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 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 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. 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.

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 the microorganisms themselves. Increasing the number of plants able to benefit from symbiotic relationships with soil microbes 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. 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 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 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 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. 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.

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

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 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.

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 selects a specific DNA 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 world wide. 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.

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 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.[1] In this way conclusions 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

[1] 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.

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 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 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). 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).[2] 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 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).

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

[2] Crops considered to be persistent and sometimes invasive include barley, rapeseed, rice, sorghum, sunflower, and wheat. Crops considered to be persistent but not invasive include apple, asparagus, blueberry, cranberry, pear, poplar, spruce, and strawberry (Hancock and Hokanson 2001).

(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.

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.

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.

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 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.

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. 200*1).

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*, 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 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.

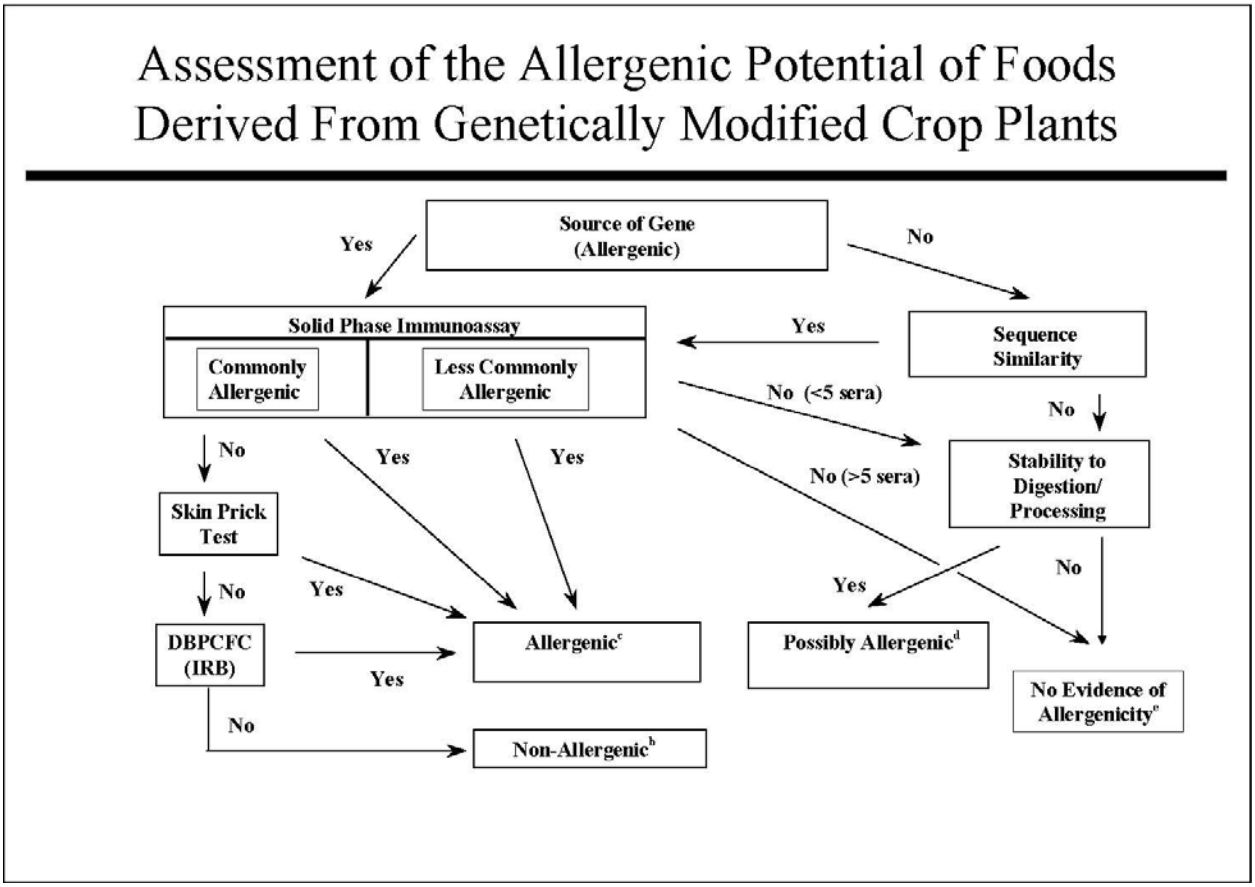

Figure 1 Decision tree for the assessment of the allergenic potential of foods derived from GE crop plants Source: Joint FAO/WHO Expert Consultation on Allergenicity of Foods Derived from Biotechnology, 2000.[3]

---

[3] (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.
(e) A novel protein with no sequence similarity to known allergens and that was not stable

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.[4] 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.

---

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.

[4] 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 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).

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). 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.

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. 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. 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.

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

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

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:

- 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

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).

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.

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; 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.

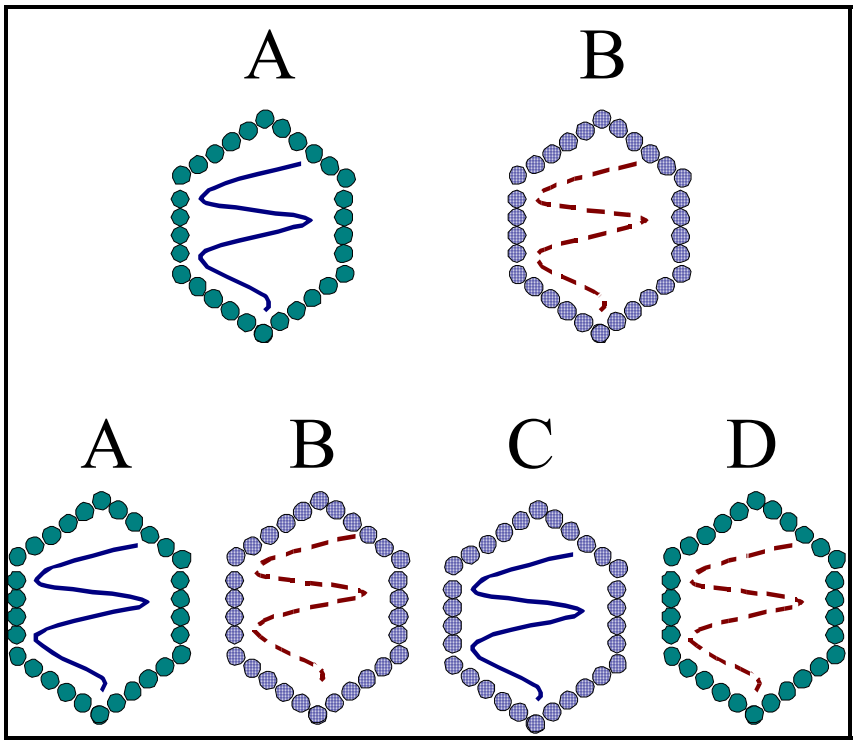

Figure 2 Heterologous Encapsidation. Possible outcomes of heterologous encapsidation interactions as previously described by Rochow 1977 and Falk 1995. A and B represent the parental viruses. When two viruses co-infect the same cell, the progeny can include virions that are identical to the parental 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 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.

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 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.

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 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.

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, 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.

Herbicide Tolerant crop plants

Introduction[5]

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.

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.

---

[5] 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 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[6].

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, however the desirable traits being selected are different.

---

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

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 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 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.

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 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[7] (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.

[7] 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**.

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. *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.

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

References

Aaziz, R., and M. Tepfer. 1999. Recombination in RNA viruses and in virus-resistant transgenic plants. *Journal Of General Virology* 80(Pt 6):1339-1346.

Abdalla, O.A., P.R. Desjardins, and J.A. Dodds. 1985. Survey of pepper viruses in California by the ELISA technique. *Phytopathology* 75:1311.

Abel, P.P., R.S. Nelson, B. De, N. Hoffmann, S.G. Rogers, R.T. Fraley, and R.N. Beachy. 1986. Delay of disease development in transgenic plants that express the tobacco mosaic virus coat protein gene. *Science* 232(4751):738-743.

Adams, J. M., G. Piovesan, S. Strauss, and S. Brown. 2002. The case for genetic engineering of native and landscape trees against introduced pests and diseases. *Conservation Biology* (4):874-879.

AIBS. 1995. *Transgenic Virus-Resistant Plants and New Plant Viruses*. American Institute of Biological Sciences. Workshop Sponsored by USDA APHIS and the Biotechnology Industry Organization.

Al Mouemar, A., and H. Darmency. 2004. Lack of stable inheritance of introgressed transgene from oilseed rape in wild radish. *Environmental Biosafety Research* 3:209-214.

Albrecht, D. 2003. *Public Perceptions of Agricultural Biotechnology: An Overview of Research to Date*. National Agricultural Biotechnology Council. http://nabc.cals.cornell.edu/pubs/occ_paper_1/Albrecht.html. Accessed on May 3, 2006.

Alexander, N. and C. Toner. 2004. *IFIC Survey: Support for Food Biotechnology Stable Despite News on Unrelated Food Safety Issues*. International Food Information Council.

Allard, R.W. 1964. *Principles of Plant Breeding*. New York: John Wiley and Sons, Inc.

Anderson, W.P. 1977. *Weed Science: Principles*. St. Paul, MN: West Publishing Company.

Angle, J.S., 1994. Release of transgenic plants: biodiversity and population-level considerations. *Molecular Ecology* 3:45-60.

Anonymous. 2004. Drugs in crops-the unpalatable truth. *Nature Biotechnology* 22:133.

AOSCA. 2003. *Operational Procedures, Crop Standards and Service Programs Publication*. Meridian, ID: Association of Official Seed Certifying Agencies,.

APHIS.2003. Field Testing of Plants Engineered to Produce Pharmaceutical and Industrial Compounds, *Federal Register* 68:11337.

Apse, M.P., G.S. Aharon, W.A. Snedden, and E. Blumwald. 1999. Salt tolerance conferred by overexpression of a vacuolar Na+/H+ antiport in *Arabidopsis*. *Science* 285:1256-1258.

Arriola, P.E. 1997. Risks of escape and spread of engineered genes from transgenic crops to wild relatives. *AgBiotech News and Information* 9:157N-160N.

Astwood, J.D., and R.L. Fuchs. 1996. Allergenicity of foods derived from transgenic plants. *Monographs In Allergy* 32:105-120.

Astwood, J.D., J.N. Leach, and R.L. Fuchs. 1996. Stability of food allergens to digestion *in vitro*. *Nature Biotechnology* 14(10):1269-1273.

Ayers, M.P., R.T. Wilkens, J.J. Ruel, M.J. Lombardero, and E. Vallery. 2000. Nitrogen budgets of phloem-feeding bark beetles with and without symbiotic fungi. *Ecology* 81:2198-2210.

Badosa, E., C. Moreno, and E. Montesinos., 2004. Lack of detection of ampicillin resistance gene transfer from Bt176 transgenic corn to culturable bacteria under field conditions. *FEMS Microbiology Ecology* 48(2):169-178.

Baker, H. G. 1965. The characteristics and modes of origin of weeds. Pages 147-172 in *The Genetics of Colonizing Species*. Edited by H.G. Baker and G. Stebbins. New York: Academic Press.

Baker, H.G. 1974. The evolution of weeds. *Annual Review of Ecology and Systematics* 5:1-24.

Bashan, Y., and G. Holguin., 1998. Proposal for the division of plant growth-promoting rhizobacteria into two classifications: biocontrol-PGPB (plant growth-promoting bacteria) and PGPB. *Soil Biology and Biochemistry* 30:1225-1228.

Basim, H., R.E. Stall, G.V. Minsavage, and J.B. Jones. 1999. Chromosomal gene transfer by conjugation in the plant pathogen *Xanthomonas axonopodis* pv. *vesicatoria*. *Phytopathology* 89:1044-1049.

Basu, C., M.D. Halfhill, T.C. Mueller, and C.N. Stewart, 2004. Weed genomics: new tools to understand weed biology. *Trends in Plant Science* 9(8):391-398.

Baucher, M., B. Chabbert, G. Pilate, J. Van Doorsselaere, M. T. Tollier, M. Petit-Conil, D. Cornu, B. Monties, M. Van Montagu, D. Inzé, L. Jouanin, and W. Boerjan. 1996. Red xylem and higher lignin extractability by down-regulating a cinnamyl alcohol dehydrogenase in poplar. *Plant Physiology* 112:1479-1490.

Beachy, R.N. 1999. Coat-protein-mediated resistance to tobacco mosaic virus: discovery mechanisms and exploitation. *Philosophical Transactions Of The Royal Society Of London. Series B, Biological Sciences* 354(1383):659-664.

Bentz, J., and A. M. Townsend. 2001. Leaf element content and utilization of maple and elm as hosts by the potato leafhopper (Homoptera: Cicadellidae). *Environmental Entomology* 30:533-539.

Bertolla, F., A. Frostegard, B. Brito, X. Nesme, and P. Simonet. 1999. During infection of its host, the plant pathogen *Ralstonia solanacearum* naturally develops a state of

competence and exchanges genetic material. *Molecular Plant-Microbe Interactions* 12:467-472.

Bessin, R. 2004. *Bt-corn for corn borer control*. University of Kentucky Entomology. www.uky.edu/Agriculture/Entomology/entfacts/fldcrops/ef118.htm. Accessed on May 3, 2006.

Betz, F.S, B.G. Hammond, and R.L. Fuchs. 2000. Safety and advantages of *Bacillus thuringiensis*-protected plants to control insect pests. *Regulatory Toxicology and Pharmacology* 32(2):156-173.

Bewley, J.D. and M. Black. 1994. *Seeds: Physiology of Development and Germination*, 2nd Ed. New York and London: Plenum Press.

Bhatti, M.A., J. Duan, G. Head, C. Jiang, M.J. McKee, T.E. Nickson, C.L. Pilcher, and C.D. Pilcher. 2005a. Field evaluation of the impact of corn rootworm (Coleoptera: Chrysomelidae)-protected Bt corn on ground-dwelling invertebrates. *Environmental Entomology* 34:1325-1335.

Bhatti, M.A., J. Duan, G. Head, C. Jiang, M.J. McKee, T.E. Nickson, C.L. Pilcher, and C.D. Pilcher. 2005b. Field evaluation of the impact of corn rootworm (Coleoptera: Chrysomelidae)-protected Bt corn on foliage-dwelling invertebrates. *Environmental Entomology* 34:1336-1345.

Bidwell, R.G.S. 1974. *Plant Physiology*. New York: Macmillan Publishing Company.

Bitzer, R.J., M.E. Rice, C.D. Pilcher, C.L. Pilcher, and W-K. F. Lam. 2005. Biodiversity and community structure of epedaphic and euedaphic springtails (*Collembola*) in transgenic rootworm Bt corn. *Environmental Entomology* 34:1346-1376.

Björklöf, K., A. Suoniemi, H. Haahtela, and M. Romantschuk. 1995. High frequency of conjugation verses plasmid segregation of RP1 in epiphytic *Pseudomonas syringae* populations. *Microbiology* 141:2719-2727.

Black, M. and J.D. Bewley. 2000. *Seed Technology and Its Biological Basis*. Boca Raton, FL: CRC Press.

Blum, S.A.E., M.G. Lorenz, and W. Wackernagel. 1997. Mechanism of retarded DNA degradation and prokaryotic origin of DNases in nonsterile soils. *Systematic and Applied Microbiology* 20:513-521.

Boerjan, W. 2005. Biotechnology and the domestication of forest trees. *Current Opinion in Biotechnology* 16:1-8.

Bohnert, H. J., D. E. Nelson, and R. G. Jensen. 1995. Adaptations to environmental stresses. *Plant Cell* 7:1099-1111.

Booth, B.D., S.D. Murphy, and C.J. Swanton. 2003. *Weed Ecology in Natural and Agricultural Systems*. Wallingford, Oxon, U.K.: CABI Publishing.

Borja, M., T. Rubio, H.B. Scholthof, and A.O. Jackson. 1999. Restoration of wild-type virus by double recombination of tombusvirus mutants with a host transgene. *Molecular Plant-Microbe Interactions* 12(2):153-162.

Brady, N.C. 1974. *The Nature and Properties of Soils*. 8th Edition. New York: Macmillan Publishing Company.

Brammall, R.A., and V.J. Higgins. 1987. The effect of glyphosate on resistance of tomato to *Fusarium* crown and root rot disease and on the formation of host structural defensive barriers. *Canadian Journal of Plant Pathology* 66:1547-1555.

Bren, Linda. 2003. Genetic Engineering: The Future of Foods? *FDA Consumer Magazine*. http://www.fda.gov/OHRMS/DOCKETS/ac/02/briefing/3886b1_Discussion%20Paper%20Allergenicity.pdf. Accessed on May 3, 2006.

Brodbeck, B.V., and D.R. Strong. 1987. Amino acid nutrition of herbivorous insects and stress to host plants. In *Insect outbreaks: ecological and evolutionary perspectives*. Edited by P. Barbosa and J. Schultz. New York: Academic Press.

Brown, B.J., and R.J. Mitchell. 2001. Competition for pollination: Effects of pollen of an invasive plant on seed set of a native congener. *Oecologia* 129:43-49.

Burris, J.S. 2001. *Adventitious pollen intrusion into hybrid maize seed production fields*. Association of Official Seed Certifying Agencies [AOSCA]. Accessed electronically June 9, 2004, http://www.amseed.com/govt_statementsDetail.asp?id=69.

Busch, J.W., and L. Phelan. 1999. Mixture models of soybean growth and herbivore performance in response to nitrogen-sulphur-phosphorus nutrient interactions. *Ecological Entomology* 24:132-145.

Cao-Guo, Y., M.A. Mullins, G.W. Warren, M.G. Koziel, and J.J. Estruch. 1997. The *Bacillus thuringiensis* vegetative insecticidal protein VIP3A lyses midgut epithelium cells of susceptible insects. *Applied and Environmental Microbiology* 63(2):532-536.

Carpenter, J. and L. Gianessi. 1999. Herbicide tolerant soybeans: Why growers are adopting Roundup Ready varieties. *AgBioForum* 2(2):65-72.

Castaldini, M., A. Turrini, C. Sbrana, A. Benedetti, M. Marchionni, S. Mocali, A. Fabiani, S. Landi, F. Santomassimo, B. Pietrangeli, M.P. Nuti, N. Miclaus, and M. Giovannetti. 2005. Impact of Bt corn on rhizospheric and soil eubacterial communities and on beneficial mycorrhizal symbiosis in experimental microcosms. *Applied and Environmental Microbiology* 71(11):6719-6729.

Chen, Y.H., and S.C. Welter. 2002. Abundance of a native moth *Homoeosoma electellum* (Lepidoptera: Pyralidae) and activity of indigenous parasitoids in native and agricultural sunflower habitats. *Environmental Entomology* 31(4):626-636.

Chess, C. 1998. Fearing fear: communication about agricultural biotechnology. *AgBioForum* 1(1):17-21.

Clancy, K.M., and R.M. King. 1993. Defining the western spruce budworm's nutritional niche with response surface methodology. *Ecology* 74:442-454.

CODEX. 2003. *Assessment of Possible Allergenicity (Proteins)*. Annex to Draft Guideline for the Conduct of Food Safety Assessment of Foods Derived from Recombinant-DNA Plants. Codex Ad Hoc Intergovermental Task Force on Foods Derived from Biotechnology.

CODEX. 2003. *Principles for the Risk Analysis of Foods Derived from Modern Biotechnology*. CODEX Alimentarius.

Colautti, R.I., and H.J. MacIsaac. 2004. A neutral terminology to define 'invasive' species. *Diversity and Distributions* 10:135-141.

Conner, A.J., T.R. Glare, and J.-P. Nap. 2003. The release of genetically modified crops into the environment. *Plant Journal* 33:19-46.

Conservation Tillage Study. 2002. The Cotton Foundation. http://www.cotton.org/tech/biotech/contill-study.cfm. Accessed on May 3, 2006.

Copeland, L.O. and M.B. McDonald. 1995. *Principles of Seed Science and Technology*, 3rd ed. New York: Chapman & Hall.

Crawley, M.J., S.L. Brown, R.S. Hails, D.D. Kohn, and M. Rees. 2001. Transgenic crops in natural habitats. *Nature*, 409:682-683 and Supplementary Table 1.

Culver, James N. 2002. Tobacco mosaic virus assembly and disassembly: determinants in pathogenicity and resistance. *Annual Review Of Phytopathology* 40:287-308.

Daly, T. and G.D. Buntin. 2005. Effect of *Bacillus thuringiensis* transgenic corn for lepidopteran control on nontarget arthropods. *Environmental Entomology* 34:1292-1301.

Day, P.R. 1996. Genetic modification of proteins in food. *Critical Reviews In Food Science And Nutrition* 36(S):S49-S67.

De Bruyn, L., J. Scheirs, and R. Verhagen. 2002. Nutrient stress, host plant quality and herbivore performance of a leaf-mining fly on grass. *Oecologia* 130:594-599.

Dearman, R.J., and I. Kimber. 2001. Determination of protein allergenicity: studies in mice. *Toxicology Letters* 120(1-3):181-186.

Delauney, A.J., and D.P.S. Verma. 1993. Proline biosynthesis and osmoregulation in plants. *Plant Journal* 4:215-223.

Department of Agriculture, Hawaii. 2002. *Papaya Ringspot Virus Reappearance Confirmed in Several Areas on Maui*. www.hawaiiag.org/hdoa/newsrelease/02-07.htm. Accessed on May 3, 2006.

Devernoe, D.L. 2000. Substantial equivalence: A valid international sanitary and phytosanitary risk assessment objective for genetically modified foods. *Case Western Law Review* 51:257.

Dively, G.P. 2005. Impact of transgenic VIP3A x Cry1Ab lepidopteran-resistant field corn on the nontarget arthropod community. *Environmental Entomology* 34:1267-1291.

Donaldson, L., and R. May. 1999. *Health Implications of Genetically Modified Foods*. http://www.dh.gov.uk/assetRoot/04/06/50/90/04065090.pdf. Accessed on May 3, 2006.

Donegan, K.K., C.J. Palm, L.A. Fieland, L.M. Porteous, D.L. Ganio, L.Q. Schaller, L.Q. Bucao, and R.J. Seidler. 1995. Changes in levels, species, and DNA fingerprints of soil microorganisms associated with cotton expressing the *Bacillus thuringiensis* var. *kurstaki* endotoxin. *Applied Soil Ecology* 2:111-124.

Donegan, K.K., R.J. Seidler, V.J. Fieland, D.L. Schaller, C.J. Palm, L.M. Ganio, D.M. Cardwell, and Y. Steinberger. 1997. Decomposition of genetically engineered tobacco under field conditions: Persistence of the proteinase inhibitor I product and effects of soil microbial respiration and protozoa, nematode and microarthropod populations. *Journal of Applied Ecology* 34(3):767-777.

Douville, M., F. Gagné, L. Masson, J. McKay, and C. Blaise. 2005. Tracking the source of *Bacillus thuringiensis* Cry1Ab endotoxin in the environment. *Biochemical Systematics and Ecology* 33:219-232.

Downing, J.A. 1997. Marine nitrogen:phosphorus stoichiometry and the global N:P cycle. *Biogeochemistry* 37:237-252.

Dröge, M., A. Pühler, and W. Selbitschka. 1998. Horizontal gene transfer as a biosafety issue: a natural phenomenon of public concern. *Journal of Biotechnology* 64:75-90.

Duan, J.J., G. Head, A. Jensen, and G. Reed. 2004. Effects of transgenic *Bacillus thuringiensis* potato and conventional insecticides for Colorado Potato Beetle (Coleoptera: Chrysomelidae) management on the abundance of ground-dwelling arthropods in Oregon potato ecosystems. *Environmental Entomology* 33(2):275-281.

Dunfield, K.E., and J.J. Germida. 2001. Diversity of bacterial communities in the rhizosphere and root interior of field-grown genetically modified *Brassica napus*. *FEMS Microbiology Ecology* 38:1-9.

Dunfield, K.E., and J.J. Germida. 2004. Impact of genetically modified crops on soil- and plant-associated microbial communities. *Journal of Environmental Quality* 33:806-815.

Duvick, D.N. 1999. Consequences of classical plant breeding for pest resistance. Pages 37-42 in *Proceedings of a Workshop on Ecological Effects of Pest Resistance Genes in Managed Ecosystems*. Edited by P.L. Traynor and J.H. Westwood. Blacksburg, Virginia: Information Systems for Biotechnology, Virginia Polytechnic Institute and State University.

Ecobichon, D. 1993. Book Review: Food poisoning: Handbook of Natural Toxins. Vol 7. *Food Research International* 26(4):313-314.

Ellstrand, N.C., and K.A. Schierenbeck. 2000. Hybridization as a stimulus for the evolution of invasiveness in plants? *Proceedings of the National Academy of Sciences* 97:7043-7050.

Ellstrand, N.C., H.C. Prentice, and J.F. Hancock. 1999. Gene flow and introgression from domesticated plants into their wild relatives. *Annual Review of Ecology and Systematics* 30:539-563.

Elser, J.J., W.F. Fagan, R.F. Denno, D.R. Dobberfuhl, A. Folarin, A. Huberty, S. Interlandi, S.S. Kilham, E. McCauley, K.L. Schulz, E.H. Siemann, and R.W. Sterner. 2000. Nutritional constraints in terrestrial and freshwater food webs. *Nature* 408:578-580.

EPA. 1998. *Potato Leaf Roll Virus Resistance Gene (also known as orf1/orf2 gene)* (006469) Fact Sheet: Environmental Protection Agency.

EPA. 2000. *Background Document for the FIFRA Scientific Advisory Panel on Mammalian Toxicity Assessment Guidance for Protein Plant-Pesticides*, Environmental Protection Agency.

EPA. 2000. *Report - FIFRA Scientific Advisory Panel Meeting*, June 6-7: Session II - Mammalian Toxicity Assessment Guidelines for Protein Plant Pesticides. Environmental Protection Agency.

EPA. 2004. *Issues associated with the development of a type of plant-incorporated protectant (PIP), specifically those based on plant viral coat proteins (PVCP-PIPS).* Environmental Protection Agency.

EPA-BPPD. 2001. *Biopesticides Registration Action Document - Bacillus thuringiensis plant incorporated protectants*. Environmental Protection Agency.

EPA-SAP. 1998. *Subpanel on Bacillus thuringiensis (B.t.) Plant-Pesticides and Resistance Management*. Environmental Protection Agency.

EPA-SAP. 2000. *Characterization and non-target organisms data requirements for protein plant-pesticides*. Environmental Protection Agency.

EPA-SAP. 2001. *Bt plant-pesticides risk benefit assessments*. Environmental Protection Agency.

EPA-SAP. 2002. *Corn rootworm plant-incorporated protectant insect resistance management and non-target insect issues*. Environmental Protection Agency.

Ermel, R.W., M. Kock, S.M. Griffey, G.A. Reinhart, and O.L. Frick. 1997. The atopic dog: a model for food allergy. *Laboratory Animal Science* 47(1):40-49.

Esau, K. 1977. *Anatomy of Seed Plants*, 2nd ed. New York: John Wiley & Sons.

Falk, B.W. and G. Bruening. 1994. Will transgenic crops generate new viruses and new diseases? *Science* 263(5152):1395-1396.

Falk, B.W. and J.E. Duffus. 1981. Epidemiology of persistent helper-dependent aphid transmitted virus complexes. In *Plant Diseases and Vectors: Ecology and Epidemiology*. Edited by K. Maramorosch and K. F. Harris. New York: Academic Press.

Falk, B.W., B.K. Passmore, M.T. Watson, and L.-C. Chin. 1995. The specificity and significance of heterologous encapsidation of virus and virus-like RNAs. In *Biotechnology and Plant Protection: Viral Pathogenesis & Disease Resistance*. Edited by D.D. Bills and S.-D. Kung. Singapore: World Scientific Publishing Co.

Falk, B.W., J.E. Duffus, and T.J. Morris. 1979. Transmission, host range and serological properties of the viruses causing lettuce speckles disease. *Phytopathology* 69:612-617.

FAO. 2004. *Preliminary review of biotechnology in forestry, including genetic modification*. Forest Genetic Resources Working Papers, FRG/59E. Rome: FAO.

FAO. 2004. *The State of Food and Agriculture 2003-2004: Agricultural Biotechnology: Meeting the Needs of the Poor?*

http://www.fao.org/documents/show_cdr.asp?url_file=/docrep/006/y5160e/y5160e00.htm. Accessed on May 3, 2006.

FAO/WHO. 2000. *Safety Aspects of Genetically Modified Foods of Plant Origin*. Geneva, Switzerland: Food and Agriculture Organization of the United Nations and the World Health Organization.

FAO/WHO. 2001. *Evaluation of Allergenicity of Genetically Modified Foods*. January 22-25, 2001. Rome, Italy Food and Agriculture Organization of the United Nations and the World Health Organization. http://www.fao.org/documents/show_cdr.asp?url_file=/docrep/007/y0820e/y0820e00.htm. Accessed on May 3, 2006.

FAS. 2003. *Planting Seed Trade Statistics and Analysis*. http://www.fas.usda.gov/seeds/seedstats.html. Accessed on May 3, 2005.

Fawcett, R.D. and D. Towery. 2002. *Conservation Tillage and Plant Biotechnology*. West Lafayette, IN: Conservation Technology Information Center.

FDA. 2002. *Discussion Paper - Evaluation of Allergenicity of Proteins Introduced into Bioengineered Foods*. Food and Drug Administration.

Fehr, W.R. 1987. *Principles of Cultivar Development*. New York: Macmillan Publishing Company.

Fernandez-Cornejo, J. 2004. *Agricultural Biotechnology: Adoption of Biotechnology and its Production Impacts*. Economic Research Service/USDA. http://www.ers.usda.gov/Briefing/biotechnology/chapter1.htm#adoption. Accessed on May 3, 2006.

Fernandez-Cornejo, J., and S. Jans. 1995. Quality-adjusted price and quantity indices for pesticides. *American Journal of Agricultural Economics* 77:645-659.

Fernandez-Cornejo, J., and W.D. McBride. 2002. *Adoption of Bioengineered Crops*. Washington, DC: USDA, Economics Research Service.

Fischer, M. and M. van Kleunen. 2002. On the evolution of clonal plant life histories. *Evolutionary Ecology* 15:565-582.

Fitt, G.P. 2000. An Australian approach to IPM in cotton: integrating new technologies to minimize insecticide dependence. *Crop Protection* 19:793-800.

Flores, S., D. Saxena, and G. Stotzky. 2005. Transgenic Bt plants decompose less in soil than non-Bt plants. *Soil Biology and Biochemistry* 37(6):1073-1082.

Fonseca, A.E., J.I. Lizaso, M.E. Westgate, L. Grass, and D.L. Dornbos, Jr. 2004. Simulating potential kernel production in maize hybrid seed fields. *Crop Science* 44(5):1696-1709.

Frary, A. and S. Do□anlar. 2003. Comparative genetics of crop plant domestication and evolution. *Turkish Journal of Agriculture and Forestry* 27:59-69.

Freeman, B.L. and R.H. Smith. 1997. *Insect scouting in Bt-transgenic cotton*. Alabama Cooperative Extension System. www.aces.edu/pubs/docs/A/ANR-1032/. Accessed on May 3, 2006.

Frewer, L., R Shepherd, and P Sparks. 1994. The interrelationship between perceived knowledge, control, and risk associated with a range of food related hazards targeted at the self, other people, and society. *Journal of Food Safety* 14:19-40.

Fuchs, M., E.M. Chirco, and D. Gonsalves. 2004(a). Movement of coat protein genes from a commercial virus-resistant transgenic squash into a wild relative. *Environmental Biosafety Research* 3(2004):5–16.

Fuchs, M., E.M. Chirco, J.R. McFerson, and D. Gonsalves. 2004(b). Comparative fitness of a wild squash species and three generations of hybrids between wild × virus-resistant transgenic squash. *Environmental Biosafety Research* 3(2004):17-28.

Fuchs, R.L. and R.E. Goodman. 1998. Products from plant biotechnology. *Allergy* 53(46):93-97.

Fuchs, R.L., and J.D. Astwood. 1996. Allergenicity assessment of foods derived from genetically modified plants. *Food Technology* 50:83-88.

Garcia, M.C., J.M. Figueroa, R.L. Gomez, R. Townsend, and J. Schoper. 1998. Pollen control during transgenic hybrid maize development in Mexico, *Crop Science* 38:1597-1602.

Gay, P. 2001. The biosafety of antibiotic resistance markers in plant transformation and the dissemination of genes through horizontal gene flow. In *Safety of Genetically Engineered Crops*. Edited by R. Custers. Zwijnaarde, Belgium: Flanders Interuniversity Institute for Biotechnology.

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.

Gebhard, F. and K. Smalla. 1998. Transformation of *Acinetobacter* sp. strain BD413 by transgenic sugar beet DNA. *Applied and Environmental Microbiology* 64:1550-1554.

Gebhard, F. and K. Smalla. 1999. Monitoring field releases of genetically modified sugar beets for persistence of transgenic plant DNA and horizontal gene transfer. *FEMS Microbiology Ecology* 28:261-272.

Gendel, S.M. 1998. Sequence databases for assessing the potential allergenicity of proteins used in transgenic foods. *Advances In Food And Nutrition Research* 42:63-92.

Gendel, S.M. 1998. The use of amino acid sequence alignments to assess potential allergenicity of proteins used in genetically modified foods. *Advances In Food And Nutrition Research* 42:45-62.

Gepts, P. 2002. A comparison between crop domestication, classical plant breeding, and genetic engineering. *Crop Science* 42:1780-1790.

Gepts, P. 2004. Crop domestication as a long-term selection experiment. *Plant Breeding Reviews* 24(2):1-44.

Gershenzon, J. 1984. Changes in the levels of plant secondary metabolites under water and nutrient stress. *Recent Advances in Phytochemistry* 18:273-320.

Gianessi, L.P., C.S. Silvers, S. Sankula, and J.E. Carpenter. 2002. Plant biotechnology: current and potential impact for improving pest management. In *U.S. Agriculture: An Analysis of 40 Case Studies*. Washington, DC: National Center for Food and Agricultural Policy.

Gibbs, M.J. and J.I. Cooper. 1995. A recombinational event in the history of luteoviruses probably induced by base-pairing between the genomes of two distinct viruses. *Virology* 206(2):1129-1132.

Gill, S.S., E.A. Cowles, and P.V. Pietrantonio. 1992. The mode of action of *Bacillus thuringiensis* endotoxins. *Annual Review of Entomology* 37:615-636.

Giller, K.E., M.H. Beare, P. Lavelle, A.-M.N. Izac, and M.J. Swift., 1997. Agricultural intensification, soil biodiversity and agroecosystem function. *Applied Soil Ecology* 6:3-16.

Gleeson, D., M.-A. Lelu-Walter, and M. Parkinson. 2004. Influence of exogenous L-proline on embryogenic cultures of larch (*Larix leptoeuropaea* Dengler), sitka spruce (*Picea sitchensis* (Bong.) Carr.) and oak (*Quercus robur* L.) subject to cold and salt stress. *Annals of Forest Science* 61:125-128.

Gleeson, D., M.-A. Lelu-Walter, and M. Parkinson. 2005. Overproduction of proline in transgenic hybrid larch (*Larix* x *leptoeuropaea* (Dengler)) cultures renders them tolerant to cold, salt and frost. *Molecular Breeding* 15:21-29.

Gonsalves, D. 1998. Control of papaya ringspot virus in papaya: a case study. *Annual Review Of Phytopathology* 36:415-437.

Gooding, G.V. 1985. Relationship between strains of potato virus Y and breeding for resistance, cross protection and interference. *Tobacco Science* 29:99-104.

Goregaoker, S.P., L.G. Eckhardt, and J.N. Culver. 2000. Tobacco mosaic virus replicase-mediated cross-protection: contributions of RNA and protein-derived mechanisms. *Virology* 273(2):267-275.

Greaves, M.P., and M.J. Wilson. 1970. The degradation of nucleic acids and montmorillonite-nucleic acid complexes by soil microorganisms. *Soil Biology and Biochemistry* 2:257-268.

Greene, A.E., and R.F. Allison. 1994. Recombination between viral RNA and transgenic plant transcripts. *Science* 263(5152):1423-1425.

Greene, A.E., and R.F. Allison. 1996. Deletions in the 3' untranslated region of cowpea chlorotic mottle virus transgene reduce recovery of recombinant viruses in transgenic plants. *Virology* 225(1):231-234.

Griffiths, B.S., I.E. Geoghegan, and W.M. Robertson. 2000. Testing genetically engineered potato, producing the lectins GNA and Con A, on non-target soil organisms and processes. *Journal of Applied Ecology* 37:159-170.

Guadagnuolo, R., D. Savova-Bianchi, and F. Felber. 2001. Gene flow from wheat (*Triticum aestivum* L.) to jointed goatgrass (*Aegilops cylindrica* Host.), as revealed by RAPD and microsatellite markers. *Theoretical and Applied Genetics* 103:1-8.

Guéritaine, G., M. Sester, F. Eber, A.M. Chèvre, and H. Darmency. 2002. Fitness of backcross six of hybrids between transgenic oilseed rape (*Brassica napus*) and wild radish (*Raphanus raphanistrum*). *Molecular Ecology* 11:1419-1426.

Guéritaine, G., S. Bazot, and H. Darmency. 2003. Emergence and growth of hybrids between *Brassica napus* and *Raphanus raphanistrum*. *New Phytologist* 158:561 567.

Hadidi, A., R.K Khetarpal, and H. Koganezawa. 1998. *Plant Virus Disease Control*. Edited by A. Hadidi, R. Khetarpal and H. Koganezawa. St. Paul, Minnesota: The American Phytopathological Society.

Hagendorn, C, and S Allender-Hagendorn. 1995. *Public Perception Issues in Agricultural and Environmental Biotechnology*. Blacksburg, VA: Virginia Cooperative Extension.

Hails, R.S., and K. Morley. 2005. Genes invading new populations: A risk assessment perspective. *Trends in Ecology and Evolution* 20:245-252.

Halfhill, M.D., B. Zhu, S.I. Warwick, P.L. Raymer, R.J. Millwood, A.K. Weissinger, and C.N. Stewart, Jr. 2004. Hybridization and backcrossing between transgenic oilseed rape and two related weed species under field conditions. *Environmental Biosafety Research* 3:73-81.

Hallman, W.K., W.C. Hebden, C.L. Cuite, H.L. Aquino, and J.T. Lang. 2004. *Americans and GM Food: Knowledge, Opinion and Interest in 2004*. New Brunswick, NJ: Food Policy Institute - Cook College.

Hammond, J., H. Lecoq, and B. Raccah. 1999. Epidemiological risks from mixed virus infections and transgenic plants expressing viral genes. Advances. *Virus Research* 54:189-314.

Hancock, J.F. 2003. A framework for assessing the risk of transgenic crops. *BioScience* 53(5):512-519.

Hancock, J.F., and K.E. Hokanson. 2001. Invasiveness of transgenic vs. exotic plant species: How useful is the analogy? Pages 187-192 In *Proceedings of the First International Symposium on Ecological and Societal Aspects of Transgenic Plantations*. Edited by S.H. Strauss and H.D. Bradshaw. Corvallis, Oregon: College of Forestry, Oregon State University.

Hanson, L.A., E. Telemo, U. Wiedermann, A. Dahlman, R. Saalman, S. Ahlstedt, V. Friman, J. Holmgren, C. Czerkinsky, and U. Dahlgren. 1993. Sensitization and development of tolerance via the gut. *Pediatric Allergy And Immunology* 4(3):16-20.

Hassett, R.P., B. Cardinale, L.B. Stabler, and J.J. Elser. 1997. Ecological stoichiometry of N and P in pelagic ecosystems: comparison of lakes and oceans with emphasis on the zooplankton-phytoplankton interaction. *Limnology and Oceanography* 41:648-662.

Hawksworth, D.L., 1991. The fungal dimension of biodiversity: magnitude, significance, and conservation. *Mycological Research* 95:641-655.

Head, G., W. Moar, M. Eubanks, B. Freeman, J. Ruberson, A. Hagerty, and S. Turnipseed. 2005. A multiyear, large-scale comparison of arthropod populations on commercially managed Bt and non-Bt cotton fields. *Environmental Entomology* 34:1257-1266.

Heimlich, R.E., J. Fernandez-Cornejo, W. McBride, C. Klotz-Ingram, S. Jans, and N. Brooks. 2000. Genetically engineered crops: Has adoption reduced pesticide use? *Agricultural Outlook* August:13-17.

Heinemann, J.A, and T. Traavik. 2004. Problems in monitoring horizontal gene transfer in field trials of transgenic plants. *Nature Biotechnology* 22(9):1105-1109.

Höfte, H. and H.R. Whiteley. 1989. Insecticidal crystal proteins of *Bacillus thuringiensis*. *Microbiological Reviews* 53:242-255.

Hossain, F., C.E. Pray, Y. Lu, J. Huang, C. Fan, and R. Hu. 2004. Genetically modified cotton and farmers' health in China. *International Journal of Occupational and Environmental Health* 10:296-303.

Hsiao, T.C. 1973. Plant responses to water stress. *Annual Review of Plant Physiology* 24:519-570.

Huang, J., R. Hu, S. Rozelle, and C. Pray. 2005. Insect-resistant gm rice in farmers' fields: assessing productivity and health effects in china. *Science* 308(5722):688-690.

Hull, R. 2004. *Matthew's Plant Virology*. San Diego, CA. Elsevier Academic Press.

Hull, R.N, and A.N. Adams. 1968. Groundnut rosette and its assistor virus. *Annals of Applied Biology* 62:139-145.

Inbar, M., H. Doostdar, and R.T. Mayer. 2001. Suitability of stressed and vigorous plants to various insect herbivores. *Oikos* 94:228-235.

International Seed Federation, *The world seed statistics*. http://www.worldseed.org/. Accessed on May 3, 2006.

ISAAA. 2006. *Global Area of Biotech Crops*. International Service for the Acquisition of Agri-Biotech Applications. http://www.isaaa.org/kc/CBTNews/press_release/images/briefs34/figures/acres/figure%20cover_acres.jpg. Accessed on May 31, 2006.

ISB. 2006. Information Systems for Biotechnology - Field Test Release Permits for the U.S. http://www.isb.vt.edu/cfdocs/fieldtests1.cfm. Accessed on May 3, 2006

Ito, Y., K. Katsura, K. Maruyama, T. Taji, M. Kobayashi, M. Seki, K. Shinozaki, and K. Yamaguchi-Shinozaki. 2006. Functional analysis of rice DREB1/CBF-type transcription factors involved in cold-responsive gene expression in transgenic rice. *Plant and Cell Physiology* 47:141-153.

Janick, J., R.W. Schery, F.W. Woods, and V.W. Ruttan. *Plant Science: An Introduction to World Crops*. New York: W.H. Freeman and Company.

Janssen, J.A.M. 1994. Impact of the mineral composition and water content of excised maize leaf sections on fitness of the African armyworm, *Spodoptera exempta* (Lepidoptera: Noctuidae). *Bulletin of Entomological Research* 84:233-245.

Jenczewski, E., J. Ronfort, and A. Chèvre. 2003. Crop-to-wild gene flow, introgression and possible fitness effects of transgenes. *Environmental Biosafety Research* 2:9-24.

Jepson, P.C., B.A. Croft, and G.E. Pratt. 1994. Test systems to determine the ecological risks posed by toxin release from *Bacillus thuringiensis* genes in crop plants. *Molecular Ecology* 3:81-89.

Kainulainen, P., J. Holopainen, V. Palomaki, and T. Holopainen. 1996. Effects of nitrogen fertilization on secondary chemistry and ectomycorrhizal state of Scots pine seedlings and on growth of grey pine aphid. *Journal of Chemical Ecology* 22(4):617-636.

Karasev, A.V. 2000. Genetic diversity and evolution of closteroviruses. *Annual Review of Phytopathology* 38:293-324.

Kavi Kishor, P. B., Z. Hong, G.-H. Miao, C. Hu, and D. P. S. Verma. 1995. Overexpression of D1-pyrroline-5-carboxylate synthetase increases proline production and confers osmotolerance in transgenic plants. *Plant Physiology* 108:1387-1394.

Keeler, K.H. 1985. Implications of weed genetics and ecology for the deliberate release of genetically-engineered crop plants. *Recombinant DNA Technical Bulletin* 8:165-172.

Keeler, K.H. 1989. Can genetically engineered crops become weeds? *Bio/Technology* 7:1134-1139.

Keeler, R.F. and A.T. Tu, eds. 1991. *Toxicology of Plant and Fungal Compounds, Vol. 6*. New York: Marcel Dekker.

Kelly, A.F. and R.A.T George. 1998. *Encyclopedia of Seed Production of World Crops*. New York: John Wiley & Sons.

Kerslake, J.E., S.J. Woodin, and S.E. Hartley. 1998. Effects of carbon dioxide and nitrogen enrichment on a plant- insect interaction: the quality of *Calluna vulgaris* as a host for *Operophtera brumata*. *New Phytologist* 140(1):43-53.

Kessler, D.A., M.R. Taylor, J.H. Maryanski, E.L. Flamm, and L.S. Kahl. 1992. The safety of foods developed by biotechnology. *Science* 256:1747-1749.

Khetarpal, R.K., B. Maisonneuve, Y. Maury, S. Chalhoub, S. Dinant, H. Lecoq, and A. Varma. 1998. Breeding for Resistance to Plant Viruses. In *Plant Virus Disease Control*. Edited by R. K. K.A. Hadidi, and H. Koganezawa. St. Paul, MN: The American Phytopathological Society.

Kidambi, S.P., S. Ripp, and R.V. Miller. 1994. Evidence for phage-mediated gene transfer among *Pseudomonas aeruginosa* strains on the phylloplane. *Applied and Environmental Microbiology* 60:496-500.

Kimber, I., C.E. Lumley, and D.D. Metcalfe. 1997. Allergenicity of proteins. *Human and Experimental Toxicology* 16(9):516-518.

King, L.J. 1966. *Weeds of the World: Biology and Control*. Aberdeen, U.K.: University Press.

Knippels, L.M., A.H. Penninks, and G.F. Houben. 1998. Continued expression of anti-soy protein antibodies in rats bred on a soy protein-free diet for one generation: the importance of dietary control in oral sensitization research. *Journal Of Allergy And Clinical Immunology* 101(6, Part 1):815-820.

Kong, P., L. Rubio, M. Polek, and B.W. Falk. 2000. Population structure and genetic diversity within California Citrus tristeza virus (CTV) isolates. *Virus Genes* 21(3):139-145.

Lamkey, K.R. 2002. *GMO's and Gene Flow: A Plant Breeding Perspective*. http://www.plantbreeding.iastate.edu/pdf/GeneFlow_2002.pdf. Accessed on May 3, 2005

Larsson, S. 1989. Stressful times for the plant stress-insect performance hypothesis. *Oikos* 56:277-283.

Lee, M.K., F.S. Walters, H Hart, N. Palekar, and J-S Chen. 2003. The mode of action of the *Bacillus thuringiensis* vegetative insecticidal protein Vip3A differs from that of Cry1Ab d-endotoxin. *Applied and Environmental Microbiology* 69(8):4648-4657.

Légère, A. 2005. Risks and consequences of gene flow from herbicide-resistant crops: Canola (*Brassica napus* L.) as a case study. *Pest Management Science* 61:292-300.

Lehrer, S.B., W.E. Horner, and G. Reese. 1996. Why are some proteins allergenic? Implications for biotechnology. *Critical Reviews In Food Science And Nutrition* 36(6):553-564.

Li, X.M, G. Kleiner, C.K. Huang, S.Y. Lee, B. Schofield, N.A. Soter, and H.A. Sampson. 2001. Murine model of atopic dermatitis associated with food hypersensitivity. *Allergy and Clinical Immunology* 107(4):693-702.

Lin, H.-X., L. Rubio, A. Smythe, M. Jiminez, and B.W. Falk. 2003. Genetic diversity and biological variation among California isolates of Cucumber mosaic virus. *Journal of General Virol*ogy 84(1):249-258.

Lopez, M.D., J.R. Prasifka, B.J. Bruck, and L.C. Lewis. 2005. Utility of ground beetle species in field tests of potential nontarget effects of Bt crops. *Environmental Entomology* 34: 1317-1324.

Lu, B., and A.A. Snow. 2005. Gene flow from genetically modified rice and its environmental consequences. *BioScience* 55:669-678.

Lung, S.C., W.L. Chan, W. Yip, L. Wang, E.C. Yeung, and B.L. Lim. 2005. Secretion of beta-propeller phytase from tobacco and *Arabidopsis* roots enhances phosphorus utilization. *Plant Science* 169:341-349.

Lynch, J.M., and J.M. Whipps. 1991. Substrate flow in the rhizosphere. In *The Rhizosphere and Plant Growth*. Edited by D. Keister and P. Cregan. New York: Kluwer Publishers.

Macintosh, S.C., T.B. Stone, S.R. Sims, P.L. Hunst, J.T. Greenplate, P.G. Marrone, F.J. Perlak, D.A. Fischhoff, and R.L. Fuchs. 1990. Specificity and efficacy of purified *Bacillus thuringiensis* proteins against agronomically important insects. *Journal of Invertebrate Pathology* 56:258-266.

Marvier, M. 2001. Ecology of transgenic crops. *American Scientist* 89:160-167.

Masuta, C., S. Kuwata, T. Matzuzaki, Y. Takanami, and A. Koiwai. 1992. A plant virus satellite RNA exhibits a significant sequence complementarity to a chloroplast tRNA. *Nucleic Acids Research* 20(11):2885.

Matthews, R.E.F. 1981. *Plant Virology*. 2nd ed. New York: Academic Press.

Mattson, W.J. 1980. Herbivory in relation to plant nitrogen content. *Annual Review of Ecology and Systematics* 11:119-161.

Mattson, W.J. and R.A. Haack. 1987. The role of drought in outbreaks of plant-eating insects. *BioScience* 37:110-119.

Mattson, W.J. and R.A. Haack. 1987. The role of drought stress in provoking outbreaks of phytophagous insects. In *Insect outbreaks*. Edited by P. Barbosa and J. Schultz. New York: Academic Press.

Maynard, F., R. Jost, and J.M. Wal. 1997. Human IgE binding capacity of tryptic peptides from bovine alpha-lactalbumin. *International Archives Of Allergy and Immunology* 113(4):478-488.

Mayo, M.A. and C.A. Jolly. 1991. The 5'-terminal sequence of potato leafroll virus RNA: evidence of recombination between virus and host RNA. *Journal Of General Virology* 72(Pt 10):2591-2595.

McDonald, M.B. and Copeland, L.O. 1997. *Seed Production: Principles and Practices*, New York: Chapman & Hall.

McDonald, M.B. and Copeland, L.O. 2001. *Principles of Seed Science and Technology*, 4th ed. Boston, MA: Kluwer Academic Press.

McNeill, S., and T.R.E. Southwood. 1978. The role of nitrogen in the development of insect/plant relationships. In *Biochemical aspects of plant and animal coevolution*. Edited by J. B. Harborne. New York: Academic Press.

Meerts, P., and C. Lefèbvre. 1989. [Genecological observations on a hybrid population of *Agrostis capillaris* L. and *A. stolonifera* L.] Observations génécologiques sur une population hybridée d'*Agrostis capillaris* L. et *A. stolonifera* L. *Bulletin de la Société Royale de Botanique de Belgique* 122:161-169.

Mendelsohn, M., J. Kough, Z. Vaituzis, and K. Matthews. 2003. Are Bt crops safe? *Nature Biotechnology* 21(9):1003-1009.

Metcalfe, D.D. 2003. Introduction: what are the issues in addressing the allergenic potential of genetically modified foods? *Environmental Health Perspectives* 111(8):1110-1113.

Metcalfe, D.D., J.D. Astwood, R. Townsend, H.A. Sampson, S.L. Taylor, and R.L. Fuchs. 1996. Assessment of the allergenic potential of foods derived from genetically engineered crop plants. *Critical Reviews In Food Science And Nutrition* 36:S165-S186.

Metcalfe, D.D., R.L. Fuchs, R. Townsend, H.A. Sampson, S.L. Taylor, and J.R. Fordham. 1996. Allergenicity of Foods Produced By Genetic Modification. *Critical Reviews in Food Science and Nutrition* 36:165-186.

Metz, P.L. J., W.J. Stiekema, and J. Nap. 1998. A transgene-centered approach to the biosafety of transgenic phosphinothricin-tolerant plants. *Molecular Breeding* 4:335-341.

Metz, P.L.J. and J.P. Nap. 1997. A transgene-centered approach to the biosafety of transgenic plants: an overview of selection and reporter genes. *Acta Botanica Neerlandica* 46:25-50.

Miller, H.I. 2003. Will we reap what biopharming sows? *Nature Biotechnology* 21:480-481.

Miller, W.A., G. Koev, and B.R. Mohan. 1997. Are there risks associated with transgenic resistance to luteoviruses? *Plant Disease* 81(7):700-710.

Milling, A., K. Smalla, F.X. Maidl, M. Schloter, and J.C. Munch. 2004. Effects of transgenic potatoes with an altered starch composition on the diversity of soil and rhizosphere bacteria and fungi. *Plant and Soil* 266:23-39.

Mitchell, C.E. and A.G. Power. 2003. Release of invasive plants from fungal and viral pathogens. *Nature* 421:625-627.

Moonan, F., J. Molina, and T.E. Mirkov. 2000. Sugarcane yellow leaf virus: an emerging virus that has evolved by recombination between luteoviral and poleroviral ancestors. *Virology* 269(1):156-171.

Morrison, L.A., O. Riera-Lizarazu, L. Cremieux, and C.A. Mallory-Smith. 2002. Jointed goatgrass (*Aegilops cylindrica* Host) × wheat (*Triticum aestivum* L.) hybrids: Hybridization dynamics in Oregon wheat fields. *Crop Science* 42:1863-1872.

Muchaonyerwa, P., S. Waladde, P. Nyamugafata, S. Mpepereki, and G.G. Ristori. 2004. Persistence and impact on microorganisms of *Bacillus thuringiensis* proteins in some Zimbabwean soils. *Plant and Soil* 266:41-46.

Mumm, R.H. and D.S. Walters. 2001. Quality control in the development of transgenic crop seed products. *Crop Science* 41:1381-1389.

Myers, Jr., O., M. Schmidt, D. Lightfoot, V. Njiti, M. Shenaut, and R. Buehler. 1999. *Interaction between application of Roundup Ultra to Roundup Ready soybean and subsequent response to sudden death syndrome (SDS).* Champaign, IL: Proceedings of the World Soybean Research Conference, August 4-7, 1999.

Naranjo, S.E. 2005a. Long-term assessment of the effects of transgenic Bt cotton on the abundance of nontarget arthropod natural enemies. *Environmental Entomology* 34:1193-1210.

Naranjo, S.E. 2005b. Long-term assessment of the effects of transgenic Bt cotton on the function of the natural enemy community. *Environmental Entomology* 34:1211-1223.

Naranjo, S.E., G. Head, and G.P. Dively. 2005. Field studies assessing arthropod nontarget effects in Bt transgenic crops: Introduction. *Environmental Entomology* 34:1178-1180.

Nielsen, K.M. and J.P. Townsend. 2004. Monitoring and modeling horizontal gene transfer. *Nature Biotechnology* 22(9):1110-1114.

Nielsen, K.M., A.M. Bones, K. Smalla, and J.D. van Elsas. 1998. Horizontal gene transfer from transgenic plants to terrestrial bacteria - a rare event? *FEMS Microbiology Reviews* 22:79-103.

Nielsen, K.M., and J.D. van Elsas. 2001. Stimulatory effects of compounds present in the rhizosphere on natural transformation of *Acinetobacter* sp. BD413 in soil. *Soil Biology and Biochemistry* 33:345-357.

Nielsen, K.M., J.D. van Elsas, and K. Smalla. 2000. Transformation of *Acinetobacter* sp. BD413(pFG4□nptII) with transgenic plant DNA in soil microcosms and effects of kanamycin on selection of transformants. *Applied and Environmental Microbiology* 66(3):1237-1242.

Nielsen, K.M., K. Smalla, and J.D. van Elsas. 2000. Natural transformation of *Acinetobacter* sp. strain BD413 with cell lysates of *Acinetobacter* sp., *Pseudomonas fluorescens*, and *Burkholderia cepacia* in soil microcosms. *Applied and Environmental Microbiology* 66:206-212.

Nilsson, C., H. Hallden, G. Lilja, M. Lundberg, and B. Harfast. 1999. A case of allergy to cow's milk hydrolysate. *Allergy* 54(12):1322-1326.

NRC. 2004. *Safety of Genetically Engineered Foods - Approaches to Assessing Unintended Health Effects*. Edited by Committee on Identifying and Assessing Unintended Effects of Genetically Engineered Foods on Human Health. National Research Council. Washington, DC: National Academies Press.

NRC. 2002. *Environmental Effects of Transgenic Plants: The Scope and Adequacy of Regulation.* Edited by National Research Council. Washington, DC: National Academy Press.

NRC. 2000. *Genetically modified pest-protected plants: Science and regulation.* Edited by Board on Agriculture and Natural Resources. Washington, DC: National Academy Press.

NRC. 1989. *Field testing genetically modified organisms: Framework for decisions.* Edited by National Research Council. Washington, DC: National Academy Press.

NRCS. 1999. *Soil Taxomony: A Basic System of Soil Classification for Making and Interpreting Soil Surveys*. Washington, DC. Natural Resources Conservation Service, United States Department of Agriculture.

OECD. 2004. *Consensus Documents for the work on the Safety of Novel Foods and Feeds*. http://www.oecd.org/document/9/0,2340,en_2649_34391_1812041_1_1_1_1,00.html. Accessed on May 3, 2006.

OECD. 2002. *Consensus document on compositional considerations for new varieties of maize (Zea mays): Key food and feed nutrients, anti-nutrients and secondary plant metabolites*. Paris, OECD.

OECD. 1996. *Consensus document on general information concerning the biosafety of crop plants made virus resistant through coat protein gene-mediated protection*. Series on Harmonization of Regulatory Oversight in Biotechnology No. 5. OECD Environment Directorate.

Ohta, M., Y. Hayashi, A. Nakashima, A. Hamada, A. Tanaka, T. Nakamura, and T. Hayakawa. 2002. Introduction of a Na+/H+ antiport gene from *Atriplex gmelini* confers salt tolerance to rice. *FEBS Letters* 532:279-282.

Oliveira, I.C., T. Brears, T.J. Knight, A. Clark, and G.M. Coruzzi. 2002. Overexpression of cytosolic glutamine synthetase. Relation to nitrogen, light, and photorespiration. *Plant Physiology* 129:1170-1180.

Osbourne, J.K., S. Sarkar, and T.M. Wilson. 1990. Complementation of coat protein-defective TMV mutants in transgenic tobacco plants expressing TMV coat protein. *Virology* 179:921-925.

OSTP. 2002. *Proposed Federal Actions to Update Field Test Requirements for Biotechnology Derived Plants and to Establish Early Food Safety Assessments for New Proteins Produced by Such Plants*. Washington, DC: Office of Science and Technology Policy.

Pappu, H.R. 1999. Biosafety issues of genetically engineered virus-resistant plants. In *Biotechnology, Biosafety, and Biodiversity: Scientific and Ethical Issues for Sustainable Development*. Edited by S. Shantharam and J. F. Montgomery. Enfield, New Hampshire: Science Publishers, Inc.

Parker, I.M., and P. Kareiva. 1996. Assessing the risks of invasion for genetically engineered plants: Acceptable evidence and reasonable doubt. *Biological Conservation* 78:193-203.

Pastorello, E.A., A. Conti, V. Pravettoni, L. Farioli, F. Rivolta, R. Ansaloni, M. Ispano, C. Incorvaia, M.G. Giuffrida, and C. Ortolani. 1998. Identification of actinidin as the major allergen of kiwi fruit. *Journal Of Allergy And Clinical Immunology* 101(4, Part 1):531-537.

Penninks, A.H., and L.M. Knippels. 2001. Determination of protein allergenicity: studies in rats. *Toxicology Letters* 120(1-3):171-180.

Perkins, M.C., H.A. Woods, J.F. Harrison, and J.J. Elser. 2004. Dietary phosphorus affects the growth of larval *Manduca sexta*. *Archives of Insect Biochemistry and Physiology* 55:153-168.

Persley, G.J. 2000. Agricultural Biotechnology and the Poor: Promethean Science. In *Agricultural Biotechnology and the Poor: An International Conference of Biotechnology*. Edited by G.J. Persley and M.M. Lantin. http://www.cgiar.org/biotech/rep0100/biocover.pdf. Accessed on May 3, 2006.

Pilate, G., E. Guiney, K. Holt, M. Petit-Conil, C. Lapierre, J.C. Leplé, B. Pollet, I. Mila, E. A. Webster, H.G. Marstorp, D.W. Hopkins, L. Jouanin, W. Boerjan, W. Schuch, D. Cornu, and C. Halpin. 2002. Field and pulping performances of transgenic trees with altered lignification. *Nature Biotechnology* 20:607-612.

Pilcher, C.D., M.E. Rice, and J.J. Obrycki. 2005. Impact of transgenic *Bacillus thuringiensis* corn and crop phenology on five nontarget arthropods. *Environmental Entomology* 34:1302-1316.

Pinçon, G., S. Maury, L. Hoffmann, P. Geoffroy, C. Lapierre, B. Pollet, and M. Legrand. 2001. Repression of O-methyltransferase genes in transgenic tobacco affects lignin synthesis and plant growth. *Phytochemistry* 57:1167-1176.

Poehman, J.M. and D.A. Sleper. 1995. *Breeding Field Crops*, 4th ed. Ames, IA: Iowa State University Press.

Polis, G.A. 1999. Why are parts of the world green? Multiple factors control productivity and the distribution of biomass. *Oikos* 86:3-15.

Popp, M.C., R.C. Wilkinson, E.J. Jokela, R.B. Harding, and T.W. Phillips. 1989. Effects of slash pine phloem nutrition on the reproductive performance of *Ips calligraphus* (Coleoptera: Scolytidae). *Environmental Entomology* 18:795-799.

Powell, W. 2006. *Chestnut Project: Restoration of an American heritage tree.* http://www.esf.edu/efb/powell/research.html#chestnut. Accessed on May 3, 2006.

Prasifka, J.R., R.L. Hellmich, G.P. Dively, and L.C. Lewis. 2005. Assessing the effects of pest management on nontarget arthropods: the influence of plot size and isolation. *Environmental Entomology* 34:1181-1192.

Pruss, G., X. Ge, X.M Shi, J.C. Carrington, and V.B. Vance. 1997. Plant Viral Synergism: The potyviral genome encodes a broad-range pathogenicity enhancer that transactivates replication of heterologous viruses. *Plant Cell* 9:859-868.

Pyšek, P., D.M. Richardson, M. Rejmánek, G.L. Webster, M. Williamson, and J. Kirschner. 2004. Alien plants in checklists and floras: Towards better communication between taxonomists and ecologists. *Taxon* 53:131-143.

Raney, T. 2004. Agricultural Biotechnology: Meeting the Needs of the Poor? In *The State of Food and Agriculture 2003-2004.* Food and Agriculture Organization of the United Nations.

Raven, J. A. 1983. Phytophages of xylem and phloem: a comparison of animal and plant sapfeeders. *Advances in Ecological Research* 13:135-234.

Raven, P.H., R.F. Evert and H. Curtis. 1981. *Biology of Plants*, 3rd ed. New York: Worth Publishers, Inc.

Raybould, A.F, L.C Maskell, M.-L. Edwards, J.I. Cooper, and A.J. Gray. 1999. The prevalence and spatial distribution of viruses in natural populations of *Brassica oleracea*. *New Phytologist* 141:265-275.

Richardson, D.M., P. Pyšek, M. Rejmánek, M.G. Barbour, F.D. Panetta, and C.J. West. 2000. Naturalization and invasion of alien plants: Concepts and definitions. *Diversity and Distributions* 6:93-107.

Richardson, S.J., M.C. Press, A.N. Parsons, and S.E. Hartley. 2002. How do nutrients and warming impact on plant communities and their insect herbivores? A 9-year study from a sub-Arctic heath. *Journal of Ecology* 90(3):544-556.

Rissler, J., and M. Mellon. 1996. *The Ecological Risks of Engineered Crops*. Cambridge, Massachusetts: MIT Press.

Rochow, W.F. 1977. Dependent virus transmission from mixed infections. In *Aphids and Virus Vectors*. Edited by K.F. Harris and K. Maramorosch. New York: Academic Press.

Rochow, W.F. and A F. Ross. 1955. Virus multiplication in plants doubly infected by potato viruses X and Y. *Virology* 1:10-27.

Rodenmeyer, M., W. H. Banzhaf, *et al.* 2001. *Biotech Branches Out: A look at the Opportunities and Impacts of Forest Biotechnology*, Proceedings from a workshop sponsored by the Pew Initiative on Food and Biotechnology, The Society of American Foresters, and The Ecological Society of America. Washington, DC: Pew Charitable Trusts.

Roossinck, M.J. 1997. Mechanisms of plant virus evolution. *Annual Review of Phytopathology* 35:191-209.

Rossi, A.M., and D.R. Strong. 1991. Effects of host-plant nutrition on the preference and performance of laboratory populations of *Carneocephala floridana* (Homoptera: Cicadellidae). *Environmental Entomology* 20:1349-1355.

Rossi, A.M., B.V. Brodbeck, and D.R. Strong. 1996. Response of xylem-feeding leafhopper to host plant species and plant quality. *Journal of Chemical Ecology* 22:653-671.

Roy, K.W., G.W. Lawrence, H.H. Hodges, K.S. McLean, and J.F. Killebrew. 1989. Sudden death syndrome of soybean: *Fusarium solani* as incitant and relation of *Heterodera glycines* to disease severity. *Phytopathology* 79:191-197.

Rubio, L., M. Borja, H. Scholtoff, and A. Jackson. 1999. Recombination with host transgenes and effects on virus evolution: an overview and opinion. *Molecular Plant Microbe Interactions* 12(2):87-92.

Rubio, L., M.A. Ayllon, P. Kong, A. Fernandez, M. Polek, J. Guerri, P. Moreno, and B.W. Falk. 2001. Genetic variation of Citrus tristeza virus isolates from California and Spain: evidence for mixed infections and recombination. *Journal of Virology* 75(17):8054-8062.

Salim, M. and R. C. Saxena. 1991. Nutritional stresses and varietal resistance in rice: effects on whitebacked planthopper. *Crop Science* 31:797-805.

Sampson, H.A. and D.D. Metcalfe. 1992. Food allergies. *Journal Of The American Medical Association* 268(20):2840-2844.

Sanford, J.C, and S.A. Johnston. 1985. The concept of pathogen derived resistance: deriving resistance genes from the parasite's own genome. *Journal of Theoretical Biology* 113:395-405.

Sankula, S. and E. Blumenthal. 2004. *Impacts on U.S. Agriculture of Biotechnology-Derived Crops Planted in 2003 - An Update of Eleven Case Studies*. Washington, DC: National Center for Food and Agricultural Policy.

Sanogo, S., X.B. Yang, and H. Schrem. 2000. Effects of herbicides on *Fusarium solani* f. sp. *glycines* and development of sudden death syndrome in glyphosate-tolerant soybean. *Phytopathology* 90(1):57-66.

Sawahel, W.A., and A.H. Hassan. 2002. Generation of transgenic wheat plants producing high levels of the osmoprotectant proline. *Biotechnology Letters* 4:721-725.

Saxena, D., and G. Stotzky. 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.

Saxena, D., and G. Stotzky. 2003. Fate and effects in soil of the insecticidal toxins from *Bacillus thuringiensis* in transgenic plants, Collection of Biosafety Reviews. International Centre for Genetic Engineering and Biotechnology, Trieste.

Saxena, D., S. Flores, and G. Stotzky. 1999. Insecticidal toxin in root exudates from Bt corn. *Nature* 402:480.

Schade, J.D., M. Kyle, S.E. Hobbie, W.F. Fagan, and J.J. Elser. 2003. Stoichiometric tracking of soil nutrients by a desert insect herbivore. *Ecology Letters* 6:96-101.

Schnepf, E., N. Crickmore, J. Van Rie, D. Lereclus, J. Baum, J. Feitelson, D.R. Zeigler, and D.H. Dean. 1998. *Bacillus thuringiensis* and its pesticidal crystal proteins. *Microbiology and Molecular Biology Reviews* 62(3):775-806.

Schuler, T.H., G.M. Poppy, B.R. Kerry, and I. Denholm. 1999. Potential side effects of insect-resistant transgenic plants on arthropod natural enemies. *Science* 17:210-215.

Sedjo, R.A. 2004. *Genetically Engineered Trees: Promise and Concerns*. Washington, DC: Resources for the Future.

Seefeldt, S.S., R. Zemetra, F.L. Young, and S.S. Jones. 1998. Production of herbicide-resistant jointed goatgrass (*Aegilops cylindrica*) × wheat (*Triticum aestivum*) hybrids in the field by natural hybridization. *Weed Science* 46:632-634.

Sherwood, J.L. 1987. Mechanisms of cross-protection between plant virus strains. In *Plant Resistance to Viruses*. Edited by D. Evered and S. Harnett. New York: Wiley.

Siemann, E. and W.E. Rogers. 2001. Genetic differences in growth of an invasive tree species. *Ecology Letters* 4:514-518.

Skinner, R.H. and A.C. Cohen. 1994. Phosphorus nutrition and leaf age effects on sweetpotato whitefly (Homoptera: Aleyrodidae) host selection. *Environmental Entomology* 23:693-698.

SL-100 (Soybean Seed Morphology), and SL-101 (Maize Seed Morphology). 1992. Prepared by ISU Seed Laboratory Staff. Ames, IA: Seed Testing Laboratory, Seed Science Center, University Extension, Iowa State University.

Slanksy, F. and P. Feeny. 1977. Stabilization of the rate of nitrogen accumulation by larvae of the cabbage butterfly on wild and cultivated food plants. *Ecological Monographs* 47:209-228.

Slovic, P. 2000. *The Perception of Risk*. Sterling, VA: Earthscan Publications, Ltd.

Smalla, K., S. Borin, H. Heuer, F. Gebhard, J.D. van Elsas, and K. Nielsen. 2000. Horizontal transfer of antibiotic resistance genes from transgenic plants to bacteria - are there new data to fuel the debate? Saskatoon, Canada: Proceedings of the 6th International Symposium on the Biosafety of Genetically Modified Organisms.

Smith, J.M., C.G. Dowson, and B.G. Spratt. 1991. Localized sex in bacteria. *Nature* 349:29-31.

Snow, A.A., M.R. Reagon, S.M. Selbo, D. Pilson, M.J. Paulsen, N. Pleskac, L.H. Rieseberg, and D.E. Wolf. 2003. A Bt transgene reduces herbivory and enhances fecundity in wild sunflowers. *Ecological Applications* 13:279-286.

Stanturf, J.A., R.C. Kellison, F.S. Broerman, and S.B. Jones. 2003. Innovation and forest industry: domesticating the pine forests of the southern United States, 1920-1999. *Forest Policy and Economics* 5(4):407-419.

Stewart, C., M.D. Halfhill, and S.I. Warwick. 2003. Transgene introgression from genetically modified crops to their wild relatives. *Nature Reviews Genetics* 4:806-817.

Stotzky, G. 2000. Persistence and biological activity in soil of insecticidal proteins from *Bacillus thuringiensis* and of bacterial DNA bound on clays and humic acids. *Journal of Environmental Quality* 29:691-705.

Stotzky, G. 2002. Release, persistence, and biological activity in soil of insecticidal proteins from *Bacillus thuringiensis*. In *Genetically Engineered Organisms: Assessing Environmental and Human Health Effects*. Edited by D.K. Letourneau and B.E. Burrows. Boca Raton, FL: CRC Press.

Stotzky, G. 2004. Persistence and biological activity in soil of the insecticidal proteins from *Bacillus thuringiensis*, especially from transgenic plants. *Plant and Soil* 266:77-89.

Strauss, S.H. 2003. Genomics, Genetic Engineering, and Domestication of Crops. *Science* 300:61-62.

Strauss, S.H., P. Coventry, M.M. Campbell, S.N. Pryor, and J. Burley. 2001. Certification of genetically engineered forest plantations. *International Forestry Review* 3(2):85-102.

Strong, D.R., J.H. Lawton, and T.R.E. Southwood. 1984. *Insects on plants: community patterns and mechanisms*. Cambridge, MA: Harvard University Press.

Superak, T.H, B.T. Scully, M.M. Kyle, and H.M. Munger. 1993. Interspecific transfer of plant viral resistance in *Curcurbita*. In *Resistance to Viral Diseases of Vegetables: Genetics and Breeding*. Edited by M.M. Kyle. Portland, Oregon: Timber Press.

Swiezynski, K.M. 1994. Inheritance of Resistance to Viruses. In *Potato Genetics*. Edited by J. Bradshaw and G. Mackay. Dundee: CAB International.

Tabashnik, B.E., Y.B. Liu, N. Finson, L. Masson, and D.G. Heckel. 1994. One gene in diamondback moth confers resistance to four *Bacillus thuringiensis* toxins. *Proceedings of the National Academy of Science* 94:1640-1644.

Tapp, H., and G. Stotzky. 1998. Persistence of the insecticidal toxin from *Bacillus thuringiensis* subsp. *kurstaki* in soil. *Soil Biology and Biochemistry* 30:471-476.

Taylor, M.R., J.S. Tick, D.M. Sherman. 2004. *Tending the Fields: State and Federal Roles in the Oversight of Genetically Modified Crops*. Pew Initiative on Food and Biotechnology. http://pewagbiotech.org/research/fields/report.pdf. Accessed on May 3, 2006.

Taylor, S.L. 1987. Food allergens: structure and immunologic properties. *Annals of Allergy* 59(5):93-99.

Tepfer, M. 2002. Risk assessment of virus-resistant transgenic plants. *Annual Review Of Phytopathology* 40:467-491.

Torres, J.B. and J.R. Ruberson. 2005. Canopy- and ground-dwelling predatory arthropods in commercial Bt and non-Bt cotton fields: patterns and mechanisms. *Environmental Entomology* 34:1242-1256.

Torsvik, V., J. Goksøyr, F.L. Daae, R. Sørheim, J. Michalsen, and K. Salte. 1994. Use of DNA analysis to determine the diversity of microbial communities. In *Beyond the biomass: Composition and Functional Analysis of Soil Microbial Communities*. Edited by K. Ritz, J. Dighton and K. Giller. Chichester, U.K.: John Wiley and Sons.

Traynor, P.L., and J.H. Westwood, editors. 1999. *Proceedings of a Workshop on Ecological Effects of Pest Resistance Genes in Managed Ecosystems*. Blacksburg, Virginia: Information Systems for Biotechnology, Virginia Polytechnic Institute and State University.

Urabe, J., and Y. Watanabe. 1992. Possibility of N or P limitation for planktonic cladocerans: an experimental test. *Limnology and Oceanography* 37:244-251.

Vacher, C., A.E. Weis, D. Hermann, T. Kossler, C. Young, and M.E. Hochberg. 2004. Impact of ecological factors on the initial invasion of Bt transgenes into wild populations of birdseed rape (*Brassica rapa*). *Theoretical and Applied Genetics* 109:806-814.

Van Doorsselaere, J., M. Baucher, E. Chognot, B. Chabbert, M.T. Tollier, M. Petit-Conil, J. C. Leple, G. Pilate, D. Cornu, B. Monties, M. Van Montagu, D. Inze, W. Boerjan, and L. Jouanin. 1995. A novel lignin in poplar trees with a reduced caffeic acid/5-hydroxyferulic acid O-methyltransferase activity. *Plant Journal* 8:855-864.

van Emden, H.F. 1966. Studies on the relations of insect and host plant. III. A comparison of the reproduction of *Brevicoryne brassicae* and *Myzus persicae* (Hemiptera: Aphididae) on brussels sprout plants supplied with different rates of nitrogen and potassium. *Entomologia Experimentalis et Applicata* 9:444-460.

van Frankenhuyzen, K., and T. Beardmore. 2004. Current status and environmental impact of transgenic forest trees. *Canadian Journal of Forest Research* 34:1163-1180.

Vance, C.P., C. Uhde-Stone, and D.L. Allan. 2003. Phosphorus acquisition and use: critical adaptations by plants for securing a nonrenewable resource. *New Phytologist* 157:423-447.

Vance, V.B, P.H. Berger, J.C. Carrington, A.G. Hunt, and X.M. Shi. 1995. 5' proximal potyviral sequences mediate potato virus X/potyviral synergistic disease in transgenic tobacco. *Virology* 206(1):583-590.

Vance, V.B. 1991. Replication of potato virus X RNA is altered in coinfections with potato virus Y. *Virology* 182(2):486-494.

Van der Have, D.J. 1979. *Plant Breeding Perspectives*. Wageningen: Centre for Agricultural Publishing and Documentation.

Verpoorte, R., A. Contin, and J. Memelink. 2002. Biotechnology for the production of plant secondary metabolites. *Phytochemistry Reviews* 1:13-25.

Vilà, M., E. Weber, and C.M. D'Antonio. 2000. Conservation implications of invasion by plant hybridization. *Biological Invasions* 2:207-217.

Vogt, D.U. and M. Parish. 2001. *Food Biotechnology in the United States: Science, Regulation, and Issues*. Congressional Research Service Report for Congress. The Library of Congress. Order Code RL30198. January 19, 2001. http://www.ncseonline.org/nle/crsreports/science/st-41.pdf. Accessed on May 3, 2006.

Wang, Z.Y., M. Scott, J. Bell, A. Hopkins, and D. Lehmann. 2003. Field performance of transgenic tall fescue (*Festuca arundinacea* Schreb.) plants and their progenies. *Theoretical and Applied Genetics* 107:406-412.

Wareing, P.F. and I.D.J. Phillips. 1981. *Growth and Differentiation in Plants*. Oxford: Pergamon Press.

Waterhouse, P.M, and A.F. Murant. 1983. Further evidence on the nature of the dependence of carrot mottle virus on carrot red leaf virus for transmission by aphids. *Annals of Applied Biology* 103:455-464.

Watson, M.T., T. Tian, E .Estabrook, and B.W. Falk. 1998. A small RNA resembling the beet western yellows luteovirus ST9-associated RNA is a component of the California motley dwarf complex. *Phytopathology* 88(2):164-170.

Wauchope, R.D., T.L. Estes, R.A., J.L. Baker, A.G. Hornsby, R.L. Jones, R.P. Richards, and D.I. Gustafson. 2001. Predicted impact of transgenic, herbicide-tolerant corn on drinking water quality in vulnerable watersheds of the mid-western USA. *Pest Management Science* 58:146-160.

White, T.C.R. 1993. *The inadequate environment: nitrogen and the abundance of animals*. New York, New York: Springer-Verlag.

White, T. C. R. 1969. An index to measure weather-induced stress of trees associated with outbreaks of psyllids in Australia. *Ecology* 50:905-909.

Whitehouse, M.E.A., L.J. Wilson, G.P. Fitt. 2005. A comparison of arthropod communities in transgenic Bt and conventional cotton in Australia. *Environmental Entomology* 34:1224-1241.

Widmer, F., R.J. Seidler, K.K. Donegan, and G.L. Reed. 1997. Quantification of transgenic plant marker gene persistence in the field. *Molecular Ecology* 6(1):1-7.

Williamson, M. 1993. Invaders, weeds, and the risk from genetically manipulated organisms. *Experientia* 49:219-224.

Williamson, M. 1996. *Biological Invasions*. London and New York: Chapman & Hall.

Willis, A.J., J. Memmott, and R.I. Forrester. 2000. Is there evidence for the post-invasion evolution of increased size among invasive plant species? *Ecology Letters* 3:275-283.

World Health Organization. 2004. *General Information about Biotechnology (GM Foods).* World Health Organization. http://www.who.int/foodsafety/biotech/general/en/. Accessed on May 3, 2006.

Worobey, M., and E.C. Holmes. 1999. Evolutionary aspects of recombination in RNA viruses. *Journal Of General Virology* 80(Pt 10):2535-2543.

Wrather, J.A., W.C. Stienstra, and S.R. Koenning. 2001. Soybean disease loss estimate for the United States from 1996 to 1998. *Canadian Journal of Plant Pathology* 23:122-131.

Wu, W., Q. Ye, and H. Min. 2004. Effect of straws from Bt-transgenic rice on selected biological activities in water-flooded soil. *European Journal of Soil Biology* 40(1):15-22.

Wu, Y.-Y., Q.-J. Chen, M. Chen, J. Chen, and X.-C. Wang. 2005. Salt-tolerant transgenic perennial ryegrass (*Lolium perenne* L.) obtained by *Agrobacterium tumefaciens*-mediated transformation of the vacuolar Na+/H+ antiporter gene. *Plant Science* 169:65-73.

Wych, R.W. 1998. Production of hybrid seed corn. In: *Corn and Corn Improvement*, 3rd ed., Edited by G.F. Sprague and J.W. Dudley. Madison, WI: American Society of Agronomy.

Xiao, K., H. Katagi, Z. Y. Wang, and M. Harrison. 2006. Improved phosphorus acquisition and biomass production in *Arabidopsis* by transgenic expression of a purple acid phosphatase gene from M. truncatula. *Plant Science* 170:191-202.

Yamada, M., H. Morishita, K. Urano, N. Shiozake, K. Yamaguchi-Shinozaki, K. Shinozaki, and Y. Yoshiba. 2005. Effects of free proline accumulation in petunias under drought stress. *Journal of Experimental Biology* 56:1975-1981.

Yamaguchi, T., and E. Blumwald. 2005. Developing salt-tolerant crop plants: challenges and opportunities. *Trends in Plant Science* 10:615-620.

Yang, X., Z. Liang, and C. Lu. 2005. Genetic engineering of the biosynthesis of glycinebetaine enhances photosynthesis against high temperature stress in transgenic tobacco plants. *Plant Physiology* 138:2299-2309.

Yeo, A. 1998. Predicting the interaction between the effects of salinity and climate change on crop plants. *Scientia Horticulturae* 78:159-174.

Yu, C-G, M.A. Mullins, G.W. Warren, M.G. Koziel, and J.J. Estruch. 1997. The *Bacillus thuringiensis* vegetative insecticidal protein Vip3A lyses midgut epithelium cells of susceptible insects. *Applied and Environmental Microbiology* 63(2):532-536.

Zechendorf, B. 1994. What the public thinks about biotechnology. *Bio/Technology* 12:870-875.

Zhang, H.X., and E. Blumwald. 2001. Transgenic salt-tolerant tomato plants accumulate salt in foliage but not in fruit. *Nature Biotechnology* 19:765-768.

Zhang, H.X., J.N. Hodson, J.P. Williams, and E. Blumwald. 2001. Engineering salt-tolerant *Brassica* plants: characterization of yield and seed oil quality in transgenic plants with increased vacuolar sodium accumulation. *Proceedings of the Natural Academies of Science* 98:12832-12836.

Zvomuya, F., and C. J. Rosen. 2002. Biomass partitioning and nitrogen use efficiency of 'Superior' potato following genetic transformation for resistance to Colorado potato beetle. *Journal of the American Society of Horticulture Science* 127:703-709.

---

i

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.