

Standard Review

Genetic engineering, ecosystem change, and agriculture: an update

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Genetically modified organisms (GMOs), alternatively called biotech crops, dominate soybean and cotton production and are rapidly increasing their fraction of market share for maize and rice in the U.S. Engineered canola is important in Canada, soybeans are dominant in Argentina and Brazil, and cotton is prominent in China and India. Adoption is much slower elsewhere, in large part due to concerns for potential ecosystem effects that may occur through development of weedy plants, by selection of herbicide resistant weeds and by effects of insecticidal proteins on nontarget insects. The precautionary principle is invoked by critics concerned that one must know in advance the effects of GMOs before releasing them. Alteration of weed species composition of agricultural fields is well documented to occur under herbicide selection pressure. Gene flow to wild relatives of crop plants can be shown under herbicide selection, and one instance (sunflower) is provided for insect resistance transfer leading to increased seed production by a weedy relative. Detailed stewardship programs have been developed by seed producers to minimize risks of gene flow. Although herbicides and insecticides are known to have major effects on agroecosystems, the ecosystem impacts of GMOs per se, thus far appear to be small.

Key words: gene-flow, herbicide-resistant weeds, genetically engineered crops, Bt maize, Roundup Ready soybeans

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1. Introduction

Recent advances in genetic technology and molecular biology have allowed greater molecular level understanding of many biochemical pathways, particularly for several model organisms and agricultural crops including rice and maize. Modification of pathways and products holds great potential for enhancing agriculture. Even prior to the recent sequencing successes, there was an effort to enhance the capabilities of crop plants through introduction or alteration of genes. In this review a number of examples are considered, mostly dealing with herbicide resistance and natural pesticide proteins. There is no discussion of animals or microbes, and wherever possible peer-reviewed literature from the past four years used. Earlier works may be found cited therein. Some information is only available on internet sites, and a significant fraction of articles are open-access. Tested URLs as of July 2006 are given.

Considering herbicide resistance as an example, there are both instances where gene transfer has been effected by recombinant DNA techniques (Laurent et al., 2000; Dill, 2005; Duke, 2005), and instances where selective breeding has been used to produce comparable results (Sebastian et al., 1989; Tan et al., 2005). There are thus far few indications that recombinant DNA per se will lead to outcomes that are qualitatively different from those that are available with conventional advanced breeding strategies.

When examining genetic engineering and ecosystem change in relation to agriculture, there are both events for which probabilities can be defined and socio-political considerations related to perceptions of risk. A probability of 1 in a million is small, but during a billion events, such as seed pollination, there will be about 1000 occurrences. Some individuals, organizations and governments have expressed concern that through recombinant DNA techniques it may be possible to produce genetically engineered plants that may have a qualitatively different impact on ecosystems and people than conventionally bred and selected plants. Some of the perceived risks are discussed by Madsen and Sandoe (2005) and Devine (2005). Perceptions of risk, and frequencies of actual events are not linearly correlated but the topics are inextricably entwined. This review focuses mainly on what is being done through genetic engineering and advanced plant breeding, for which probabilities of occurrence (such as gene flow frequency) may be established, but one cannot avoid some discussion of perceived risks. Major perceived risks include potentially catastrophic ecosystem alterations such as have occurred with invasive weeds. For more extensive discussion of both perceived risks and quantitatively measured events see for instance the published proceedings of the 8th International Symposium on Biosafety of Genetically Modified Organisms (GMOs) (ISBR, 2004).

Although thousands of transgenic crop plants have been approved for field testing in the U.S., the rate of

approval and commercial release of engineered plants has decreased greatly in the past five years. Duke (2005) indicates that there were only seven new approvals for herbicide resistance world-wide from 2000-2003, compared to 37 in the six years prior to that. High costs for research and development are considerations but costs of regulatory approval and trade restrictions may be larger factors (Devine, 2005). Few species of modified crop plants have been submitted for approval in Canada since 2000. They include alfalfa, cotton, lentil, maize, potato, rice, soybean, sunflower, sugar beet, and wheat (Canada, 2006).

In Table 1, I have attempted to summarize some of the perceived risks of genetically modified organisms (GMOs), specifically agricultural crop plants. Recent research that addresses these risks is discussed throughout the body of this review. Where it is known, a quantitative assessment is given.

2. Historical perspective on ecosystem change

Humans have always had effects on ecosystems. The use of fire, and effective hunting weapons, has produced profound changes in the flora and fauna across whole continents. As human populations increased, their impact increased disproportionately. While settlement and the development of agriculture allowed greater populations to survive on smaller areas, it also increased the ecological impact in those settled areas. Domestication of plant species and establishment of large areas of uniform cultivars of only a few species greatly decreased biodiversity in cultivated areas. Even a century ago, there were few large landscape areas that were not affected by the human presence.

Very recently in an evolutionary time scale, systematic plant breeding and highly mechanized agriculture which makes use of fossil fuels, together have resulted in huge areas being converted to production of relatively few species over large areas. During the mid to late 20th century, herbicides and pesticides further narrowed the abundance of species living in cultivated areas. This occurred prior to the advent of genetic engineering. Now it is not uncommon to find areas up to 1000 ha with >90% of the planted consisting of one cultivar of one species, such as wheat or maize. Few borders, hedgerows, woodlots or pastures remain in large portions of the U.S. and other highly mechanized agricultural production areas. This naturally supports fewer kinds of microbes, insects and animals than would a more diverse plant population. In the 21st century, genetic engineering may be used in a number of ways to once more alter the modes of production being used, possibly over even larger areas. In this review I will look at a few examples that may help us gain an appreciation of how genetic engineering might affect ecosystems, in comparison to current practices.

Table 1. A Guide to Comments on Perceived Risks of GMOs

GMO Category	Perceived risk	Section discussing the perceived risk
Herbicide resistance genes	weediness of crop	Crops as weeds
Herbicide resistance genes	gene and trait transfer to weedy relatives	Gene flow from desired plants to others; selecting natural herbicide resistance vs genetically engineered resistance
Herbicide resistance genes	overuse of herbicides	Fire, tillage and herbicides as management tools; ecosystem impacts of herbicides
Herbicide resistance genes	unanticipated emergent traits of GMOs	Producing herbicide resistance in crop plants without genetic engineering; risk of novel traits vs risk of genetic engineering; perceived risk vs quantifiable effects
Insect resistance genes	toxicity to nontarget insects	Potential insect population shifts
Insect resistance genes	gene transfer to weedy relatives, upsetting predator control	Gene flow from desired plants to others; demonstrable ecosystem impact of a transgene migration
Insect resistance genes	human sensitivity to introduced protein	not addressed here
Insect resistance genes	unanticipated emergent traits of GMOs; altered plant composition	Risk of novel traits vs risk of genetic engineering; perceived risk vs quantifiable effects
Phytoremediation genes	unanticipated emergent traits of GMOs	Enhanced phytoremediation

3. Fire, tillage, and herbicides as management tools

Fire has long been used as a tool to manage ecosystems for human benefit (O'Neill 2006). Fire suppression rapidly leads to huge changes in a landscape such as the prairies and forests of the U.S. Within prairies, lack of fire results in invasion by woody species and suppression of the grassland species (Konza, 2002). Few herbicides have ever been used to produce such extensive changes in species composition as occurs with repeated fires, with the possible exception of mangrove extermination during the Vietnam war where close to 5 million ha of land were treated up to three times (Buckingham, 1983). In forests, fire alters species composition, yet fire suppression ultimately results in more extreme wildfires, yielding major upsets in seemingly stable ecosystems (O'Neill, 2006). Slash and burn agriculture obviously has some comparable effects. Both natural and controlled burns release above-ground mineral nutrients quickly, which herbicides do not. Fire lowers N content of the remaining material, selecting for plants that demand low N (Konza, 2002). Herbicide application results in nutrients remaining tied up in biomass, until natural decomposition releases them to become available for new plant growth. Changes of N content are less. Tillage is intermediate between fire and herbicide in rate of nutrient release. Compared to the impacts of presence or absence of fire, or application of herbicides, the impact of genetically modified organisms per se is likely to be relatively small, though different, depending on how the modified organisms are applied. To the extent that it allows substitution of herbicides for tillage, it will reduce the rate of nutrient turnover.

4. Some present applications of genetically modified plants

Among the earliest transgenic plants that were introduced to commercial markets there were potatoes resistant to the Colorado potato beetle, herbicide resistant flax, a tomato with delayed ripening, and squash with multiple virus resistance. Only the last of these is still on the market (Byrne et al., 2006). Cost/profit considerations and consumer acceptance issues have focused the market in a few countries and on large acreage crops where there are not large numbers of different cultivars that must be tested one by one. Prominent examples are discussed below.

Lentils, alfalfa, sugar beet and wheat have been submitted for approval in Canada but commercial application is very limited or non-existent thus far.

4.1 Engineered herbicide resistance

Weedy plants are characterized by their abilities to grow on disturbed areas, rapidly, with low inputs, displacing more desirable species. When agriculturally desirable species are not able to out-compete weeds, herbicides and cultivation have typically been used to reduce weed populations. Some weeds, such as nutsedge (*Cyperus esculentus*) (CWMA, 2006) or Tropical Spiderwort (*Commelina benghalensis*) (Ferrell et al. 2005), have growth habits that make them relatively resistant to cultivation as a means of control. Selective herbicides are often more effective and less expensive than mechanical means. For about half a century broad

spectrum herbicides such as 2,4-dichlorophenoxyacetic acid (2,4-D) have proven useful to control whole genera of weeds. But with 2,4-D, control of many grassy weeds is not possible, only broad-leafed species and sedges are reasonably susceptible. Typically, the weed population shifts in response to herbicide treatments, giving rise to a different set of troublesome weeds, when some are removed (Itoh, 1994; Owen and Zelaya, 2005; Purecelli and Tiesca, 2005).

An alternative strategy for weed control is to protect the desired crop plant against a herbicide, either through selection of resistance, or engineering of resistance genes, and then to apply a broad-spectrum herbicide. In principle this can produce a clean crop with no weeds. The best example of this strategy is use of "Roundup Ready" soybeans and other crops within the U.S. Use of resistance to the broad spectrum herbicide glyphosate (trade name Roundup) presupposed acceptance of genetically engineered plants, because producing resistance to that herbicide required introduction of a bacterial gene for aromatic amino acid synthesis, resistant to the herbicide. The advantage to farmers was so obvious from their standpoint that this herbicide strategy was rapidly adopted to the large majority of U.S. soybean production within a few years.

Five years after introduction, 70% of all U.S. soybean production was glyphosate resistant. In Argentina adoption was more rapid and extensive, reaching 98% (Dupont, 2006). In Brazil, the number two exporter of soybeans, GM crops were illegal prior to 2004 (but were grown). It is estimated that in Brazil over 9 million ha were planted with glyphosate resistant soybeans in 2005 (James, 2006). For the U.S. the area exceeded 30 million ha (Monsanto, 2006). Soybeans are the majority of all biotech crops planted world-wide (>50 million ha), with maize having about 40 % as much area (James, 2006). For maize, over half of all planted area in the U.S. contains introduced genes, about 1/4 contains herbicide resistance (USDA, 2006). Insect resistance is a more important in maize (see below). Triazine herbicides are generally effective in maize plantings as it is inherently tolerant to that herbicide class, so no engineering is required.

Recently a patent application shows that glyphosate resistance can be obtained by direct selection of soybeans using glyphosate as a seed selection (Davis, 2005a). Now we can no longer distinguish genetically engineered vs no-engineered glyphosate-resistant plants at the phenotypic level, although there will be an obvious difference at the DNA level, because the engineered plants contains DNA from other, bacterial species.

4.2 Engineered herbicide detoxification

An alternative strategy of detoxifying glyphosate by acetylation is now being announced, in combination with resistance to another herbicide, targeting acetolactate

synthase (ALS) under the name of Optimum GAT (Dupont, 2006). The acetyl transferase uses another bacterial enzyme which has been engineered in the laboratory to increase its effectiveness (Castle et al., 2004).

Transgenic plants with introduced cytochrome P450 genes have been shown to degrade various pesticides more efficiently than their unmodified counterparts (Inui and Ohkawa, 2005). This may be useful both for phytoremediation of sites with contamination and for enhancing resistance of plants to herbicides or insecticides. Some of the P450 enzymes (mixed function oxidases) have broad substrate range specificity; others are more restricted in their activity. "Stacking" resistance by introduction of multiple P450 genes has been reported by Kawahigashi et al. (2005), while one particular P450 (CYP2B6) alone gives resistance to 13 of 17 herbicides tested from several classes (Hirose et al., 2005). In these two instances, rice is the plant being modified and the potential for transfer of genes to weedy wild rice is a consideration for concern. Because the CYP2B6 is a human enzyme, selection of a comparable form directly from those naturally present in plants is not likely, although increased metabolism of herbicides could perhaps be selected directly.

Introduction of a somewhat more selective oxidase was used to produce cotton resistant to 2,4-D as described by Laurent et al. (2000). In this instance a dioxygenase from a bacterial species was able to oxidize 2,4-D to dichlorophenol, which in turn was rapidly conjugated by endogenous enzymes within the plant to yield more polar, less toxic products including a glucoside, malonyl glucoside and sulfatoglucoside. In this instance, transfer of the gene to weedy relatives of cotton is unlikely, because they are not indigenous to many cotton growing areas. Of course comparable engineering of oilseed rape would be more problematic, because weedy relatives are usually common in areas where it is grown (Lutman et al., 2005).

4.3 Introduction of natural pesticide

In the area of insect control, initially very broad spectrum, persistent insecticides such as DDT or arsenate of lead were often used. This resulted in a depauperate insect fauna in treated areas because they killed many nontarget and predator species. Introduction of specific insecticidal proteins into plants, rather than applying general insecticides, may well yield a richer fauna, even within a crop monoculture. The most prominent example of this approach is introduction of *Bacillus thuringiensis* Cry protein genes into maize to prevent the infestation by European corn borer and corn earworm that is common in unprotected fields. (Product name commonly Yieldgard in U.S.). Specific stewardship agreements were developed in 2001, to assure that selective pressure does not elicit resistant forms of the targeted insects (Sloderbeck,

2006). Other lepidopterans including rootworms are also inhibited by related Cry proteins, and products have been introduced (e.g. Herculex XTRA by DuPont).

Similar benefits have been observed when Cry genes are introduced into cotton to prevent bollworm feeding. Monsanto Company markets Bollgard varieties of cotton, containing Cry genes, which are planted on a large fraction of all the cotton area in the U.S. In most of that area, the Roundup Ready trait is also present. India and China are rapidly adopting the same technology (James, 2006). Monsanto reported an estimated 8 million ha of Bollgard cotton in India and 2 million ha in China for 2006. China has other locally developed sources of Bt cotton (Huang et al., 2002), so two-thirds of all cotton grown there now is Bt cotton (China Daily, 2006). The advent of Bt cotton has resulted in increased concern for formerly minor insect pests in cotton in China, presumably because of decreased insecticide use (Jia, 2006).

4.4 Enhanced phytoremediation

Thus far a minor area of genetic engineering is the modification of plants to enhance phytoremediation. Plants have inherent capacity to deal with a wide range of chemical classes because they are exposed to numerous chemicals in their environment, whether from other plants or predators. Typically, for degradation, xenobiotics are hydroxylated by cytochrome P450 type oxidases and then conjugated to glutathione or sugars or organic acids to increase their polarity. Then they are transferred to the vacuole or out of the plant cell into the region of the cell wall. Many compounds end up incorporated into the insoluble wall material, often by lignification enzymes (Davis et al., 2002). If plants are deliberately used to speed up the degradation or sequestration of undesirable chemicals by these routes it is termed phytoremediation (Burken, 2003).

The actual mechanism of action for any particular instance of phytoremediation or herbicide detoxification has to be determined experimentally and only a few pathways are known in detail (Harms et al., 2003; Schwitzguebel and Vanek, 2003). Until the likely path is known, it is difficult to enhance it by engineering. In many cases of phytoremediation at the field scale, plants simply supply substrate for microbes that are the actual effectors of remediation (Davis et al., 2002).

The uptake and metabolism of heavy metals by plants has been extensively studied. Some species accumulate metals or metalloids to concentrations above those found in the soil and sequester the metal so that it does not produce toxicity to the plant. Specific examples are accumulation of cadmium with phytochelatins & metallothioneins, volatilization of metallic mercury or selenium, and accumulation of arsenate. Overexpression of the phytochelatins & metallothioneins may allow a plant to accumulate higher concentrations of the heavy metals (Cobbett and Goldsbrough, 2002; Eapen and D'Souza,

2005; Gratao et al., 2005). Expression of bacterial reductases, the Mer genes, has been shown to promote mercury volatilization, and by engineering the Mer genes into the chloroplast, uniparental inheritance is assured (Ruiz et al 2003), because chloroplast genes are generally not transmitted by pollen. Selenium volatilization has been enhanced too (Berken et al., 2002) though many plants are fairly effective in this process without any engineering.

Because in each instance the engineered plants used for phytoremediation are to be applied to very specific relatively small areas, they are unlikely to have anywhere near the environmental impact that would be had with a plant species engineered to be generally resistant to one or more herbicides. Nor does it appear that transfer of the genes in question to native populations is likely to happen, unless the engineered species is indigenous to the location being treated. Then the chloroplast transformation strategy of Ruiz et al. (2003) may be applied to prevent movement of engineered genes by pollen flow. A third concern would be if the introduced genes produced a significant advantage to the plants carrying them so that they might become more invasive. With the species used thus far, that is not likely to be an issue. Usually the engineered genes only provide an advantage to the plant under the conditions of contamination, if at all.

5. Ecosystem impacts of herbicides

Use of broad spectrum herbicides must of necessity have impact on more species than use of individual selective herbicides. However, when the objective is a clean crop, multiple applications of multiple fairly selective herbicides, necessary to control different classes of weeds, could lead to equivalent or more damage than use of a single broad-spectrum herbicide, depending on the residual action and the drift of the applied herbicides (Karthikeyan et al., 2004a,b). There is not a large amount of literature on herbicide effects on nontarget species, but there are some ecological studies, cited by Karthikeyan et al. (2004a,b) indicating that spray drift significantly alters species composition in areas not part of the intentionally managed area.

Large changes of the weed population occur within a herbicide treated crop area, as reviewed by Owen and Zelaya (2005). Use of herbicide may actually increase weed species diversity within treated areas, at least by some measures. Purecelli and Tuesca (2005) found that in Argentina, application of glyphosate did decrease the numbers and diversity of early-emerging weeds, but promoted the appearance of late-season broad-leaved weeds. In one instance the weed was known to be present at low levels prior to initiation of the study and became abundant, while another weed was not identified at the study location until after application of the herbicide. It has inherent glyphosate resistance, and the authors

predict that with continued use of glyphosate in crop rotations the abundance of such weeds will increase. Similar trends are forecast for other herbicides in other areas (Owen and Zelaya, 2005).

There are suggestions that use of herbicide resistant crops will hasten changes already occurring under mechanized large-scale agriculture. For instance, modeling of production systems suggests that glyphosate resistant sugar beets may allow elimination of weeds that are essential food sources for birds in the U.K. (Watkinson et al., 2000). Although such sugar beets have been available since the late 1990s, no U.S. processor had accepted any transgenic beets through 2001 (Gianassi et al., 2002) and none are presently grown commercially in Europe, although they have been widely studied at the field scale (Teagasc, 2006).

As an example of how herbicide application may radically alter a weedy ecosystem consider the example of Tropical Spiderwort (*Commelina benghalensis* L.) in Florida (Ferrell et al., 2006). This weed was uncommon until about a decade ago and now is a major concern. It has an inherent high tolerance to glyphosate so that it is not well controlled in "Roundup Ready" crops, or in minimum tillage systems that depend on the herbicide to clear fields before planting. Because the plant rapidly reproduces from fragments, ordinary cultivation is relatively ineffective against it also. This makes it a serious difficult to manage weed, as an indirect effect of genetic engineering of crop species. It is not the GMO per se that alters the ecosystem, but rather its interaction with the herbicide management strategy that does so.

Reddy (2004) has examined the relative shift in weed populations under different herbicide regimes with cotton resistant to either bromoxynil (BR) or glyphosate (GR). Continuous BR led to increase of three main weeds ~15 to 350 fold over 3 seasons, compared to continuous GR. The most dramatic increase was with nutsedge (*Cyperus esculentus*) which had 373 plants per m² in BR vs 1 in GR. This weed is difficult to control by cultivation also as noted above.

6. Crops as weeds from carryover of herbicide resistant crops

A non-trivial problem in some cropping sequences is the appearance of volunteer seedlings of the crop plant which may allow survival and multiplication of plant pests, including insects and viruses, even if the intended following crop in a rotation is not a host for the insect or virus. Herbicide resistance interacts with this in a subtle way. If we have "Roundup Ready" brassica or wheat, we can no longer control spontaneous seedlings with that herbicide which has often been used in minimum tillage systems to clear the field before planting. In particular with *B. rapa* or *B. napus*, herbicide tolerance of spontaneous seedlings can have significant impact on following crops. Lutman et al (2005) found that an

average of 3575 seeds per m² was lost at harvest of *B. napus*, due to shattering of the seed pods. While 60% of these spilled seeds lost viability within a few months, the decline was slower over following years with 5 % persisting to 5 yr at several sites in the U.K. The volunteer seedlings that arise from these may be strongly competitive weeds for another crop grown in rotation, such as sugar beets. Their inherent herbicide resistance may increase the difficulty of control, unless the following crop is also herbicide resistant (for a different herbicide) as could be the case with sugar beets if the transgenic versions were accepted. Both plants genetically engineered for glyphosate or glufosinate resistance, and those conventionally selected for tolerance to imidazolinones had the same behavior in the study of Lutman et al. (2005). So it is not the genetic engineering per se that makes these "weeds" troublesome, but rather the dependence on a single herbicide.

Owen and Zelaya (2005) consider the problem of glyphosate resistant maize and soybeans in rotations of those crops. While maize may sometimes be a problem in a following crop of soybeans, soybeans have poor winter survival and are not competitive with a following crop of maize. Deployment of glyphosate resistant spring wheat has been delayed for economic and management reasons, including potential weediness which would have to be controlled by a comprehensive stewardship program (Dill, 2005).

7. Selecting natural herbicide resistance vs genetically engineered resistance

Many people express concern that genetically engineered crops may transfer genes to wild relatives resulting in either increased competition from the undesirable wild relatives, as for instance with shattering sorghum or rice, or that they may give new combinations of genes that yield unexpected results such as extreme competitiveness of a weed (e.g. Garcia and Altieri, 2005). In most instances it can be shown that for herbicide resistance at least, the resistant form is unlikely to out-compete the susceptible in the absence of strong selective pressure. With the exception of the deliberately introduced genes for resistance to, or degradation of, glyphosate and glufosinate, most forms of herbicide resistance arise spontaneously so far as we can tell. They only emerge with detectable frequency in a population under strong selective pressure. For instance in South-East Asia, there were four biotypes of various species resistant to paraquat and three resistant to 2,4-D after 20 years of intensive use of these herbicides (Itoh, 1994). It was also noted that a change to direct seeding of rice has resulted in a whole suite of different problem weeds, with a shift to grassy weeds, which have similar herbicide resistance as the crop plant.

As of June 2006, there are known to be three hundred biotypes of nearly 200 species with herbicide resistance

that has resulted specifically from selective pressure of herbicide application (Heap, 2006). Just two dozen biotypes have an identified resistance to synthetic auxins, although the auxins have been in use for about 50 years. So far, 95 biotypes, representing 70 species, have developed resistance to acetolactate synthase inhibitors. There are five classes of such chemical inhibitors targeting one enzyme (Dupont, 2006). They are known as sulfonyl ureas, imidazolinones, triazolopyrimidines, pyrimidyl thiobenzoates and sulfonylamino- carbonyl-triazolinones. Some resistance includes multiple chemical classes, some does not. The high number of resistant types may relate to the popularity of these herbicides, or the single amino acid change required to produce resistance without detriment to the plant (Tranel and Wright, 2002). There are 65 biotypes of weeds resistant to triazine type herbicides that work at photosystem II. In this instance the altered plant is at a significant disadvantage in the absence of selective pressure from herbicide (Jordan, 1999). Twenty-three biotypes are resistant to photosystem I inhibitors like paraquat and 21 resist inhibition at photosystem II by chlortoluron or its relatives. The geographic distribution of resistant biotypes is related to the intensity of herbicide use with the U.S. having the greatest number of reports (112), followed by Australia with 47, Canada with 44, France with 30, Spain with 27, the U.K with 24 and Israel with 20.

Mutant forms of herbicide-binding proteins may arise (or be identified) repeatedly within one species, or at an equivalent site of the receptor or enzyme protein within different species. In some cases different amino acids at the herbicide binding site are altered in different biotypes. This is particularly clear and common for the ALS inhibitors (Tranel and Wright, 2002). Because the selected populations are not mutagenized, one must assume that the selected resistant biotypes are pre-existent within the population. They must be present at very low frequency or resistance would be observed more quickly than is typical for successfully introduced herbicides. Tranel and Wright (2002) discussed likely causes for the relatively high incidence of resistant ALS which include its dominant character, variety of active site modifications possible, and low fitness penalty to plants carrying mutant forms of the enzyme.

Some forms of resistance depend on changes in translocation or metabolism of the herbicide. For instance, the conjugation of atrazine to glutathione is enhanced in foxtail millet (*Setaria italica*) resistant to atrazine (Gimenez-Espinosa et al., 1996). Cross-resistance of rigid ryegrass (*Lolium rigidum*) to quite different herbicides (diclofop-methyl and chlorsulfuron) depends on increased metabolism using a mixed function oxidase (Christopher et al., 1991). Wheat possesses sufficient activity of this enzyme that it is naturally resistant to chlorsulfuron at certain (field application rate) doses, although it is still sensitive to other inhibitors of acetolactate synthase beside chlorsulfuron. The newly

reported resistance of downy brome (*Bromus tectorum*) to chlortoluron, a photosystem electron transport inhibitor (Menendez et al., 2006) also depends on induction of oxidase(s). This kind of resistance is similar to that induced by use of safeners.

The nature of glyphosate resistance in two weed species has been characterized. For *Conyza canadensis* (horseweed), decreased translocation of ¹⁴C labeled glyphosate has been shown to be associated with the resistant phenotype (Koger and Reddy, 2005). Uptake is not altered in the source leaf. For *Lolium rigidum* (rigid rye) translocation is also affected (Lorraine-Colwill et al., 2003). The mutant rye was identified in a field after 15 years of repeated glyphosate use (Powles et al., 1998). In neither species is the detailed mechanism of resistance understood. There is no evidence for selective advantage of the weed in absence of the herbicide.

8. Producing herbicide resistance in crop plants without genetic engineering

In some countries, the major objections to GMOs are based on the construct rather than the consequence. Hence, advanced breeding strategies not using recombinant DNA have been applied. Sebastian et al. (1989) described successful selection of a soybean line resistant to sulfonyl urea herbicides, whose site of action is the acetolactate synthase (ALS) enzyme. They used chemical mutagenesis of 400,000 soybean seeds and obtained one line after selection with chlorsulfuron. This laborious strategy was used because at the time there was no reliable way to regenerate soybean plants from tissue culture, so neither engineering and transformation, nor selection in tissue culture were viable options to obtain resistance.

Recent patent applications claims that wheat resistant to glyphosate can be obtained by direct selection from hard red winter wheat cultivars (Davis, 2005b). Genes allowing the resistance are identified by name. In this instance a strong selective pressure was applied to identify uncommon genes already present in the population. If these genes are deployed, glyphosate can no longer be effectively used to control volunteer wheat seedlings (Lyon et al., 2002).

One large chemical company, BASF, already markets non-engineered (non-GM) crop plants with high resistance to a particular herbicide family that acts on the enzyme ALS. The Clearfield production system for wheat makes use of wheat that was selected for resistance to field application levels of Beyond herbicide (active ingredient imazamox, a member of the imidazolinone family). The resistance arises as a natural, selected mutation of the enzyme. Note that wheat is inherently resistant to another ALS inhibitor, chlorsulfuron, through oxidation and glycosylation (Christopher et al., 1991). The broad-spectrum herbicide Beyond is specifically intended to allow control of jointed goatgrass, (*Aegilops cylindrica*)

a close relative of wheat, as well as other grasses and broad-leaf weeds. The entire stewardship program of the Clearfield system includes a number of restrictions on the grower, including purchase of certified seed each year, management with appropriate rotations to avoid selection of resistant weeds, and judicious use of other herbicides (Clearfield wheat, 2004).

The same general strategy, with slightly different herbicides and doses can be had for maize, canola and rice according to company literature, available on-line (Clearfield, 2006). Application of this production strategy promises great advantages in some specific regions. For instance, the Clearfield rice cultivars are grown on 30-40% of the entire acreage of rice in Arkansas and Louisiana after only a few years of availability. A weedy rice relative called red rice is a serious problem, because it freely interbreeds with commercial cultivars, and competes for space, greatly lowering yields and market quality of the desired cultivars wherever it is present. Thus far the red rice, which shatters, dropping seed prior to harvest, is susceptible to the herbicide, named Newpath, an imidazolinone. Under the stewardship agreement enforced by BASF, only certified seed free of red rice is to be grown with the Clearfield gene. Carelessness in using the herbicide, or reutilization of seed contaminated with red rice that has picked up the resistance, can lead to a rapid breakdown of the control strategy (Bennett, 2006). Outcrossing has happened within the first two years of growing the rice large-scale (Boyd, 2005). The weedy relative with resistance is highly competitive and produces a large yield of shattering seed. (See further discussion of gene flow below.)

Since 2004 a similar Clearfield production system has been available for sunflower (NSA 2005). A wild sunflower with resistance to herbicides of this class was found in Kansas in 1996. From this, a USDA breeder in Fargo, ND was able to breed by backcrossing and selection to produce oil-seed sunflowers with the desirable traits of the cultivated form. Use of Beyond herbicide in fields of resistant sunflower permits effective control of problem weeds like cocklebur and of course wild sunflower volunteers. However, as with rice, out-crossing to the weedy relative is a serious concern. Failure to use the herbicide at appropriate dose in a field with infestation by the wild relative will lead to development of a resistant population of the weedy form through natural gene flow by pollination. It may be that these advanced strategies as exemplified by rice and sunflower ALS inhibitor programs are only usable under highly mechanized, advanced agriculture with viable crop rotations and alternatives.

The Clearfield technology has captured a significant fraction of the Canadian market for canola (low erucic *Brassica*) with about a million hectares, and up to several million hectares of maize in the U.S. Equivalent mutations have been identified in other crops including sugarbeet, cotton, lettuce, tomato and tobacco indicating a potential for application to a further range of crops (Tan et al., 2005). Whether their development is economically

justified in the view of herbicide manufacturers remains to be seen, because even non-engineered crops require major investment in regulatory compliance testing for use in some jurisdictions, such as Canada (Devine, 2005).

Very recently CIMMYT has developed imidazolinone-resistant (IR) maize for use in Africa (CIMMYT, 2006). Seed is now under offer for testing at research centers for control of *Striga*, a parasitic plant that is not, thus far, resistant to this class of herbicides. The same company that deployed Clearfield crops in the U.S. and Europe is offering to develop treated seed processes for Kenya, and in future presumably in other countries. As with the crops previously deployed, there are specific stewardship agreements to be signed so that the technology does not experience a quick breakdown through development of *Striga* resistance to the herbicide. The African Agricultural Technology Foundation, CIMMYT and BASF provide details for the StrigAwayR technology (AATF, 2006). Coating seed with imidazolinone herbicide allows normal growth of the maize crop while inhibiting any parasite that attaches to the treated seedling.

9. Risk analysis for modified crops

9.1. Gene flow from desired plant to others

One issue repeatedly raised as a concern is gene flow from engineered crops (Ellstrand and Hoffman, 1990; Eastham and Sweet, 2002). Engineering or selecting herbicide resistance is unlikely to make a cultivar into a troublesome weed, although volunteer wheat and brassica seedlings have raised some concerns. Much more likely is introgression of the trait into weedy relatives as mentioned above for red rice and sunflower. In those instances, where the weedy relative could be troublesome for following crops, a detailed stewardship program may be needed. For the Clearfield technologies, such a stewardship program has been designed by the company marketing the modified crop plant and all producers are supposed to sign and abide by the requirements of the agreement. Similarly, there are stewardship agreements for use of Bt maize (Sloderbeck 2006). Insect resistance may give weedy relatives an advantage (see discussion of Snow et al. 2003 below).

So far there is little evidence for differential gene flow. Rates of trait transfer are presumed to be independent of the nature of the gene being transferred (e.g. for virus, herbicide or insect resistance), but too few studies have been done to assure this. As discussed below (Halfhill et al., 2004), different constructs of the same resistance gene, with different chromosomal locations, do appear to migrate at different rates in the case of insect resistance (Bt toxin gene) during hybridization of different brassica species. Gene flow is one of the many topics considered extensively at meetings of the International Society for Biosafety Research. For the most recent published proceedings see ISBR (2004), available at their web site.

9.1.1. Virus resistance

One species containing virus resistance traits is in extensive production. That is the papaya resistant to ringspot virus, growing in Hawaii. There the need was extremely strong because the virus was rapidly devastating the standard cultivars being grown in intensive agricultural settings (Perry, 2005). Trees are traditionally grown from saved seed using repeated selection for desirable cultivars and landraces. Trees take only a few years to reach peak production. Little information was available on gene flow for that species, until a recent study undertaken by the organization GMO Free Hawaii, to detect contamination of traditional cultivars by the transgenes from pollen of the transgenic cultivars, which include a GUS gene amenable to rapid screening tests. (Bondera and Query, 2006). Results were different for various islands in the chain but indicated high levels of contamination. Later PCR tests for the 35-S promoter (driving expression of the virus coat protein transgene) ranged up to 50% of seeds in some bulked samples of organic and home garden fruits. While there is no evidence of human health hazard associated with the GMO, loss of markets such as Japan which did not accept GMO fruits, and loss of purity in traditional land races of papaya, have caused considerable dissatisfaction amongst parts of the local population.

As noted for herbicide resistance in brassicas below, contamination of certified seed stocks is an issue. It appears that between one and 10 seeds per 10,000 of the traditional non-GMO cultivars being distributed by the University of Hawaii are contaminated (transgenic). The widespread small-scale cultivation of papaya by individuals and the wide-spread, perhaps long-range, gene flow made evident by the studies of Bondera and Query (2006) indicate that this will be a very difficult system to control. Mechanisms and frequencies of gene flow could not be determined from the sampling design used. The selective advantage of resistant trees under virus infection pressure will encourage their spread, both in cultivation and as weedy feral trees. Views on the benefits and costs of transgenic papaya are highly divergent, with the Hawaii Papaya Industry Association very positive (Perry, 2005) and the GMO Free Hawaii group quite negative (Bondera and Query, 2006).

Another species complex with virus resistance genes introduced is the summer squash (*Cucurbita pepo*). As with the papaya, a viral coat protein is used to introduce viral resistance in the plants. Multiple genes, specific to several viruses were simultaneously introduced. Fuchs et al (2004a) monitored movement of the protein genes from commercial squash to a wild relative, *Curcubita pepo* ssp *ovifera* var *texana* (*C. texana*). In field settings, gene transfer occurred only when the wild relative was not under severe virus selection. Once transferred, the genes were expressed, yielding resistance to the three viruses for which coat proteins had been introduced into the transgenic form. Under low disease pressure the wild *C*

texana out-performed all of the various hybrids and backcrosses (F1, BC1, BC2). Under high pressure, the transgenic backcrosses to *C. texana* outperformed both parents, and only one back cross was needed to recover most traits of *C. texana*. So it appears that timing of the introgression event relative to viral disease pressure may be important to whether it spreads in the wild population or not.

The insect-pollinated cucurbits provide a complex pattern of natural gene flow with low frequencies at distances >1 km, but not exceeding 5% even when plants are close together (Kirkpatrick and Wilson 1988). Pollen of the pistillate parent received preference (Wilson and Payne 1994). Other species: such as rice and maize discussed below show different spatial effects on gene flow because they are wind pollinated. Implications of gene flow within the *C. pepo* complex between cultivated and feral types was discussed in some detail by Wilson (1993) during the time of approval and review for release of the transgenic cultivars. Issues raised at that time included extensive documentation that *C. texana* is a common noxious weed in many specific areas (soil types) over areas including those in which the transgenic squash would be grown. This raises a significant concern of increased weediness under viral disease pressure, where plants with the introgressed genes might well have a reproductive advantage (Fuchs et al., 2004b). The market acceptance (by producers) of transgenic squash is relatively low (<15 %) because it does not prevent all viral diseases and the seed is 78 % more costly (Sankula et al., 2005). Commercial production only partially overlaps the range of the feral gourd type. There is thus far no report of increased weediness of the wild *C. texana* due to introgression of viral resistance.

9.1.2. Herbicide resistance

In Canada, large quantities of oilseed brassicas are grown with herbicide resistance genes present. Beckie et al. (2003) examined gene flow between commercial fields of glyphosate and glufosinate resistant cultivars at distances up to 800 m. Eleven sites were studied in 1999, sampling seeds and testing for resistance. In the following year, volunteer seedlings that escaped herbicide control were tested for double resistance at three locations. Rates of gene flow at field edges were above 1%, but only 0.04% at 400 m in the 1999 sampling. However doubly-resistant volunteer plants were found to the maximum distance of 800 m. In two of the three locations sampled in 2000, it was concluded that the glyphosate-resistant seed used the previous year was adventitiously contaminated with glufosinate resistance. This provides an example of why a vigorous stewardship program is essential to maintain the integrity of herbicide resistant crops.

The central U.S. is an area with large production of sunflower for oil, and in addition the source of diversity of

the crop species *Helianthus annuus*. An imidazolinone-resistant (IMI) biotype of wild sunflower was first identified in Kansas, and the group making that identification examined the facility of gene flow from improved, domesticated strains to wild relatives (Massinga et al., 2003). Both common sunflower (*H. annuus*) and prairie sunflower (*H. petiolaris*) were highly receptive to pollen from the IMI type in controlled crosses. In field studies, 11-22 % of seedling progeny were IMI resistant when wild sunflower was grown 2.5 m from a dense patch of IMI-resistant domestic sunflower while at 30 m 0.5 - 3% were resistant during one season. Somewhat lower levels of resistance gene transfer were seen in a second year of study. The maximum distance over which gene flow is likely was not determined in these studies, nor was the distance needed to reduce transfer below 0.1% which is significant for acceptance of a transgenic crop in the E.U., where contamination of food or feed grains is a major concern.

The likelihood of gene flow from modified crop plants to unmodified cultivars and weedy relatives has been examined in some considerable detail for both maize and oilseed rape (*Brassica* spp) by the U.K Department for Environment, Food and Rural Affairs (DEFRA). Extensive reports are available (Henry et al., 2003; Ramsay et al., 2003). For forage maize, some gene flow was detected at distances greater than 200 m, although the level of gene flow dropped rapidly within the first 20 m (Henry et al., 2003). With rapeseed there was some gene transfer detected at distances up to 26 km, although relatively short distances (tens of meters) were required to lower the level of contamination of surrounding crops to below 0.1% (Ramsay et al., 2003). The long distance transfer was attributed to a particular insect, the pollen beetle which travels over long distances compared to bees which forage over a few km. One important finding of this study was that for this crop, insect pollination is predominant over wind pollination.

DuPont Company, through its subsidiary Pioneer Seed, maintains a highly informative website which provides extensive reviews of several issues concerning genetically engineered plants, including concerns for gene flow. As discussed on that site, gene flow is a much smaller issue for a plant such as soybean which is almost exclusively self-pollinated prior to opening of the flower (DuPont, 2006). Presumably the same would be true of herbicide resistant lentils developed by BASF.

For rice, which is a major food crop for about half of the world, detailed studies of gene flow have been done (Zhang et al., 2003; Chen et al., 2004; Lu and Snow, 2005). Zhang et al. (2003) studied a glufosinate resistant (bar gene) cultivar of rice grown in Louisiana, and monitored several traits in spontaneous crosses with red (weedy) rice and a purple leafed rice grown in all combinations as 1:1 mixtures in large plots. All three types bloomed at the same time in the initial year. An out-crossing rate of 0.3 % was observed with the bar gene but the hybrid progeny showed decreased fitness, and

most bloomed too late to produce seed in a field. The purple marker trait was transferred at a frequency of <1%. Chen et al. (2004) studied rice in Korea and China. With weedy rice (called red rice in the U.S.) gene flow, as measured by appearance of marker genes, was relatively low at less than 1/1000 seeds, when the cultivated and wild rice were growing close together. For different types of weedy rice and different commercial cultivars the degree of introgression varied as a function of the timing of anthesis and height of plants. Wind pollination of plants with short pollen viability is expected to show such a pattern. With wild perennial rice (*Oryza rufipogon*), a higher frequency of >1/100 was observed. Chen et al. (2004) cite several earlier studies indicating that gene flow to weedy rice may also approach 1% or higher in some cropping systems. The observations on red rice in the U.S. discussed above under Clearfield technologies are consistent with this. If red rice is not fully controlled by herbicide application in the first year, it is likely that in the next year there will be resistant plants in herbicide-treated fields. If a plant produces 1000 seeds of which 10 are herbicide resistant, a major problem could rapidly ensue, unless an alternative herbicide is used. However, few of the hybrids may survive and establish if the observations on flowering time reported by Zhang et al. (2003) are of general application. They observed that hybrid plants bloomed too late to set seed.

Lu and Snow (2005) have provided a table showing that already by 2004 there were several dozen transgenic rice cultivars being tested for a wide range of applications beyond herbicide resistance. However, the gene flow properties of these transgenes are likely to be essentially the same as those for herbicide resistance. In some cases, only a strong selection would give an advantage to the form carrying the transgene. Lu and Snow suggest that a systematic examination of ecological risks is urgently needed, because few studies have examined changes of population fitness that might accrue from introduction of new traits into wild or weedy populations.

9.1.3. Insect resistance

Halfhill et al. (2002) tested the effect of Bt toxin gene movement from oilseed rape (*Brassica napus*, 2n=38) to its wild relative *B. rapa* (2n=20). Following repeated backcrosses to the *B. rapa*, progeny from half of the six transformant *B. napus* lines that had been tested as donors had lost the Bt trait, along with the phenotypic characters of the donor parent. Ploidy level also declined to near that of the *B. rapa* parent. Because *Agrobacterium* transformation was used to introduce the Bt genes into the *B. napus*, it was expected that some lines (with different locations of DNA insertion) would give more effective gene transfer to *B. rapa* than others. Also the levels of expression of the Bt toxin protein varied between lines. Generally, the surviving progeny expressed the protein at levels comparable to the *B. napus* donor. This

level was sufficient to deter feeding by corn earworm in controlled feeding tests. The general fitness of these transgenic *B. rapa* was not directly compared to that of its progenitors, so it is not possible to speculate on fitness in the absence of insect predation.

No studies were done under natural conditions by Halfhill et al (2002) to assess the extent to which expression of the Bt toxin protein might protect from insect predation in natural settings. However, field studies were done to show that *B. rapa* growing in the midst of the oilseed *B. napus* did produce hybrids with the resistance gene. The frequency varied from <1 % to nearly 17 %, depending on the *B. napus* line used as donor. Further study of deliberately constructed hybrids and their backcrosses to the weedy *B. rapa* showed that the Bt gene is stably expressed at levels comparable to those in the donor *B. napus* (Zhu et al., 2004). Thus under insect pressure, the transgenic wild plants might have a significant fitness advantage. The implications for weediness in natural ecosystems are unclear. Because no brassicas containing the Bt toxin have been released commercially, no large scale studies have been done in agricultural settings.

In the U.S., part of the stewardship plan for use of Bt maize crops is to provide refuges for susceptible insects by planting non-Bt crops as a proportion of the entire acreage (Sloderbeck, 2006). In the northern U.S. the requirement is 20%, for cotton-growing regions it is 50% because maize is the alternate host of the cotton bollworm. Chilcutt and Tabashnik (2004) documented gene flow from transgenic maize carrying Bt genes. Pollen-mediated gene flow resulted in kernels containing the Bt gene at distances up to 31 m. At 3 m (three rows), over 15% of the kernels carried the trait, and at 8 m it was near 10%. This raises the concern that insects will be exposed to low levels of Bt within the refuge areas, giving a selective advantage to heterozygous insects which would be exposed to non-lethal doses of the Bt toxin. Refuge strips are permitted to be as narrow as four rows (4 m) (Sloderbeck, 2006) and must be within ½ mile of the Bt crop. The extent of gene flow reported here indicates that maize from refuge areas, or adjacent fields, not intended as refuge, and perhaps with a different owner, may well carry the Bt gene at significant levels, so that it ought not to enter the food chain as unengineered grain. In addition, many of the particular combinations of stacked insect resistance alone or stacked with herbicide resistance, do not qualify for food and feed in the E.U. Companies selling those products provide detailed stewardship plans and producer instructions (e.g. Monsanto, 2006b).

9.2. Potential insect population shifts in a Bt containing crop

When the Cry proteins (BT toxins) are expressed in all parts of the maize or cotton plant, there is some risk that

other nontarget insects coming into contact with the protein could be affected. For instance the pollen of maize, which drifts a considerable distance from the plant, could potentially be toxic to lepidopteran larvae feeding on plants in the vicinity of maize. It was suggested that this could be a hazard for monarch butterflies consuming leaves of milkweed near fields of transgenic maize. However, the initial suggestion, though published in a prominent magazine, could not be reproduced, and there is likely to be only a small risk, as shown by a thorough risk analysis (Sears et al., 2001).

The work of Tabashnik and Carriere (2004) documents the important point that presence of Bt toxin protein in crops does not give a selective advantage to resistant insects. They reviewed studies that used natural populations selected in responses to sprayed Bt bacteria on crops. The authors cite two cases in which there was no difference between normal and resistant biotypes, and eight studies in which the resistant strains are disadvantaged when growing on Bt plants compared to unmodified plants. The most reasonable conclusion is that the resistance is not complete and transgenic Bt plants have on average much higher doses of Bt than applied by direct spraying of the bacteria.

Studies in the southeastern parts of the U.S. have indicated no adverse effect of cotton containing Cry genes on natural predators of the bollworm (Moar et al., 2002). Compared to fields sprayed with regular insecticides, predation on bollworm eggs was significantly increased. Populations of nontarget insects were not significantly affected in any of several test areas. More recent papers have confirmed and extended these observations (Sisterson et al., 2004; Hagerty et al., 2005). Sisterson et al (2004) scored all arthropods (except pink bollworm and nymphs of whitefly) on Bt and non-Bt cotton grown alone or as a mixture of 75 % Bt:25 % non-BT. The greatest abundance and diversity was observed in the mixed plot but there was not a significant difference between the Bt and non-Bt plots, although the Bt plots did have a lower abundance and diversity of arthropod families. Over 3300 individuals were found during 3 sampling dates (over 2 years at 2 sites) with a final total of 120 plants for each treatment. Thus about 10 arthropods per plant were found, even though some of the fields had been treated with insecticide for control of some insects other than pink bollworms.

Hagerty et al. (2005) considered the impact of transgenic cotton, with and without insecticide application, on arthropod abundance. Predators that feed on the bollworm were of particular interest. In both Bollgard (containing Cry1Ac) and Bollgard II (containing Cry1Ac + Cry2Ab) plots the populations of predators were as high as or higher than in the non-Bt cotton, when no insecticide was used early in the cropping season. This may have been because the non-Bt plants were severely damaged and could support only a smaller population of prey insects. Disruption of the predator population by broad spectrum insecticide treatment in one season

resulted in the bollworm population reaching an economic threshold, even when Bollgard II was used, because the moths producing worms invade from maize, sometimes in great numbers. The authors concluded that their results were consistent with earlier cited studies in showing that the presence of Cry proteins does not reduce predator populations, and less use of insecticides leads to an increase in generalist predators (as well as prey).

Dutton et al (2003) describe a general approach to assessing the risk to nontarget insects from use of Bt transgenic plants. Their work is done in the European context, where there are relatively stringent assessments required for transgenic crops, with potentially a near infinite number of predators, competitors, symbionts and parasitoids to be considered. They considered in detail one entomophagous insect, the green lacewing (*Chrysoperla carnea*), on Bt-maize. Using a tiered approach they first tested the direct toxicity of Bt toxin protein, then the toxicity of prey insects fed with Bt toxin, and then the behavioral preferences of the predator. The preferred prey insects, aphids and spidermites, were not toxic to lacewings when feeding on Bt-maize. Nor was a nutrient solution containing the toxin. Lepidopterans, which are affected by the toxin, were not a preferred prey. Hence the risk to lacewings of Bt-maize is minimal. Field tests have confirmed that observation, as cited by the authors. In the case of the lacewing, testing could have stopped once it was shown that the toxin had no effect when fed directly at levels higher than likely to be encountered in the field. The authors propose that formal analysis can be done for other predators, by identifying those that feed on lepidopterans and hence would be exposed, and then determining their sensitivity to the toxin first in the laboratory, then if needed in plants in controlled environments and finally in field studies.

9.3. Demonstrable ecosystem impact of a transgene migration

Jenczewski et al. (2003) reviewed the literature on crop to wild plant gene flow and found that there were few examples providing unambiguous evidence on the relative fitness of specific genes. Examples were cited in which the hybrids showed increased vigor in the F1 and other instances of decreased fertility in the F2. Few studies have monitored a population beyond that time.

One example where movement of a transgene from cultivated plant to a wild relative has a demonstrable effect is a study by Snow et al. (2003) of wild sunflower carrying a Bt insect resistance gene. In this instance, insect predation was reduced and hence reproductive fitness was increased in a wild population. A similar scenario might be envisioned for a rice transgene enhancing insect resistance, if insect predation were a strong limiting factor in the weedy population. Snow et al. (2003) found that wild sunflower produced 55% more seeds at one site in Nebraska when carrying the *cry1Ac*

gene which reduced lepidopteran damage. Weevil and fly damage was unaltered. At a second site in Colorado the seed increase was 14% (but not significantly). This appears to be the first experiment to show at a field scale that a transgene confers a clear fitness advantage in a natural (non-agricultural) setting. Whether seed production is a limiting factor in wild sunflower populations is not discussed.

9.4. Risk of novel traits vs risk of genetic engineering

All of the above engineered or custom-tailored crops, such as the imidazolinone resistant maize can be found described in more detail at the Canadian food inspection agency website under "Decision documents-determination of environmental and livestock feed safety" (Canada, 2006). In Canada, it is a government policy to examine novel traits whether they were produced by gene transfer to constitute GMOs or if they arose by selection of induced or spontaneous mutation. The rationale is that appearance of a novel trait is the key consideration, rather than if it was engineered by use of recombinant DNA techniques. Thus one can find somaclonal variants, induced and spontaneous mutants, and engineered modifications (typically via *Agrobacterium*-mediated transformation) all receiving the same review. At that site one finds that lentils (*Lens culinaris*) have been selected for resistance to imidazolinone, although none are indicated as commercially available yet. BASF has also reported to the Canadian authorities a maize line resistant to sethoxydim, a herbicide of a different family specific for grasses (acetyl-CoA carboxylase inhibitors). There are a number of crops with glufosinate resistance (bar gene, Basta or Liberty herbicide), sugar beet resistant to glyphosate, cotton resistant to bromoxynil, and various insect resistant crops. A few viruses are on the list also, as well as a few crops with altered lipid composition. The last of these do not appear to be marketed yet.

10. Perceived risks vs quantifiable effects

10.1. Altered composition

A fundamental difference in viewpoints between different parties to the process of development and deployment of modified crops may be seen in the following example. The organization Greenpeace responded to a notification for placing on the market glufosinate-tolerant rice (Monsanto LibertyLink LLRice62). They invoked the precautionary principle that because there was "no proof of no adverse effects on genome function", there should be no release of the rice. A quantitatively more substantial comment pertained to the risk of red rice acquiring herbicide resistance. Zhang et al. (2003) put this risk at about 0.3% per year. Also Greenpeace indicated that because the composition (protein, starch,

lignin etc) of the rice was not reported, it could have an altered composition.

An altered composition of the plant not obviously related to the transgene in question had been posited for the impact of Bt toxin transgenes in maize. Jung and Schaeffer (2004) undertook an extensive study of lignification and digestibility of maize stover because there had been suggestions that the hybrids contain Cry1Ab might contain more lignin. Using four locations and 12 commercial hybrids (paired, half with and half without the Bt toxin) they examined yield, digestibility and lignin content (by three different assays). No consistent differences between Bt/ non-Bt pairs were observed in any of the measures, and for two pairs there was no difference in lignin at any location. There were environment, and hybrid x environment interactions as expected but not related to the presence or absence of the Bt trait. Differences in composition are anticipated for hybrids derived by conventional breeding and are often sought out deliberately.

10.2. Altered survival in natural conditions

Two early studies in the U.K. by Crawley et al.(1993, 2001) showed that transgenic crops had no increase in invasive potential compared to their unmodified counterparts, and none survived in natural conditions over several years. Included were sugar beet with Roundup Ready character, potato with the Bt insect resistance gene, maize and rapeseed with glufosinate tolerance (bar gene). This latter trait is for resistance to the herbicides Basta or Liberty. A dozen habitats in four regions of the U.K were studied up to 10 years, until extinction of the introduced plants. There are no reports that there is increased survival of transgenic plants in the absence of selective pressure for the trait in question.

For a virus-resistant transgenic cucurbit, summer squash, studies were done by the developer to show that it is not more likely to overwinter than non-transgenic cultivars of the same type (NRC, 2002). It is not considered a weed and so would not show increased weediness due to virus resistance.

10.3. Potential emergent traits

The European Union initially accepted a number of GM crops, including herbicide resistant tobacco, oilseed rape, soybean, chicory, carnations, and maize. However, a reaction set in and by 1999 there was a moratorium on approval of new crops. In 2002 a new directive on deliberate release of GM crops was in effect (Madsen and Sandoe, 2005). At this point there are specific regulations on labeling and traceability of GM in food and feed products. Although considerable quantities are imported, only a relatively small area of GM crops was grown by 2005 (~100,000 ha of maize in aggregate for five

countries) (James, 2006). Public perceptions and lack of trust in government have led to this situation (Madsen and Sandoe, 2005). With more than a dozen GM crops approved for growth, few are actually grown. It is suggested that both gene technology and herbicide use prompt a "dread" response, amongst the public in Europe. Concern for human health effects, such as allergies, and fears of invasiveness seem to be the major factors. There is thus little economic incentive to develop or introduce new crops even in those countries in which the activities are not expressly prohibited. The surveys upon which the attitudinal information is based (Eurobarometers) did not include the "novel traits" obtained without genetic engineering, so it is unclear how the general public in E.U. states might view Clearfield technology.

Quite recently, the E.U. has accepted Herculex I maize for import to use in animal and human food, although not to grow in Europe (Dupont, 2006). The Herculex trait is a Bt gene introduced with a bar gene so that the plants are resistant to European corn borer and the glufosinate herbicide. Not yet approved are maize lines with stacked resistance to glyphosate herbicide, or a Bt gene for root worm resistance alone (Herculex RW) or in combination with the previous Bt gene (Herculex XTRA). The most recent literature from Monsanto indicates that the majority of their modified maize lines are also awaiting approval (Monsanto, 2006b). It should be noted that small amounts of Bt maize (<100,000 ha) are already being grown in several member states of the E.U. (James, 2006).

11. Concluding comments

During the preparation of this review, many transgenic plants were noted in searches of websites and formal databases. Many agricultural and horticultural crop plants have been engineered for expression of genes that may enhance their resistance to insects or fungi, increase salt and drought tolerance, increase levels of essential nutrient and vitamin accumulation, amongst other traits. However, very few have been introduced to the commercial development stage. Almost all of those that have been are all mentioned above. As discussed by Devine (2004) with regard to herbicide resistance, high costs of regulatory clearance, and international trade issues are likely to delay introduction, perhaps indefinitely for many traits. As noted in a recent review of prospects for India (Bhat and Chopra, 2005), crops for which there is little or no external trade may be more amenable to engineering until such time as regulatory acceptance becomes more routine and less costly. Thus for Basmati rice which is extensively traded to Japan, transgenic forms might not be useful at this time because of market resistance, whereas for tomatoes there might be considerable benefit in using an already available technology to delay ripening (even though it was not a

commercial success in the U.S. and was withdrawn from the European market in 1999 because of concerns over GMO foods).

Until such time as the general public in many countries is more willing to accept GMOs, further studies are needed at a field scale in the U.S. and elsewhere to provide additional documentation of the extent to which GMOs pose risks not strictly comparable to those of non-GMOs. Many GMOs have proven of great value in research, enhancing our understanding of metabolic pathways. In some cases, "traditional" breeding strategies may permit exploitation of that knowledge without use of GMOs per se.

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