxicity assessment should be handled on a case-by-case basis, using appropriate tests to characterize the expressed substance (CODEX 2003). Further, the assessment should ensure that genes coding for known toxins or anti-nutrients that may be present in the donor organism are not transferred to the GE plant (CODEX 2003; NRC 2004).

Exposure

As mentioned, it is not only important to consider if a substance found in a plant is potentially harmful, but it is also important to consider the level at which exposure to the substance is considered unsafe (Day 1996; NRC 2004). This can either be accomplished by considering available data based on prior exposure to the substance in the diets of humans or animals, or by using surrogate animal testing that will be discussed later in this section (CODEX 2003; NRC 2004). If the substance has previously been a part of the human or animal diet, then information should be available about what levels of exposure are considered safe or if there have been reports of any adverse effects resulting from exposure to the substance. If there has been no previous exposure then consideration should be given to how much and in what form exposure to the expressed substance will occur and whether the crop is normally consumed raw or if it is normally processed or cooked. If the crop is to be consumed raw, a determination should be made regarding whether the substance is stable to digestion; if the crop is normally processed, a determination should be made whether the processing affects stability (CODEX 2003; NRC 2004).

Amino Acid Sequence Similarity

Most proteins and other substances that are known to be mammalian toxins have been well studied (Ecobichon 1993; EPA 2000; Majak 1995). For toxic proteins, their amino acid sequences have been elucidated and entered into publicly available databases. These databases are powerful tools that can be used as part of the safety assessment for substances expressed in GE plants. The amino acid comparison can be used to identify structural and functional relatedness of a protein to known toxins and antinutrients (CODEX 2003; EPA 2000). However, while the amino acid sequence comparison can provide valuable information about the relatedness of a protein to a

known toxin, relatedness, or lack thereof would only be considered to be part of the assessment (CODEX 2003; NRC 2004).

Digestibility

Crop plants contain numerous proteins and other substances. Many of the substances are well characterized and others remain unknown or uncharacterized. For proteins found in the mammalian diet, the assumption is that these proteins are broken down into amino acids or peptides as part of the digestion process (EPA 2000). As discussed in the section on allergenicity, stability of a protein to digestion can be assessed using in vitro methods in which the protein is subjected to simulated gastric fluid, and then examined by gel electrophoresis (CODEX 2003; EPA 2000; NRC 2004). While in vitro digestion alone is not a sole determinant of the potential of a protein to be a toxin, it can contribute to the overall characterization of a protein (CODEX 2003; EPA 2000; NRC 2004).

Surrogate Animal Testing

Unlike the allergenicity assessment for proteins, surrogate animal testing can be performed when warranted to assess the potential toxicity of a protein or other expressed substance. The use of animal models is often a significant part of the risk assessment of substances, such as proteins, expressed in GE crops (CODEX 2003; EPA 2000; NRC 2004). The use of surrogate animals allows for determination of whether a substance is potentially a mammalian toxin and at what exposure levels a potential toxin can induce adverse effects upon consumption. However, there are limitations to the use of animal models, such as when they are used to assess the safety of whole foods (CODEX 2003). Feeding surrogate animals certain whole foods, or diets comprised entirely of one food can induce adverse effects on the animal that are not related to the test substance itself (CODEX 2003). Therefore, the use of surrogate animals may not be appropriate when attempting to identify any potential unintended effects that may occur as a result of a transformation.

Nutritional Quality of Genetically engineered Plants Compared to their Traditional Counterparts

Background

Agricultural food crop development through the use of modern biotechnology techniques has provided the potential to improve the amount and quality of our food supply (Fuchs and Goodman 1998; Kessler *et al.* 1992; Metcalfe, Astwood *et al.* 1996). Crops have been developed to resist damage caused by insects and microorganisms that in turn allows for a reduction in the amount of chemical pesticides used on these crops. Other food crops have been developed that may provide improved nutritional quality, or produced improved oils purified from those crops. In addition to crops GE for use as food and feed, some agricultural crops have been developed to produce pharmaceutical or industrial products (ISB 2004).

Food and feed crops have been and will continue to be engineered to intentionally alter the nutritional quality of that crop. In such cases, additional analysis is required to determine what impact the intentional composition change may have (CODEX 2003; Kessler *et al.* 1992; NRC 2004). Beyond the food safety aspects of GE crops, the nutritional quality of these crops should be analyzed to ensure that they are as nutritious as the same crop that was developed through traditional methods and grown under the same conditions (NRC 2004). When performing the overall assessment of a GE crop, a determination is made about whether the engineered crop is substantially equivalent to its traditional counterpart (NRC 2004). Substantial equivalence in terms of plant composition, along with the other safety testing, helps address the issue of whether there has been any unintended changes resulting from the transformation process (NRC 2004). It should be seen as a key step in the safety assessment process although it is not a safety assessment in itself; it does not characterize hazard, rather it is used to structure the safety assessment of a GE food relative to a conventional counterpart. (OECD 2000)

Composition

Each line of crops developed via genetic engineering should be analyzed to determine the composition of the transformed plants. In others words, the concentrations of key components (*e.g.* nutrients, anti-nutrients, metabolites, toxins and allergens) normally found in a particular crop should be determined and compared to what are considered to be normal levels of these components in parental and/or non-transformed lines of the same crop (CODEX 2003; NRC 2004). CODEX describes key nutrients and key anti-nutrients as those found in a particular food that may have a substantial impact on the overall diet (CODEX 2003). If this analysis identifies any differences in composition of statistical significance, then an analysis should be performed to determine if the differences have biological significance (CODEX 2003; NRC 2004).

Examples of assessing potential impacts of GE plants on the environment.

Genetically Engineered Insect-Resistant crop plants

Introduction

In general, all plants have the ability to repel, destroy or mitigate pests. While the mechanisms of plant pest resistance remain a mystery in most cases, all plants are resistant to most pests. In other words, plant pest susceptibility is generally the exception (CAST 1998). For centuries, farmers and plant breeders have used insect and disease resistance genes from wild relatives to improve crop plants, an ongoing process because often insects overcome the resistance. Agricultural biotechnology has increased the number of ways in which crops can made resistant to pests. Since the early 1990s, many biotech companies and public institutions (*e.g.*, government and universities) have invested considerable research and development efforts on GE crops

resistant to insect pests. To date, only insect-resistant plants expressing genes from *Bacillus thuringiensis* (Bt) have been deregulated by the United States Department of Agriculture Animal Plant Health Inspection Service (USDA/APHIS) and registered for commercial use by the EPA. Bt proteins have been used for more than 40 years as microbial insecticides, which are sprayed on crop plants. However, their use in commercial agriculture has been limited because the proteins are short-lived in the environment, and sprays can protect only above-ground portions of the plant. Genetic engineering of plants that contain Bt proteins in all tissues continuously throughout the growing season has overcome many of the limitations of Bt microbial insecticides.

Bt is a naturally occurring Gram-positive bacterium found in many environments including soil, insects, stored-product dust, and deciduous and coniferous leaves. There are two current types of Bt proteins used as insecticides: "crystal" proteins and "vegetative insecticidal" proteins. Crystal proteins, called Cry toxins or delta-endotoxins, form within the spores of Bt bacteria. When ingested by a susceptible insect, these proteins readily bind to receptors on the midgut, insert into its membrane (Gill, Cowles, and Pietrantonio 1992; Schnepf *et al.* 1998), and form pores causing destruction of cells, leading to starvation, gut paralysis, septicemia (blood poisoning) and death of the insect (Schnepf *et al.* 1998). **Error! Main Document Only.**

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Commercialization of Bt crops has resulted in fewer insecticide applications and thus lower management costs (Fitt 2000; Schnepf *et al.* 1998). Also, one notable advantage of GE insecticidal crops over conventional insecticides is their high specificity, such that potential toxic effects on non-target insects are minimal (Betz, Hammond, and Fuchs 2000; Macintosh *et al.* 1990)(restricted within an order?). Bt crops may also reduce the need for synthetic insecticides which, in turn, would decrease risks to the environment and effects on non-target organisms including beneficial insects.

Assessment of environmental effects

Issues that are typically considered in risk assessment of Bt crops include:

- Potential effects on non-target organisms
- Potential unintended effects on the target organism
- Potential changes in toxicity and allergenicity.

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Potential effects on non-target organisms[i]

As the inserted genes code for insecticidal toxins, there is reason to consider in the risk assessment the question of potential effects on non-target organisms, including beneficial organisms. The scenarios that would be considered are (1) direct effects in the case of other insects or other animals eating the GE plants with the Bt gene, and (2) indirect effects in the case of other animals that consume the target insects due to (a) indirect consumption of the Bt toxin or (b) reduced numbers of prey. In the cases of the GE crops with Bt genes to date, the gene products are well known to specifically target a small group of Lepidoptera. The likelihood of those Lepidoptera insects being

directly affected by the Bt toxin depends on the size of crop, i.e., in cases of small scale field trials, any impact at the population level of affected Lepidoptera insects is very unlikely. In cases of large scale commercial use, the estimation of likelihood should consider the presence and feeding behavior of those Lepidoptera insects, which depends on those insects and on the crops involved. When those insects are not present in the area of planting or do not use the crop involved as main source of food, then an impact at the population level of those insects is very unlikely. But not impossible and in fact Bt corn has a demonstrable effect on a nontarget lepidopteran that does not in fact utilized the GE crop (Sears et al. 2001, Zangerl et al. 2001; Obrycki et al. 2001).When they are present and do use the crop involved as main source of food, then additional testing may be required.

Potential unintended effects on the target organism

The continuous production of Bt proteins may increase the potential for target insects to become resistant to Bt proteins through constant selection pressure upon target and non-target susceptible insects. This type of sustained exposure can increase the probability for pest resistance to develop. These concerns resulted in the requirement of insect resistance management (IRM) strategies for good stewardship of these crops, and EPA has been the lead government agency regulating IRM for Bt crops. Written reports on various aspects of IRM are submitted to EPA to aid in the evaluation of the success of resistance management for Bt crops. Although information is often shared between EPA and USDA/APHIS, most of the IRM materials and reports are not submitted to or reviewed by APHIS as part of deregulation.

An insect resistance management strategy is developed by incorporating (1) knowledge of Bt proteins, their targets, and their alternative modes of action, (2) knowledge of pest ecology and biology, (3) appropriate dosages for Bt proteins, (4) appropriate refuge design, and integrated pest management (IPM) of the refuge and Bt crop, (5) plans for monitoring, reporting and mitigating incidents of insect resistance, and (6) communication and educational strategies on the use of the product (SAP 1995, see Office of Pesticide Program (OPP) docket, OPP-00401) into a single plan to delay resistance of target pests to Bt crops.

Thorough knowledge of pest biology is essential to the effective use of crops expressing Bt proteins and to the management of insect resistance to Bt proteins. For example, feeding behavior of the target pest may influence the optimal location within the plant for Bt protein expression, as well as dosage expression (see 'Dose of Bt Insecticidal Protein' section). Larval and adult movement (within and between fields, and overwintering habitats) may affect the types, sizes, and management of refuges developed for IRM (see 'Refuges' section). Reproduction (egg-laying habits, mating preferences, and generations per year) will also influence the development of resistance management plans, particularly when implemented to encourage random mating of insects residing in Bt and non-Bt crops (see 'Refuges' section below for further discussion).

Another important component of IRM is determining the effective and appropriate dosage of Bt protein. The February, 1998 FIFRA Scientific Advisory Panel (SAP) Subpanel on *Bacillus thuringiensis* (Bt) Plant-Pesticides and Resistance Management determined that a high dose strategy (together with a refuge strategy, see 'Refuges' below) is necessary to mitigate resistance of stalk boring Lepidoptera (e.g., moths) in Bt corn (meeting held on February 9-10, 1998. Docket # OPPTS-00231). A "high dose" is defined as 25 times the protein dose necessary to kill all susceptible lepidopteran insects (EPA-SAP 1998). For coleopteran (beetle)-active Bt products, the definition of a high dose has not been determined, nor has it been concluded that a high dose is necessary to mitigate resistance.

The August 2002 SAP suggested that it is adequate to differentiate between high dose and non-high dose products when determining an effective IRM. A high dose strategy would involve killing a high proportion of the target pest population, whereas a non-high dose has limited impact on the fitness, survival and selection pressure of the target pest. Products with a non-high dose may not require a refuge and may have a longer period of durability. In general, IRM plans for a non-high dose product are not well understood. With a non-high dose event, it may be difficult to sufficiently control the pest while limiting effect on its fitness (http://www.epa.gov/scipoly/sap/1998/february/finalfeb.pdf).

Refuge development is another significant component of an IRM program. Structured refuges are non-Bt host plants that are managed to provide sufficient Bt-susceptible adult insects to mate with potentially Bt-resistant adult insects. These matings result in Bt-susceptible offspring, which decreases the number of resistant insects and dilutes the frequency of resistance genes.

Refuge size, proximity to the GE crop and refuge management are believed critical for resistance management. Refuge size and location must be structured to maximize the potential for mating between susceptible insects (from the refuge) and possible resistant survivors (from the Bt field). Currently, refuges are planted with a similar hybrid, in close proximity to, and concurrently with, the Bt crop. Refuges are treated as needed to control insect pests with non-Bt insecticides or other appropriate IPM practices and managed according to practices in the Bt field.

Consideration should also be given to the use of temporal, alternative host, and spatial refuges to delay resistance. Adjusting the planting time and/or availability of alternative host-plants, may increase the attractiveness of the Bt field by manipulating the insects' life history strategies. Currently, there is no evidence that refuges can be temporally implemented to delay resistance.

As more Bt products are commercialized, it is theoretically possible for insect pests to come into contact with multiple Bt insecticidal proteins during their development. If the insecticidal proteins produced by the Bt plants all have similar modes of action, pests may develop cross-resistance (resistance to all proteins using that mode of action) (Tabashnik *et al.* 1994). One potential method to circumvent or delay cross-resistance is to plant two or more Bt crops, each of which produces a Bt protein with a

mode of action different from the others. The theory behind spatial refuges is that it is very unlikely that a pest population would develop resistance to multiple unrelated proteins. However, for many pests, a single individual will only experience a single plant, and therefore a single Bt protein (mode of action), during its development. Because many pest larvae do not move from plant to plant, and would not be exposed to multiple Bt proteins, spatial refuges have not been implemented.

Monitoring and Mitigation

Identifying populations of resistant insects through a comprehensive resistance monitoring plan is one method to test the effectiveness of resistance management programs and detect the onset of resistance before widespread crop failure occurs. However, monitoring and detecting pest resistance to a Bt protein is a difficult and imprecise task requiring a high level of sensitivity and accuracy. Appropriate resistance monitoring requires baseline susceptibility data prior to initiation of a monitoring program. In addition to baseline susceptibility data, information is needed to determine how many individuals need to be sampled and in how many locations. The chances of finding resistant larvae in a Bt crop depend on the level of pest pressure, the frequency of resistant individuals, the location and number of samples that are collected and the sensitivity of the detection technique.

Because there have been no confirmed instances of pest resistance to Bt crops currently planted, there has been no need to implement mitigation measures, and their success has not been evaluated Tabashnik et al 2000 PNAS?. Mitigation may involve informing customers and extension agents in the affected areas of suspected or confirmed resistance, increasing monitoring in the affected areas, implementing alternative means to reduce or control target pest populations in the affected areas, implementing a structured refuge in the affected areas, and cessation of Bt seed sales in the affected and bordering counties until an effective local management plan has been implemented.

Grower Stewardship

Growers are an essential element for the implementation and success of an IRM plan as they are responsible for planting refuges according to guidelines, and for monitoring fields for unexpected pest damage. Therefore, an education program that informs growers why IRM is needed and provides guidance how to implement appropriate strategies is necessary. Growers are required by the EPA to sign a technology use agreement that outlines IRM requirements and acknowledges the growers responsibility to comply with them. The agreement states that growers received a Product Use Guide provided by the company selling the Bt seed. Technical bulletins, grower guides, sales materials, training sessions, websites, toll-free numbers for questions or further information and educational publications have been recommended as tools to educate growers. Educational materials should be consistent and reflect the most current resistance management guidelines to help ensure compliance with IRM requirements. It takes time and money to comply with IRM requirements, and there is a concern that if IRM requirements are too complex or time consuming, growers may avoid planting Bt crops or not adhere to IRM strategies.

EPA-registered Cry proteins have been considered safe because the intestinal walls of mammals do not have the endotoxin receptor necessary for the toxic effect, and the proteins are degraded quickly in the stomach. Vegetative insecticidal proteins (VIPs) are secreted proteins derived from the vegetative growth stage of Bt. When ingested, the protein binds to midgut cells, attacks the epithelial layer of the midgut, and eventually causes death (Lee *et al.* 2003). VIPs have a similar mode of action as Cry proteins, but VIPs associate with different midgut binding sites (Cao-Guo *et al.* 1997; Lee *et al.* 2003; Yu *et al.* 1997). Both Bt Cry proteins and VIPs have been deregulated by the USDA and Cry proteins have been registered for commercial use by EPA.

As part of the ecological risk assessment, the EPA also considers potential risks to mammal, avian and aquatic (e.g., fish) organisms. Although wildlife may be exposed to Bt protein, there is no evidence to date that shows toxicity to wild or domesticated mammals, fish, or avian species, and there are no reports of adverse effects from the commercial poultry industry after several years of using Bt corn in poultry feeds. Accidental aquatic exposure from Bt crops is extremely small, and there is no evidence for sensitivity of aquatic species to Bt proteins (EPA-BPPD 2001). USDA/APHIS, as part of their ecological risk assessment, also considers potential risks of GE crops to migratory birds under the Migratory Bird Act and threatened and endangered species under the Threatened and Endangered Species Act.

Genetically engineered drought and saline tolerant crop plants

Introduction

All plants have in various degrees tolerances against abiotic stresses such as drought, and salinity. However, while some specialized wild plants such as succulents can have high tolerances against stresses, most crop plants have low tolerances to drought and salinity.

There is intense interest in the development of drought- and salinity-tolerant plants. The size of the human population is increasing, creating a need for increased agricultural production. At the same time, most prime farmland is already under cultivation, and growers are considering the use of more marginal lands. Decreasing availability of fresh water and changes in rainfall patterns due to climate change provide some of the impetus to modify plants for drought-tolerance. Soil salinity is increasing in irrigated cropland, with approximately 20 percent of irrigated cropland experiencing salt stress at some level (Yamaguchi and Blumwald 2005; Yeo 1998). The increased probability of salt-stress conditions is promoting the development of salt-tolerant plants. The objectives much biotechnological research, therefore, is to obtain plants that can be grown under dry conditions and on marginal, saline land, resulting in increase in production and reduction of the use of water in agriculture, which is of particular importance to developing countries.

Assessment of environmental effects

As the appearance of abiotic stress tolerant GE plants is a relatively new development in biotechnology, this section is only a sampling of the potential effects, and does not exhaustively address risk assessment.

Issues that would be considered in risk assessment of drought or saline tolerant crop plants include:

- Potential effects on plant-insect interactions
- Potential changes in weediness

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Potential effects on plant-insect interactions

When plants experience abiotic stress, a multitude of physiological changes occur. For example, when plants experience drought and salinity stress, protein metabolism and amino acid synthesis are impaired (Hsiao 1973). Under such conditions, existing proteins may be broken down resulting in increased levels of available nitrogen and amino acids (Brodbeck and Strong 1987); (Bohnert, Nelson, and Jensen 1995; Delauney and Verma 1993). These stress-induced changes in plant physiology are widely thought to positively influence insect herbivores due to favorable modifications in plant nutrient content, particularly increases in amino acids and nitrogen (Bentz and Townsend 2001; Busch and Phelan 1999) or increase its concentration in food resources (Chen and Welter 2002; Richardson *et al.* 2002).

However, not all stress-induced changes in plant physiology will positively affect insect herbivores. Defensive chemical compounds, collectively called "allelochemicals," are produced by the plant to affect insect herbivores in a negative manner, such as decreasing survival (Brodbeck and Strong 1987; White 1993; Gershenzon 1984; Inbar, Doostdar, and Mayer 2001; Mattson and Haack 1987, 1987). Different insects feed on plants in different ways: for example, some chew plant tissues, while others suck plant fluids from vascular tissue. Because allelochemicals are much less concentrated in vascular tissue compared to leaf tissue (Raven 1983), insect species that feed in vascular tissue (sap-feeding insects such as aphids) may respond more positively to plant stress than chewing insects, such as caterpillars, that feed on leaf tissue with higher levels of defensive chemicals (Larsson 1989). As plants are engineered to become tolerant of abiotic stress, there is a need to consider the complex interactions between genetically-engineered stress-tolerant plants and insect herbivores.

Potential changes in weediness

A primary reason behind stress tolerance traits is to enable crop survival in areas or under conditions under which it could not survive before. Therefore, one of the key questions for the risk assessments of these traits will be whether they will also change the potential for crop plants to become weeds or cause the crop to become more

invasive. The weediness or invasiveness of a plant depends on many different characteristics, such as persistence, reproductive strategy, and dispersal and other factors such as the receiving environment and its climate. In general, it is not very likely that a change in one particular trait would suddenly make a plant become more weedy. However, it is theoretically conceivable that a change in abiotic stress resistance may incrementally increase the weediness of a crop that already had a number of weedy characteristics. Whether or not this may be the case will depend on the characteristics of the crop itself, the phenotypic changes, and the receiving environment.

Genetically engineered crop plants tolerant to low levels of nutrient availability.

Introduction

Another stress-tolerant phenotype being developed is the ability of plants to withstand low levels of nutrient (fertilizer) availability through increased nutrient assimilation and/or utilization. Increases in nitrogen utilization efficiency of crop plants have the potential to decrease fertilizer costs to farmers by decreasing the nitrogen applications required for adequate yield production, as well as reducing nitrogen run-off and subsequent water contamination (Oliveira *et al.* 2002). Tobacco modified to increase ammonium assimilation and thus increase nitrogen efficiency resulted in plants with greater biomass and leaf soluble protein compared with the non-GE tobacco plants (Oliveira *et al.* 2002), indicating that increased nitrogen utilization efficiency is possible. Nutrient utilization for phosphorus is also being examined as a potential modification for crops plants. Over 30 percent of crop land experiences phosphorus deficiency (Vance, Uhde-Stone, and Allan 2003). GE tobacco plants modified for increased phosphorus utilization exhibited significantly greater growth and higher phosphorus concentrations in phosphorus-deficient conditions than non-transformed plants (Lung *et al.* 2005) and *Arabidopsis* (Xiao *et al.* 2006). Thus, genetic modifications that alter the assimilation and utilization efficiency of nutrients can result in increased nutrient content of the plant.

Assessment of environmental effects

Issues that would be considered in risk assessment of drought or saline-tolerant crop plants include:

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- Potential effects on plant-insect interactions
- Potential changes in weediness

Potential effects on plant-insect interactions

GE plants engineered for stress tolerance show an increase in nutrient content, either nitrogen or phosphorus, through either an accumulation of nitrogen-based osmoprotectants during drought and salinity or an increased nutrient use efficiency.

Although nitrogen has traditionally been recognized as a limiting nutrient for insect herbivores (Mattson 1980; McNeill and Southwood 1978; White 1993), from the few studies that have investigated the effects of phosphorus limitation on insects, there is evidence showing that it can be an important determinant of survivorship (Ayers *et al.* 2000; Clancy and King 1993), fecundity (Popp *et al.* 1989), body size (Busch and Phelan 1999; Janssen 1994), oviposition (Skinner and Cohen 1994), growth rate (Perkins *et al.* 2004) and population density (Schade *et al.* 2003). Thus, genetic modifications that result in plants increasing in either nitrogen or phosphorus content may potentially affect insect herbivore populations on stress-tolerant plants because of the sensitivity of insect herbivores to nitrogen and phosphorus content.

Potential changes in weediness

Because plants frequently inhabit environments where water and nutritional resources are limited, the ability to more efficiently exploit these resources may enable a plant to outcompete its neighbors. This could result in the development of plants with invasive or weedy characteristics, and the assessment of the impacts from these characteristics would need to include considerations of the impacts of the movement of stress-tolerance traits to the wild relatives of GE crop plants.

Conclusion

Of the more than 11,000 permits and notifications that have been issued, acknowledged or are pending over the last 18 years, only 315 applications include plants that are GE for stress-tolerance. As this area of biotechnology research continues to moves from strictly experimental to product development, stress-tolerant phenotypes and the mechanisms underlying the tolerance will become more refined and better understood. Subsequently, because insect herbivores show such significant sensitivity toward even minute changes in plant nutrient content, future field studies involving stress-tolerant plants and other GE phenotypes that change plant nutrient or defensive chemical content may have the ability to establish any positive or negative relationships between these GE plants and their insect herbivores.

Genetically Engineered Virus-Resistant Crop Plants

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Introduction

Plant viruses represent a significant threat to global agriculture because of their ability to reduce the quality and, more important, the yield of food and fiber crops (Hull 2004; Pappu 1999). Hundreds of plant viruses have been described, affecting a wide range of plants and trees. In general, most plant viruses consist of genetic material – either RNA or DNA – enclosed by a protective coat. This coat, which is made from many individual protein molecules ("coat proteins") plays an important role in protecting the genetic material, as well as determining how the virus spreads. Most plant viruses are obligate parasites (parasites requiring a living host) which move from plant to plant via insect vectors (Hull 2004; OECD 1996). Additional means of plant virus transmission

include fungal transmission, seed transmission, mechanical transmission and grafting (Hull 2004; OECD 1996).

In cases where plants are susceptible to viruses, common control or management strategies have included the use of pesticides for control of insect vectors; cultural practices, that include removal of infected plants and plant material serving as sources of viruses; use of virus-free planting material; and/or use of resistant varieties (Gooding 1985; Khetarpal *et al.* 1998; OECD 1996; Superak *et al.* 1993; Swiezynski 1994). While the use of these control strategies has been effective in parts of the world, the overall effectiveness of these strategies can vary significantly from crop to crop and year to year (Hadidi, Khetarpal, and Koganezawa 1998; OECD 1996; Pappu 1999).

Another control strategy shown to be effective is cross protection (Gonsalves 1998; Gooding 1985; Hull 2004; Sherwood 1987). Cross protection involves the ability of a mild strain of a virus to prevent or delay infection by a second more virulent strain of virus (Culver 2002; Gooding 1985; Hull 2004; Sherwood 1987). Cross protection has been attributed to various mechanisms (Culver 2002; Goregaoker, Eckhardt, and Culver 2000); (Beachy 1999; Culver 2002; Goregaoker, Eckhardt, and Culver 2000; Sherwood 1987). Coat protein mediated cross protection, for example, relies upon the coat protein to properly associate with and block disassembly of the virulent virus (Culver 2002). While cross protection has proven to be effective with some viruses, because of the labor and time needed to infect plants with the mild virus strain, cross protection is generally not a practical means of controlling virus disease in large scale or agricultural systems.

In recent years, much of the research and development for plant virus disease control has focused on development of GE virus-resistant plants. Using the concept of pathogen-derived resistance (Sanford and Johnston 1985) and cross protection, genetic modifications of host plants and trees are made that allow for expression of viral genes or proteins in the plant and tree tissue. Plant expression of viral genes or proteins often acts to delay or prevent infection by the same or related viruses. This form of pathogen-derived resistance was first accomplished in 1986 by Roger Beachy and colleagues (Abel *et al.* 1986) in which tobacco plants engineered to express tobacco mosaic tombusvirus (TMV) coat protein were resistant to TMV infection.

Numerous other virus-resistant plants have been developed and field-tested (ISB 2004; Tepfer 2002). Most of the virus resistance is based on so-called "pathogen-derived resistance," most often using viral coat protein (VCP) or VCP gene expression as the basis for resistance (ISB 2004; Tepfer 2002). Over the past 15 plus years, nearly 900 virus-resistant plants (including trees) have been authorized by USDA–APHIS for field testing in the U.S. In addition, several virus-resistant crop plants have been deregulated by APHIS and have been grown commercially. GE virus-resistant plants deregulated by APHIS to date include those that express viral coat protein genes (*e.g.*, papaya ringspot virus-resistant papaya) or the replicase protein gene of potato leafroll virus-resistant potato (EPA 1998; Gonsalves 1998; ISB 2004).

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Assessment of environmental effects

While the development and deployment of genetically-modified plants has proven to be effective in controlling targeted virus diseases, some concern has been raised about the potential risks associated with agricultural use of genetically-modified virus-resistant plants (NRC 2002, 2000). The safety of these plants has been the subject of numerous scientific meetings and workshops, as well as scientific articles written by members of the U.S. government, academia and industry that address potential risks associated with these plants (AIBS 1995; Falk and Bruening 1994; Miller, Koev, and Mohan 1997; OECD 1996; Tepfer 2002).

Potential adverse effects that have been identified and studied in detail include:

- The development of new virus diseases, through either:
  - Heterologous Encapsidation (transcapsidation) – the phenomenon where the coat protein of one virus is able to enclose ("encapsidate") the nucleic acid of a separate virus. When heterologous encapsidation occurs, there is some potential for altered phenotypes and/or host range.
  - Virus Recombination – exchange of the genetic material between two or more different viruses. If recombination is possible, there is some potential for the generation of new viruses.
- Synergy – increase in severity of infection or symptoms when two or more viruses infecting the same plant. If synergy occurs, the potential result is increased virus disease severity.
- Change in weediness due to gene flow between cultivated crops and weeds, there is some potential for a weedy relative to acquire virus resistance from the crop plant.

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While the technology, as well as the analysis of potential adverse effects, continues to evolve, currently available scientific data has been used to study these risks (AIBS 1995; OECD 1996; Tepfer 2002). This data and information will be discussed in this section to briefly explore the possible risks and other concerns that have been raised with regard to large-scale deployment of genetically-modified virus-resistant plants.

Many of the issues that will be discussed in this section are similar for both RNA and DNA plant viruses. However, to date, the development of GE virus-resistant plants has been mostly limited to plants developed for resistance to RNA viruses (ISB 2004; Tepfer 2002), and most GE virus-resistant plants that have been commercialized express the VCP's or VCP genes. Therefore, the focus of this section will be GE plants expressing VCP's or VCP genes from RNA viruses. Over 30 different species of plants have been developed for virus resistance and subsequent field testing, with many developed to express genes other than VCP, including: replicase protein; nuclear inclusion protein; movement protein; nucleocapsid protein; N gene; helper component;

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as well as other virus-specific proteins (ISB 2004). As crops continue to be developed to express proteins/genes other than VCP, the agency will assess the safety of these crop/gene combinations on a case-by-case basis, using the same level of scrutiny that has been used for VCP's.

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Development of new virus diseases through Heterologous Encapsidation

There are many instances in nature and in agricultural settings where a single host plant is infected by two or more viruses. In fact, some reports about naturally infected plants have identified individual plants that were infected by as many as six different viruses (Abdalla, Desjardins, and Dodds 1985; Falk *et al.* 1995). Viruses in a naturally-occurring mixed infection could interact in a number of scenarios. One potential scenario that can occur in plants co-infected with two or more viruses is a phenomenon involving the viral coat protein known as heterologous encapsidation (Falk *et al.* 1995; Miller, Koev, and Mohan 1997; Waterhouse and Murant 1983).

Heterologous encapsidation occurs when the coat protein of one virus is able to encapsidate the nucleic acid of a second virus (Figure 2). Heterologous encapsidation was first described by Rochow (1970) and has been the subject of numerous reviews (Falk and Duffus 1981; Falk *et al.* 1995; Miller, Koev, and Mohan 1997; Rochow 1977; Tepfer 2002. These interactions occur naturally in both agricultural crop and weed plants, and are a natural part of virus-virus and virus-plant interactions (Falk and Duffus 1981; Falk *et al.* 1995; Rochow 1977). In some cases, heterologous encapsidation is a specific interaction between two viruses that plays an important role in both virus biology and survival.

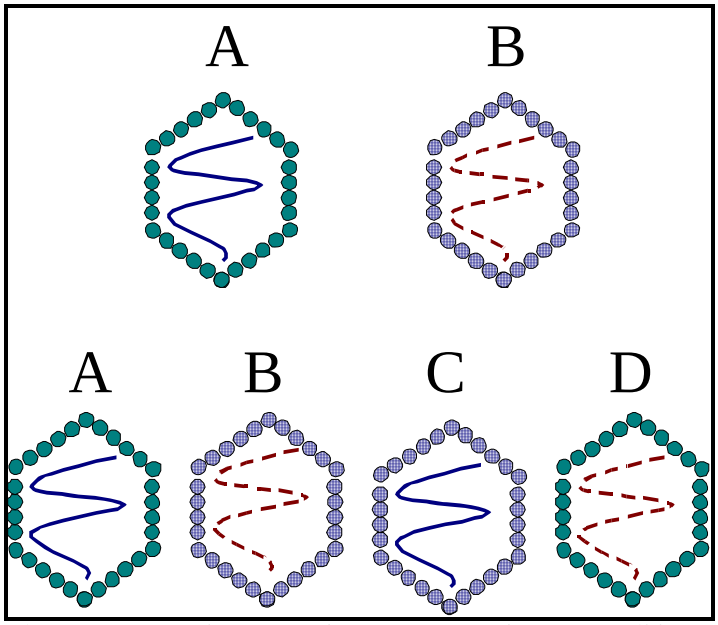

Figure Error! Main Document Only. **Heterologous Encapsidation.** Possible outcomes of heterologous encapsidation interactions as previously described by Rochow 1977 and Falk 1995. A and B represent the parental viruses. When two viruses co-infect the same cell, the progeny can include virions that are identical to the parental viruses (A and B), or progeny that are composed of the capsid protein of one virus and the RNA of the second virus (C and D).

Because the viral coat protein may determine which insect vector is capable of transmitting a particular virus, when heterologous encapsidation occurs, the RNA of one virus, essentially acquires the phenotypic properties of the second virus for insect transmission (virions C and D in Figure 2). This observation means that the insect vector recognizes the coat protein, not the RNA inside the coat protein. In other words, the progeny viruses that are the result of heterologous encapsidation often temporarily exhibit new or altered biological properties differing from those of the parental viruses (Falk *et al.* 1995). The impact on vector specificity is likely limited because many of the heterologous encapsidation interactions that have been identified occur between viruses that are closely related enough that they are transmitted by the same vectors (Hull 2004).

Once heterologous encapsidation occurs, the potential exists for new or different "exposure" or host range for the RNA of the encapsidated RNA via insect transmission. If such a scenario occurs, the result may be one of the following:

13. The inoculated plant is not a host for the virus.

14. The inoculated plant is a host for virus, but there no insect vector is available to transmit the virus from that plant.

15. The inoculated plant is a host for virus and an insect vector is available to transmit the virus from that plant.

If scenario one occurs and the new plant is not a host for virus whose RNA is encapsidated, it represents a "dead-end" for the virus. This is because the virus will likely not be able to replicate and/or move in the new plant. Without the ability to establish an infection—due to its inability to move and/or replicate—the virus would not likely be transmitted from that plant. Heterologous encapsidation that occurs according to this scenario would not likely be of any ecological significance (Falk *et al.* 1995; OECD 1996).

If scenario two occurs, the virus whose RNA is encapsidated, could replicate and move within the plant to which it was transmitted, and would subsequently be encapsidated in its own coat protein. However, the virus would not be able to move from that plant via insect transmission because the plant would not be a typical host for an insect vector of this virus. In general, the significance of this scenario is likely only transient because once the encapsidated RNA is injected into the host plant, the biological properties of the virus will take over and determine its subsequent fate (Falk *et al.* 1995; OECD 1996).

If scenario three occurs, the result would be similar to a typical infection of the encapsidated virus, if the virus is transmitted to a normal host of the virus. Once the virus is inoculated into the plant, its normal replication mechanisms would take over and the virus would then produce its own coat protein. However, because the viral RNA was initially transmitted by a different insect vector, it is possible that the virus could be introduced into a host to which it has not been previously exposed. Whether or not the virus would be subsequently transmitted from this host plant would depend upon whether an insect vector was available to transmit the virus. In terms of virus biology, this scenario would not be significantly different from what naturally occurs, other than the fact that the primary inoculation occurred with virus RNA that was the product of heterologous encapsidation. Subsequent virus biology would reflect that of the naturally-occurring virus

Overall, despite the potential for heterologous encapsidation to readily occur in nature, there have only been a few cases in which heterologous encapsidation has been shown to be important in agricultural situations (Falk *et al.* 1995; OECD 1996). The likelihood of heteroencapsidation would not be significantly different in VCP-expressing plants (Hull 2004).

Helper-Dependent Transmission

There are a limited number of cases where heterologous encapsidation is a natural part of plant virus epidemiology (Falk *et al.* 1995). Helper-dependent transmission occurs when one virus exclusively relies upon another virus for heterologous encapsidation for subsequent insect transmission from mixed infections (Falk and Duffus 1981; Falk *et al.* 1995; Hull and Adams 1968; Rochow 1977). In cases such as carrot motley dwarf, groundnut rosette, and lettuce speckles, the virus diseases are

caused by co-infection of the plant by two or more viruses (Falk, Duffus, and Morris 1979; Falk *et al.* 1995; Hull and Adams 1968; Waterhouse and Murant 1983). In each of these, and other virus disease complexes, one of the viruses is insect transmissible and the other is not independently insect transmissible. The non-independently transmissible virus relies upon the insect transmissible virus, via heterologous encapsidation, for insect transmission. In each of the complexes that has been characterized, the insect transmissible virus is able to replicate and move within the host plant in the absence of the non-insect transmissible virus. The non-insect transmissible virus is also able to replicate and move within the host in the absence of the insect transmissible virus, however, it lacks a coat protein and therefore must rely on the insect transmissible virus for encapsidation and insect transmission (Falk *et al.* 1995). While the non-insect transmissible virus is able to be spread by mechanical inoculation by itself, it benefits greatly by being associated with the insect transmissible virus that provides for more efficient dissemination and potentially a wider host range.

Given the crucial role that the coat protein plays in insect transmission and natural plant virus epidemiology, some concern has been raised about whether constitutive expression of viral coat protein in GE plants would increase heterologous encapsidation interactions. Because the amount of coat protein available in GE plants is so dramatically less than the amount of coat protein in virus infected plants, the potential for heterologous encapsidation is reduced

Reducing the Risk

Even if one assumes that there is risk associated with heterologous encapsidation interactions occurring in virus-resistant GE plants, the potential exists to reduce ecological impact via modification of the viral coat protein gene (Tepfer 2002). Research has shown that mutations in the coat protein gene can result in loss of insect transmissibility of the virus (Tepfer 2002). By incorporating such mutations into the coat protein gene that is expressed in GE plants, the potential for insect transmission of viruses that may have been encapsidated by the plant-expressed coat protein can be eliminated, without affecting the effectiveness of the virus resistance.

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Development of new virus diseases through Recombination

Plant virus recombination occurs when the exchange of genetic material between two, or more, different viruses results in production of a new virus (OECD 1996; Tepfer 2002; Worobey and Holmes 1999). Recombination between viruses in different taxonomic groups has played a significant role in virus evolution (AIBS 1995; OECD 1996; Roossinck 1997; Worobey and Holmes 1999). In terms of virus evolution, recombination would be considered to be a frequent event (Hull 2004). Nucleotide sequence comparisons of different/unrelated viruses have identified similar segments of nucleotide sequence, suggesting that recombination has occurred (Hull 2004).

Because of the potential for recombination to occur in GE plants, consideration should be given to whether recombinants arising from GE plants would be different from those that arise from mixed infections in non-GE plants, and whether the recombinants are viable (Hull 2004). Factors affecting the rate of recombination include sequence between the two viruses, the location of the virus within the plant, and structural similarity between the nucleic acids (OECD 1996). The ability of a virus arising via recombination to persist in nature depends upon factors such as its ability to replicate, spread systemically and/or its ability to be transmitted to other host plants (Hull 2004; OECD 1996). A significant difference between the potential for recombination in non-GE plants with mixed infections versus the potential for recombination in GE plants is that the virus gene is constitutively expressed, i.e. is continuously produced in every cell in the GE plant, which allows for greater opportunity for interaction and hence recombination between the expressed gene and the infecting virus (Hull 2004).

Plant-Expressed Genes-Virus Recombination

Looking beyond virus-virus recombination, other studies have focused on whether viral transgenes present in virus-resistant plants can either complement or recombine with viruses that infect the GE plant. In the mid-90's, Greene and Allison were able to show that such recombination could occur (Greene and Allison 1994, 1996). Their experiments were the first to show the potential for recombination between plant expressed genes and viruses infecting that plant. It is not clear, however, from these and subsequent studies (*e.g.*, (Borja *et al.* 1999)) how closely these experiments performed under non-natural conditions reflect what occurs in natural/agricultural settings with either GE or non-GE virus-resistant plants. Rubio *et al.* (Rubio *et al.* 1999) suggest that the levels of recombination seen in their experiments are orders of magnitude higher than would be expected in GE plants where virus replication is reduced or prevented.

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Plant-Virus Recombination

Finally, other researchers have shown that over time, plant viruses in natural settings, have incorporated various plant cellular RNA's into their genomes as part of their evolutionary process (Karasev 2000; Masuta *et al.* 1992; Mayo and Jolly 1991). In some cases, it appears that once these cellular RNA's become incorporated into the viral genome via recombination, they are subsequently maintained as part of the viral genome.

Overall, given that mixed infections are common in nature, the opportunity for both related and unrelated viruses to interact in natural virus populations is high. Issues such as selection pressure, adaptation to changing environments, competition, fitness, etc., likely play significant roles in the various types of recombination that have been identified. These factors also help determine what role the resulting recombinants play in virus biology and epidemiology. Based upon currently available information, it appears that the potential for recombination in virus-resistant GE plants (*i.e.*, virus-

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virus & plant-virus) would be similar to the natural occurrence of recombination in virus infected, non-GE plants (Falk and Bruening 1994; OECD 1996; Rubio *et al.* 1999).

Synergy – increase in severity of infection or symptoms when two or more viruses infecting the same plant

Synergy occurs when two independent viruses infect a plant simultaneously and the resulting disease symptoms are more severe than when either virus infects the plant individually (OECD 1996; Pruss *et al.* 1997; Tepfer 2002). Several naturally-occurring synergistic virus interactions have been described (OECD 1996; Pruss *et al.* 1997; Rochow and Ross 1955; Tepfer 2002; Vance 1991; Vance *et al.* 1995). Vance and colleagues have shown that when plants are co-infected with two different viruses, the disease symptoms are significantly worse than plants infected with either of the viruses alone (Vance 1991; Vance *et al.* 1995).

Subsequent studies have shown that the potyvirus helper-component-protease (HC-Pro) mediates the increase in PVX (Pruss *et al.* 1997; Tepfer 2002; Vance *et al.* 1995). Pruss *et al.* also showed that the potyvirus HC-Pro can enhance pathogenicity and virus accumulation of other viruses including cucumber mosaic virus (CMV) and tobacco mosaic virus (TMV) (Pruss *et al.* 1997). Researchers continue to explore whether other viral proteins or genes play similar roles in virus synergy.

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What, if any, risk synergy poses on the environment as a result of the use of GE virus-resistant plants is not entirely clear. However, current scientific data suggests that any impact would be minimal for several reasons. The first consideration is that any effect of synergy associated with a particular GE crop will be limited to the GE plants themselves (OECD 1996). Additionally, it is not likely that the potential for synergy occurring in GE plants expressing virus genes would be greater than in natural mixed infections (Hull 2004). Consideration of this type of effect on the GE plant should be included as a part of product development by the plant developer (OECD 1996; Tepfer 2002). Potential synergistic interactions could be identified during development of a plant line by inoculating GE plants with widely prevalent viruses of that host plant.

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Given the knowledge of the roles that different virus genes play in synergy, developers can also select only those genes that likely would not contribute to synergism, or include mutations in such genes so that their potential impact is limited. Genes such as the potyvirus HC-Pro should be avoided given what is known about its ability to enhance disease development and virus titer of some co-infecting viruses (AIBS 1995; Tepfer 2002). Other genes such as those that might aid in virus replication, movement and/or symptom severity might also be avoided because of their potential to facilitate virus disease development (AIBS 1995; Tepfer 2002).

Change in Weediness due to gene flow between cultivated crops and weeds

Weeds and other non-cultivated plants are one of the primary sources of pest and pathogen resistance genes. In general, most pest and pathogen resistance genes used in traditional breeding for resistance have been found in the centers of origin and areas of diversification of cultivated plants (Khetarpal *et al.* 1998). These are the areas in the environment where plants have been exposed to selective pressure of pests and pathogens over thousands of years and therefore have developed resistance as a mechanism of survival (Khetarpal *et al.* 1998).

The potential for introgression of a virus resistance transgene into a wild or weedy species is another possible outcome of large-scale agricultural use of GE virus-resistant plants. The primary concern is whether transgene introgression would result in a wild or weedy species becoming invasive because introgression of the virus transgene has made the wild or weedy species resistant to a virus disease that normally plays a role in control of the species (Fuchs, Chirco, and Gonsalves 2004(a); Fuchs *et al.* 2004(b); Tepfer 2002). To consider the potential risk, several aspects of virus and plant biology should be considered.

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As discussed earlier in this section, plant viruses cause significant problems by limiting the amount and quality of agricultural products. Most virus epidemics are the result of a virus and/or a vector moving from non-crop plants located adjacent to production areas into cultivated crops. Plant viruses are obligate parasites, and, as such, total destruction of their plant hosts would lead to the extinction of that virus. Therefore, it is assumed that there is a certain level of tolerance by some hosts - possibly wild or weedy hosts – that allow for persistence of the virus. In fact, many virus infections do not produce visible symptoms in weeds (Hull 2004). Because of this, there likely exists a number of wild or weedy plant species that contain resistance genes that allow these plants to survive virus infection and serve as reservoirs for the virus (Raybould *et al.* 1999).

This is somewhat different than the relationship between cultivated crops and plant viruses. Most of the major crop species used in today's agriculture (*e.g.* soybean, rice, wheat, beans) have been subjected to intensive artificial selection over centuries and only have low survival under most natural conditions. The vast majority of the crops used in agriculture are much less fit, under natural conditions, than wild or weedy plants. Because of this, the impact of virus infection on crop plants is potentially more severe than on many wild or weedy plants.

It is known that gene flow from cultivated agricultural crops to wild and weedy species has occurred since the domestication of a particular crop when sexually compatible wild or weedy species are present (Fuchs, Chirco, and Gonsalves 2004(a); Stewart, Halfhill, and Warwick 2003). It is also known that gene flow occurs between virus-resistant GE crops and non-GE crops (Fuchs, Chirco, and Gonsalves 2004(a))). What is not as well understood is how much gene flow from GE virus-resistant plants to wild or weedy relatives results in introgression of the gene(s), and what ecological impact this introgression would have. Stewart and others discuss the basic difference between gene flow, mediated via pollen or other mechanisms, and introgression of genes, as well as the frequency of introgression and impacts on the frequency (Fuchs,

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Chirco, and Gonsalves 2004(a); NRC 2000; Stewart, Halfhill, and Warwick 2003). According to Stewart, there have been a relatively low number of confirmed cases of introgression (Stewart, Halfhill, and Warwick 2003).

However, there is no clear evidence to indicate that the introgression of a virus resistance transgene into a wild or weedy species would be any different than introgression of a naturally-occurring virus resistance gene from a non-GE plant (Fuchs, Chirco, and Gonsalves 2004(a); Tepfer 2002). Further, there is no evidence indicating that a weedy plant would become more competitive, if it gained virus resistance via gene flow from VCP-expressing plants.

Whether or not introgression is considered to be a significant issue with a particular crop or crops, there are steps that can be taken to reduce any potential risk. These include use of crops for which there are no sexually-compatible relatives present in the geographical region; use of crops that have been identified to exhibit low levels of gene flow/introgression; and further development of transgene containment strategies (Stewart, Halfhill, and Warwick 2003).

Current knowledge and data suggests that gene flow from a GE virus-resistant plant to a wild or weedy plant is not likely to provide different exposure from that which occurs under natural agricultural and environmental settings.

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Herbicide Tolerant crop plants

Introduction[i]

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 proportion of pesticides used in the US that are herbicides continues to grow and is now close to 75 percent of the crop protection pesticide market. While some persistent herbicides can have serious negative impacts on the environment, and in particular on soil and aquatic ecosystems, some of the newer, non-selective and non-persistent herbicides are less hazardous to the environment.

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Crops made resistant to post-emergence, non-selective herbicides, such as glyphosate and glufosinate, by biotechnology are being widely adopted in North America and other parts of the world. These products allow the farmer to more effectively use reduced- or no-tillage cultural practices, eliminate use of some of the more environmentally harmful herbicides, and use fewer herbicides to manage nearly the entire spectrum of weed species.

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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 with the concern has been raised regarding 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 section below discuses some of the issues.

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 has been documented. This problem has not yet reached the severity of insecticide resistance, but in isolated cases the impact has been severe. 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.

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*

Over the past few years, several HRCs, both transgenic and non-transgenic, have become available in North America (see Table 1). Of these, glyphosate- and glufosinate-resistant crops have been widely adopted. 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. HRCs have accounted for nearly one-third of field tests conducted under USDA authority.

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. 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).

Reliance 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. A 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, possibly resulting in ecological impacts.

There's no discussion here of the community diversity impacts of extensive use of herbicide-resistant GE crops, as documented in the British Farmscale studies; Haughton et al. 2003, e.g., documented reduction in abundance of butterflies in GE beet and oilseed rape and bees in GE beet fields (see also Roy et al. 2003)

[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.

[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.

GE crop plants producing pharmaceuticals and vaccines

A new development in biotechnology is the production of pharmaceuticals and vaccines in plants ('Plant Made Pharmaceuticals'- PMPs). This area of research has expanded over the years, because PMPs may have advantages in terms of production scale, production costs, ease of

storage, and distribution. In addition, the production of pharmaceuticals and vaccines in plants may avoid one of the major disadvantages of pharmaceuticals produced in animal cells, namely, the risk of pathogens in the animal cells that are traditionally used to produce vaccines.

While these potential advantages are generally recognized, there is also recognition that this development poses new challenges, which require that adequate confinement and segregation measures are in place. This is particularly important where there is a possibility of commingling with crops for the food or feed chain. It is not only the growing the crop itself – be it in field trials or commercially - but also the likely impact it has on surrounding crops and crops planted later in the rotation cycle that must be taken into account. Which measures are taken to ensure appropriate confinement and segregation is decided in a case by case basis.

Silviculture

Introduction

The United States forest products industry employs 1.6 million people and ranks among the top ten manufacturing employers in 46 states. The industry produces products valued in excess of $230 billion each year which includes $23 billion in exports. One third of the United States is forested which is about 747 million acres. Of this amount, 350 million acres represent commercial timberland. Over 270 million acres of federal land have been set aside for use as wildlife refuges, parks and wilderness areas[i].

Approximately 2.6 million acres are planted annually in the United States. Approximately 1.6 billion trees are produced, harvested, and shipped by forest tree nurseries annually. Forest product nurseries produce 852 million trees, private nurseries produce 366 million trees, state nurseries produce 348 million trees, and federal nurseries produce 38 million trees.

Currently most forest tree breeding programs are only in the third or fourth generation of tree improvement. The more advanced genetic improvement programs were begun in the late 1940s early 1950s. The species receiving most of the attention in the U.S. has been loblolly pine in the southern U.S., but there is considerable research with other species such as slash pine, hybrid poplar and cottonwood. In the western U.S. the dominant species is Douglas fir.

Breeding programs have historically been primarily conducted using recurrent selection where the best parents are selected to establish seed orchards. The best trees, called “mother” trees, are planted in a seed orchard and are allowed to cross among each other. The resulting seeds that are produced are of higher genetic value than seeds collected in the wild. Over time the performance of the progeny are evaluated and trees lacking the desired traits can be removed from the orchard. Most trees resulting from tree breeding programs are destined for the lumber and paper

pulping industries, and so most of the traits that have been selected are related to growth and form. Trees with rapid growth and good form that have increased volume are selected to create the next breeding generation. Wood quality has been examined in some species, most notably in wood density for pulp yield. Also traits such as disease and insect resistance are selected. For example in loblolly pine, trees are selected for resistance to southern fusiform rust, while *Populus* (poplar) species, are selected for resistance to fungal diseases such as *Septoria* leaf spot or *Melampsora* leaf rust. Resistance to white pine blister rust in pine species in the western U.S. is another important trait selected by breeders. Trees that are not intended for timber, but for landscape use or production of tree fruits, also are improved through breeding programs in a similar manner, but the desirable traits being selected are different.

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Because most tree breeding programs have been in existence only three or four generations and because little has been done to "domesticate" these species, they are not far removed from their wild progenitors when compared to agronomic crops, and many can intercross freely with their wild relatives. For example, species of *Pinus* and *Populus* are indigenous to the continental United States and have been selected from the wild for tree breeding programs.

Current forest tree breeding programs are moving toward so-called "clonal" forestry, in which all of the trees planted are genetically identical. Clonal programs are well advanced in some genera, such as hybrid poplar and cottonwood, and are under development in others, such as loblolly and slash pine. By selecting superior clones, substantial genetic gains in volume can be achieved, thus allowing more wood to be grown on less land.

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Trees, like other plants, are GE using established biotechnological methods which result in stable transgene incorporation. However, tree breeding methods differ in some ways from those used with annual crops, and these differences may affect the deployment of GE tree products. In the immediate future it is most likely that any deregulated GE trees will be deployed as clones. Assessing the stability of transgenes in GE trees over multiple generations may be more difficult since breeding cycles take several years. Also since generation times are long, it will be a number of years before breeding with GE trees occurs and new cultivars are produced. Varieties with stacked traits will initially be produced by multiple genetic transformations.

Tree breeders are currently not using GE trees as either pollen or seed parents, and, production seed orchards using GE trees for the production of GE seeds could be decades away. This also means that all of the GE trees that are produced for deployment in the near term will be produced by vegetative propagation. This will most likely be done through tissue and cell culture or rooted cutting propagation. Therefore plantations will be established by transplanting vegetative propagules into the landscape.

Forest trees are long-lived perennials. The life span of hardwoods and softwoods are decades or centuries. Thus the duration of a field test of GE trees can span a number

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of years. Depending on the trait being measured, it could require several years of testing to gather meaningful data.

Many forest trees are wind-pollinated and the pollen can travel large distances. Pollen from some tree species can live a long time compared to many crop species. Therefore pollen from GE trees could potentially travel for miles from one plantation to another or from managed plantations to unmanaged areas.

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In many forest trees seed dormancy is common. Seeds of some species can remain dormant for years and in some species stratification, *i.e.*, a cold treatment, is required for germination. Therefore seeds from GE trees could lay dormant for years following harvest and germinate in subsequent years.

Genetically improved trees are usually grown in plantations. These plantations cover hundreds to thousands of acres. Plantations are planted in large blocks or mosaics and are harvested when the trees are mature. In a plantation the rotation can range from 8-12 years for a genus such as eucalyptus to 80 years for long-lived conifers such as spruce or fir. Therefore a "crop" can last for decades.

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The control of flowering by genetically engineering sterility is under consideration. Some stakeholders have expressed the opinion that all GE trees will have to be sterile before they can be deregulated. Questions arise as to whether sterility is necessary or desirable. It is anticipated that this will be evaluated on a case-by-case basis. Questions also arise as to whether sterility over a long period of time is achievable and whether redundant methods will be required to insure long term stability of a sterile trait.

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Tree traits under development

Forest trees have a number of insect pests. Some insects attack young trees and others attack older trees. Annual crops engineered for insect resistance using Bt toxins are grown with refugia to delay the development of resistant insects. For trees engineered for insect resistance, refugia may be useful when grown in a plantation setting. For some species, these resistance genes will need to be effective in plantations for 20 or more years. This could argue for incorporating multiple mechanisms for resistance into these trees. The potential impact of species that feed on insects that are no longer present in resistant plantations may also have to be considered.

Forest trees are also subject to a number of devastating disease pests. For example, in 1900, the American chestnut was deemed the "Redwood of the East Coast," standing 100 feet tall and comprising approximately thirty percent of the eastern seaboard forests. A fungal blight, accidentally introduced in the late 1800's, resulted in the death of approximately 3.5 billion trees, and relegated the species to low growing sprouts, unusable as timber. Resistance to tree diseases, such as fusiform rust in southern pines, is being researched using genetic markers and genomics. There is also a potential for engineering resistance to disease using existing genes.

Projects are underway to engineer resistance to blight in American chestnut and Dutch elm disease in American elm.

Modification of lignin levels and types through genetic engineering is a project that is well on its way in forest trees. These projects are aimed at improving pulping traits or solid wood properties. This will result in a change in wood chemistry and secondary compounds. These changes could affect resistance to insects and diseases or the ability of the trees to respond to adverse environments. These alterations could also accelerate or slow wood decay rates. This would not likely be an issue in a plantation where almost all the wood is harvested but could be an issue if the gene were to escape into native stands.

Lignin is a chemical compound that is an integral part of the cell walls of plants, providing strength. When trees are used for paper production, lignin must be removed from pulp before manufacturing the paper. This extra step is costly both economically and environmentally (Pilate et al. 2002); thus, poplar trees have been genetically-engineered to reduce lignin content (Baucher et al. 1996; Van Doorsselaere et al. 1995). Lignin also has a secondary function as a plant defensive chemical; it reduces the leaf digestibility in insect herbivores and functions as a barrier to some pathogens. By decreasing lignin content in GE trees, a possible outcome could be an increase in insect herbivore pest populations (Van Frankenhuyzen and Beardmore 2004) as well as an increase in disease incidence (Pinçon et al. 2001). However, a small plot study investigating herbivore and pest pressures on trees genetically-engineered for reduced lignin content found no change in insect abundance, a similar variety of insect species within the GE and non-GE plots, and no difference in phytopathogen occurrence (Pilate et al. 2002).

Forest certification

Large amounts of forest land are bought and sold between companies and other institutions every year. If APHIS adopts a conditional approval process and requires data to be provided once large acreages of GE trees are planted, there will need to be some mechanism to monitor long-term tracking of GE trees as they move from one owner to another.

There are different organizations that currently certify forestry operations. Currently there is no one industry standard and often programs compete with each other. Organizations such as Forest Stewardship Council (FSC, http://www.fscus.org), Sustainable Forestry Initiative (SFI, http://www.aboutsfi.org), Pan European Forest Certification (PEFC, http://www.perf.org), and Canadian Forest Certification System (CFCS) are the more prominent. These competing organizations have differing certification standards. In particular some certification programs allow GE trees and others do not. For example the FSC (Forest Stewardship Council) does not allow the planting of any GE trees, even for testing purposes, on certified plantations. SFI has no such restriction. This could cause problems both within the U.S. and between the U.S. and other countries, if certified and non-certified products were to become co-mingled.

Currently no premium is given for products with certification by FSC, SFI, PEFC, CFCS and others. However, more and more companies are looking at purchasing forest products with a "seal" of certification that can have an effect on the price of a resource or product. Increasingly, outlets such as Staples and Home Depot are indicating that they will stock products only that have one or the other seal of approval. Consequently mills that have a seal will be the preferred supplier for these outlets. More and more outlets, producers and forest-based companies are taking the position that they will deal only in goods from certified forest operations.

Currently the number and size of field tests of GE forest trees are small compared to other crops. The current acreages of field tests are small but the duration of the field tests cover multiple years. APHIS currently has allowed a limited number of GE trees in field tests to flower. The number of trees that are field tested and the size of field trials is limited by the ability to monitor for flowering. It is also limited in that few companies or institutions are at the point of producing large numbers of GE trees for testing.

No forest tree product has been deregulated. The possible time frame anticipated for the agency to be approached for deregulation of a forest tree engineered in the United States is within 3 to 7 years. However, entities outside the United States could possibly approach APHIS earlier for deregulation. Worldwide over 210 field trials with GE trees have taken place, mainly on species such as *Eucalyptus*, *Populus*, and *Pinus*. With the exception of China, none have been deregulated. China has reported the commercial release of GE poplar, with approximately 1.4 million insect-resistant trees planted on 300-500 ha[i] (FAO 2004).

Summary

The major difference between forest trees and crop plants is that most of the tree species that are being considered for deregulation and deployment in the United States are not far removed from their wild progenitors. Many can intercross freely with their wild relatives, thus gene flow can occur from plantations into surrounding forests.

There are a limited number of studies that examine how far pollen can move within and from a tree plantation, and there are few studies that have looked at potential gene flow from GE trees in a plantation to trees in native forests; however there are some studies that have looked at the movement of non-GE markers from an established field test. Therefore the best available data APHIS will have to evaluate the potential for transgenes to move into native forests may be gene flow models from other perennial species, like grasses. APHIS will need to evaluate the applicability of these data on a case by case basis, when considering deregulation or conditional approval of GE trees.

For some of the traits that are being engineered into trees, it may not be possible to gather data on the effect of the trait on the environment over many years. A good example would be genes for lignin modification. It will take years to produce such

data. Therefore for some traits, APHIS may need to consider whether particular assumptions can be made without hard data when petitioned for deregulation.

Appendices

Appendix B

Glossary

Abiotic stress. Stress due to non-living, environmental factors such as cold, heat, drought, flooding, salinity, toxic substances, and ultraviolet light.

Adventitious presence, AP. Low-level and intermittent occurrence in commerce of biotechnology-derived material that has not completed all applicable reviews.

Agrobacterium tumefaciens. A bacterium that causes crown gall disease in some plants. The bacterium characteristically infects a wound, and incorporates a piece of its own DNA into the host plant genome, causing the host cell to grow into a tumor-like structure. This DNA-transfer mechanism is commonly exploited in the genetic engineering of plants.

***Agrobacterium tumefaciens*-mediated transformation.** The process of DNA transfer from *Agrobacterium tumefaciens* to plants, that occurs naturally during crown gall disease, and can be used as a method of transformation.

Allele. One of several alternate forms of a gene occupying the same location on the chromosome.

Allelochemical. A chemical produced by a plant of one species that has a detrimental effect on plants of other species.

Allergen. Any substance that causes an allergic reaction.

Antibiotic resistance marker gene. Genes (usually of bacterial origin) used as selection markers in transformation, because their presence allows cell survival in the presence of normally toxic antibiotic agents.

Anti-nutritional compound. A compound in food or animal feed that has a negative impact on nutrition or the absorption of nutrients.

Antisense DNA. The DNA strand complementary (hence "anti") to the mRNA, *i.e.* the non-transcribed strand.

Antisense gene. A gene that produces an mRNA complementary to the transcript of a normal gene, usually constructed by inverting the coding region relative to the promoter. See also **antisense RNA**.

Antisense RNA. An RNA sequence that is complementary to all or part of a functional mRNA molecule, to which it binds, thereby blocking its translation.

Arabidopsis thaliana. A small plant in the mustard (Brassicaceae) family. *Arabidopsis* is used as a model for studying plant genetics.

Backcrossing. Crossing an individual with another organism that is genetically identical to its parent. The offspring of such a cross are referred to as the backcross generation or backcross progeny.

***Bacillus thuringiensis* (Bt).** A common soil bacterium, notable for its ability to produce proteins which are toxic to certain categories of insects. See also ***Cry* proteins**.

Bioaccumulation. The increase in the concentration of a chemical in biological systems over time as compared to the chemical's concentration in the environment.

Biological control agent, biocontrol agent. Any enemy, antagonist, or competitor used to control a plant pest or noxious weed (Plant Pest Act of 2000).

Biolistics. A technique to generate genetically engineered cells, in which DNA-coated microscopic metal particles, usually tungsten or gold, are propelled by various means fast enough to puncture target cells (a "gene gun"). Provided that the cell is not killed, the DNA may be taken up by the cell and incorporated into the cell's genome. *Synonym*: microprojectile bombardment.

Biomagnification. The process that results in the accumulation of a chemical in an organism at higher levels than are found in its food; occurring when a chemical becomes more and more concentrated as it moves up the food chain.

Biotechnology. Techniques based on molecular biology, such as gene manipulation, gene transfer, DNA typing and cloning of plants and animals, for making specific modifications to the genome of an organism.

Breeding. The process of sexual reproduction and production of offspring.

Breeder Seed. Seeds of a particular plant variety maintained by a plant breeder, usually at a very high level of purity, which serves as the source for all subsequent generations of seed production. See also **foundation seed**.

Bt proteins, Bt toxins. See ***Cry* proteins**.

Codex Alimentarius Commission. An international food safety standard setting body (part of the Food and Agriculture Organization of the United Nations) responsible for defining a set of international food standards. The Commission periodically determines, then publishes a list of food ingredients and maximum allowable levels (the *Codex Alimentarius*) deemed to be safe for human consumption.

Coding sequence. That portion of a gene which directly specifies the amino acid sequence of its product. Non-coding sequences of genes include introns and control regions, such as promoters, operators, and terminators.

Competent bacteria. Bacteria able to take up and stably maintain foreign DNA.

Conservation tillage. A broad range of soil tillage systems that leave crop residue on the soil surface, substantially reducing the effects of soil erosion from wind and water.

Constitutive expression. Describing a gene that is expressed (i.e. "turned on") at a relatively constant level in all cells of an organism without regard to cell environmental conditions.

Construct. An engineered piece of DNA designed to be transferred into a cell or tissue. Typically, the construct comprises a gene or genes of interest, a marker gene and appropriate control sequences, often from different organisms, as a single package. A repeatedly used construct may be called a "cassette."

Cross protection. Resistance to one disease that also confers resistance to other diseases.

***Cry* proteins.** A class of crystalline proteins produced by strains of the soil bacterium *Bacillus thuringiensis*, and engineered into crop plants to give resistance against insect pests. These proteins are toxic to certain categories of insects (*e.g.* corn borers, corn rootworms, mosquitoes, black flies, armyworms, tobacco hornworms, some types of beetles, etc.), but are harmless to mammals and most beneficial insects (depending on order). *Synonyms*: delta endotoxins, *Bt* toxins.

Dicot. A flowering plant with two embryonic seed leaves. Examples include oaks, maples, roses, beans, mustards, and cacti. See also **monocot**.

Dietary toxicity. The toxicity of a substance when eaten.

Dietary toxicity tests. Methods used to determine whether and how much a substance is toxic if eaten.

Digestion stability. The ability of a substance to resist degradation by acids and digestive enzymes. A property often associated with allergens.

Disease resistance. The genetically determined ability to prevent the reproduction of a pathogen, thereby allowing the resistant individual to remain healthy. Some

resistances operate by pathogen exclusion, some by preventing pathogen spread, and some by tolerating pathogen toxin.

DNA (deoxyribonucleic acid). A nucleic acid that carries the genetic information of a cell. The structure of DNA is two long chains of chemical building blocks called 'nucleotides,' twisted into a double helix. The order of nucleotides determines hereditary characteristics.

Donor. An organism that provides a gene or gene fragment used in the genetic transformation of another organism, which is called the "recipient."

Down regulation. The action of a DNA sequence or other chemical compound that causes a given gene to express less of the protein that it normally would.

ELISA (enzyme-linked immunosorbent assay). A sensitive assay that is used to detect a specific protein.

Encapsidation. The process by which the genetic material of a virus is enclosed in a protein coat (the capsid).

Endospermatic seed. Seed having an endosperm, the nutritive tissue surrounding the seed embryo.

Epistatic effects. The result of one gene suppressing the effect of a different gene.

Expression. The means by which a gene's information stored in DNA (or RNA in some viruses) is turned into biochemical information such as RNA or protein.

Fecundity. The capacity for producing offspring.

Flanking region. The DNA sequences extending on either side of a specific sequence.

Foundation seed. Seed of a particular plant variety that is produced from breeder seed in order to generate enough quantity for commercial sale. See also **breeder seed**.

Gene. The basic unit of heredity transmitted from generation to generation during sexual or asexual reproduction; an ordered sequence of nucleotide bases comprising of a segment of DNA. A gene contains the sequence of DNA that encodes an individual RNA or protein.

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Gene expression. The process by which a gene produces mRNA and protein, and hence exerts its effect on the phenotype of an organism.

Gene flow. The spread of genes from one population to another, by the movement of individuals, gametes, or spores.

Gene gun. See **biolistics**.

Gene insertion. The incorporation of one or more copies of a gene into a chromosome.

Gene product. A RNA or a protein (*e.g.* an enzyme), the production of which is directed by the corresponding gene.

Gene silencing. Loss of gene expression either through an alteration in the DNA sequence of a structural gene or its regulatory region; or because of interactions between its transcript and other mRNAs present in the cell. See also **antisense RNA**.

Gene splicing. The enzymatic attachment of one gene or gene fragment to another.

Genetic engineering. Modifying the genotype, and hence phenotype, of an organism by genetic transformation.

Genetically engineered organism. An organism that has been modified by genetic engineering.

Gene stacking. The use of plant breeding to combine two or more genetic traits into a single plant variety.

Genetic marker. A gene that is a reliable indicator that a particular organism possesses a specific trait of interest. Markers may be used to select certain individual organisms, *e.g.*, cells that have inherited resistance to an antibiotic will be the only ones in a population that survive an antibiotic treatment.

Genetic transformation. See: transformation.

Genome. All of the hereditary material in a cell, including DNA present in the cell nucleus as well as in other locations, such as plant chloroplasts and mitochondria.

Genomics. The study of the entire genome of an organism, often in comparison to the entire genome of another organism (*i.e.* comparative genomics).

Genotype. The total genetic makeup that an individual receives from its parents.

Halophyte. A plant adapted to living in very salty soils.

Herbicide resistance/tolerance. The ability of a plant to remain unaffected by the application of what would otherwise be a toxic dose of a herbicide.

Heterologous encapsidation. The phenomenon where the coat protein of one virus is able to encapsidate (*i.e.* package) the nucleic acid of a different virus. See also *encapsidation.*

High dose. Twenty-five times the dose necessary to kill all susceptible insects.

Homologous recombination. The physical exchange of genetic material between two closely-related or similar genetic sequences.

Horizontal gene transfer. The transfer of genetic material from one organism (the donor) to another organism (the recipient) which is not sexually compatible with the donor.

Hybrid. The offspring of two genetically dissimilar plant varieties.

Industrial Gene. A gene whose effect on an organism or product is primarily of industrial use, as opposed to an agricultural or nutritional purpose.

Inserted gene. A piece of DNA that has been inserted into an organism using recombinant DNA technology and that contains sufficient heritable information to direct the production of a particular gene product in that living organism.

Instar. A stage in the development of an insect between two successive molts.

Interfertile. Two plants or groups of plants capable of interbreeding and producing offspring.

Introgression. The introduction of genes from one species into the gene pool of another, via sexual crossing. The process begins with hybridization between the two species, followed by repeated backcrossing to one of the parent species.

LD_{50}. Median lethal dose, or dose needed to kill 50 percent of a population of organisms.

$LD_{99.9}$. The dose that kills 99.9 percent of a population of organisms.

Macronutrient. An element or compound required in a relatively high proportion for normal growth and development. In animals, the term refers to carbohydrates, fats, and proteins. In plants, the term refers to elements such as nitrogen, phosphorous, and potassium.

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Marker gene. A gene of known function or known location that is inherited in Mendelian fashion and facilitates the study of inheritance of a trait or a linked gene.

Marker-assisted selection. The use of DNA markers to select the organisms that possess genes for a particular phenotype desired for subsequent breeding/propagation. This allows selection without having to screen for the performance trait itself, which may be difficult.

Monocot. A flowering plant with only one embryonic seed leaf. Examples include grasses, irises, lilies, and onions. See also **dicot**.

Mutagen. A chemical or dose of radioactivity capable of producing a genetic mutation, by causing changes in the DNA of living organisms.

Mutagenesis. Induction of heritable change(s) in the genetic constitution of a cell through alterations to its DNA, most often via treatments with chemicals or ionizing radiation.

Non-homologous recombination. The physical exchange of genetic material between two unrelated or dissimilar genetic sequences.

Non-propagative. See **non-viable plant material**.

Non-viable plant material. Broadly speaking, all plant tissues other than viable propagules, such as seeds, bulbs, tubers, etc. That is, all tissues dead or alive which cannot directly result in the propagation of a new plant.

Noxious weed. Any plant or plant product that can directly or indirectly injure or cause damage to crops (including nursery stock or plant products), livestock, poultry, or other interests of agriculture, irrigation, navigation, the natural resources of the United States, the public health, or the environment (Plant Protection Act of 2000).

Obligate parasite. A parasite that cannot live independently of its host.

Open reading frame (ORF). A sequence of nucleotides in a DNA molecule that has the potential to encode a peptide or protein. The term is generally applied to sequences of DNA for which no function has yet been determined. The number of ORFs provides an estimate of the number of genes transcribed from the DNA sequence.

Osmoprotectant. Compounds accumulated by plants to reduce water stress, such as caused by drought.

Outcrossing. The tendency of a plant species to produce offspring that result from the mating of two different individual plants. Compare to **self-pollinated**.

Pathogen-derived resistance. Resistance to a disease conferred by something derived from the disease-causing agent itself. Example: coat protein mediated viral resistance.

Performance-based standards. A form of regulation in which required *outcomes* are defined by regulation, but the actions or conditions necessary to attain the outcomes are not defined by regulation. Contrast with a *prescriptive* standard, which specifies actions or conditions that must be followed to attain the required outcome.

Phenotype. The appearance or other externally manifested characteristics of an organism, resulting from the interaction of its genetic constitution with the environment.

Phytoremediation. The use of plants to remove or reduce pollutants in soil by production of compounds that stimulate their degradation or by uptake through roots and accumulation in plant tissues.

Plant incorporated protectants (PIPs). The term PIP was designated by the Environmental Protection Agency (EPA) to describe substances that are incorporated into plants to protect them from damage caused by insect pests and diseases. PIPs are defined as the pesticidal substance that is produced in a plant and the genetic material necessary for the production of those substances (Federal Register, Volume 66, No. 139, July 19, 2001).

Plant pest. Any living stage (including active and dormant forms) of insects, mites, nematodes, slugs, snails, protozoa, or other invertebrate animals, bacteria, fungi, other parasitic plants or reproductive parts thereof; viruses; or any organisms similar to or allied with any of the foregoing; or any infectious agents or substances, which can directly or indirectly injure or cause disease or damage in or to any plants or parts thereof, or any processed, manufactured, or other products of plants (7 CFR 340.1). Vertebrates aren't considered plant pests (e.g. starlings, voles)?

Pleiotropic effects. A phenomenon in which a single genetic alteration affects multiple phenotypic characteristics, such as a single gene affecting flowering, leaf shape, and growth rate.

Deleted: h

Post-translational modification. The addition of specific chemical residues to a protein after it has been translated. Common residues are phosphate groups (phosphorylation) and sugars (glycosylation).

Primer. A short, single-stranded piece of DNA that, when annealed to a long template of single-stranded DNA, provides a doubled-stranded structure from which DNA polymerase will synthesize a new DNA strand to produce a duplex molecule.

Proline. An amino acid.

Promoter. A region of DNA location upstream of a gene that controls to what degree, where, and/or when a gene is expressed.

Propagules. A propagule is any part of a plant that can be detached from the organism and propagated in order for it to grow into a new plant in a new environment.

Proteomics. An approach that seeks to identify and characterize complete sets of protein, and protein-protein interactions in a given species.

Protoxin. A precursor of a toxin that requires additional modification before acquiring its toxic properties.

Pyramid. In the context of PIPs, the presence of multiple resistance genes that are targeting the same pests with possible overlap in the mode of action. For example, a corn or cotton plant containing a Cry1A protein and a Cry2A protein active against the same lepidopteran pest such as the European corn borer or tobacco budworm is termed a "pyramid."

Recombinant DNA organism. An organism in which the genetic material has been changed through gene splicing techniques, using recombinant DNA and direct injection of nucleic acid into cells or organelles.

Recombination. The physical exchange of genetic material between two genetic sequences that produces new combinations of genetic information. See also *homologous recombination* and *non-homologous recombination.*

Refuges. Non-Bt host plants that are managed to provide sufficient susceptible adult insects to mate with potentially Bt-resistant adult insects to decrease the number of resistant insects and dilute the frequency of resistance genes. This is a very specific definition—a refuge is more generically defined as the part of a habitat where an organism can avoid or escape a mortality agent

Regulated article. That which is subject to APHIS regulation under 7 C.F.R. Part 340.

Rhizosphere. The root surface together with that region of the surrounding soil in which the microbial population is affected by the presence of the root and root exudates.

Risk analysis. A process consisting of three components, risk assessment, risk management, and risk communication, performed to understand the nature of unwanted, negative consequences to human and animal health, or the environment.

Risk assessment. A scientifically based process consisting of the following steps: (i) hazard identification; (ii) hazard characterization; (iii) exposure assessment; and iv) risk characterization.

Risk communication. The interactive exchange of information and opinions throughout the risk analysis process concerning hazards and risks, risk-related factors, and risk perceptions, among risk assessors, risk managers, consumers, industry, the academic community, and other interested parties, including the explanation of risk assessment findings and the basis of risk management decisions.

Risk management. The process, distinct from risk assessment, of weighing policy alternatives, in consultation with all interested parties, considering risk assessment and other factors relevant for the health protection of consumers and for the promotion of fair trade practices, and, if needed, selecting appropriate prevention and control options.

RNA (ribonucleic acid). A nucleic acid composed of a long, often single-stranded chain of chemical building blocks called 'nucleotides.' RNA has multiple functions in the process of translating information stored in genes (DNA) into proteins.

Rotation. The number of years required to establish and grow trees to a specified size, product, or condition of maturity. A pine rotation may range from as short as 20 years for pulpwood to more than 60 years for sawtimber. Full rotation is the total time from planting to harvest. Half rotation would be approximately half the time to reach maturity or harvest.

Secondary metabolism. The production by living organisms of substances not essential for primary metabolic functions or physiology. Their role is associated with interaction with the environment, for example for defense, as elicitors, or as attractants. Some of these have useful pharmacological or nutritional properties, while others are toxic.

Self-pollinated. The tendency of a plant species to produce offspring that result from a flower pollinating itself. Compare to **outcrossing**.

Stratification. Seed stratification is a time for moist seeds to sit preparing to germinate. Many seeds require a cold stratification period in order to germinate while some require a warm stratification prior to the cold stratification. Cold stratification is usually at the temperature of 34-41 degrees F., while warm stratification is at the temperature of 68-86 degrees F.

Stress tolerance gene. A gene which confers upon a plant an increased ability to withstand an environmental stress, such as drought, temperature extremes, or soil salinity.

Synergy. The interaction of two or more agents so that their combined effect is greater than the sum of their individual effects.

Trait. A characteristic of an organism that manifests itself in the phenotype. Traits may be the result of a single gene or may be polygenic, resulting from the simultaneous expression of more than one gene.

Transcription. The process by which a messenger RNA (mRNA) is created from the nucleotide sequence of a gene (DNA).

Transencapsidation. See **heterologous encapsidation.**

Transformant. A cell or organism that has been genetically altered through the integration of a transgene(s). A “primary” transformant is the first generation following the transformation event.

Transformation. The uptake and integration of DNA in a cell’s genome, in which the introduced DNA is intended to change the phenotype of the recipient organism in a predictable manner.

Transgene. A gene that is inserted into the genome of a cell via gene splicing techniques.

Transgenic. An organism whose genome has been modified via the stable incorporation of a piece of foreign DNA (a transgene).

Translation. The process by which the sequence of nucleotides in a messenger RNA (mRNA) directs the sequence of amino acids in a new protein during protein synthesis.

Trophic. Relating to the feeding habits or food chain of different organisms in a food chain.

Up regulate. The action of a DNA sequence or other chemical compound that causes a given gene to express more of the protein than it normally would.

Vector. The agent, such as a plasmid, used by researchers to carry new genes into cells.

Viral coat protein. A protein produced by a virus that forms a protective layer, or *capsid*, around the genetic material of the virus.

Volunteer. Plants resulting from crop seed that escapes harvest and remains in the field until subsequent seasons, where it germinates along with the succeeding crop.

Weediness. The ability of a plant to colonize a disturbed habitat and compete with cultivated species.

Appendix H

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Biotech crops expressing Bt toxins have been used for a decade, and have allowed farmers all over the world to use reduced amounts of insecticides. The ubiquity of such crops has also allowed the Bt toxin class to become the most widely used in the world. What does this bode for regulation and safety assessment, now that more Bt crops are being developed, and will soon be released in the market?

Jorg Romeis and colleagues of the Swiss Federal Research Station for Agroecology and Agriculture offer their perspectives on "Transgenic Crops Expressing Bacillus thuringiensis Toxins and Biological Control." Their article appears in the latest issue of Nature Biotechnology.

The researchers look at studies already conducted on the safety of Bt crops by reviewing the results of those performed in the laboratory or glasshouse environment; experiments performed to assess the effects of the toxin on predators and parasitoids; and those conducted in a field or semi-field situation. They conclude that semi-field studies are likely to be the most accurate, as they offer some control over the environment, while at the same time keeping plants and predators in close-to-field conditions. They also perceive field studies to be less accurate, as such conditions are harder to keep under control, and their results thus cannot be interpreted conclusively.

In the studies reviewed, Bt plants have been shown to have no direct adverse effects on non-target species. Such data, however, cannot predict the effects of other insecticidal proteins in future GM crops. Thus, the writers recommend that: 1) researchers take great care in designing experiments, such that their results will be relevant for field situations; 2) insecticide treatments should be considered as the baseline for risk assessment; and 3) standards and protocols should be established regarding issues of non-target effects of Bt crops.

Subscribers to Nature Biotechnology can read the complete article at http://dx.doi.org/10.1038/nbt1180.

Reviewer Report 3

Introduction

Following the USDA/APHIS 'Charge to Peer Reviewers of Influential Scientific Information Contained in the APHIS BRS Draft Programmatic Environmental Impact Statement, this review report addresses the following parameters: Completeness, Currency, Accuracy, Uncertainty, Objectivity, and Clarity and ends with a final recommendation.

Completeness

All potential factors and relevant issues known to me and of relevance to the statement have been considered in the document that I was provided for review.

Currency

Although the information presented in the document is in general current, the references to the introductory section (pages 1-5 inclusive) tend to be older textbooks and reviews (Allard, 1964; Bidwell, 1974; Brady, 1974; Esau, 1977, and others). While it might not be expected that such introductory information would have changed significantly in recent decades, the authors are advised to consult more recent texts and update the introductory information as necessary to reflect current thinking.

Accuracy

Page 3, second paragraph under Physical Environment. Change from "roots release organic acids" to "roots release organic compounds." Roots are known to release many compounds that impact nutrient availability and soil development.

Page 4, first paragraph. Add a citation after the final sentence, which relates transpirational water loss over a season to several inches of rainfall.

Page 5, first paragraph, next to last sentence – Rather than stating that altered photosynthetic efficiency may require "additional" water and fertilizer and "increase" overall environmental fitness, consider emphasizing that these factors may be altered in either direction—increased or decreased. I know of no evidence that altered photosynthetic efficiency works in only one direction—to increase.

Page 5, last paragraph – State that the purpose is either unknown or not apparently related to growth. In the absence of data, it is not advisable to state definitively that there is no relationship to growth.

Page 8, discussion of flower structure and pollination strategies. This section does not give an accurate overview of pollination as it relates to crop plants. There is discussion of self-pollinating crops with perfect flowers and cross-pollinating crops with imperfect flowers (corn).

Missing is discussion of crop plants that have perfect flowers yet cross pollinate to a significant extent. Alfalfa is an example, as is canola (APHIS' own website describes the latter as about 35% cross-pollinating). I suggest that attention be given to the second paragraph on this page. Indicate that some important crop plants have perfect flowers yet outcross. There is abundant information on this phenomenon in the plant genetics and breeding literature.

Page 9, third paragraph – Some seeds can be stored for more than 100 years and remain viable. The phrase "several years" vastly understates this fact.

Page 9, fourth paragraph – Proteins, carbohydrates, and lipids are indeed stored in parts of plants other than seeds. Witness the potato or the watermelon.

Page 13, third paragraph – Replace "work in a new place" with "work in progeny." This keeps the focus on the scientific level.

Page 21, second paragraph, first sentence – The crop could also become established in an unmanaged habitat if crop land is allowed to return to an unmanaged state. Farms are abandoned quite often in some parts of the country, and when this happens, there is no need for the physical transport of seeds or other plant parts.

Page 22, first paragraph. It would be helpful if the authors would state whether or not standard growing conditions have been established by AOSCA for all crop seeds or just some of them. This point is ambiguous in the text.

Page 31, second paragraph. All methods of genetic modification not only have the *potential* to alter a plant's genetic makeup. They are designed to do so. This should be stated explicitly. This paragraph also confuses the issue of what can and cannot be detected with the issue of what is intentional and what is unintentional. This confusion is unfortunate, because it implies (last sentence) that intentional changes are easier to identify and that unintentional changes are more difficult to identify. In reality, these are unlinked phenomena. Often the desired change is exceptionally difficult and costly to measure (elevated levels of some functional food chemical, for example), while unintentional changes are easy to spot as gross alterations in plant form. The authors should make this point explicit.

Page 33, second paragraph. In context, the second sentence should state "the allergic potential of every protein."

Page 47 and 48, bulleted list of issues. I believe it inaccurate to omit the potential effects of drought or saline tolerant crop plants on pathogens, including viruses. In theory, these could be as significant as effects on insects and weediness.

Page 50, first paragraph. Grafting is a form of mechanical transmission. It would be better to state "mechanical transmission such as grafting" than "mechanical transmission and grafting."

Uncertainty

Page 1, paragraph 2, sentence as follows: "The agency recognizes, however, that in a scientific approach, it cannot make general conclusions about the safety of GE organisms." I find this sentence to be grammatically awkward and to be inadequate in dealing with the uncertainty surrounding GE organisms. The emphasis should be on the state of current scientific knowledge and the extent to which it allows specific and more generalized conclusions to be/not be made.

Page 26, fourth paragraph. In the absence of data on persistence of nonmodified DNA in soil, the data on persistence of GE DNA are, as the writer points out, largely meaningless—at least from the standpoint of whether or not GE DNA is any more persistent than unmodified DNA. On the other hand, if the question is whether or not plant DNA persists for up to two years in the soil, then the answer is yes. It might be helpful to explicitly separate these two issues as they are dealt with here.

Page 27, fourth paragraph. To state that "soil- and plant-associated microbial communities and their interactions are not completely understood" is to paint a very bright picture indeed. Most microbial ecologists would write in terms of very poor understanding. We are nowhere close to complete in our understanding of these communities.

Page 29, third paragraph. It would be helpful if the writers would draw two conclusions from this paragraph. The first is that the potential for generalized HGT in soil is vanishingly low, and the second is that the potential for HGT of a specific, small segment of a genome is vanishingly smaller still. It would be logical to invert the final sentence of this paragraph (top, page 30) to indicate what is significantly smaller rather than what is significantly greater.

Objectivity

Page 2, last paragraph. The text here should be revised to indicate that there are thousands of associations, many beneficial and many detrimental. As written, there is a subtle bias that most such interactions are detrimental (hundreds of negative), but only a few (certain) are positive. Also, please italicize the genus *Rhizobium* to be consistent with the style on page 5.

Page 3, first paragraph. Emphasize that GE traits could impact both positive associations and negative associations. There is undue emphasis here (see comment above) on negative associations.

Page 6, first paragraph – Altering secondary metabolite production may also have negative effects—it is not just an issue of more fit and higher value. These changes can work in either direction.

Page 9, second paragraph – In reality, no crop plant "perfectly" holds its seed until harvest. I suggest changing the last sentence of this paragraph as follows: "No crop does this perfectly, and thus some grain is inevitably lost prior to harvest."

Page 11, first paragraph, last sentence – It is unclear why the requirement for science based policies and regulations merits special emphasis (the word "especially") when GE varieties are traded. This sentence implies a kind of bias that allows decisions to drift away from science so long as the issue is unrelated to GE varieties—yet be strictly held to science when GE varieties are considered. I believe that these agencies have a commitment to science-based decisions, regardless of the details of the kind of seeds.

Page 19, first paragraph, last sentence. Did Keeler imply that GE crops are as likely or no more likely to be a source, Etc? The construction of the sentence leads me to believe that the intent was one of no more likely, but the words state otherwise. This is an issue of accurate representation of a citation.

Page 48, second paragraph. The second sentence should indicate that nitrogen is not the only nutrient with this potentially beneficial effect.

Page 48, third paragraph. The statement that "such significant sensitivity toward even minute changes" seems overly dramatic relative to the much more general and tempered verbiage earlier in this section. If even tiny changes in nutrients content have such dramatic effects, the authors might want to insert a series of citations documenting the effect.

Page 61, third paragraph. Consider beginning the first sentence as follows: "Extensive reliance….". I believe that this qualifier is valid—the concern is more with vast acreages treated with the same herbicide than with small spots.

Page 62, second paragraph. Transfer of herbicide resistance genes into related weed species would be of concern not only to farmers, but to many others—homeowners, natural resource managers, municipalities, Etc.

Clarity

Throughout the manuscript – The authors should adopt a standard convention for citing references. The current version of the manuscript sends mixed signals—sometimes multiauthored works are cited as first author et al, other times multiple names are listed. Reference sometimes are cited as "Jones (1999) showed," and other times in the form "Jones (Jones, 1999) showed." These are issues of careful editing so as to convey the impression of careful content.

Page 1, paragraph 5: "these features control." Consider replacing the word control with a phrase such as "take into account." I am not sure that regulations ever are able to truly control environmental interactions.

Page 2, paragraph 2: "compete so effectively with crop plants to cause economic harm." Clarify by changing to "compete so effectively with crop plants that they cause economic harm."

Page 3, first paragraph under Physical Environment: "A wild plant which is more able to exploit limited resources may be more likely to survive and reproduce, while a highly efficient crop plant may be more likely to produce large yields." The reason for including this sentence is unclear. Don't large yields more or less equate with survival and reproduction of all plant species?

Page 4, last paragraph, next to last sentence, typo: "sunlight may grow (not grown) successfully. See also page 5, second paragraph, third line, typo: "absorbed by plant (not plants) roots."

Page 7, third paragraph: "When seed is used to reproduce plants, it has genetic and other quality characteristics that differentiate it from grain." I suggest that this sentence be rewritten to emphasize that the characteristics are constant. The issue is not that these characteristics sometimes are absent; rather, it is that depending on the use, these characteristics can be important or not important.

Page 7, last paragraph – replace "man" with people. Ditto for page 9, paragraph 5.

Page 9, sixth paragraph – Include a citation to support the statement that starch and hemicellulose are the two main forms of carbohydrates stored in seeds.

Page 10, third paragraph – I do not believe that transforming seeds for these purposes always involves (as is implied here) genes not currently present in plants. This may be the case, but there are other instances of gene transfer from one plant species to another.

Page 12, fourth paragraph: "Their use is supported by decades of industrial experience." Consider changing this sentence to: "Their use is supported by decades of experience by plant breeders and the seed industry."

Page 14, second paragraph. Replace "giants" with "wonders." The former word emphasizes size, while the issue is one of flexibility and adaptability. Delete the word "anti-disease" from the sentence at the very end of this paragraph. The phrase is clear without this cumbersome modifier.

Page 17, second paragraph – replace the phrase "wild free-living" with "noncultivated." This enhances clarity and follows parallel construction.

Page 17, third paragraph: Also several definitions (insert the word of) "invasive….

Page 17, third paragraph – "Transformative." Avoid this term if possible in discussing environments—many readers could confuse thae common definition with the scientific definition involving DNA transfer. If the word must be retained, then define the context explicitly in this paragraph.

Page 17, fourth paragraph – Consider replacing "will be considered a weed in some particular instance" with "could be considered a weed under some particular conditions." This adds clarity.

Page 19, second paragraph: Should be Williamson (1993) at the beginning of the sentence with no separate citation at the end of the sentence.

Page 19, second paragraph – 10:10 rule. I am not sure if this rule should be presented as a model and predictor. The connotation here is one of a scientific "rule of thumb," yet words such as model and predictor imply a more detailed mathematical modeling exercise. The authors should clearly differentiate between these two possibilities.

Page 20, second paragraph: – Pea lectin, not pea leptin. This is an important difference.

Page 20, third paragraph – Replace "bred in conventionally" with "introduced by conventional breeding." The former is a jargonish phrase.

Page 21, heading in middle of the page should read: "Gene Flow via Hybridization with Wild Relatives." There also seems to be information missing (or some sort of garbling) in the fifth line of this section (and rapeseed….).

Page 23, first paragraph . Consider replacing "years of cultivation" with the more relevant "years of experience."

Page 24, third paragraph, typo: It should read, "If the transgene in question….."

Page 25, last sentence of narrative on page, typo: Should be rate of the product's entry. Also page 26, last two lines, typos: Should read "In cases where biomagnifications and bioaccumulation are likely."

Page 27, third paragraph: Should read "GE glufonisate- and glyphosate-tolerant crops." This missing hyphen is essential for clarity.

Page 28, second paragraph. I am not sure of the need for the last clause in the final sentence of this paragraph. Other than microbial and invertebrate communities in soil, what other kinds of organisms could be impacted? Could this clause simply be deleted?

Page 29, first paragraph: Insert the word conditions after the word natural in the fourth sentence from the end of this paragraph.

Page 30, fourth paragraph. There is unnecessary ambiguity in this paragraph and also a section that is garbled ("and it is thus far unclear that introducing"). An objective reading of the science as summarized in this manuscript would, I believe, allow for a stronger statement to be made—the potential for impact of GE technology on soil and its components is vanishingly small compared to the impact of traditional agriculture on these components.

Page 31, fourth paragraph, grammatical error that obscures the meaning of the sentence: More the phrase "into crop plants" from the end of the first sentence to a position right after "to move genes." Otherwise, this phrase gets lost in a whole lot of intervening verbiage.

Page 31, third paragraph – It is unclear if "new varieties of whole foods" means foods from new crop varieties (lettuce from a new variety, for example) or new kinds of whole foods (a fresh salad containing a new mixture of different greens). I think the former is meant, but the wording leads to ambiguity.

Page 32, second paragraph, last sentence. To more accurately reflect the activities of EPA and FDA, consider: "that help ensue that potential adverse effects of GE crop plants are adequately assessed."

Page 33, third paragraph. This paragraph is exceptionally convoluted and difficult to understand, especially the first sentence—which is filled with references and abbreviations. It would be helpful if it could be broken down into several simpler sentences. Consider modifying the second sentence as follows: "The scientists participating in this effort created a decision tree, termed ILSI/IFBC, for the assessment of potential allergenicity." This should make it clear that the ILSI/IFBC report referenced in the next paragraph is the same as the report discussed in this paragraph.

Page 34, section (d). Insert a comma between processing and should for clarity.

Page 35, first paragraph. It would be helpful if the authors indicated whether or not this new decision tree has been accepted. I believe that this may be the intent of the last sentence in paragraph 3 on this page, but the reader tends to get lost in the verbiage related to the various reports and recommendations. If there is any question about understandability, I suggest that wording be revised to be explicit.

Page 36, second line in first paragraph, typo: "heat and other food processing conditions, is." Also, third paragraph: "individuals who have previously"

Page 39, first paragraph. For the sake of clarity, please insert "the primary" between "in" and "published literature" in the second sentence.

Page 41, second paragraph, next to last sentence, typo: should be "whether there have been any unintended changes."

Page 42, second paragraph. The authors should consider using headings or some other mechanism to let the reader know that the issue of vegetative insecticidal proteins will be considered later (much later, actually—on page 46). Because there is so much verbiage on crystal proteins, I was quite sure that the authors had forgotten about the other type of proteins. It would help if the reader would be informed that the second type of proteins will be dealt with, even if much later in the manuscript.

Page 43, second paragraph. Consider qualifying the first sentence by stating that continuous production would have to be on extensive acreages. This is an important qualification from a practical viewpoint.

Page 43, third paragraph. After the word refuge, it might be helpful to insert a parenthetical statement alerting the reader to the fact that this term will be defined in context and dealt with later in the manuscript.

Page 44, fourth paragraph. Consider stating that "Structured refuges are areas containing non-Bt host plants."

Page 45, last paragraph, typo: "the grower's responsibility."

Page 46, third paragraph. Consider inserting "the potential of" before "accidential aquatic exposure." In addition, the final sentence in this paragraph leaves the reader wondering at the significance of APHIS assessment under the Migratory Bird Act and the Threatened and Endangered Species Act. Why is this information important for the reader of the present document? Also at the very end of the page, it should be "The objectives of much….".

Page 48, first sentence. Replace jargony phrase with "A primary rationale for development of stress tolerance…." Towards the middle of this paragraph: It is unclear to me what is meant by "receiving environment." Please make this explicit.

Page 49, Conclusion. Indicate the date as of which the statistics on permits are valid. There is also a typo in this paragraph (should be "continues to move from").

Page 50, fourth paragraph. Plant expression of viral genes has another advantage—it overcomes the time and labor problem identified in the paragraph above this one.

Page 51, first paragraph. Does 900 virus resistant plants literally mean 900 individuals or does it mean 900 different tests?

Pages 52 and 53. Heterologous encapsidation can be very difficult to understand and complicated to describe. I believe that there are several ways in which this section could be made more understandable. One is to rearrange the three scenarios and orient the narrative towards the insect. Scenario 1 (Scenario 2 in the narrative under review) would thus read: Virus does not move because no insect is available. Scenario 2 (Scenario 1 in the narrative under review) would thus read: Insect carries the heterologously encapsidated virus to a nonhost plant. Scenario 3 would read: Insect carries the heterologously encapsidated virus to a host plant. To me, at least, it is easier to think forward from insect to plant than backward from plant to insect. This structure also simplifies the narrative at the top of page 54, which is Byzantine as currently written.

In this general section, also consider using phrases such as "virus is transmitted to" rather than "virus is inoculated onto." Great attention needs to be given to this section if the reader is to truly comprehend heterologous encapsidation and its potential significance.

Page 54, last paragraph. Should be carrot mottled dwarf.

Page 55, second paragraph, typo: Period missing at end of final sentence. Also, third from last sentence, bottom of this page, consider "sequence similarity between the two viruses." Also, last sentence this page, consider "structural similarity between the polynucleotides."

Page 56, second paragraph. Who is the "their" referred to by Rubio and colleagues? Are the levels of recombination from Rubio's own experiments or from the experiments of Green and Allison and/or Borja et al.? The sentence structure does not make this point clear.

Page 56, third heading: "more viruses infect the same plant." And page 57, fourth paragraph: "given what is known about their ability."

Page 60, third paragraph, sentence beginning "Although with concern…." is garbled.

Page 62, the two footnotes in the middle of the page appear to be orphaned—antecedents not clear.

Page 67, second paragraph. I suggest that the authors point out here that life span is another major difference (this topic is amply covered in previous pages—but it is not emphasized here in the summary.)

Biological Control addendum, page 1, paragraph 1, sentence reading: "As practiced by trained professionals, biological control is a highly developed process." The purpose of this sentence within the context of this section is unclear to me. I recommend that the authors include a statement to the effect that specialized knowledge and training are needed by those actually implementing biological control. Words about "a highly developed process" can, I believe, be deleted.

Biological Control addendum, Conclusions. I do not understand the rationale behind the description of biological control as an "ethical" pest management tactic. This adjective does not appear elsewhere in the document, and its inclusion here seems awkward. I cannot imagine circumstances under which unethical pest management would be practiced.

Conclusion

The Charge to Peer Reviewers referred to above, requests the peer reviewers to explicitly answer the following question: **Does this scientific information presented accurately and objectively provide non-expert readers with a broad base of knowledge to understand the aspect of the biological and physical environment that is likely to be affected by the regulations currently administered by APHIS BRS and by possible changes of those regulations?**

After careful review of the document and after having used the criteria outlined above, I believe that the scientific information is presented accurately and objectively for non-expert readers, provided that revisions are made (as detailed above) to address a small number of specific weaknesses. Thus my response to the question in the charge to peer reviewers is: "(2) Yes, but only after revisions have been made to address specific weaknesses.

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Review Report 4

I followed the instructions and format provided to me in the Charge to Peer Reviewers. I start off with an analysis according to the six criteria of the review, Completeness, Currency, Accuracy, Uncertainty, Objectivity, and Clarity (Sections 1-6 of this report). I make a brief comment on a

supplemental text received (Section 7). I end with an overall assessment of Chapter 4.1 (Section 8).

1 Completeness

1.1 Much of the discussion on gene flow centers on gene flow between GE crops and their wild relatives. In reality, in the U.S. at least, this is a minor concern, except if one deals with crops such as sunflower, strawberries, tobacco, blueberries, and a few others. The major issue of gene flow affecting GE crops in the U.S. is between GE varieties, on the one hand, and classically bred varieties, whether in conventional agricultural systems or other systems, such as organic agriculture. This is not dealt with in this text.

1.2 Page 50: "GE plants engineered for stress tolerance show an increase in nutrient content, either nitrogen or phosphorus, through either an accumulation of nitrogen-based osmoprotectants during drought and salinity or an increased nutrient use efficiency."

I am surprised that the topic of nutrient status and disease susceptibility is not discussed here. This is a well-known phenomenon: e.g.,

Huber DM, Watson RD. 1974. Nitrogen form and plant disease. Annual Review of Phytopathology 12: 139-155

1.3 Page 53: "Therefore, the focus of this section will be GE plants expressing VCP's or VCP genes from RNA viruses. "One of the issues with the current regulatory system is that it seems to be running behind technological developments. Therefore, I would suggest that this document be more proactive and discuss potential issues with alternative GE mechanisms for virus resistance.

1.4 Page 62-63: "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."

Further information is available at:

http://www.gmsciencedebate.org.uk/topics/forum/0051.htm

http://ipm.missouri.edu/ipcm/archives/v16n7/ipmltr1.htm

1.5 Page 63: "GE crop plants producing pharmaceuticals and vaccines"
This statement is insufficient especially for a document that should be forward-looking and address future applications of genetic engineering. It does not take into account the different steps prior to commercial production, not the steps following harvest of the commercial

production up until processing, all of which represent potential for commingling of the PMP and food or feed production chains.

2 Currency

This text shows a tendency of not using the latest information, especially from the peer-reviewed literature. I have listed references in the relevant sections. I would add here one general reference:

Snow, A.A., D.A. Andow, P. Gepts, E.M. Hallerman, A. Power, J.M. Tiedje, and L.L. Wolfenbarger. 2004. Genetically engineered organisms and the environment: Current status and recommendations. Ecological Society of America Position Paper. [Online]. Available from Ecological Society of America http://www.esa.org/pao/esaPositions/Papers/geo_position.htm. And Ecol. Applic. 15: 377404 (2005).

3 Accuracy

3.1 Page 1: "The system of safeguards and controls imposed by APHIS on all GE organisms within the scope of authority has been highly effective in ensuring that releases are conducted in accordance with the conditions set by APHIS..."

This is an overstatement. Located in the introduction of this chapter, it sets the tone for everything that comes after it. If regulations have worked, then why should they be updated? Instead, APHIS should quantify this assertion. The Prodigene case is the most prominent case that made it into the public eye where regulations were not followed. Granted, the USDA was able to address the problem. However, how many other, perhaps minor cases, have not been publicized? To quantify this statement, APHIS should provide information on incidents that put into question the regulations, their nature and frequency.

3.2 Page 2: Paragraph on plants

This paragraph misses a statement about plant reproduction, not only one of the most important if not THE most important function in any organism but also a trait which can also be modified by genetic engineering and underlies one of the environmental concerns, namely gene flow by pollen and seed. Although discussed later, it seems strange not to even mention plant reproduction in this introduction.

The last sentence of this paragraph, by its omission of plant breeding, suggests that only genetic engineering can accomplish the goals mentioned, namely "can alter the value of a plant to humans and may also affect one or more of the physical and biological interactions between plants and their environment." Breeding for altered composition (e.g., canola) or disease and pest resistance have been successfully accomplished by classical breeding.

3.3 Page 5: Physiological environment

"Although breeders have been trying to alter photosynthetic efficiency for many years, the complexity and number of genes that must be expressed to coordinate efficient photosynthesis has limited breeders' ability to significantly improve photosynthetic efficiency."

This is an incomplete assessment. Plant breeders have been able to improve yield, which, by definition, is genetically more complex than photosynthetic efficiency! There are other reasons why progress has not been achieved: a) the inherent inefficiency of ribulose-bisphosphate carboxylase/oxygenase; and b) phenomena associated with source-sink partitioning. See, for example:

Richards, R.A. 2000. Selectable traits to increase crop photosynthesis and yield of grain crops. Journal of Experimental Botany 51:447-458.

3.4 Page 8: "Crop plants with perfect (complete) flowers are largely self-pollinated, and the majority of crop plants are self-pollinating. It is possible for pollen from another plant to cause fertilization in these self-pollinating plants, but the probability is very low because pollination typically occurs even before the flower petals open"

It is true that some of the major crops are self-pollinating: e.g., rice, wheat, soybean, etc. There are, however, major crops that are either cross-pollinating: e.g., maize, rapeseed, or vegetatively reproduced: e.g., sugarcane, potato. Furthermore, the text creates the impression of a simple situation where crops are either cross-pollinated or self-pollinated, with few intermediates. This is far from being the case. Self-pollinated crops do outcross, although not at the same frequency as outcrossing species. This lower level of outcrossing is nevertheless sufficient to lead to the appearance of noxious weeds: e.g., rice (see below for references), or modify the distribution of genetic diversity between populations and within a crop species' genome.

References for rice:

Arrieta-Espinoza, G., E. Sanchez, S. Vargas, J. Lobo, T. Quesada, and A.M. Espinoza. 2005. The weedy rice complex in Costa Rica. I. Morphological study of relationships between commercial rice varieties, wild Oryza relatives and weedy types. Genetic Resources and Crop Evolution 52:575-587.

Gealy, D.R., T.H. Tai, and C.H. Sneller. 2002. Identification of red rice, rice, and hybrid populations using microsatellite markers. Weed Science 50:333-339.

Gealy, D.R., D.H. Mitten, and J.N. Rutger. 2003. Gene flow between red rice (Oryza sativa) and herbicide-resistant rice (O-sativa): Implications for weed management. Weed Technology 17:627-645.

Suh, H.S., Y.I. Sato, and H. Morishima. 1997. Genetic characterization of weedy rice (Oryza sativa L.) based on morpho-physiology, isozymes and RAPD markers. TAG Theoretical and Applied Genetics 94:316-321.

Zhang, N.Y., S. Linscombe, and J. Oard. 2003. Out-crossing frequency and genetic analysis of hybrids between transgenic glufosinate herbicide-resistant rice and the weed, red rice. Euphytica 130:35-45.

References for bean:

Papa, R., and P. Gepts. 2003. Asymmetry of gene flow and differential geographical structure of molecular diversity in wild and domesticated common bean (Phaseolus vulgaris L.) from Mesoamerica. Theor. Appl. Genet. 106:239-250.

Papa, R., J. Acosta, A. Delgado-Salinas, and P. Gepts. 2005. A genome-wide analysis of differentiation between wild and domesticated Phaseolus vulgaris from Mesoamerica. Theor. Appl. Genet. 111:1147-1158.

3.5 Page 9: top of the page: The cross-pollination of maize is not due only to the separation of male and female flowers. It is also due to the fact that male flowers mature before female flowers on the same plant (called protandry). Thus, the mating system of maize of more complex than portrayed here and the statement that "pollen is released in close synchrony with maturation of female flowers" is inaccurate.

3.6 Page 9: "Fortunately, the predicable (sic) nature of flowering dynamics and improved methods for modeling pollen dispersal are making it possible to quantify loss of genetic purity under field conditions."

There are limits to flowering predictability. There are variables that influence its characteristics, including the weather during flower development and after flower opening, the level and type of cross-pollinators, etc., that diminish the predictability of flowering and seed set, including the level of cross-pollination. This provides a false image of technical control over a phenomenon with inherent variability beyond genetic determinism.

3.7 Page 9: "Some crops do not do this perfectly and some grain is lost prior to harvest."

I agree with this statement, It is important to note here that not all crops have been domesticated to the fullest (such as maize). I have provided examples with Track changes. Note that one of the crops in question is transgenic crop, oilseed rape. The lack of seed retention at maturity, combined with seed dormancy, causes herbicide-resistant, transgenic oilseed rape to become its own worst weed and can account for uncontrolled dispersal of herbicide-resistant oilseed rape in the agricultural landscape. See also comment on p. 20. Crawley et al. (2001) may not have noted an increase in weediness for rapeseed, but it should be noted that this crop is already weedy in itself!

3.8 Page 11: “When a crop is exclusively self pollinated, there is no need for pollen control in seed production, because little pollen is released into the air around the flower.”

This situation is a rarity; it is actually more appropriate to consider that most self-pollinated species have a low, but variable and non-negligible level of outcrossing, especially those whose flowers are visited by insects.

This is an awkward statement. I would say: "The ease in producing hybrid seeds makes hybrid varieties a more practical approach in cross-pollinated crops than in self-pollinated crops.

3.9 Page 11: “it is less likely that hybrids will show strong hybrid vigor.”

The authors should cite their sources on this statement, which is an over-generalization. There is actually hybrid vigor in selfing species as well. The main obstacle to commercial hybrids in selfing species is not the lack of hybrid vigor but the development of an economically feasible system for the production of hybrid seeds.

3.10 Page 13: “For outcrossing crops, unintended crossing during open pollination is the major source of unintended off-type pollinations in the field.”

Again, this is also the case for selfing species, although to a lesser extent. Furthermore, the text oversimplifies in that it only considers two categories: selfing vs. outcrossing. In reality, even selfing species can in some years and locations exhibit higher levels of outcrossing.

3.11 Page 13: “The vast majority of unintended outcrosses and of off-type plants come from adjacent fields.”

However, occasional long-distance dissemination events can assure the dispersal of a gene in spite of its relative rarity because the most important factor influencing the persistence of a transgene will be the selective advantage it confers. The migration does not have to be high (m >1) to reduce differentiation between populations. In addition, repeated gene flow will assure establishment of a transgene even if it does not confer a strong fitness advantage.

Wright, S. 1931. Evolution in Mendelian populations. Genetics 16:97-159.
Rieseberg, L.H., and J.M. Burke. 2001. The biological reality of species: gene flow, selection,and collective evolution. Taxon 50:47-67.
Neubert, M.G. and Caswell, H., 2000. Demography and dispersal: calculation and sensitivity analysis of invasion speed for structured populations. Ecology 81, 1613-1628.

3.12 Page 14: “This rapid growth has more recently slowed due to a number of issues of health and environmental concern and lack of an existing commercial marketing mechanism for plants with unique non-traditional traits and uses.”

There are a number of other reasons for this slowdown:
a) Lack of additional, economically viable transgenic traits besides Bt and herbicide resistance; for example, even new products may only be grown on small scales.
b) The inherently slow nature of new cultivar development (independent of regulatory procedures)
c) The negative economics of technology fees vs. additional genes.

3.13 Page 20: “Many participants felt that the types of pest resistance traits being tested or released commercially were not fundamentally different from those bred in conventionally and, as such, would present similar ecological risks. However, some participants disagreed and contended that some transgenes could have a much greater impact on weediness.”

One of the major justifications for genetic engineering is that it can bring in new traits not present in the native genetic diversity of the crops species. In this case, it would be difficult to compare resistances obtained by conventional breeding and genetic engineering. An example is Bt-maize with resistance to European corn borer. To my knowledge, there is no comparable level of resistance in conventionally bred maize lines. Therefore, you cannot talk about “similar ecological risk.”

3.14 Page 21: “the escape of a GE crop is not inherently more likely than the escape of any other crop.”

I suggest adding: “although it could be less although it could be of more concern than with conventionally bred crops depending on the genetically engineered trait.”

3.15 Page 22: “the Association of Official Seed Certifying Agencies has established standard growing conditions for crop seed production which result in very low levels of outcrossing (AOSCA 2003).”

But the criteria for seed purity have evolved. In pre-GE days, overall genetic purity was important and a tolerance of several % was in place. Since the introduction of GE crops, the tolerance has been reduced considerably to less than 1%. You should quantify what you mean with "very low levels".

3.16 Page 25: “Multiple introductions via repeated instances of gene flow may be necessary before a potentially invasive species can become established (Ellstrand and Schierenbeck 2000; “)

The same can be said for individual genes. Repeated gene flow can establish a (trans)gene, even it if it does not confer a strong fitness advantage or no advantage at all:

Lenormand, T. 2002. Gene flow and the limits to natural selection. Trend Ecol Evol 17:183-189.

Haygood, R., A.R. Ives, and D.A. Andow. 2003. Consequences of recurrent gene flow from crops to wild relatives. Proceedings of the Royal Society of London - Series B: Biological Sciences 270:1879-1886.

3.17 Page 31: “Each method of genetic modification, including the two most common methods currently employed, traditional breeding and genetic engineering, ...”

Genetic engineering is _not_ a method to develop new cultivars; it is a method to introduce new genetic diversity in a crop. Even when using transgenes, plant breeding remains the only comprehensive way to develop an improved variety.

Gepts, P. 2002. A comparison between crop domestication, classical plant breeding, and genetic engineering. Crop Sci. 42:1780-1790.

3.18 Page 43: “Genetic engineering of plants that contain Bt proteins in all tissues continuously throughout the growing season has overcome many of the limitations of Bt microbial insecticides.”

All tissues: This is also a problem if Bt is expressed in tissues in which its expression is not necessary, e.g., pollen

3.19 Page 43: “Commercialization of Bt crops has resulted in fewer insecticide applications and thus lower management costs (Fitt 2000; Schnepf et al. 1998). “

Is there any more recent data on this important topic, e.g., from 2005 or 2006? In addition, this should be discussed in more detail depending on the crop, particularly maize and cotton. My sense is that insecticide applications have been reduced in cotton but not in maize. Therefore, such a sweeping statement is inappropriate.

3.20 Page 44: “ In the cases of the GE crops with Bt genes to date, the gene products are well known to specifically target a small group of Lepidoptera.”

What about Coleoptera, Diptera, etc. This seems to be an almost meaningless paragraph. In the case of large-scale field trials or commercial fields, on what basis is additional testing then required? What testing should be conducted? For how many year?

3.21 Page 58: “Additionally, it is not likely that the potential for synergy occurring in GE plants expressing virus genes would be greater than in natural mixed infections (Hull 2004).

Except that with current technology, the transgenic virus resistance would be expressed constitutively throughout the plant. This might not be the case with natural infections.

3.22 Page 59: “This is somewhat different than the relationship between crops and plant viruses. Most of the major crop species used in today's agriculture (e.g. soybean, rice, wheat, beans) have been subjected to intensive artificial selection over centuries and only have low survival under most natural conditions. The vast majority of the crops used in agriculture are much less fit, under natural conditions, than wild or weedy plants. Because of this, the impact of virus infection on crop plants is potentially more severe than on many wild or weedy plants.”

This paragraph needs to be re-written. The lack of fitness of domesticated plants in the wild is due to traits such as reduced seed shedding, seed dormancy, change in photoperiod sensitivity, etc. These traits do not necessarily hae a relationship with disease resistance.. Therefore, assuming that reduced fitness of domesticated plants in the wild leads to higher disease susceptibility is a non sequitur.

3.23 Page 59: “There have been a relatively low number of confirmed cases of introgression (Stewart, Halfhill, and Warwick 2003).”

There is a different analysis of the situation by Ellstrand et al. (1999). Even assuming that one considers only the cases of introgression and not just the cases of gene flow, hybridizations between wild and domesticated plants are widespread and exist even in predominantly self-pollinated crops. The estimates of the frequency are probably underestimates because of the lack of appropriate markers to detect introgression. See Papa and Gepts (2003) and Papa et al. (2005).

3.24 Page 71: “Breeding. The process of sexual reproduction and production of offspring.”

This definition is deficient in the context of this presentation, which seeks to compare geneticengineering and (plant) breeding. A more circumstantiated definition is necessary.

I suggest the following:
"Plant breeding is an applied, multidisciplinary science, which represents the application ofgenetic principles and practices associated with the development of cultivars more suited to the needs of humans than the ability to survive in the wild. It uses knowledge from agronomy,botany, genetics, cytogenetics, molecular genetics, physiology, pathology, entomology, biochemistry, and statistics.

from Schlegel, R.H.J. 2003. Dictionary of plant breeding. Food Products Press/The Haworth Reference Press, New York.

3.25 Page 73: “Fecundity. The capacity for producing offspring.”

As defined here, this is fertility. Fecundity refers to the number of gametes or progeny of an individual.

3.26 Page 73: “Gene flow. The spread of genes from one population to another, by the movement of individuals, gametes, or spores.”

Should add seeds! “Gene flow. The spread of genes from one population to another, by the movement of individuals, gametes, seeds, or spores.”

3.27 Page 74: “Gene stacking. The use of plant breeding to combine two or more genetic traits into a single plant variety.”

Should be: “Gene stacking. The use of genetic engineering to combine two or more genetic traits into a single transformant.” In plant breeding, the practice of accumulating several genes governing a trait such as disease resistance is called pyramiding.

3.28 Page 75: “Marker gene. A gene of known function or known location that is inherited in Mendelian fashion and facilitates the study of inheritance of a trait or a linked gene.”

One should add to that: “The gene also has high heritability, i.e., its expression is not influenced by the environment.”

3.29 Page 75: Mutagenesis is, by definition, artificial. This should be added to the definition.

3.30 Page 77: It is pleiotropic and not pleiotrophic!

3.31 Page 78: “Rotation. The number of years required to establish and grow trees to a specified size, product, or condition of maturity. A pine rotation may range from as short as 20 years for pulpwood to more than 60 years for sawtimber. Full rotation is the total time from planting to harvest. Half rotation would be approximately half the time to reach maturity or harvest. “

I suggest a more complete definition: “Rotation. A) In forestry, the number of years required to establish and grow trees to a specified size, product, or condition of maturity. A pine rotation may range from as short as 20 years for pulpwood to more than 60 years for sawtimber. Full rotation is the total time from planting to harvest. Half rotation would be approximately half the time to reach maturity or harvest. B) In crop production, the cycle of crops grown in successive years in the same field. Rotations are instituted to limit the spread and accumulation of diseases (especially soil-borne diseases) and pests and to manage plant nutrients.”

4 Uncertainty

4.1 Page 26, bottom: “Obviously, soil and natural bodies of water are not sterile environments and there are a number of abiotic and biotic factors that will affect

accumulation and persistence, such as soil type, aeration, water movement, and soil biota activity."

This statement suggests that long-term studies are lacking that follow the fate of the Bt toxin over several years, especially in areas in which Bt crops (maize, cotton) are grown in short, recurring rotations.

4.2 Page 30, bottom: Horizontal gene transfer:

There is a need to cite references reporting on studies that have analyzed the presence/absence of HGT in "Bt crops ...currently grown in many countries, in many diverse environments, covering millions of acres." If this is not possible, then this should be listed as an uncertainty.

4.3 Page 47: "Genetically engineered drought and saline tolerant crop plants "

This whole section is very vague and probably reflects the lack of information on the consequences of drought- and salinity tolerance.

4.4 Page 57: "Based upon currently available information, it appears that the potential for recombination in virus resistant GE plants (i.e., virus-virus & plant-virus) would be similar to the natural occurrence of recombination in virus infected, non-GE plants (Falk and Bruening 1994; OECD 1996; Rubio et al. 1999).

But what about the results of Greene and Allison ? My sense is that this issue is still up in the air.

Zaitlin M, Palukaitis P ANNUAL REVIEW OF PHYTOPATHOLOGY 38: 117-143 2000

4.5 Page 58: "Given the knowledge of the roles that different virus genes play in synergy, developers can also select only those genes that likely would not contribute to synergism, or include mutations in such genes so that their potential impact is limited. "

But this knowledge is limited at this stage. The HC-Pro gene is an exception to this pattern.

4.6 Page 60: "Current knowledge and data suggests that gene flow from a GE virus resistant plant to a wild or weedy plant is not likely to provide different exposure from that which occurs under natural agricultural and environmental settings."

I would agree with this statement except that there is actually little data to go by because most transgenic crops have been planted outside areas of domestication, thus, in areas where wild and domesticated types do not co-exist within pollinating distance.

4.7 Page 68: "For some of the traits that are being engineered into trees, it may not be possible to gather data on the effect of the trait on the environment over many years. A good example would be genes for lignin modification. It will take years to produce such

data. Therefore for some traits, APHIS may need to consider whether particular assumptions can be made without hard data when petitioned for deregulation."

I question whether this is the right approach to regulation. I guesss it is "Let us keep our fingers crossed."

5 Objectivity

As detailed in my comments above and in the "track changes" version, the assessment in this chapter minimizes effects on the environment and human health. In a number of places, it also selectively cites both among and within articles the information that tends to minimize actual or potential impacts on the environment or human health.

6 Clarity

6.1 Page 3: "A wild plant that is more able to exploit limited resources may be more likely to survive and reproduce, while a highly efficient crop plant may be more likely to produce large yields."

This sentence sets up a false duality between wild and domesticated plants. Exploitation of limited resources (wild) and efficiency at producing a higher yield are not mutually exclusive traits. One of the most distinguishing traits is the higher harvest index in domesticated plants, i.e., a larger proportion of the photosynthates end up in the harvested part of the plant, e.g., grains. Wild plants may produce the same amount of biomass but a larger part remains in the vegetative organs. Furthermore, wild plants may not be better able to withstand stresses, whether biological or physical. The environment of wild plants is quite different from that of domesticated plants. For example, wild plants grow often at low densities where disease epidemics have more difficulty becoming established. Likewise, insect pests are kept under control not only by the lower density, but also by a suite of parasites and predators. Thus, there may be less selection pressure from diseases and pests in wild populations.

6.2 Page 8: "flowering forms"

Please make this more explicit.

6.3 Page 12: "Adaptation of outcrossing crops to the hybrid condition"
This is an awkward statement. I would say: "The ease in producing hybrid seeds makes hybrid varieties a more practical approach in cross-pollinated crops than in self-pollinated crops."

6.4 Page 12: "Hybrid seed production involves well defined production steps, and much of the structure of the commercial hybrid seed industry is influenced by the difficulty and cost of pollen control, which control and limit, but do not eliminate, outcrossing and mixtures. In the production field, the sequence of seed increases generally follows a pattern where:"

There is a confusion here between "hybrid seed production" and "the commercial hybrid seed industry". The former refers to the production of seed for F1 hybrid varieties (e.g., maize); the latter refers to seed companies that produce new varieties through hybridization, whether these are F1 hybrid varieties, pure line varieties, or any other type of variety. I suggest you just delete the word "Hybrid" at the beginning of the paragraph: "Seed production involves well-defined …."

All of these varieties go through a certification program, whether public or private, that includes the steps in the bulleted list. There is an unnecessary split in this text between hybrid seed production and the next section (Crop Improvement …) leading to text duplication.

6.5 Page 12: “Conventional breeding, at its most basic, is a process in which differences in plants are observed in small plots, the differences are compared with the needs of the person doing the selection, and the plots that most fit the selector's needs are saved and perpetuated. Other variants are eliminated from the selected gene pool.”

This is an antiquated view of plant breeding going back to the 19th century (e.g., Luther Burbank). If I could suggest another definition from Schlegel (2003) and Gepts and Hancock (2006): Plant breeding is an applied, multidisciplinary science. It is the application of genetic principles and practices associated with the development of cultivars more suited to the needs of humans than the ability to survive in the wild; it uses knowledge from agronomy, botany, genetics, cytogenetics, molecular genetics, physiology, pathology, entomology, biochemistry, and statistics (Schlegel, 2003). Of particular importance is the ability to transfer, in addition to major genes, large suites of genes conditioning quantitative traits such as productivity and other traits of interest to humans. The ultimate outcome of plant breeding is mainly improved cultivars. Therefore, plant breeding is primarily an organismal science even though it is eminently suited to translate information at the molecular level (DNA sequences, protein products) into economically important phenotypes.

Schlegel, R.H.J. 2003. Dictionary of plant breeding. Food Products Press/The Haworth Reference Press, New York.

Gepts, P., and J. Hancock. 2006. The future of plant breeding 10.2135/cropsci2005-12-0497op. Crop Sci 46:1630-1634.

6.6 Page 13: “of corn”

Clearly, corn is one of the major commercial targets for genetic engineering. However, the text would be strengthened if the perspective was broadened to address a broader range of crops: e.g., agronomic and horticultural crops.

6.7 Page 13: “In the U.S. both varieties and genes can be patented. Varieties can also be protected under plant breeder rights. The International Union for the Protection of New

Varieties of Plants coordinates a simplified plant breeder's rights system with standardized claims."

I suggest to make this text more explicit:
"In the U.S. both varieties and genes can be covered by a utility patent. Furthermore, vegetatively propagated varieties (except those of potatoes) can received a plant patent. Cropvarieties can also be protected under plant breeder's rights (Plant Variety Protection). TheInternational Union for the Protection of New Varieties of Plants (UPOV) coordinates the plantbreeder's rights system with standardized claims at the international level."

6.8 Page 14: "The first decade of commercial plant agricultural biotechnology has seen remarkable growth from a mere 6 million acres in GE crops in 1996 to over 220 million acres in 21 countries in the 2005 growing season. The year 2005 also marked the point where cumulatively over one billion commercial acres of GE crops had been grown world wide. "

These seemingly high numbers (taken from ISAAA, I suppose) need to be put in perspective to provide a more objective image to the public on the part of a public administration like USDA. Is 220 million acres a lot? It represents only 6.3% or 4.5% of total arable land in the world depending on FAO or CIA data, respectively.

http://faostat.fao.org/faostat/form?collection=LandUse&Domain=Land&servlet=1&hasblk=0&version=ext&language=EN
https://www.cia.gov/cia/publications/factbook/fields/2097.html

6.9 Page 15: "Risk assessments for GE organisms:..."

This is an important paragraph. I would be more explicit in defining terms here: transparency, explicit in addressing uncertainties,....

Same question for next page: "receiving environment": what features of this environment are considered?

6.10 Page 17: Replace cultivated by domesticated: not every cultivated plant is domesticated, but every domesticated plant is cultivated. It is important to distinguish cultivated and domesticated plants in this context because fully domesticated plants are unlikely to escape the cultivated environment. See also comment on next page!

6.11 Page 18: "crop plants often are themselves not very weedy and have a low propensity for persistence when not managed in an agricultural context, whereas wild relatives by their nature may have weedy characteristics and an ability to persist in the environment."

See text with track changes. It is important not to oversimplify by considering only two

categories: crop vs. wild. Among crop plants, the degree of domestication varies. Fully domesticated crops, such as maize, will not survive on their own in the wild; partially domesticated crops, such as oilseed rape, can survive because they shed their seeds, which have a certainly level of dormancy. Both seed shedding and dormancy are characteristics of wild plants, which increase the weediness of the crop.

On the same page, at the bottom: To this list, should be added traits such as: seed dormancy, seed sheeding, photoperiod insensitivity, etc. Same comment on p. 20, top.

6.12 Page 31: “Genetic modification”

The use of the expression "genetic modification" should be avoided. This is a misnomer best replacec by the expression "genetic engineering". All organisms, even viruses, are genetically modified in one way or the other as they are subject to mutation, recombination, … Without "genetic modification" we would not have biodiversity on this planet. Clearly this is not what we are discussing in this document.

6.13 Page 31: “Traditionally, new varieties of whole foods have not been subjected to extensive chemical, toxicological or nutritional evaluation prior to marketing.”

There is a good reason for this. Consumption of some crops dates back more than 10,000 years, from before the beginning of agriculture during the hunter-gatherer era. Even hunter-gatherers had developed technologies to detoxify plants before consumption documented by anthropologists. Thus, on this basis alone, humans have received plenty of exposure to compounds contained in these crops, including modern varieties. The difference with transgenes is that they can introduce new compounds to which human beings have not been exposed unlike the situation with naturally occurring compounds.

Johns, T., and I. Kubo. 1988. A survey of traditional methods employed for the detoxification of plant foods. Journal of Ethnobiology 8:81-129.

6.14 Page 32: It should be clarified that FDA evaluations are voluntary only.

6.15 Page 43: “Agricultural biotechnology has increased the number of ways in which crops can made resistant to pests>”

Apart from Bt, what other ways of dealing with insects pests have been commercialized?

6.16 Page 45: “Structured refuges are non-Bt host plants that are managed to provide sufficient Bt-susceptible adult insects to mate with potentially Bt resistant adult insects. These matings result in Bt-susceptible offspring.”

It should be clarified that the progeny will be susceptible to Bt only “(assuming that Bt-susceptibility is a genetically recessive trait)” Does the evidence support this? Reference(s)?

7. **Additional text on Biological Control provided:**
I was asked to comment on a separate document describing Biological Control. It would not fitin Chapter 4.1 as there is no relationship to genetic engineering other than providing a valuable alternative to disease and pest control.

8. Final conclusion: Does this scientific information presented accurately and objectively provide non-expert readers with a broad base of knowledge to understand the aspect of the biological and physical environment that is likely to be affected by the regulations currently administered by possible changes of those regulations?

(3) No, this Chapter 4.1 document has significant shortcomings in its treatment of the natural and physical environment affected by APHIS BRS regulations.

There are shortcomings on the substance as well as the form as detailed in my comments above and in the Track changes version. The text: 1) in certain parts ends up minimizing risk (see my Track changes; 2) while I acknowledge that this text is intended for non-experts, the current text presents in certain sections overly simplified information (e.g., wild vs. domesticated, self- vs. cross-pollinated) without considering intermediate situations (see my Track changes); and 3) is a combination of different sections written presumably by different individuals, with the ensuing differences in style and unnecessary duplications. In this regard, I would like to congratulate whoever wrote the section on Sylviculture, which is by far the most-balanced and best-written section of the chapter! It sets a standard for a necessary re-write of Chapter 4.1.

Page 36: [1] Comment **Government User** **9/12/2006 3:36 PM**

As another aside, {Reference Deleted}

"Direct seeding, also known as "no-till farming," saves time, energy and steel by allowing annual crops to be produced with a sprayer (for weed control), planter [that simultaneously and precisely places seed and fertilizer in the same seed furrow in a single pass over the field (figure 1)] and combine harvester, thereby eliminating the need for plows, cultivators, disks, and harrows to till the soil. As an example of the economic benefits, it takes about one-fourth as much diesel fuel (approximately 5 L/T of grain compared to 20 L/T of grain) to produce wheat with direct seeding compared to wheat production using the standard and multiple tillage operations. More importantly than just the greater use of fuel, four times the amount diesel must be visualized in terms of the additional time and steel needed to use that much more diesel fuel.

In addition to the economic benefits, leaving all residue of the previous crop on the soil surface protects against evaporative loss of water needed to grow the next crop, essentially stops soil loss from wind and water erosion, results in a buildup (sequestration) of soil carbon as organic matter, and the stubble with all weeds seeds left on the soil surface provides habitat and food for birds and other wild life. Indeed, direct-seed cropping systems can provide the same ecosystem services expected of natural ecosystems, including mitigation of floods and droughts, purification of water and air, recycling of nutrients, and protection of biodiversity. North American agriculture has been moving slowly but steadily in this direction since the days of the Dust Bowl of the 1930s."

While I am citing the above as background, it would be useful to cite the report from the Conservation Tillage Information Center CTIC. (2002) Conservation Technology Information Center. Purdue University, West Layfayette, IN
http://www.ctic.purdue.edu/CTIC/CTIC.html
More than 60% of farmers surveyed credited HT soybeans with their decision to reduce or eliminate tillage on their farm.

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I note that the perspective comes later, but would it not be preferable to make the point here as to the extremely low probability (again, likely to be in "evolutionary time," meaning thousands or hundreds of thousands of years) that a microbe will acquire and integrate funtional DNA into its genome from the substrate that it grows upon. The gene for production of chalcone synthase, a plant gene, has been shown to be part of the operon in *P flourescens* for production of phenazine antibiotics, but this is probably due to long evolutionary lineages and not a recent acquisition, especially since the gene is highly conserved in both the plant kingdom and in these soil and plant-associated bacteria world wide. Bangera, M.G. & Thomashow, L.S. (1999) *J. Bacteriol.* **181**,3155-3163.